

# Diazotroph community and activity in the Southern Indian Ocean

SUBHADEEP CHOWDHURY<sup>1</sup>, Hugo Berthelot<sup>2</sup>, Stéphane L'Helguen<sup>2</sup>, Jean-François Maguer<sup>2</sup>, Carolin Löscher<sup>3</sup>, Arvind Singh<sup>4</sup>, Nicolas Cassar<sup>5</sup>, Sophie Bonnet<sup>1</sup>, and Mar Benavides<sup>1</sup>

<sup>1</sup>Aix Marseille Université, CNRS, Université de Toulon, IRD, OSU Pythéas, Mediterranean Institute of Oceanography (MIO), UM 110, 13288, Marseille, France

<sup>2</sup>Laboratoire des sciences de l'environnement marin, IUEM, Université de Brest-UMR 6539 CNRS/UBO/IRD, Technopole Brest-Iroise, 29280 Plouzane, France

<sup>3</sup>Nordceee, Department of Biology, University of Southern Denmark, Odense 5230, Denmark

<sup>4</sup>Geosciences Division, Physical Research Laboratory, Ahmedabad 380009, India

<sup>5</sup>Division of Earth and Climate Sciences, Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

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## Abstract

Dinitrogen (N<sub>2</sub>) fixers (diazotrophs) fuel primary productivity by providing reactive nitrogen into the ocean ecosystem and promoting CO<sub>2</sub> sequestration. N<sub>2</sub> fixation has been extensively studied in the low latitudes of the Atlantic and Pacific Oceans. By comparison, the Indian Ocean remains the least explored and most enigmatic ocean basin. This is particularly the case for the Southern Indian Ocean (SIO). Here we explore N<sub>2</sub> fixation activity and diazotroph community composition, diversity, and abundance from 20 to 60°S in the SIO. While this region plays a key biogeochemical role serving as a link between the Atlantic and South Pacific Ocean waters, its N<sub>2</sub> fixation potential remains unknown. Our results provide new insights into diazotrophy in a poorly studied region and expand the range of biomes where diazotrophy may be observed.

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Aix Marseille Université, CNRS, Université de Toulon, IRD, OSU Pythéas, Mediterranean Institute of Oceanography (MIO), UM 110, 13288, Marseille, France



**INTRODUCTION**

- N<sub>2</sub> fixation (diazotrophy) is the main nitrogen-fixing pathway in the ocean, which sustains productivity of C<sub>3</sub> production.
- The Indian Ocean (IO) is warmer than the other oceans, and it could be a hot spot for diazotroph activity.
- Potential for symbiotic N<sub>2</sub> fixers (diazotrophs) and particular environments for biomass production may witness its enhancement.

**OBJECTIVES**

- Diversity and activity of diazotrophs from subtropical to subpolar waters of the Indian Ocean.
- Evaluation of gene markers to assess the diazotroph community composition.

**METHODS**

- 2009/2010 Diatoms, Cruise R/V Marion Dufresne, January, Month 2011.
- Quantitative PCR of Trichodesmium sp., UCV1, A, UCVNLK1, and UCVNLK2.
- Thermal Milieu soft and hard.
- Statistic with temperature as the dependent variable.

**RESULTS**

Fig. 1: Temporal variation of thermal based enteropelagic

Fig. 2: Location of sites sampled in the IO

Fig. 3: N<sub>2</sub> fixation rates across the subpolar waters in the IO

Fig. 4: Relationship N<sub>2</sub> fixation rates, depth of Manganese nodule distribution and the South African coast, while nearly no available seafloor sediment front.

**DISCUSSION**

- The distribution of Trichodesmium and UCVNLK1 is very complex in this area, whereas it is known to be well correlated in other oceans.
- The diazotroph groups determined south of the subpolar gyre here have different activity to the N<sub>2</sub> fixers found in the North. These groups may be using PC fixation genes for other purposes.
- Depth of manganese nodule distribution decreased only by cold phylogeny. Manganese may provide a strong cold-watering of diazotroph increasing temperature.

**CONCLUSIONS**

- Trichodesmium is more abundant in >25°C, but temperatures are constant over in low temperature gradient.
- UCVNLK1 is more abundant earlier in the SO, when was in our interest, UCVNLK1 is the most abundant in the range of 15°C-20°C.
- We observe a clear latitudinal bands between symbionts and non-symbionts diazotrophs around the front, respectively.
- Despite the dominance of the symbionts along the front, the non-symbionts took front, location is questionable. Further researches of diazotrophs in cold areas is needed.

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## INTRODUCTION

- N<sub>2</sub> fixation (diazotrophy) is the main exogenous source of nitrogen (N) to the ocean, which sustains productivity and CO<sub>2</sub> sequestration (Karl et al., 2002 (<https://link.springer.com/article/10.1023%2FA%3A1015798105851>)).
- The Indian Ocean (IO) is warming faster than the other ocean basins and it could be a hotspot for N<sub>2</sub> fixation activity, but it is largely under-sampled (Hood et al., 2009 (<https://agupubs.onlinelibrary.wiley.com/doi/abs/10.1029/2007GM000714>); Hermes et al., 2019 (<https://www.frontiersin.org/articles/10.3389/fmars.2019.00355/full>)).
- Potential for yet unknown N<sub>2</sub> fixers (diazotrophs) and particular environments for heterotrophic N<sub>2</sub> fixation activity across its subtropical to subpolar gradients (Hermes et al., 2019 (<https://www.frontiersin.org/articles/10.3389/fmars.2019.00355/full>))).

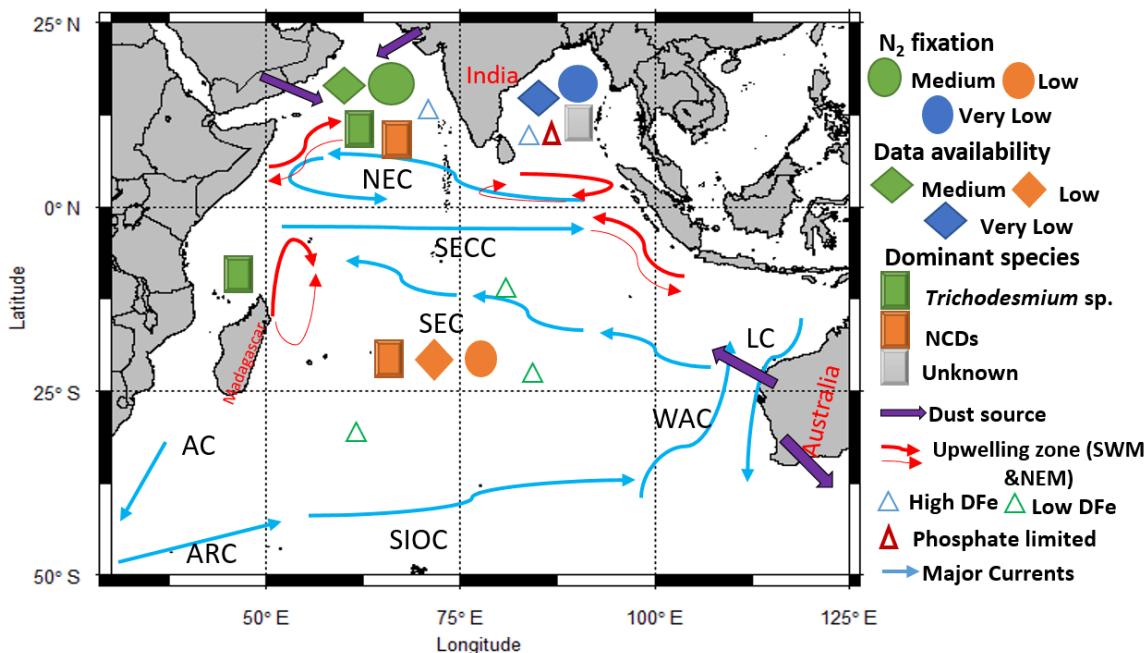


Fig 1: Summary of distribution of N<sub>2</sub> fixation, currents, biogeochemical parameters, data, dominant species, and dust source in the Indian Ocean (IO)

**Major Currents:** AC, ARC- Agulhas & Agulhas Return Current; SIOC- South Indian Ocean Current; LC- Leeuwin Current; WAC- West Australian Current; SEC, SECC- South Equatorial & South Equatorial Counter Current; NEC- North Equatorial Current

**SWM:** South West Monsoon; **NEM:** North East Monsoon

## OBJECTIVES

- Diversity and activity of diazotrophs from subtropical to subpolar waters of the Southern Indian Ocean (SIO)
- Evaluation of gene markers to assess the diazotroph community composition

## METHODS

- SWINGS Geotraces Cruise R/V Marion Dufresne, January- March 2021.
- Quantitative PCR of *Trichodesmium sp.*, UCYN-A1, UCYN-A2, Gamma A, and Gamma 4.
- Illumina MiSeq of *nifH* and *nifD*.
- Statistics with temperature as the dependent variable.

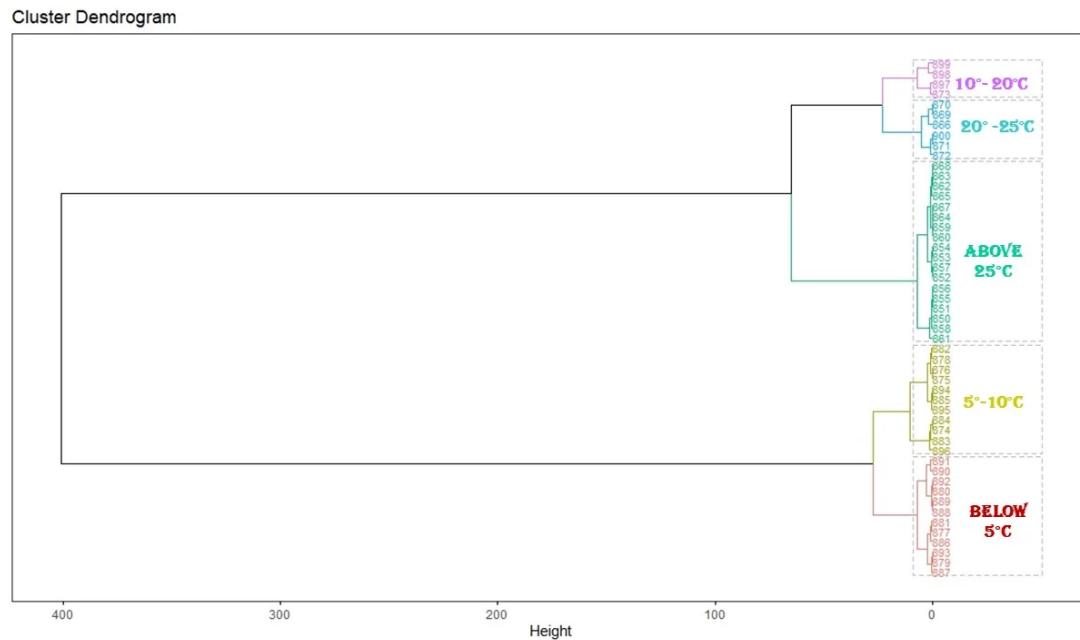


Fig 2: Sampled stations clustered based on temperature

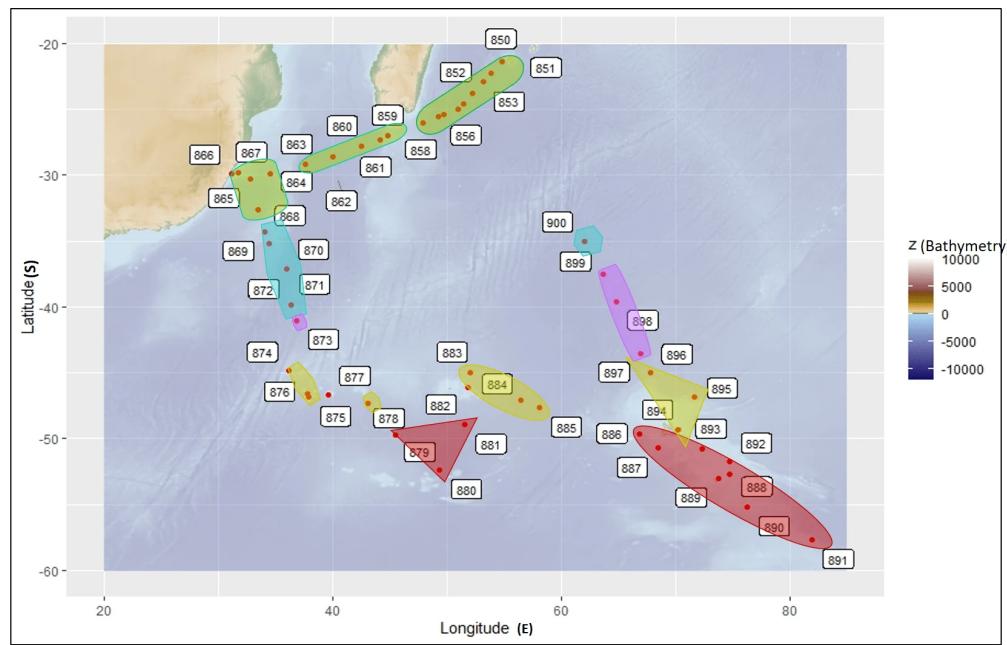


Fig 3: Location of the stations sampled in the SIO



## RESULTS

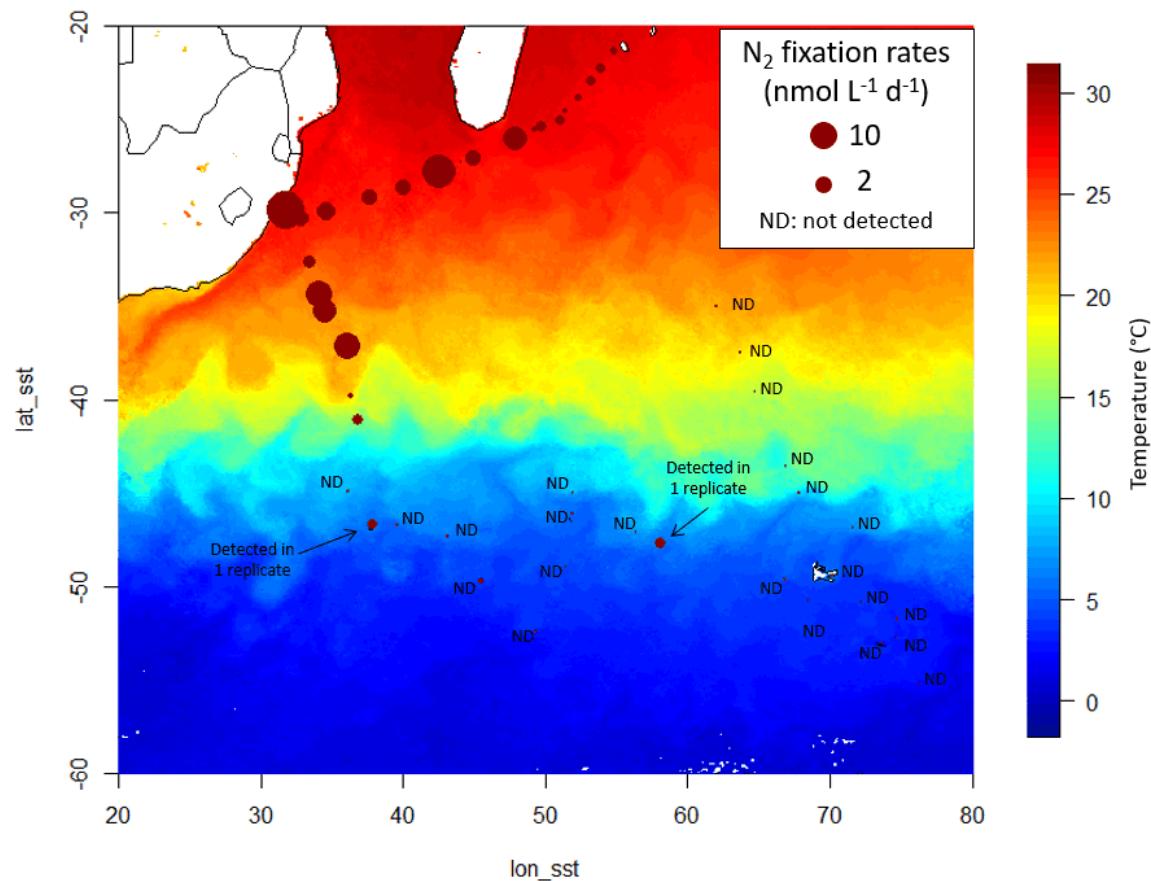
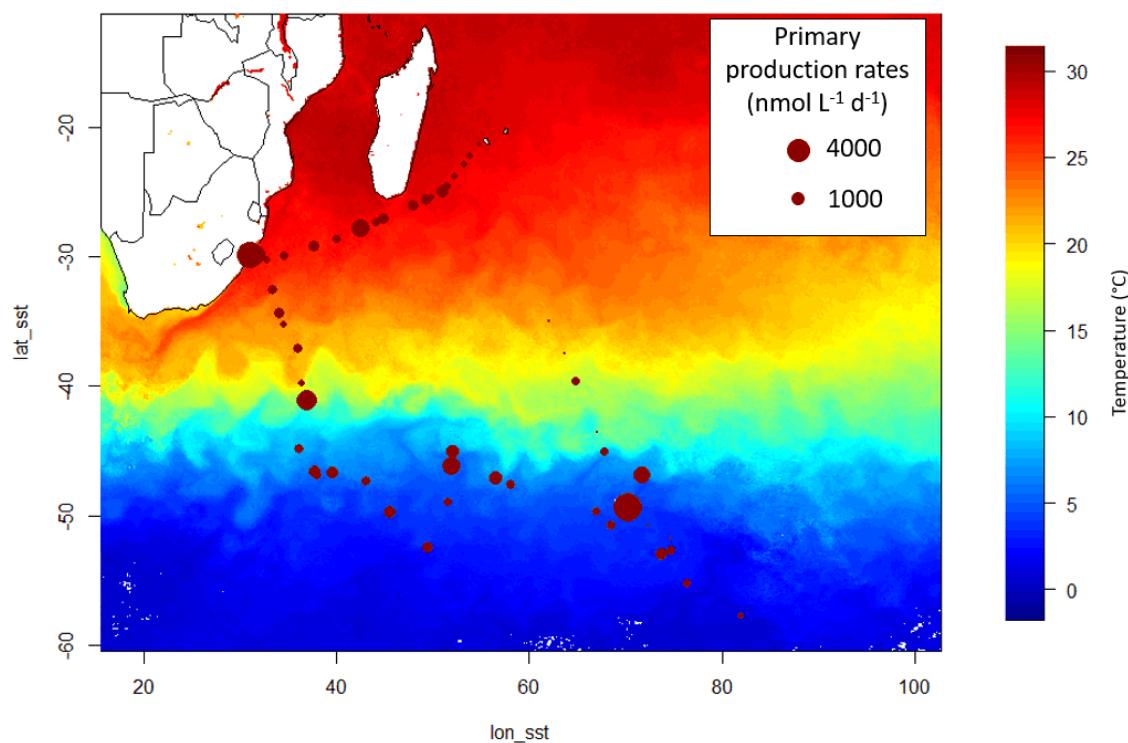
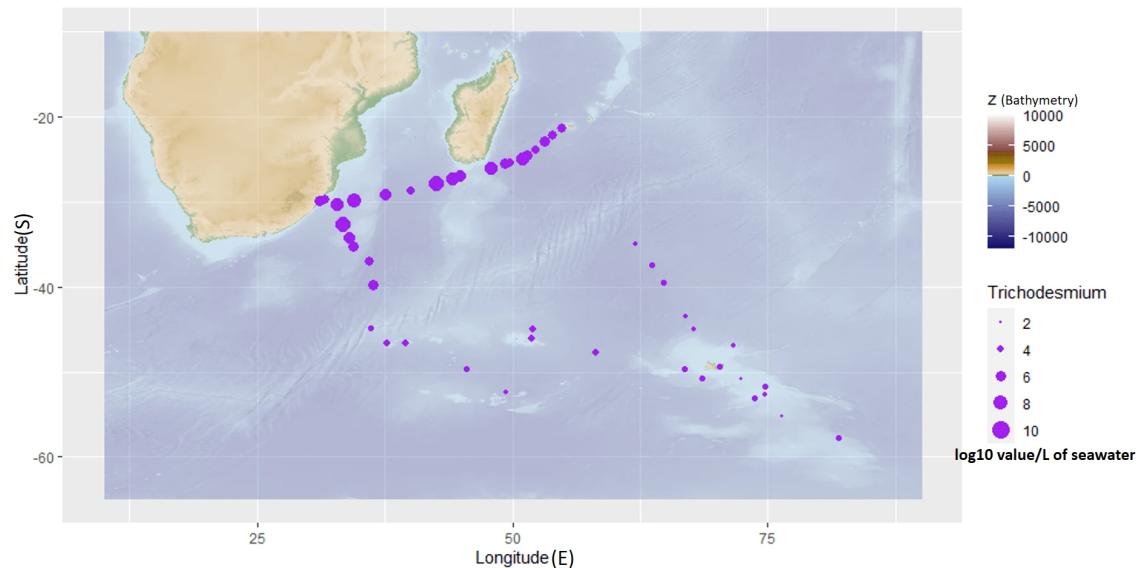


Fig 4:  $N_2$  fixation rates across the sampled stations in the SIO

- Relatively high N<sub>2</sub> fixation rates south of Madagascar coast and off the South African coast, while mostly undetectable south of the subtropical front.



*Fig 5: Primary productivity across the sampled stations in the SIO*



*Fig 6: qPCR abundance of the nifH gene of Trichodesmium in the SIO*

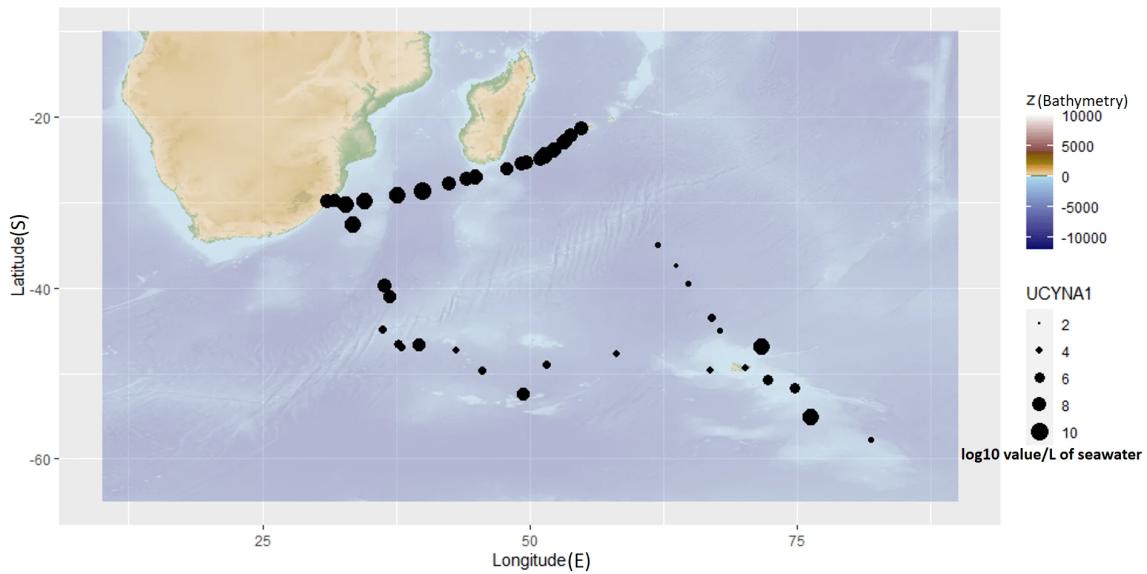


Fig 7: qPCR abundance of the *nifH* gene of UCYN-A1 in the SIO

- *Trichodesmium* and UCYN-A1 are more abundant in higher temperature

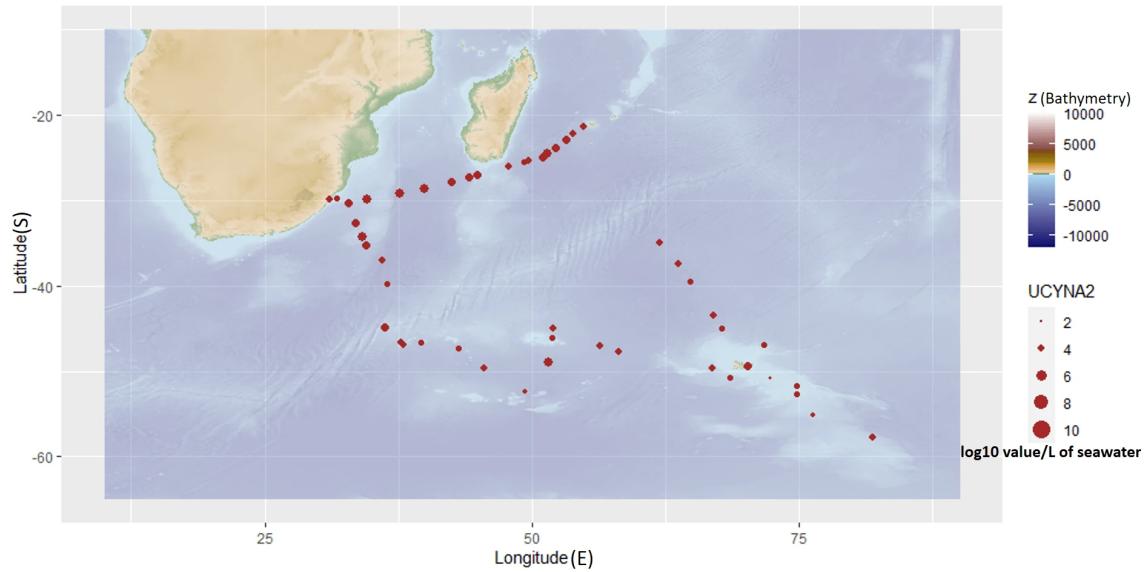
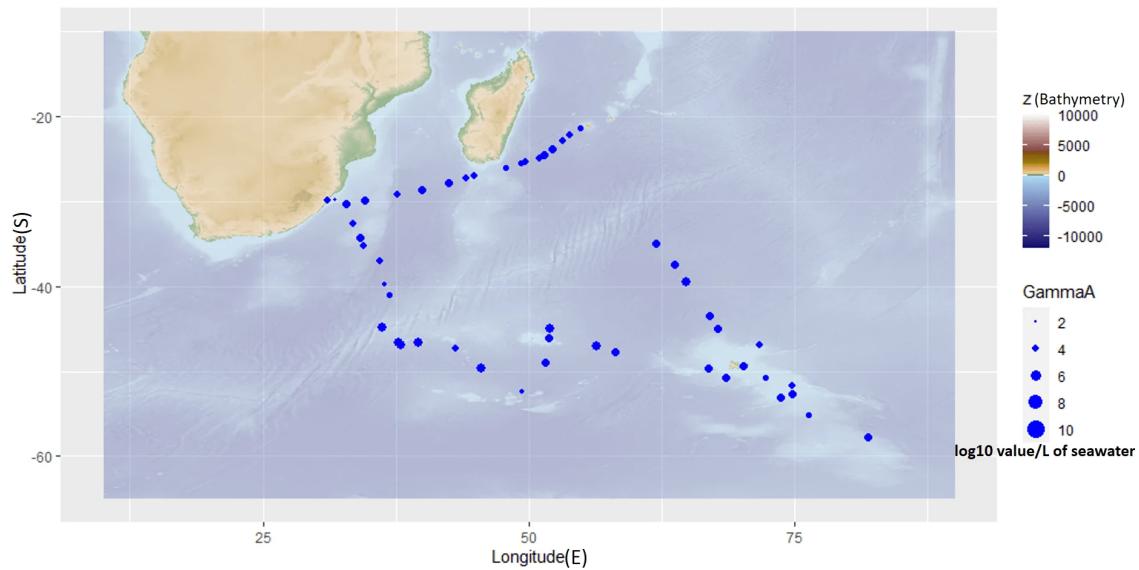
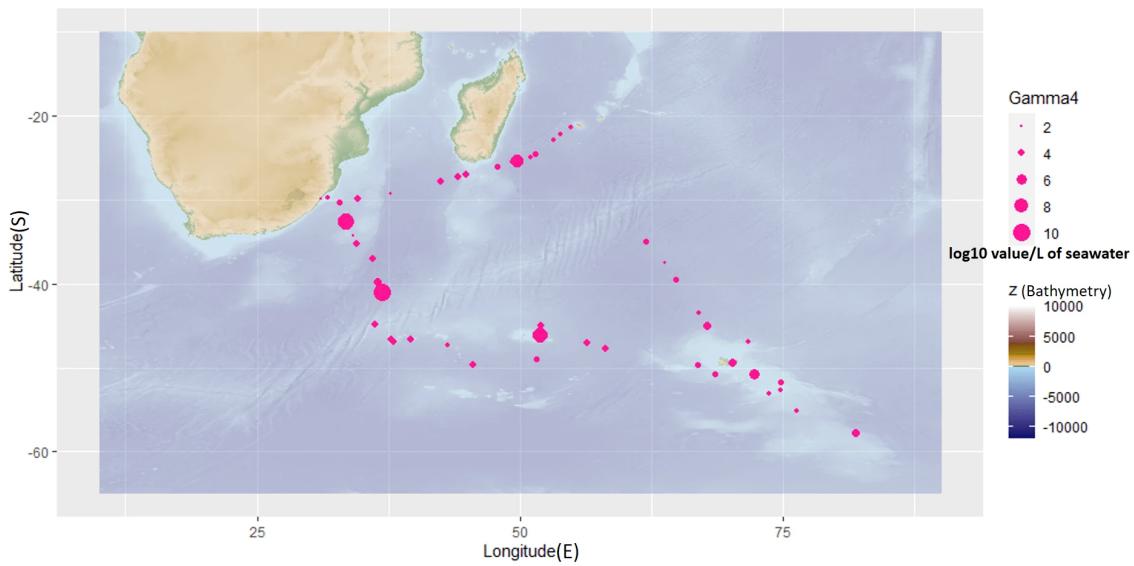


Fig 8: qPCR abundance of the *nifH* gene of UCYN-A2 in the SIO



*Fig 9: qPCR abundance of the *nifH* gene of Gamma A in the SIO*



*Fig 10: qPCR abundance of the *nifH* gene of Gamma 4 in the SIO*

- Spatial gradients of diazotrophs abundance are well observed in *Trichodesmium* and UCYN-A1 across the transect.
- UCYN-A2, Gamma A, and Gamma 4 are more evenly distributed across SIO.

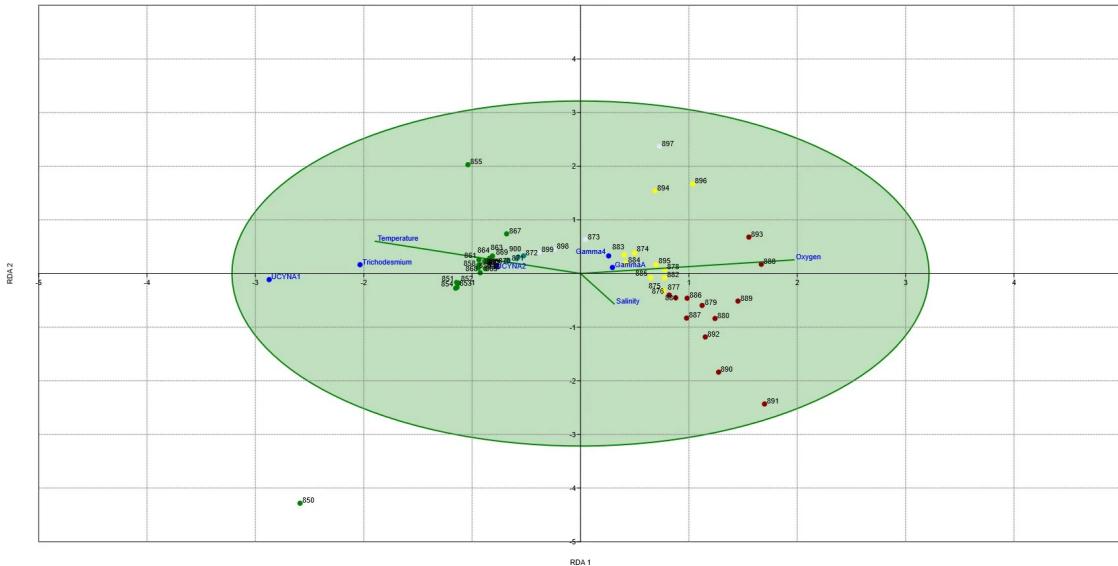


Fig 11: Redundancy analysis (RDA)

- RDA shows the most important environmental factor is temperature, influencing *Trichodesmium* abundance and Oxygen for Gamma A and Gamma 4 abundance.

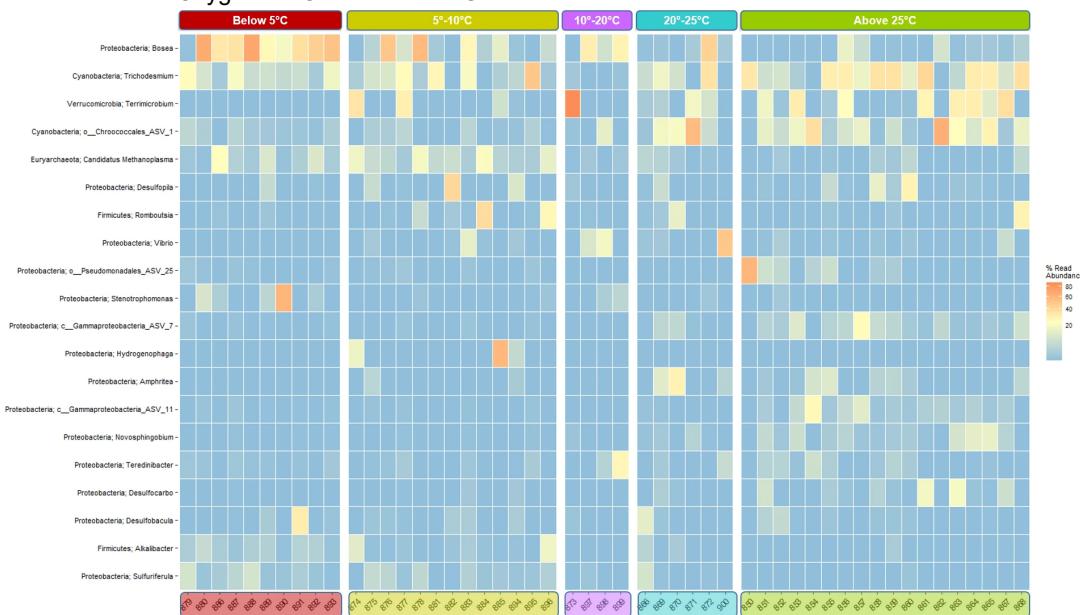
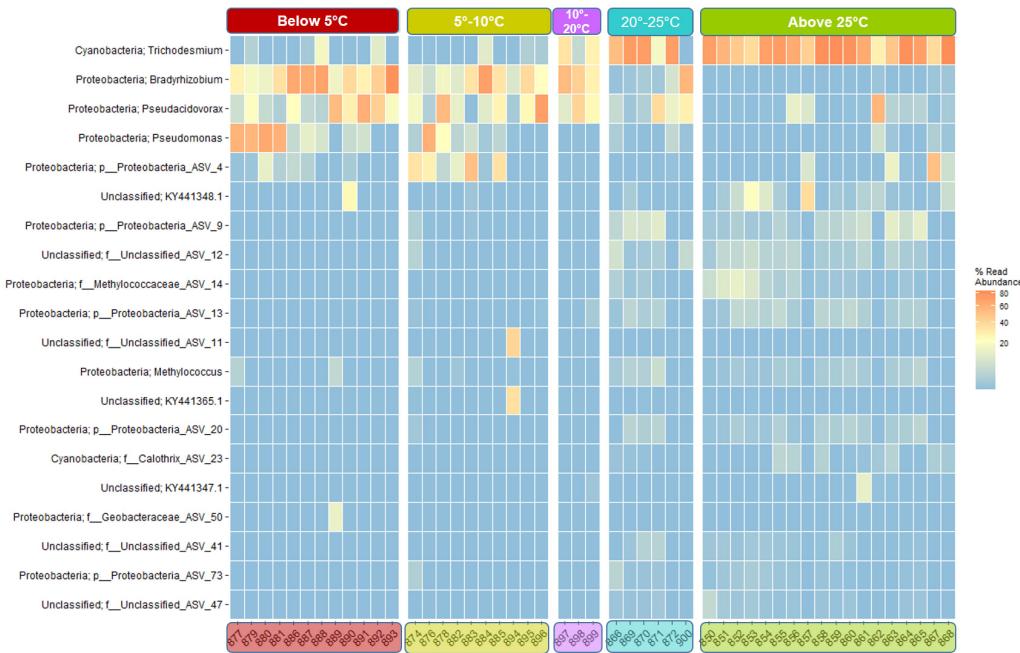
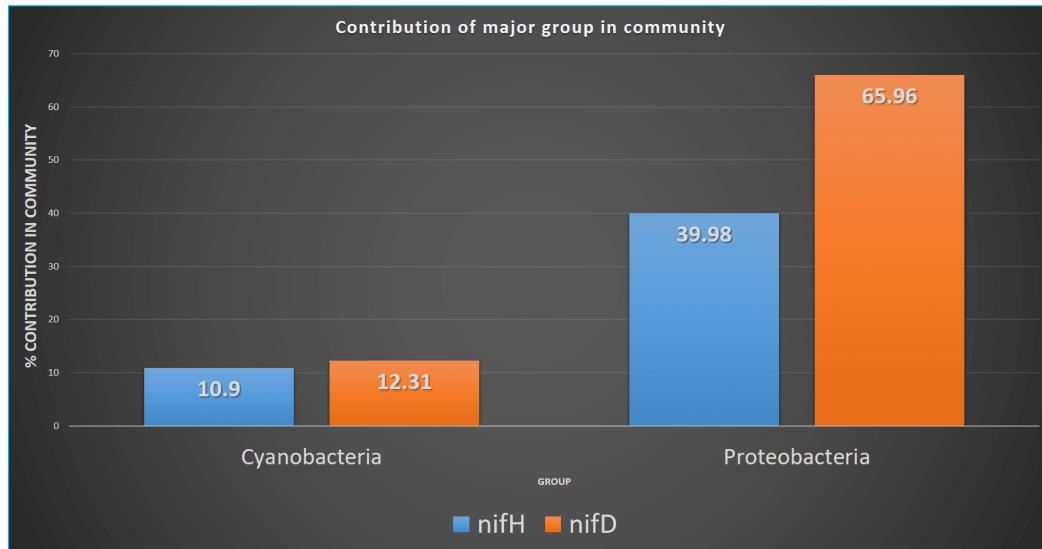


Fig 12: Diazotrophs community composition across temp. gradients in the SIO (top 20 most abundant ASVs based on Illumina nifH data)



*Fig 13: Diazotrophs community composition across temp. gradients in the SIO (top 20 most abundant ASVs based on Illumina nifD data)*

- *Trichodesmium* was dominant on the north of the subtropical front and Proteobacteria with putative sulfate and iron-reducing metabolism were dominant south of the subtropical front



*Fig 14: Comparison of major phylum group contributing to the diazotroph community in the SIO based on nifH and nifD dataset*

- The contribution of nifD is much higher for Proteobacterial diazotrophs (compared through phylum level ASVs)

## DISCUSSION

- The coexistence of *Trichodesmium* and UCYN- A1 is very unique in this area, whereas it is known to be anti-correlated in other ocean ecosystems.
- The diazotroph groups detected south of the subtropical front did not match any detectable N<sub>2</sub> fixation activity. These groups may be using N<sub>2</sub> fixation genes for other purposes.
- Many diazotroph groups cannot be detected only by *nifH* phylogeny. *nifD* sequencing may provide a deeper understanding of diazotroph community composition.

## CONCLUSIONS

- *Trichodesmium* is more abundant in >25°C, but sequences are present even in low temperatures gradient.
- UCYN-A was not detected earlier in the SIO, whereas in our transect, UCYN-A1 is the most abundant diazotroph unexpectedly at >25°C.
- We observe a clear latitudinal break between cyanobacteria and non-cyanobacterial diazotrophs north and south of the front, respectively.
- Despite the dominance of non-cyanobacterial diazotrophs south of the front, their contribution to N<sub>2</sub> fixation is questionable. Further inspections of diazotrophs in mid-and high-latitudes are needed.

## DISCLOSURES

- IFCPAR/CEFIPRA for the grants of the projects “DINDE” endorsed by IIOE-2
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## AUTHOR INFORMATION

<sup>1</sup>Presenting Author: Subhadeep Chowdhury

Aix Marseille Université, CNRS, Université de Toulon, IRD, OSU Pythéas, Mediterranean Institute of Oceanography (MIO), UM 110, 13288, Marseille, France

subhadeep.chowdhury@mio.osupytheas.fr (mailto:subhadeep.chowdhury@mio.osupytheas.fr)

www.oceanbridges.net (http://www.oceanbridges.net/)

<sup>2</sup>Hugo Berthelot, Stéphane L'Helguen, Jean-François Maguer

Laboratoire des sciences de l'environnement marin, IUEM, Université de Brest-UMR 6539 CNRS/UBO/IRD, Technopole Brest-Iroise, 29280 Plouzane, France

<sup>3</sup>Carolin Löscher

Nordceee, Department of Biology, University of Southern Denmark, Odense 5230, Denmark

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Geosciences Division, Physical Research Laboratory, Ahmedabad 380009, India

<sup>5</sup>Nicolas Cassar

Division of Earth and Climate Sciences, Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

<sup>1</sup> Sophie Bonnet, Mar Benavides (corresponding author, Email- mar.benavides@ird.fr)

Aix Marseille Université, CNRS, Université de Toulon, IRD, OSU Pythéas, Mediterranean Institute of Oceanography (MIO), UM 110, 13288, Marseille, France

## ABSTRACT

Dinitrogen ( $N_2$ ) fixers (diazotrophs) fuel primary productivity by providing reactive nitrogen into the ocean ecosystem and promoting  $CO_2$  sequestration.  $N_2$  fixation has been extensively studied in the low latitudes of the Atlantic and Pacific Oceans. By comparison, the Indian Ocean remains the least explored and most enigmatic ocean basin. This is particularly the case for the Southern Indian Ocean (SIO). Here we explore  $N_2$  fixation activity and diazotroph community composition, diversity and abundance from 20 to 60°S in the SIO. While this region plays a key biogeochemical role serving as a link between the Atlantic and South Pacific Ocean waters, its  $N_2$  fixation potential remains unknown. Our results provide new insights into diazotrophy in a poorly studied region, and expand the range of biomes where diazotrophy may be observed.

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