Biological N_2 fixation in the upwelling region off NW Iberian Peninsula: magnitude, relevance and players

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Universidade de Vigo

DO MAR
PHD PROGRAM IN MARINE SCIENCE, TECHNOLOGY AND MANAGEMENT

Grupo de Oceanografía Biolóxica

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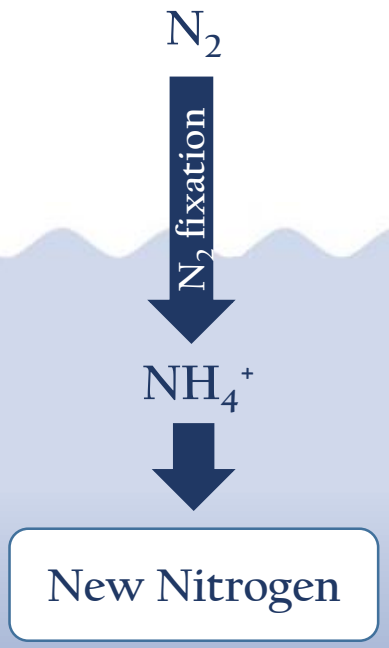
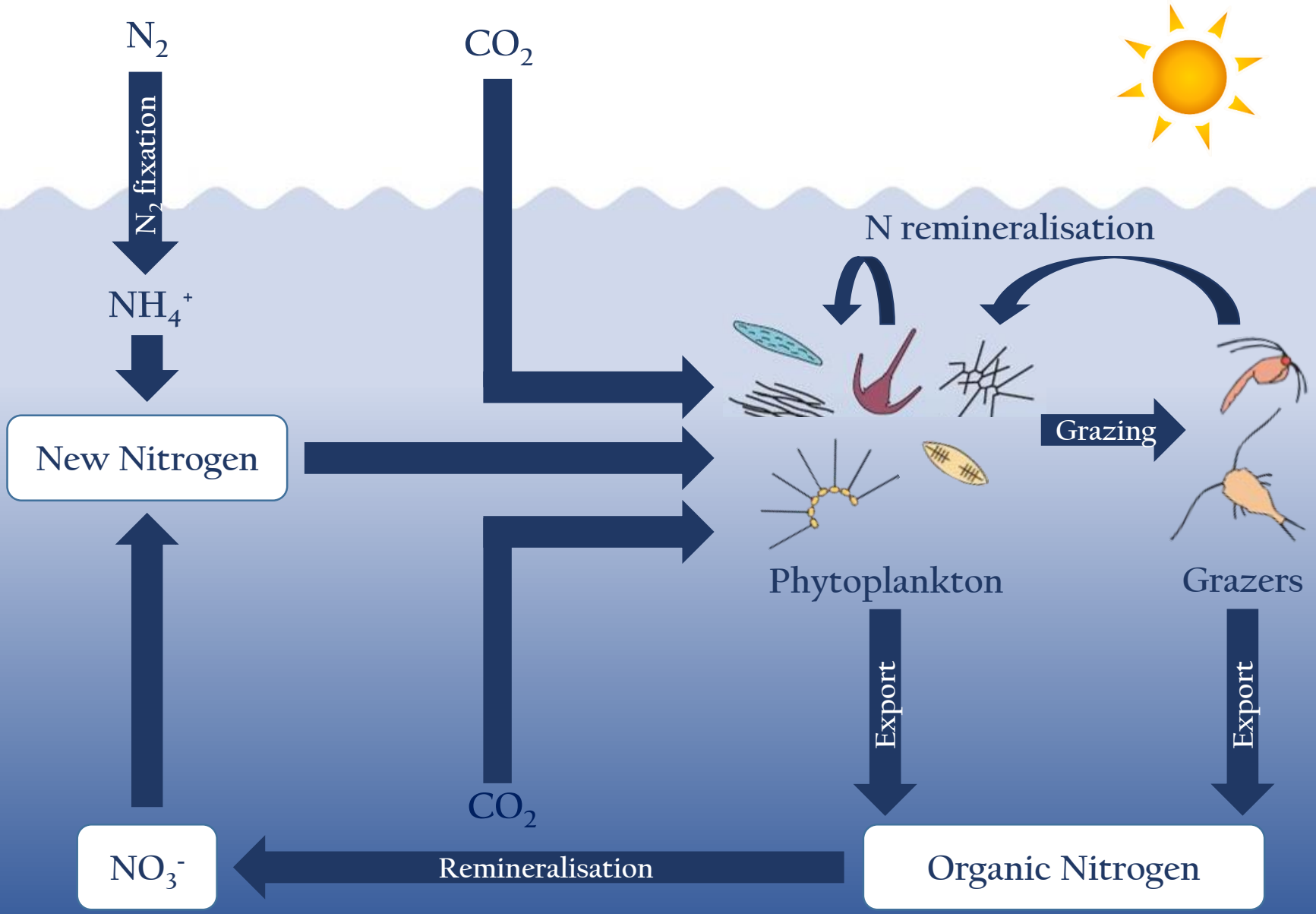
Background

What is biological N_2 fixation?



What is biological N_2 fixation?



How to measure biological N_2 fixation?

$^{15}N_2$-tracer addition technique (Montoya et al., 1996)

$^{15}N_2$ → Organic N

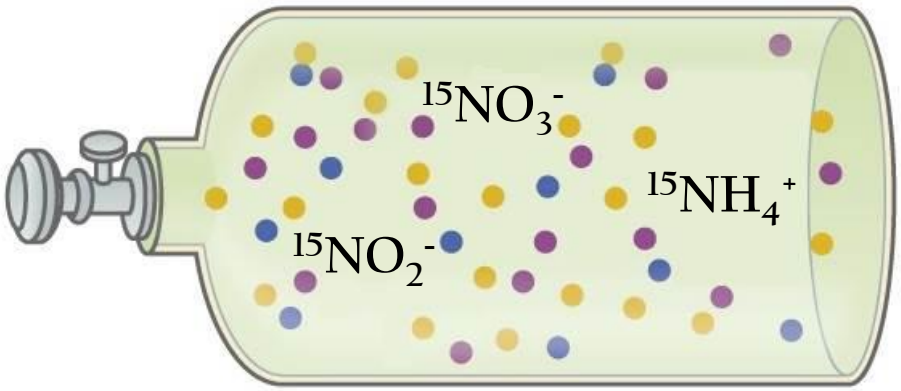
OPEN ACCESS Freely available online

PLOS | ONE

The Contamination of Commercial $^{15}N_2$ Gas Stocks with ^{15}N–Labeled Nitrate and Ammonium and Consequences for Nitrogen Fixation Measurements

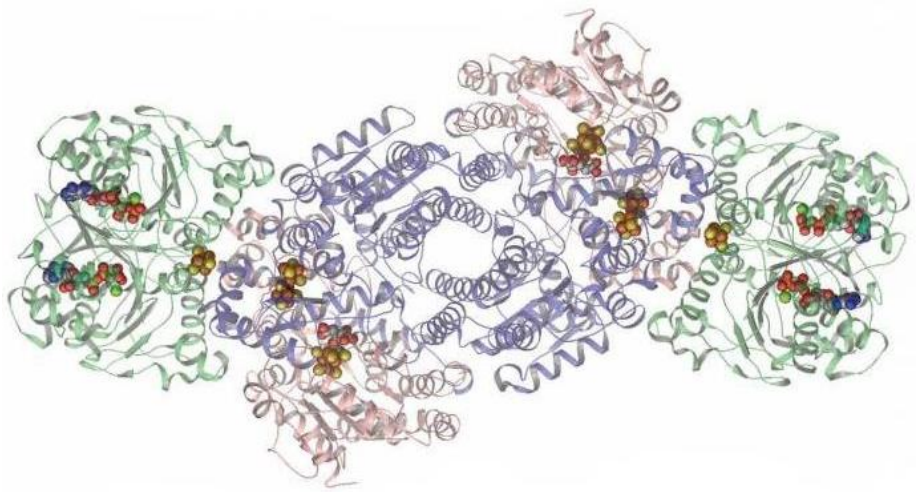
Richard Dabundo[1]*, Moritz F. Lehmann[2], Lija Treibergs[1], Craig R. Tobias[1], Mark A. Altabet[3], Pia H. Moisander[4], Julie Granger[1]

$^{15}N_2$ gas cylinder



Nitrogenase gene

Nitrogenase enzyme complex



$$N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16P_i$$

nifH gene

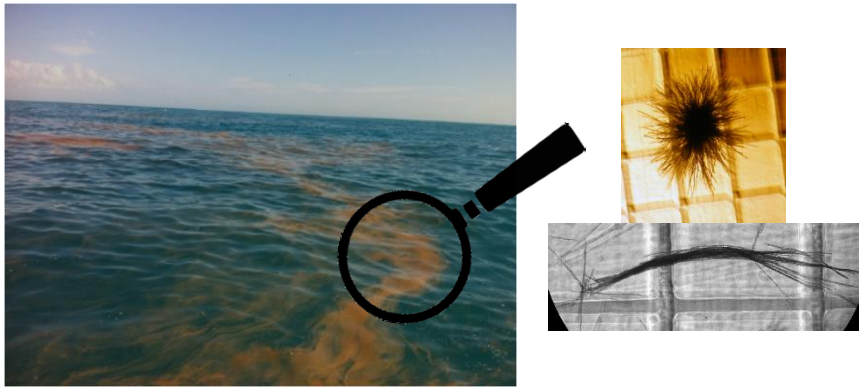
encodes

Fe proteins (dinitrogenase reductase)

Molecular structure of nitrogenase from Danyal et al., 2016

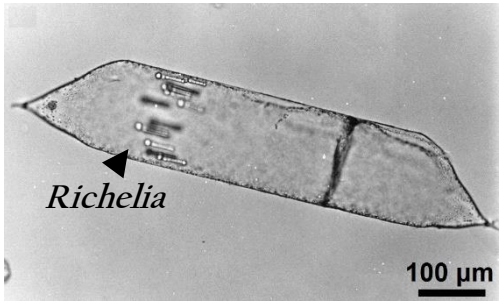
Diversity of N_2-fixing microorganisms

- *Trichodesmium* sp.

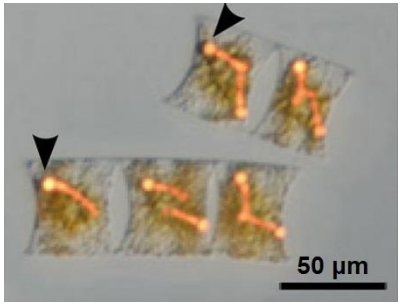


- Diatom-Diazotroph Associations (DDAs)

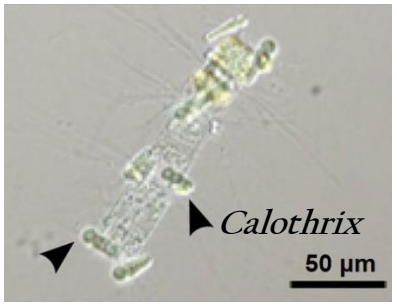
Rhizosolenia-Richelia



Hemiaulus-Richelia



Chaetoceros-Calothrix

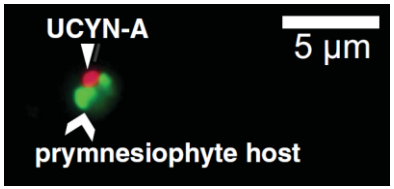


Images from TRYNITROP project, www.whoi.edu, Hilton et al. (2013) and Zehr et al. (2000)

Diversity of N_2-fixing microorganisms

- Unicellular cyanobacteria:

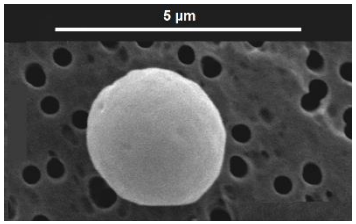
Group A (UCYN-A)



Candidatus Atelocyanobacterium thalassa

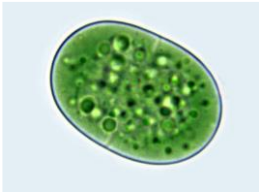
UCYN-A1 to UCYN-A6

Group B (UCYN-B)



Crocosphaera sp.

Group C (UCYN-C)

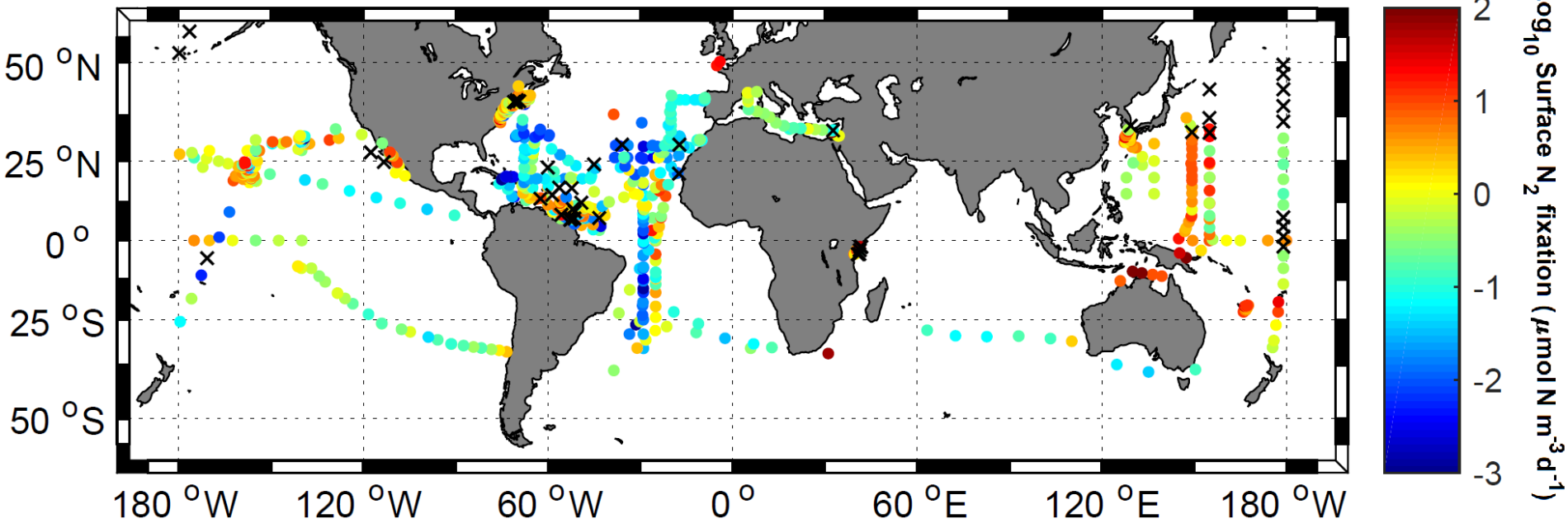


Cyanothece sp.

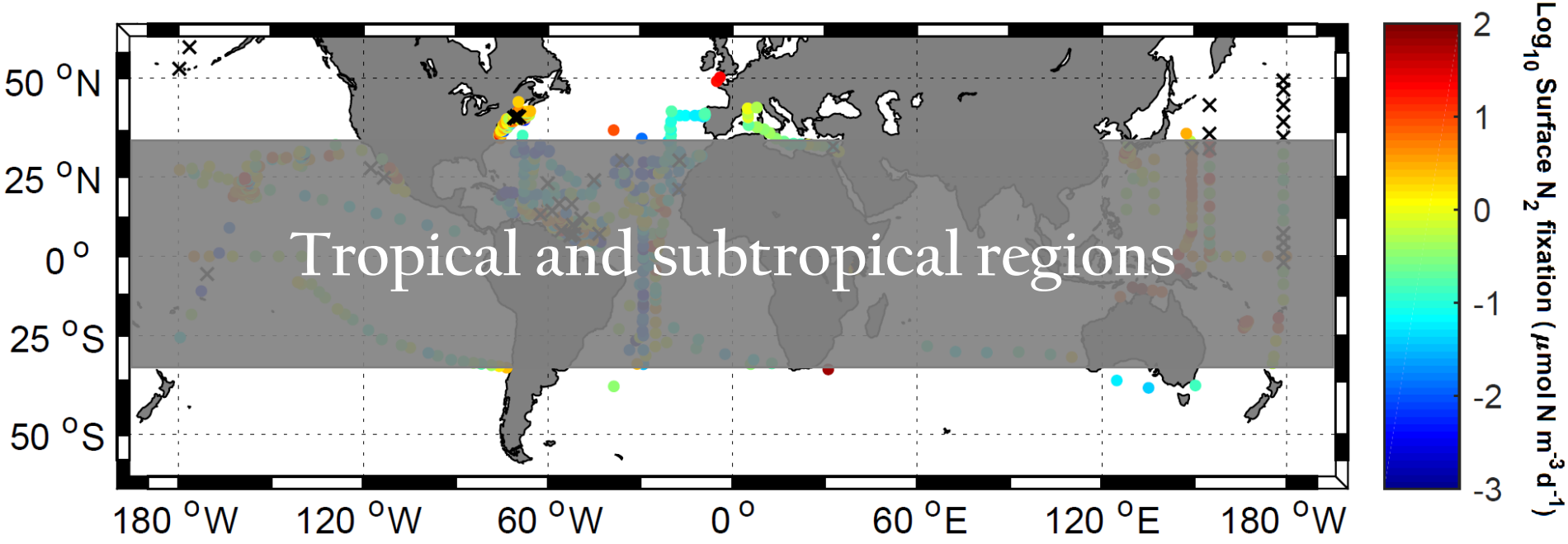
- Non-cyanobacterial diazotrophs
  - Heterotrophic bacteria (Proteobacteria, etc.)
  - Archaea

Images from Cabello et al. (2016), Jacq et al. (2014) and landcareresearch.co.nz

Oceanic N_2 fixation domain

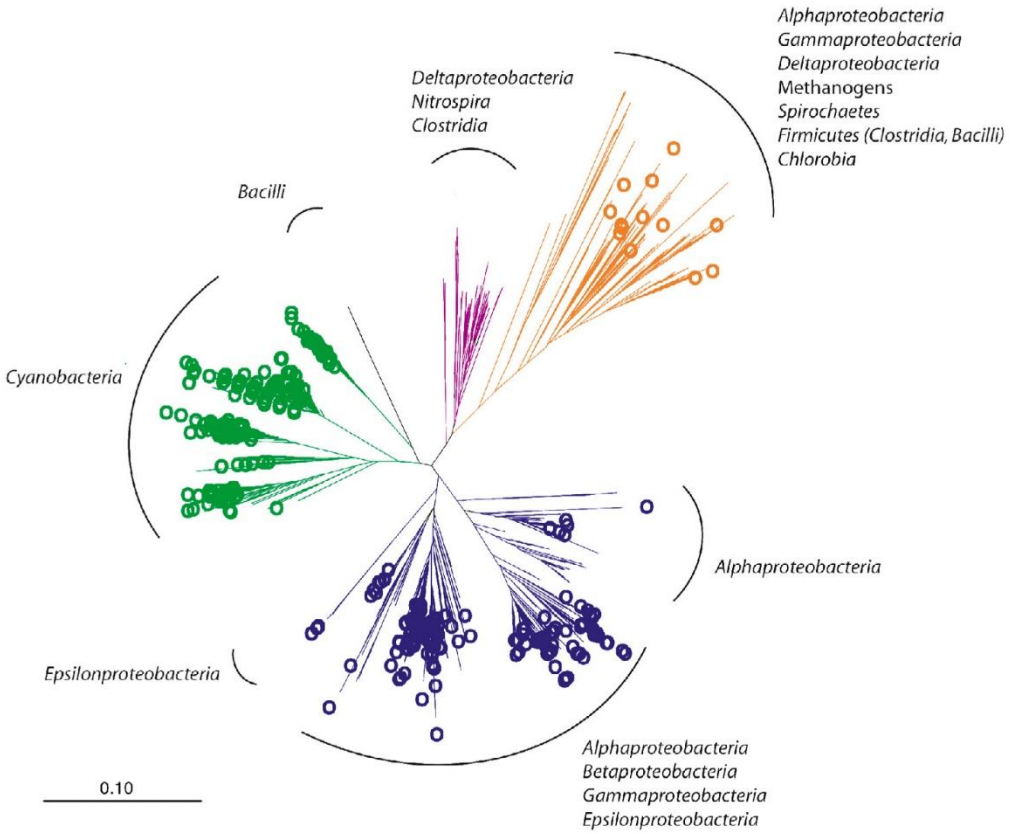


Oceanic N_2 fixation domain



Oceanic N_2 fixation domain

- Large diazotroph diversity
- Wide distribution



Phylogenetic tree from Riemann et al. (2010)

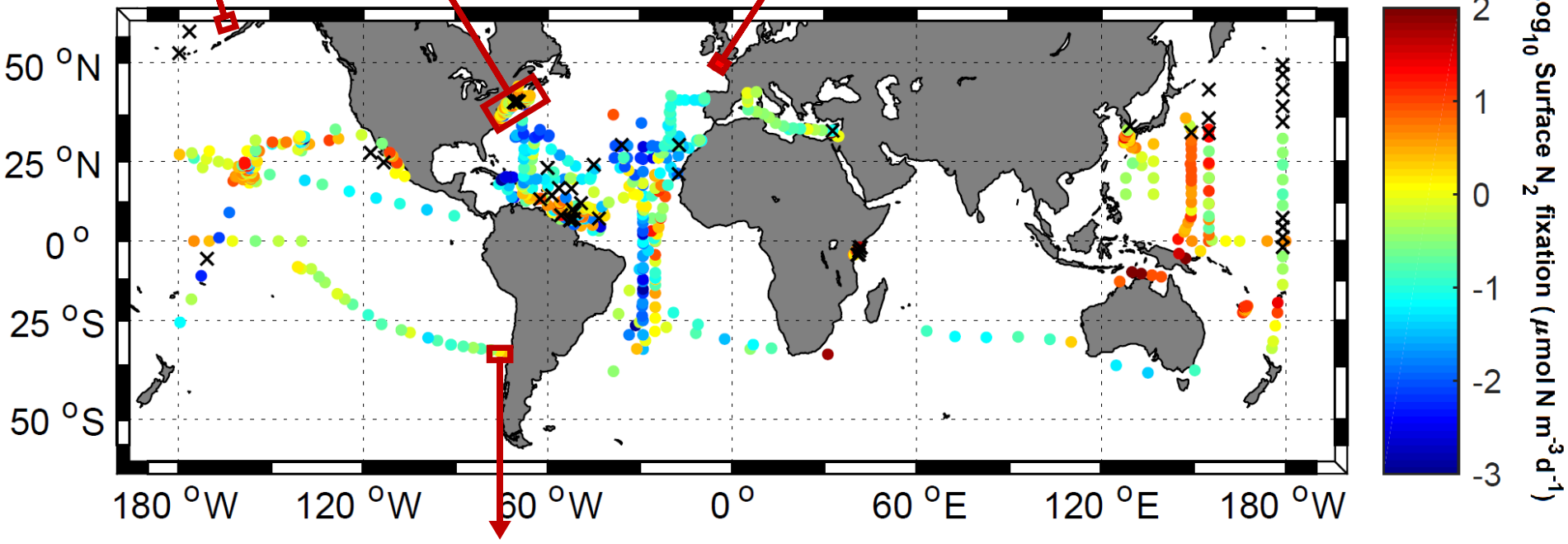
Evidences of N_2 fixation in N-rich waters

- Large diazotroph diversity
- Wide distribution
- N_2 fixation in N-rich temperate, cold and upwelling regions

NE USA coast
(Mulholland et al., 2012)

W English Channel (Rees et al., 2009)

Arctic
(Shiozaki et al., 2017, 2018)



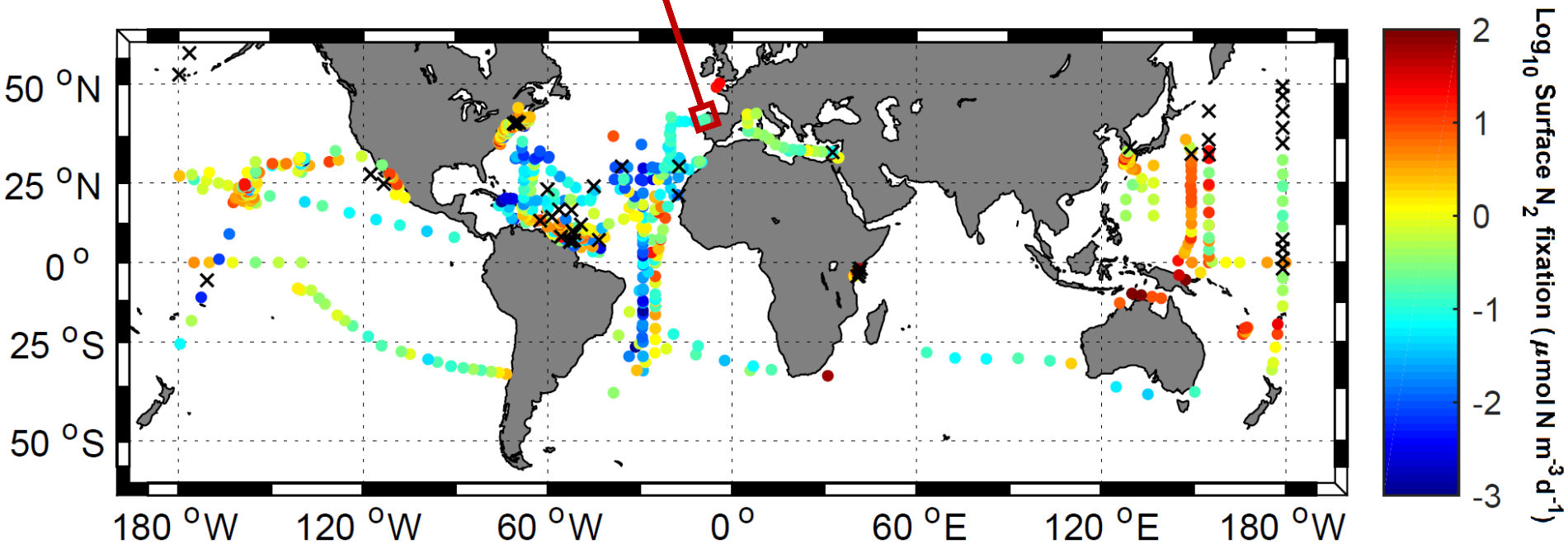
Central Chile upwelling system
(Fernández et al., 2015)

Evidences of N_2 fixation in N-rich waters

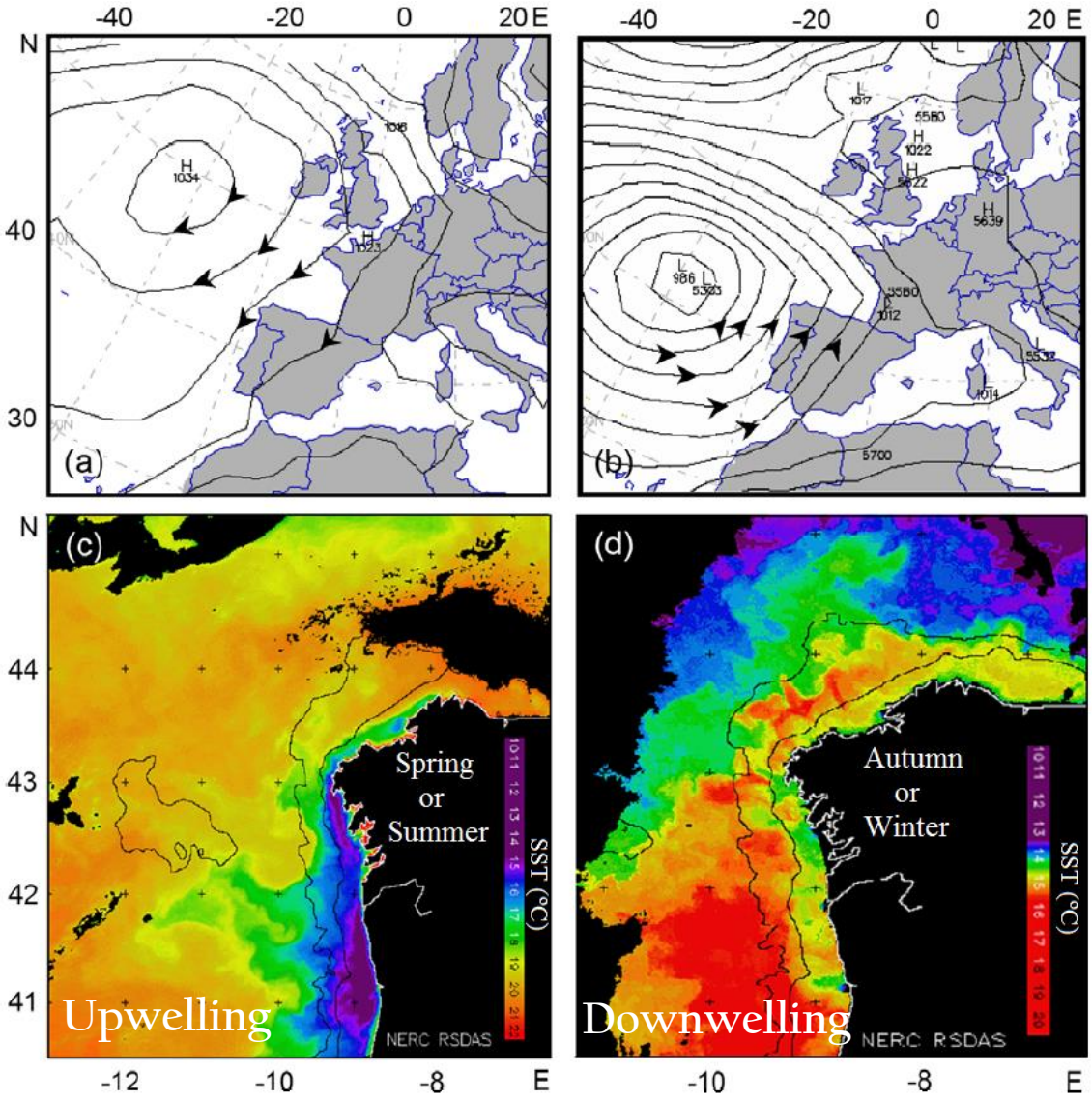
Upwelling region off NW Iberia
(Benavides et al., 2011)

Low N_2 fixation rates (0.025 – 0.36 nmol N L^{-1} d^{-1})

Attributed to UCYN-A



NW Iberian upwelling system



Modified from Pitcher et al., 2010

Hypotheses and objectives

Hypotheses

1) ^{15}N-labeled contaminants yield significant overestimations in biological N_2 fixation.

2) N_2 fixation represents a minor input of new N in the upwelling region off Northwestern Iberia.

3) Contrasting hydrodynamic forcing induces variability in diazotrophic community composition.

Objectives

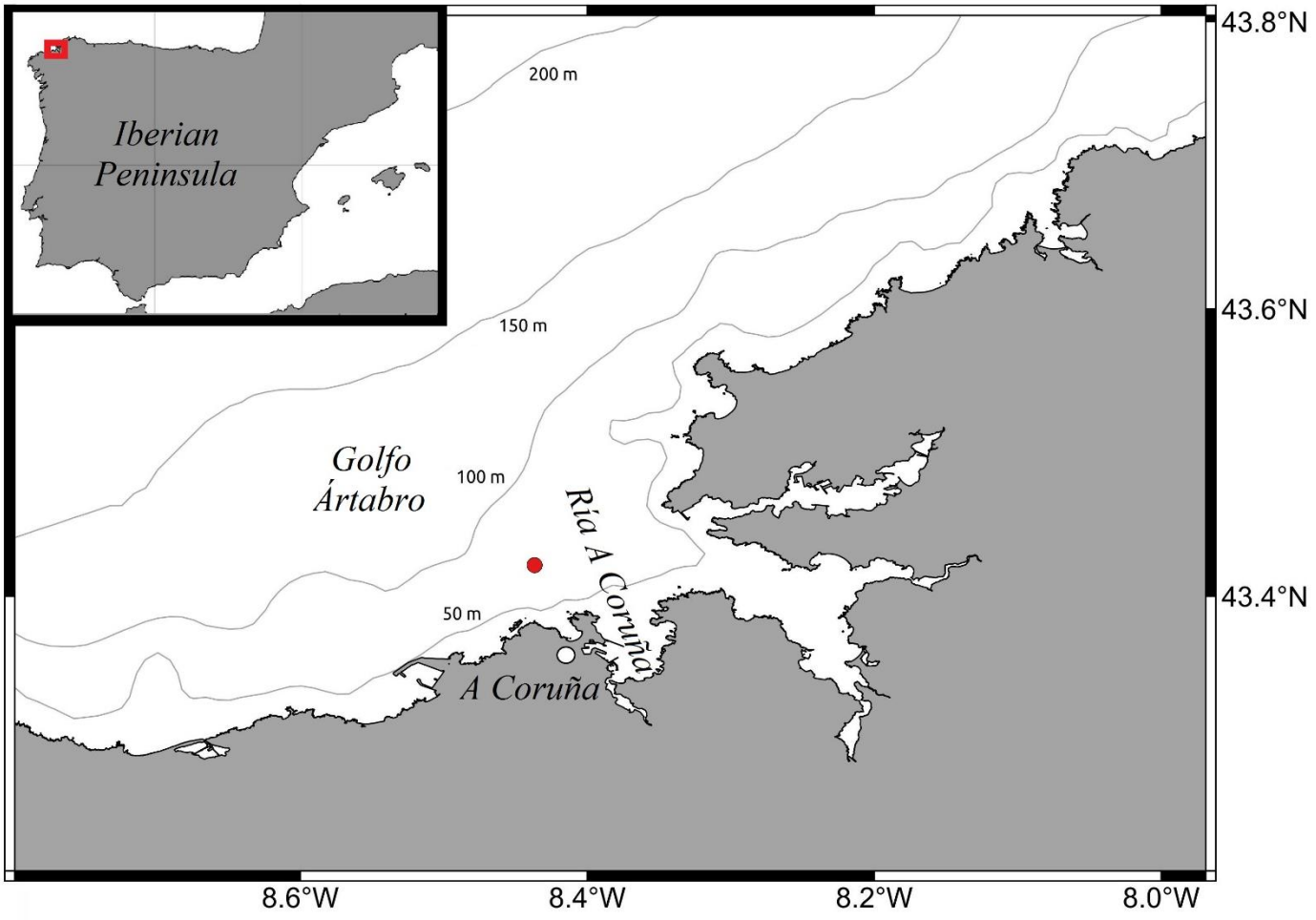
1) To test the assimilation of ^{15}N-contaminants by non-diazotroph organisms, and to determine the potential overestimation of N_2 fixation rates.

2) To describe the seasonal variability of N_2 fixation, and to quantify its role as a mechanism of new N supply.

3) To investigate the relationship between variability in hydrodynamic forcing and diazotroph abundance and community composition.

Methods

Sampling


Iberian
Peninsula
200 m
150 m
100 m
50 m
Golfo
Ártabro
Ría A Coruña
A Coruña
43.8°N
43.6°N
43.4°N
8.6°W
8.4°W
8.2°W
8.0°W

Sampling

15 samplings	
2014	2015
19 Feb	14 Apr
18 Mar	12 May
15 Apr	11 Jun
27 May	22 Jul
19 Jun	3 Sep
16 Jul	19 Nov
4 Sep	16 Dec
17 Dec	

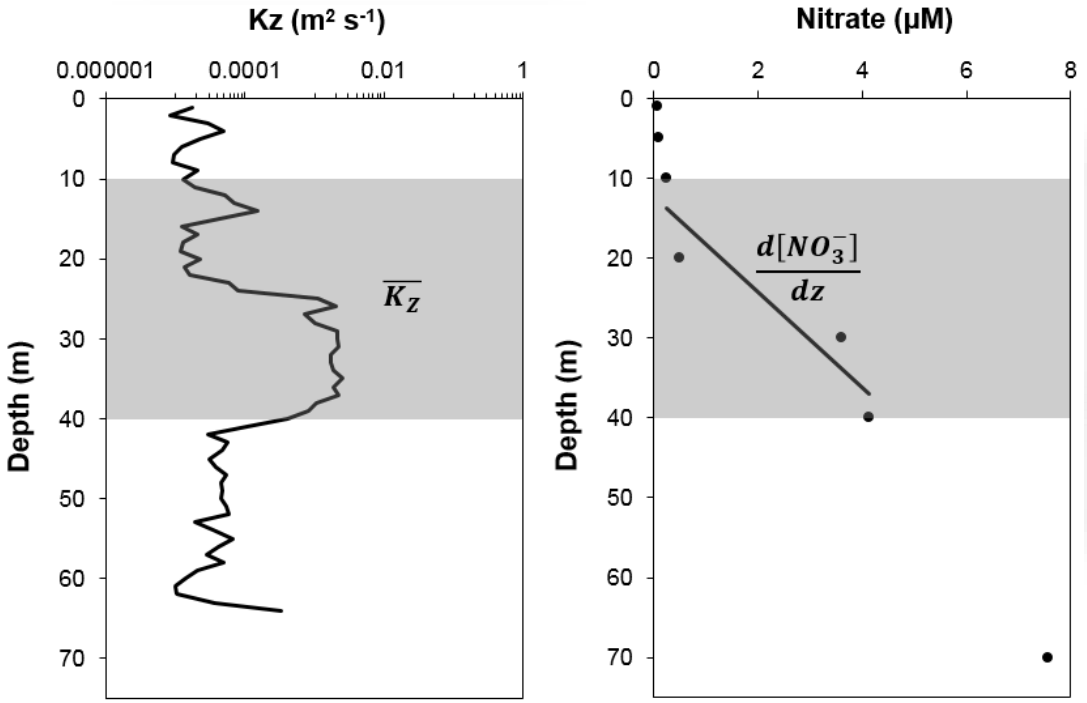
Hydrographic measurements

- Vertical CTD casts
- Microstructure turbulence (MSS profiler): $K_z = e \frac{\varepsilon}{N^2}$
- Inorganic nutrients: $\frac{d[nutrient]}{dz}$

MSS profiler

Hydrographic measurements

- Vertical CTD casts
- Microstructure turbulence (MSS profiler): $K_z = e\,\frac{\varepsilon}{N^2}$
- Inorganic nutrients: $\frac{d[nutrient]}{dz}$
- Diffusive fluxes: $Flux_{nutrient} = \overline{K_z} \times \frac{d[nutrient]}{dz}$



Biological measurements

- Chlorophyll *a* (spectrofluorometric method)
- Picoplankton community composition (flow cytometry)
- Primary production (^{14}C-uptake)
- Biological N_2 fixation rates ($^{15}N_2$-uptake) — › 10 µm; ‹ 10 µm
- Contamination tests

15 samplings	
2014	2015
19 Feb	14 Apr
18 Mar	12 May
15 Apr	11 Jun
27 May	22 Jul
19 Jun	3 Sep
16 Jul	19 Nov
4 Sep	16 Dec
17 Dec*	

Dabundo et al. (October 2014)

Contaminated $^{15}N_2$ gas bottle

Determining the potential overestimation of N_2 fixation

How? Comparing N_2 fixation rates at 0 m and 70 m depth

vs.

$^{15}N_2$ Sigma

$^{15}N_2$ Cambridge

Testing the susceptibility of ^{15}N-contaminants to assimilation by non-diazotrophs

How?

Tetraselmis suecica cultures

$^{15}N_2$ gas cylinders

Sigma-Aldrich

Cambridge

Control

Molecular study of *nifH* gene

- Diazotroph community diversity

nifH gene amplification by PCR

↓

Sequencing by Illumina®

↓

Oligotyping

- Diazotroph abundances
  - qPCR
  - CARD-FISH (UCYN-A symbiosis)

Results

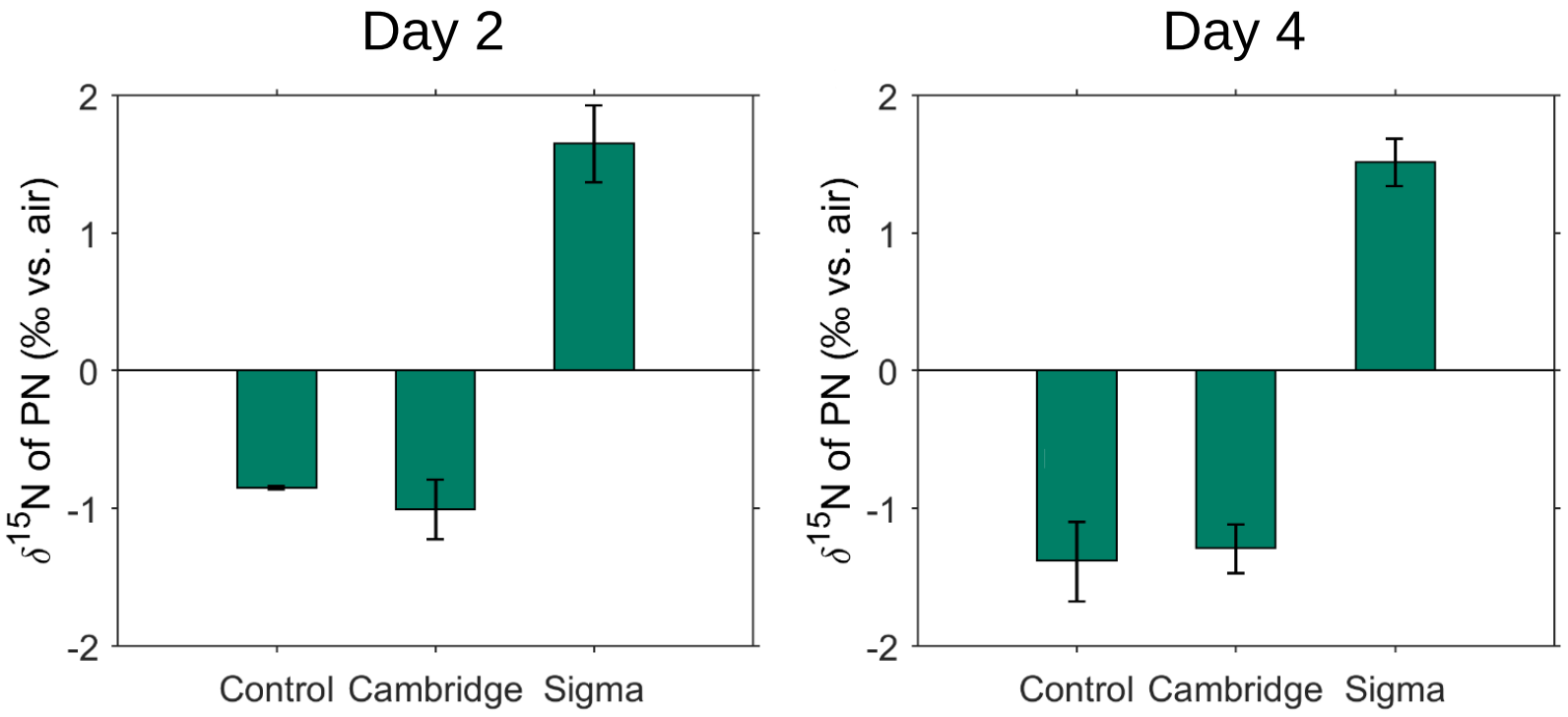
Objectives

1) To test the assimilation of ^{15}N-contaminants by non-diazotroph organisms, and to determine the potential overestimation of N_2 fixation rates.

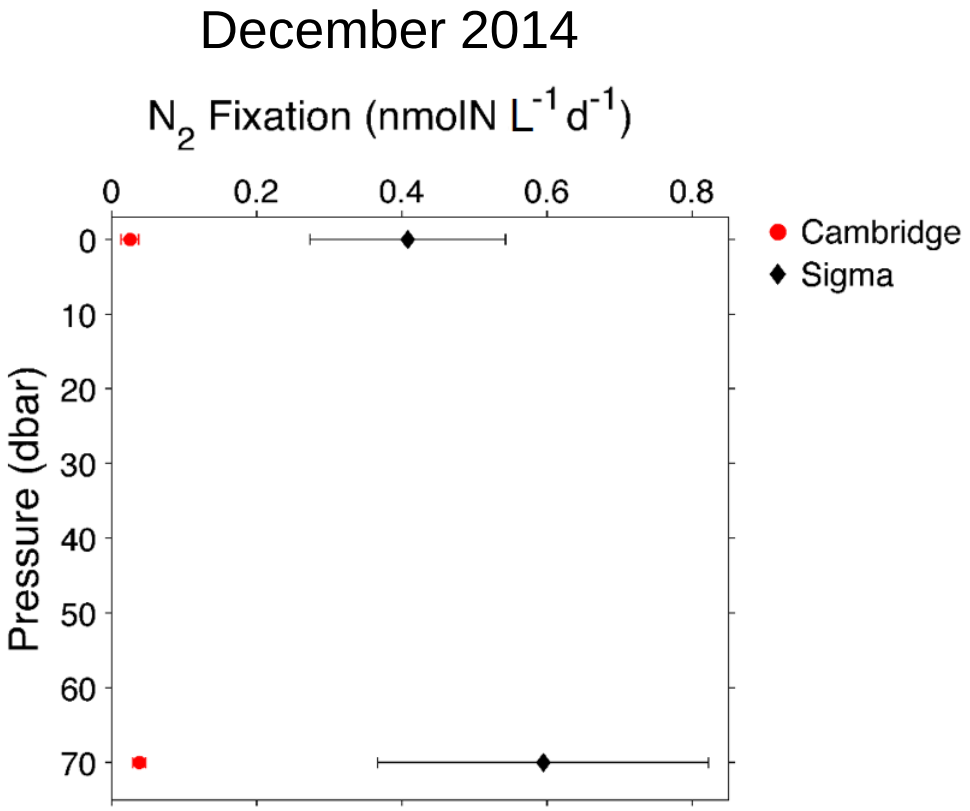
2) To describe the seasonal variability of N_2 fixation, and to quantify its role as a mechanism of new N supply.

3) To investigate the relationship between variability in hydrodynamic forcing and diazotroph abundance and community composition.

^{15}N-contaminants uptake by non-diazotrophs

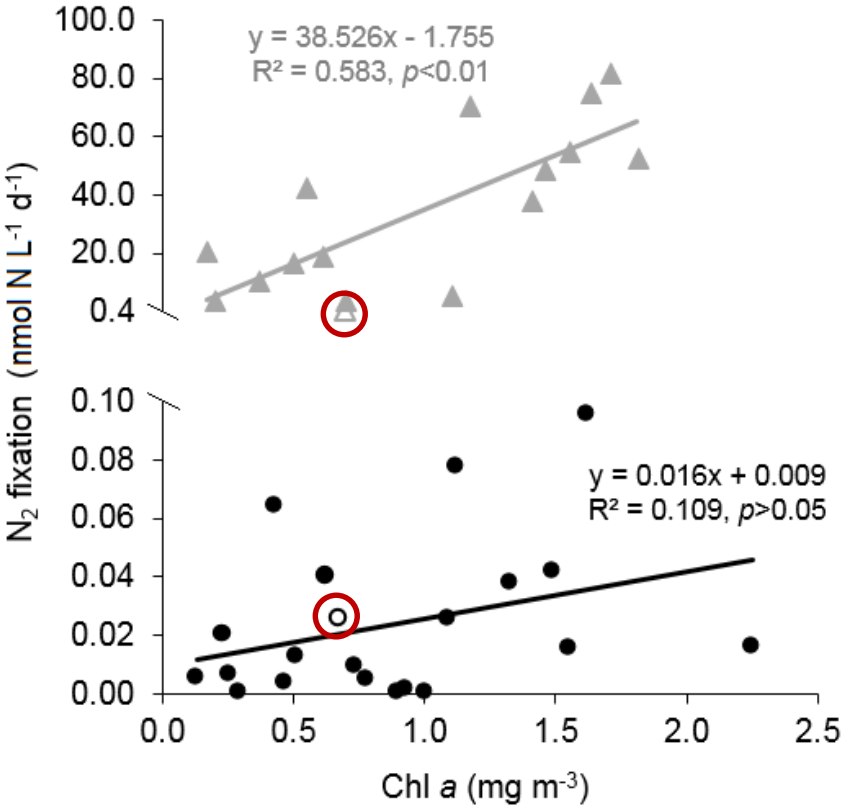


Determining the potential overestimation of N_2 fixation



Overestimation of volumetric N_2 fixation rates by a factor of 16

Consequences of contamination on N_2 fixation rates



Contaminated $^{15}N_2$ gas stock

Non-contaminated $^{15}N_2$ gas stock

↑ Chl *a*
↑ PP

Overestimation factor ›16

Objectives

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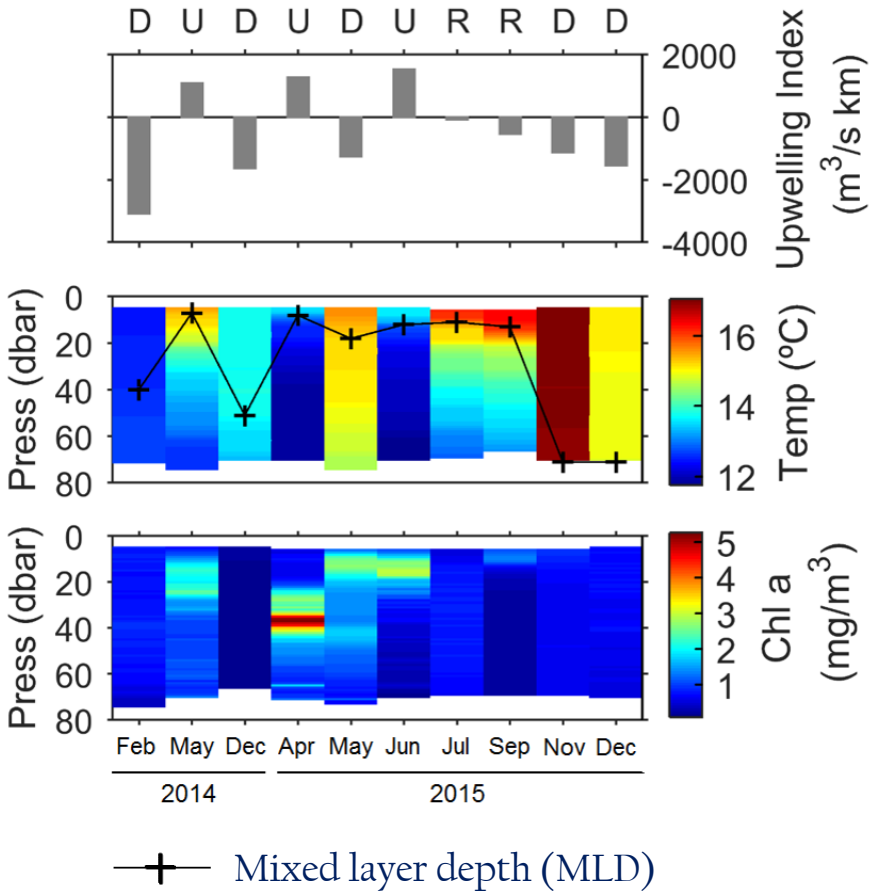
Hydrography

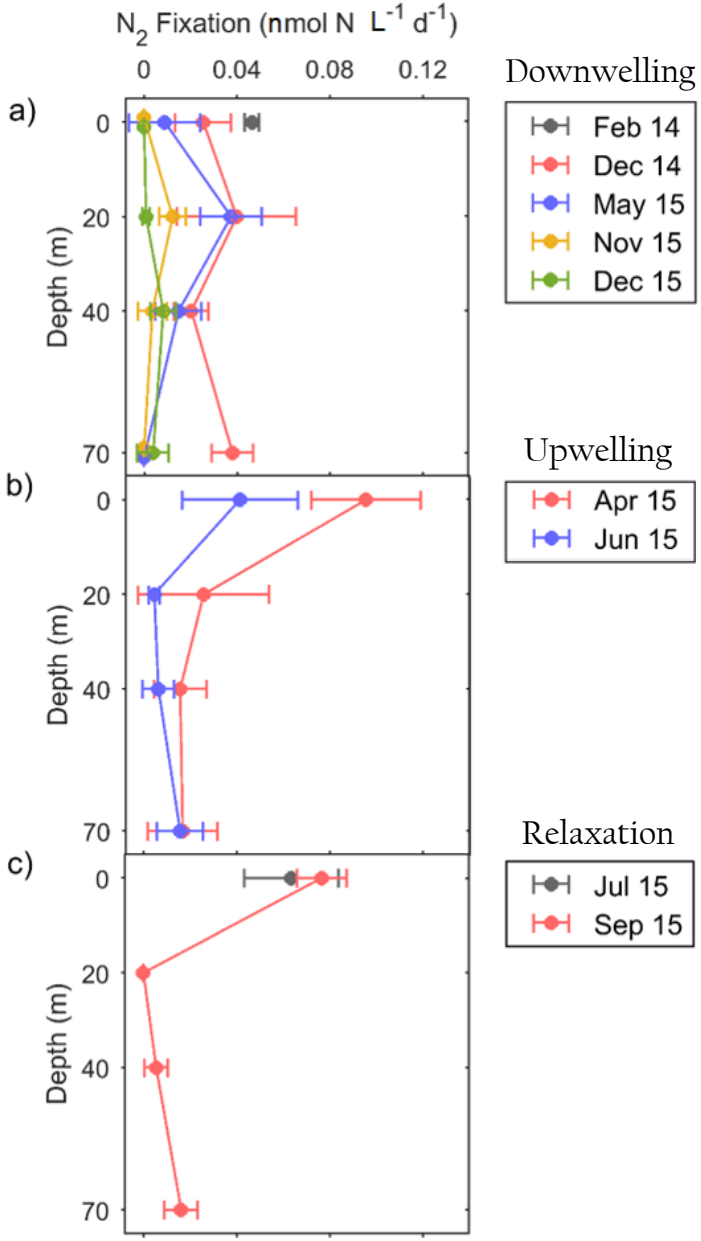


Hydrographic conditions

- Downwelling (D): ↑ MLD
- Upwelling (U): ↑ Chl *a*, ↑ PP, Bloom
- Relaxation (R): surface stratification

Seasonal and vertical variability of N_2 fixation

N_2 fixation by diazotrophs <10 µm (unicellulars)



Comparing with other studies

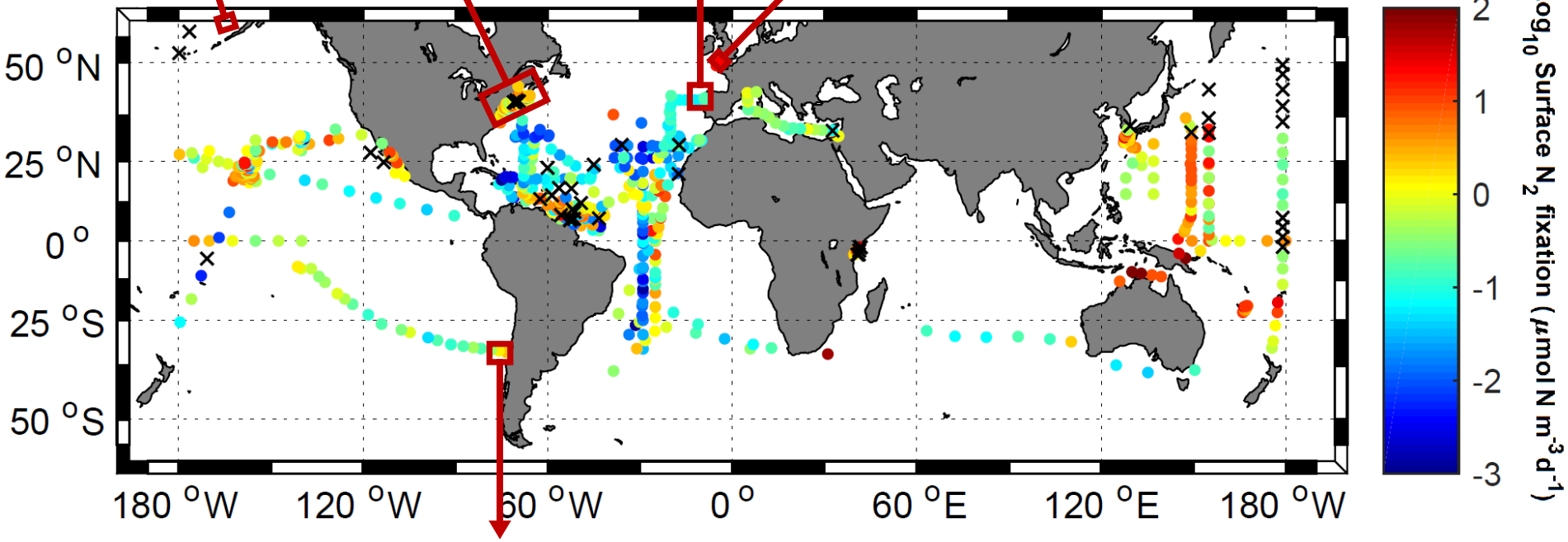
0.001 – 0.1 nmol N L^{-1} d^{-1}
(Moreira-Coello et al., 2017)

0.24 – 76.8 nmol N L^{-1} d^{-1}
(Mulholland et al., 2012)

0.025 – 0.36 nmol N L^{-1} d^{-1}
(Benavides et al., 2011)

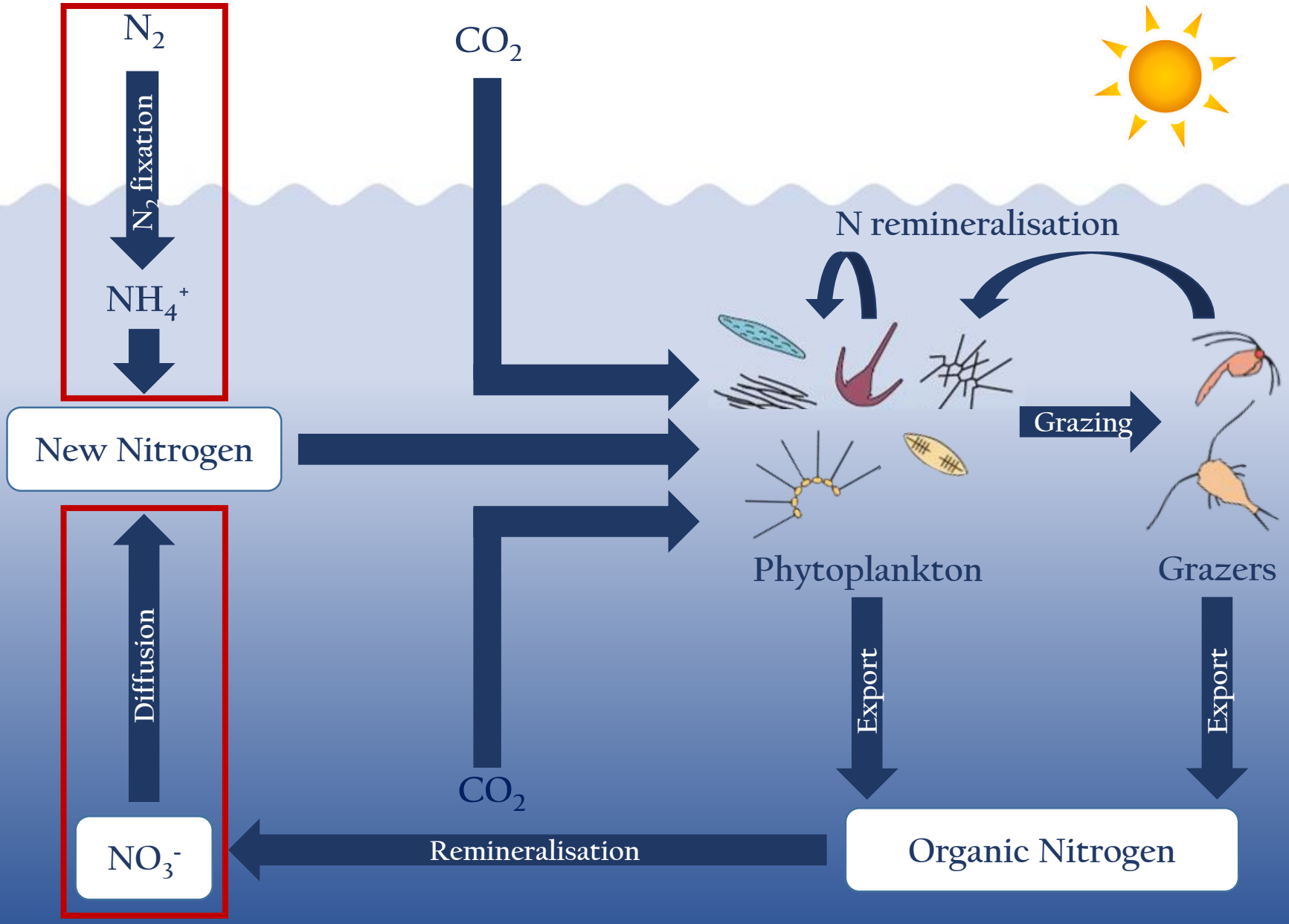
18.9 – 19.9 nmol N L^{-1} d^{-1}
(Rees et al., 2009)

0.1 – 3.6 nmol N L^{-1} d^{-1}
(Shiozaki et al., 2017, 2018)

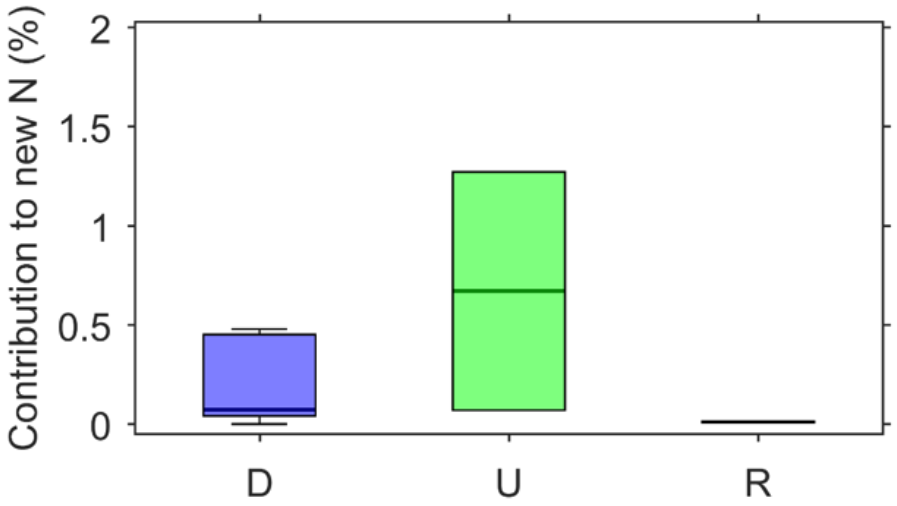


0.5 – 126.8 nmol N L^{-1} d^{-1}
(Fernández et al., 2015)

N_2 fixation vs. nitrate eddy diffusion



Biogeochemical role of N_2 fixation



Contribution of N_2 fixation to new N supply ‹2%
(New N supply = NO_3^- diffusion + N_2 fixation)

Objectives

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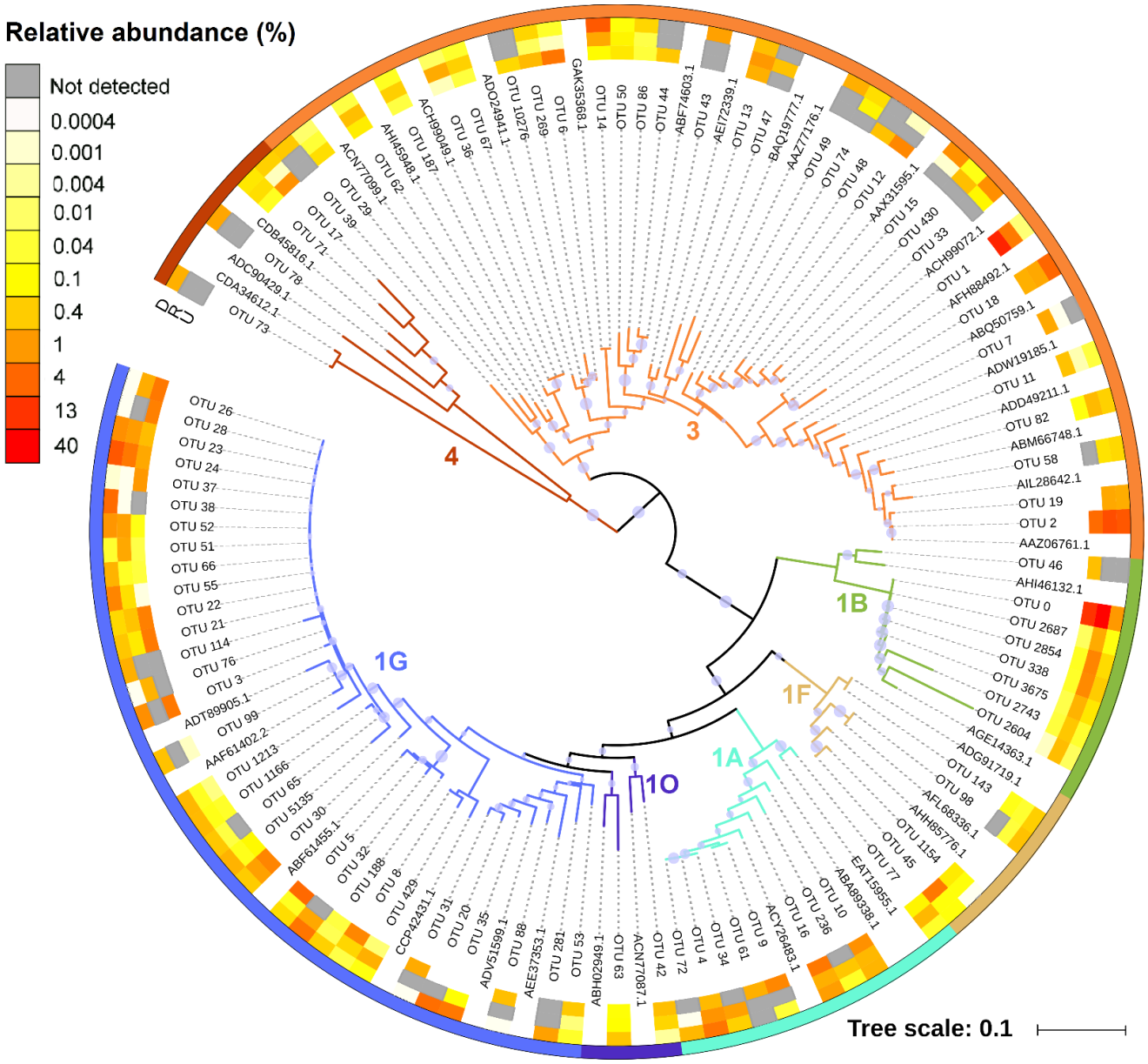
Diazotrophic community composition

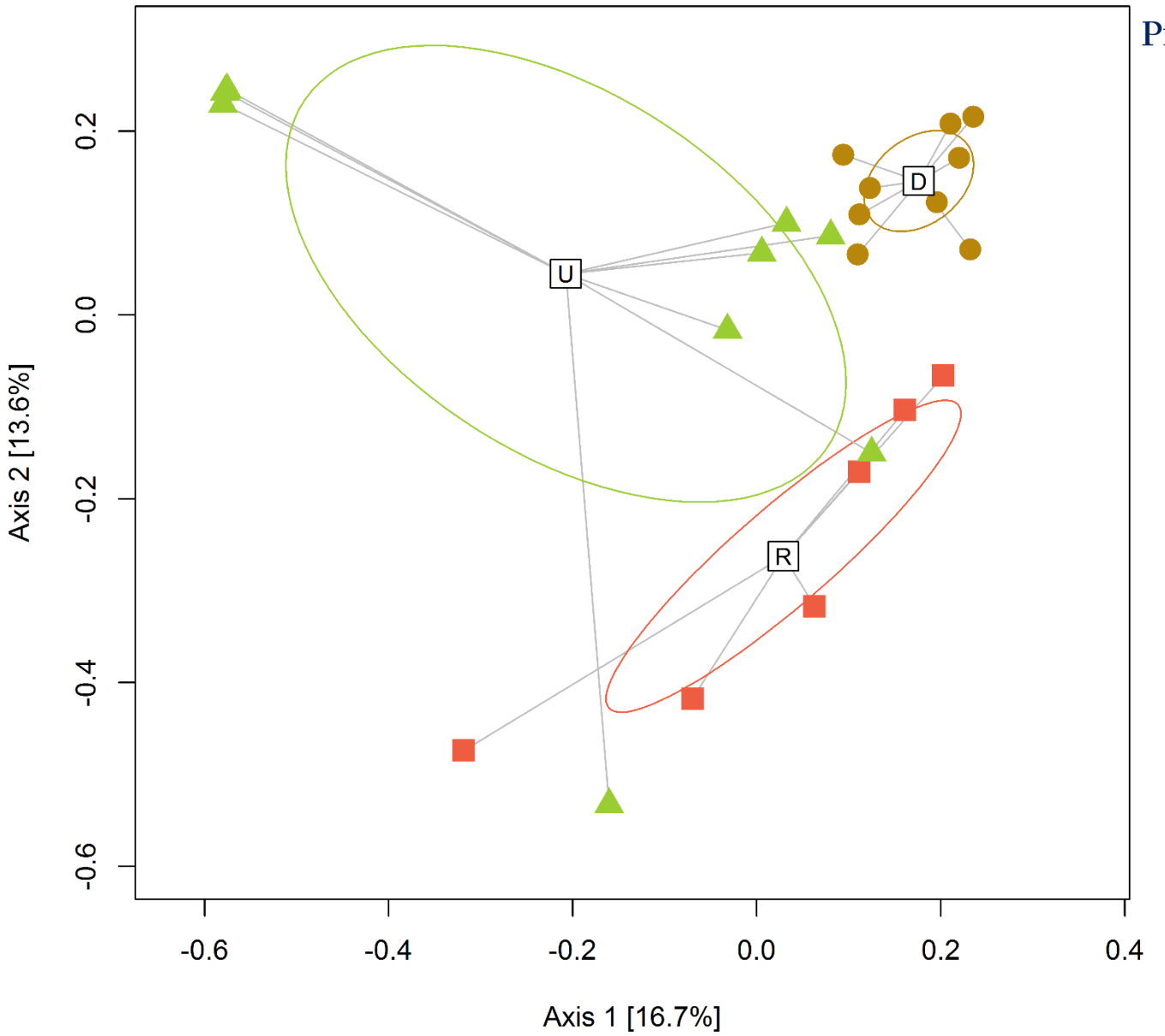
582000 *nifH* sequences

1276 OTUs (92% nucleotide similarity)

Phylogenetic tree



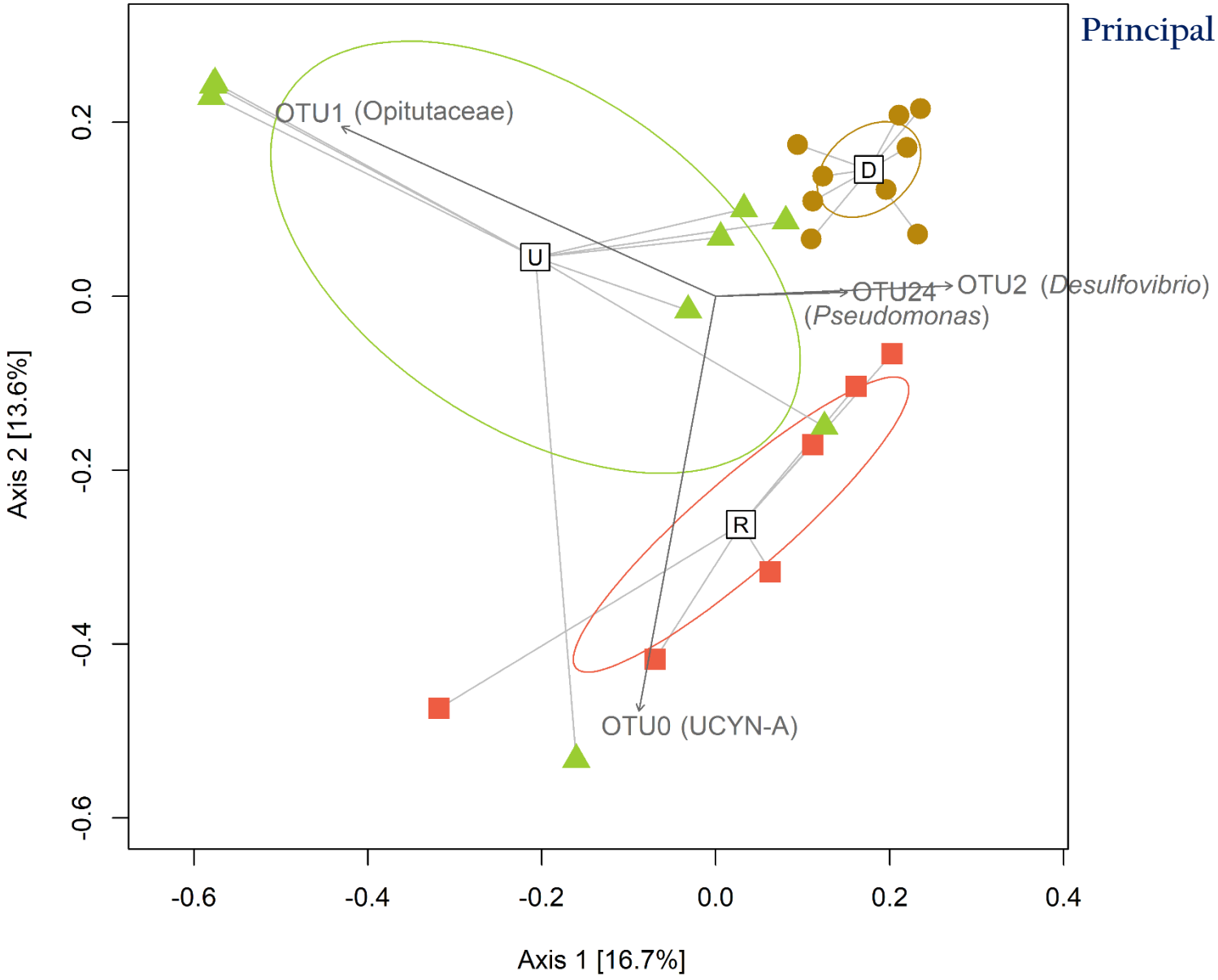
Temporal variability of diazotroph community composition



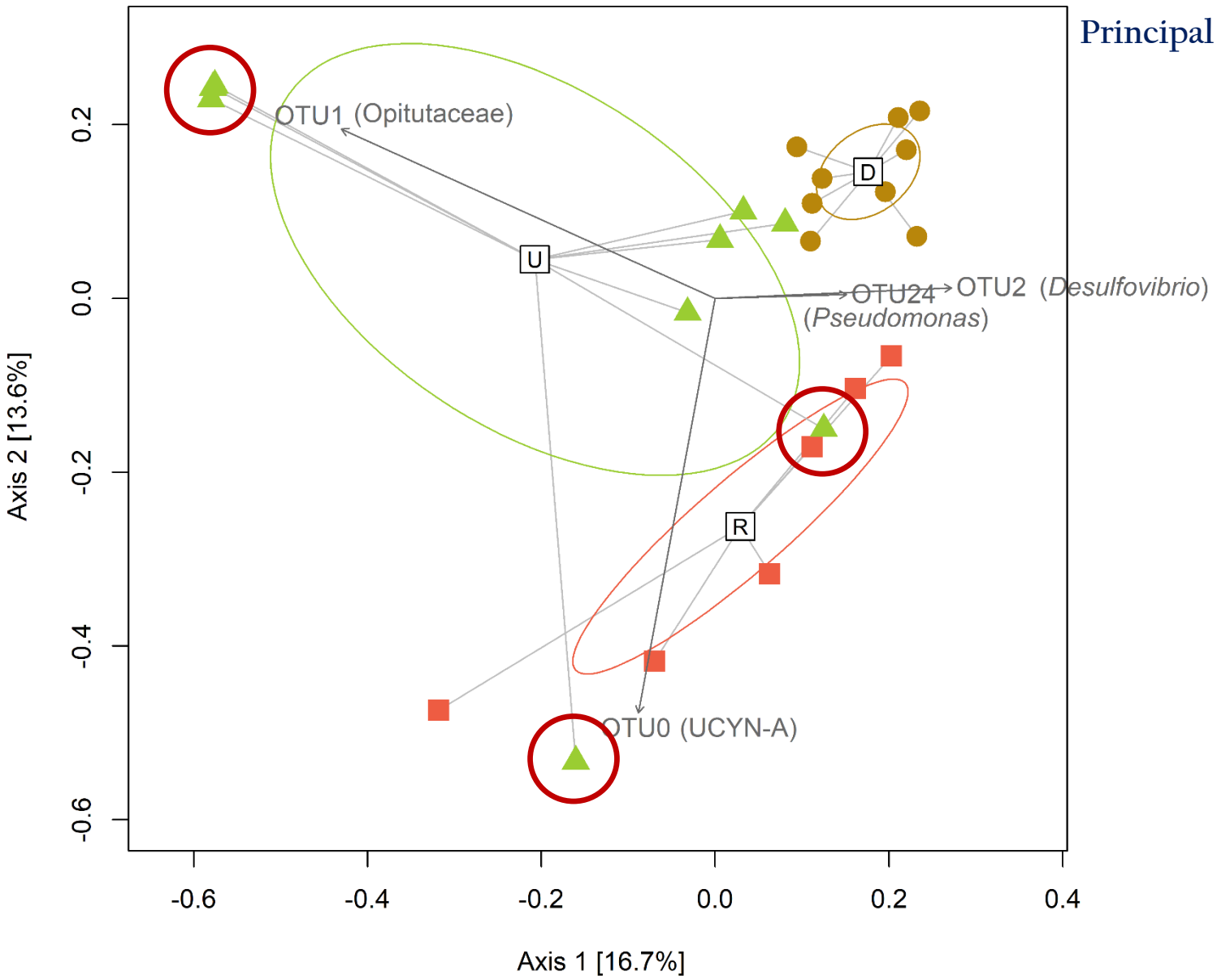
Principal Coordinate Analysis

Temporal variability of diazotroph community composition

Principal Coordinate Analysis



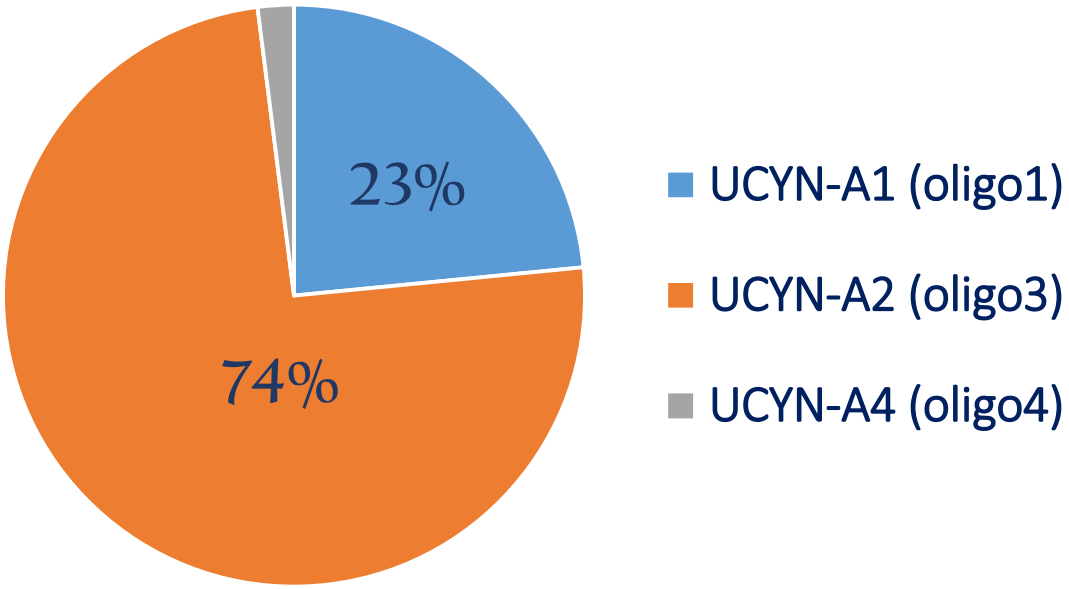
Temporal variability of diazotroph community composition



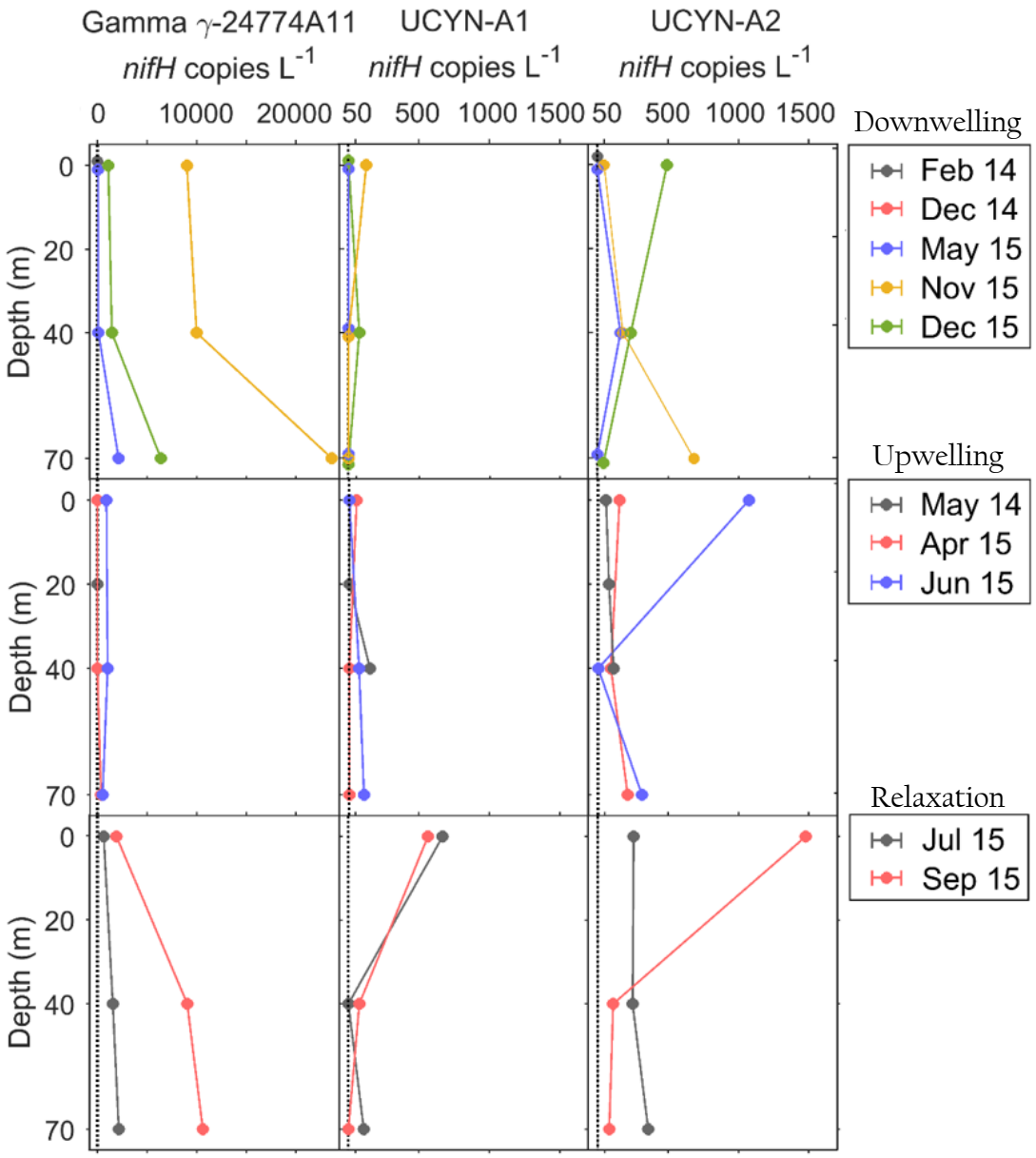
Principal Coordinate Analysis

Diversity, variability and ecology of UCYN-A sublineages

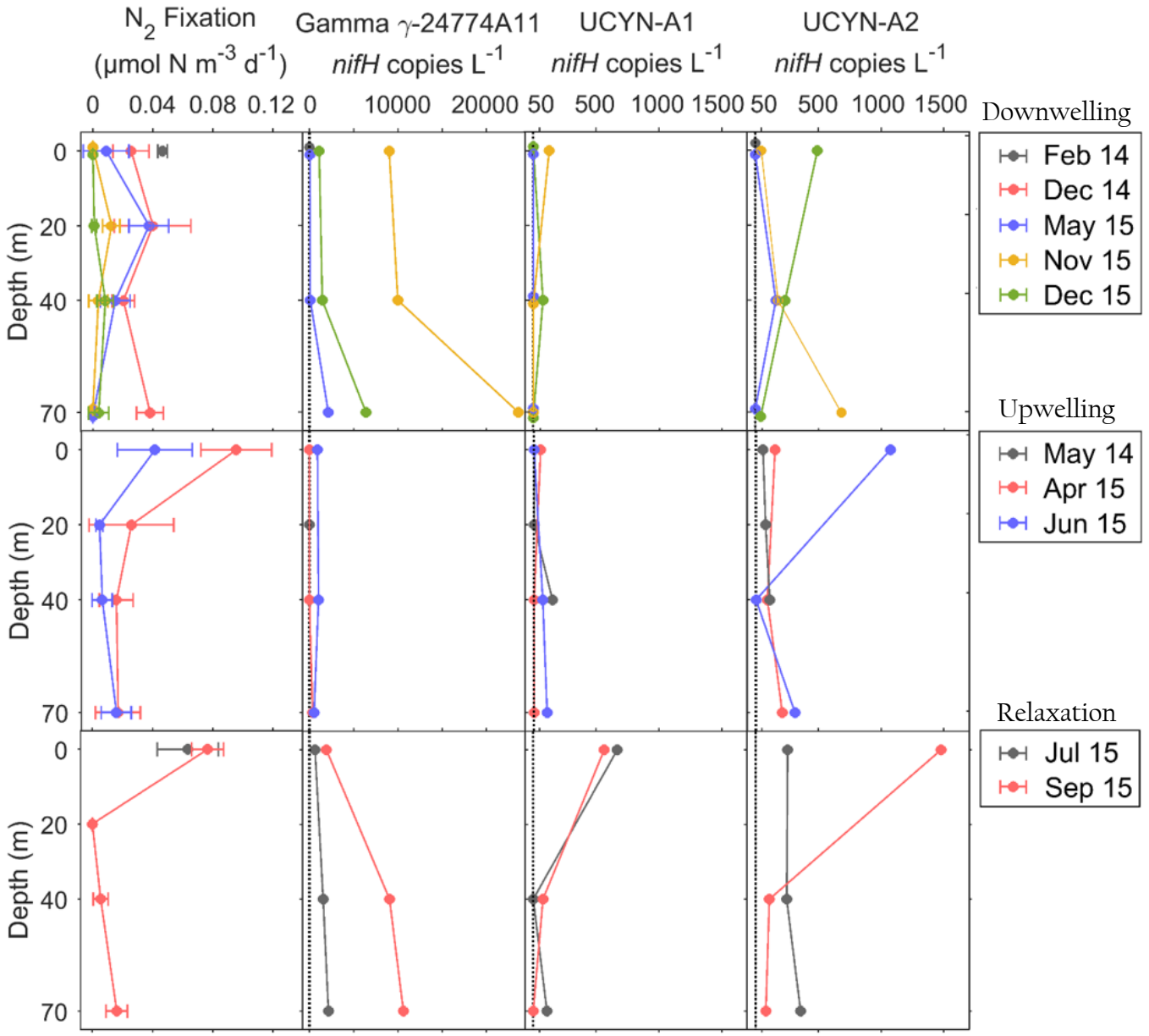
UCYN-A *nifH* sequences → Oligotyping → Oligotypes



Diazotroph abundances (*nifH* copies L^{-1})

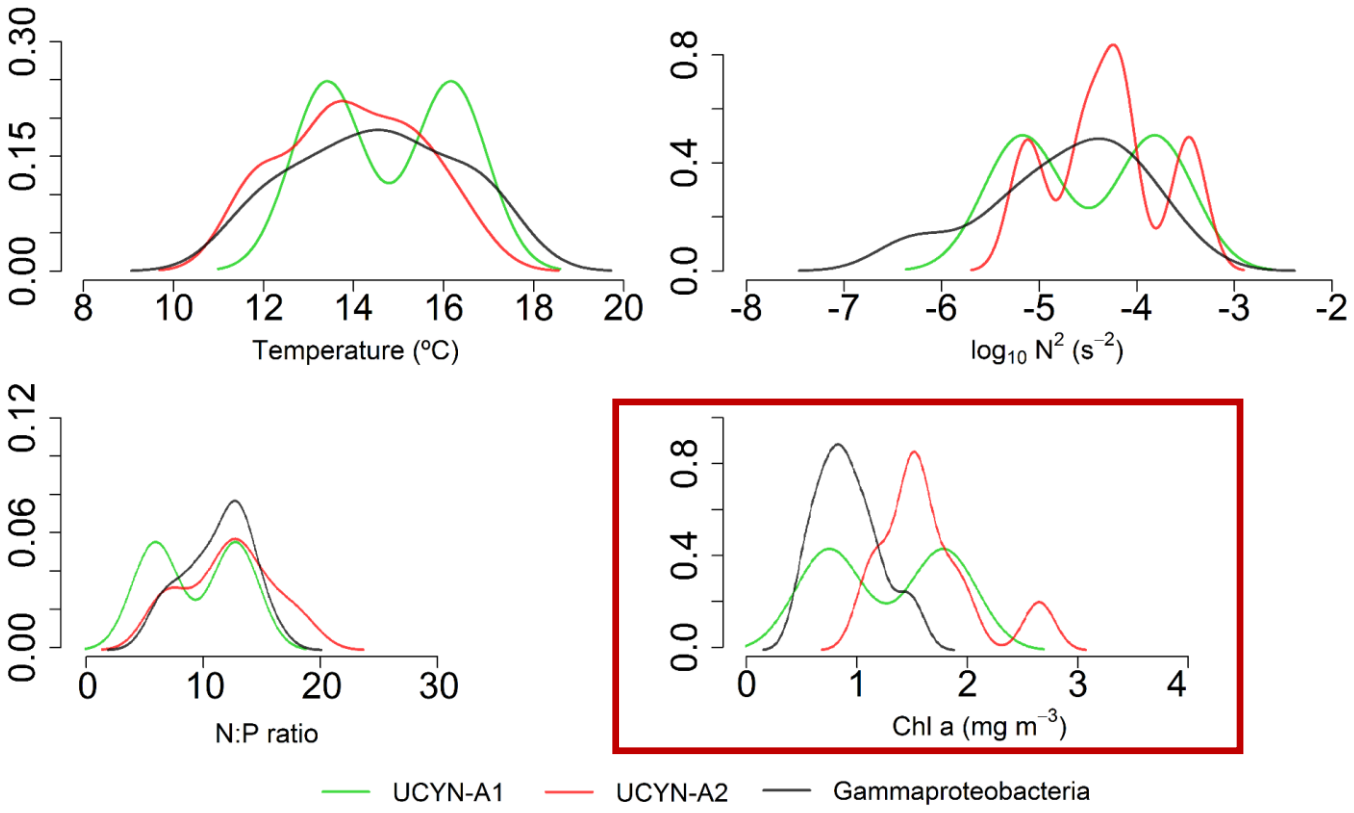


Diazotroph abundances (*nifH* copies L^{-1})



Ecological niches of diazotrophs

Overlapping of the ecological niches by kernel density functions



↑ Gamma in open ocean
↑ UCYN-A1 in open ocean
↑ UCYN-A2 in coastal regions

Conclusions

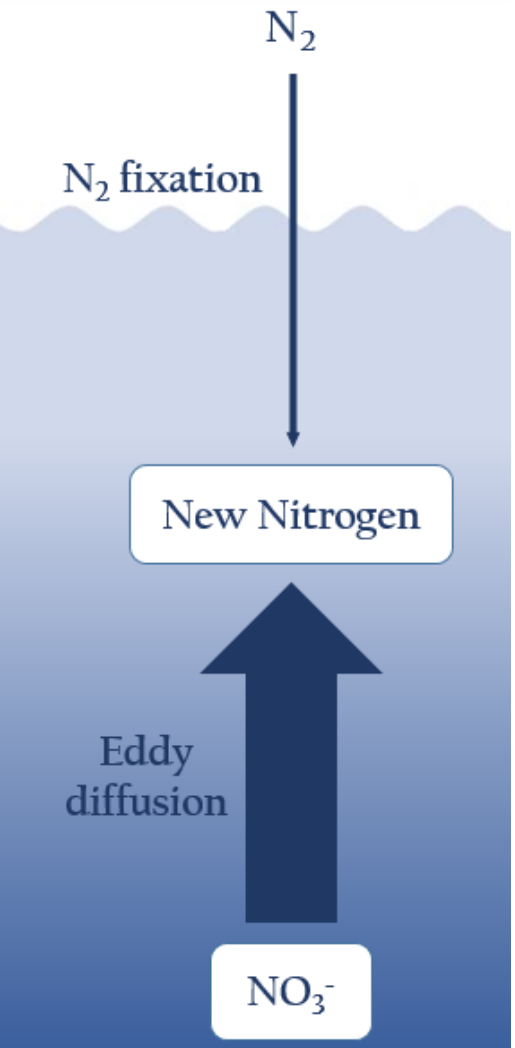
Hypothesis I

^{15}N-labeled contaminants yield overestimations in N_2 fixation.

Conclusion I

- ^{15}N-labeled contaminants assimilable by non-diazotrophs.
- N_2 fixation rates overestimated up to 16-fold.

Conclusions



Hypothesis II

N_2 fixation represents a minor input of new N in the upwelling region off Northwestern Iberia.

Conclusion II

- Low N_2 fixation rates (up to 0.1 nmol N L^{-1} d^{-1}).
- Peaking at surface waters during upwelling and relaxation.
- Minor biogeochemical role (‹2%).

Conclusions

Hypothesis III

Contrasting hydrodynamic forcing induces variability in diazotrophic community composition.

Conclusion III

- Diazotroph community composition dominated by:
  - Anaerobic bacteria (36%).
  - Gammaproteobacteria (28%).
  - UCYN-A (21%).
- Gammaproteobacteria peaked during downwelling.
- UCYN-A1 and -A2 peaked at surface in upwelling and relaxation.
- UCYN-A principal active diazotroph in the region.


Many thanks for your attention!