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**Diazotroph
succession in
VAHINE mesocosms**

K. A. Turk-Kubo et al.

Diazotroph community succession during the VAHINE mesocosms experiment (New Caledonia Lagoon)

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Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

The VAHINE mesocosm experiment, conducted in the low-nutrient low-chlorophyll waters of the Noumea Lagoon (coastal New Caledonia) was designed to trace the incorporation of nitrogen (N) fixed by diazotrophs into the food web, using large volume (50 m³) mesocosms. This experiment provided a unique opportunity to study the succession of different N₂-fixing microorganisms (diazotrophs) and calculate in situ net growth and loss rates in response to fertilization with dissolved inorganic phosphate (DIP) over a 23 day period, using quantitative polymerase chain reaction (qPCR) assays. Inside the mesocosms, the diazotroph community assemblage was dominated by the heterocyst-forming *Richelia* associated with *Rhizosolenia* (Het-1) in the first half of the experiment, and unicellular cyanobacterial Group C (UCYN-C) became the dominant diazotroph in the second half of the experiment. Decreasing DIP concentrations following the fertilization event and increasing temperatures were significantly correlated with increasing abundances of UCYN-C. Maximum net growth rates for UCYN-C were calculated to be between 1.23 ± 0.07 and $2.16 \pm 0.07 \text{ d}^{-1}$ which are among the highest growth rates reported for diazotrophs. Outside the mesocosms in the Noumea Lagoon, UCYN-C abundances remained low, despite increasing temperatures, suggesting that the microbial community response to the DIP fertilization created conditions favorable for UCYN-C growth inside the mesocosms. Maximum net growth and loss rates for nine diazotroph phylotypes throughout the 23 day experiment were variable between mesocosms, and repeated fluctuations between periods of net growth and loss were commonly observed. The field population of diazotrophs in the Noumea Lagoon, was dominated by Het-1 over the course of the study period. However, eight additional diazotroph phylotypes were present in the lagoon at lower abundances, indicating a diverse field population of diazotrophs. Two ecotypes of the *Braarudosphaera bigelowii* symbiont unicellular cyanobacterial group A (UCYN-A) were present simultaneously in the lagoon, with the recently described *B. bigelowii*/UCYN-A2 association present at higher abundances than the *B. bigelowii*/UCYN-A1 association.

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



1 Introduction

Biological nitrogen fixation (BNF), the microbially-mediated conversion of dinitrogen (N_2) gas into bioavailable nitrogen (N), is a significant source of new N in oligotrophic oceanic regions where primary productivity is N limited (Gruber and Sarmiento, 1997; Karl et al., 1997), and has the potential to directly impact carbon sequestration (Karl et al., 2012; Karl and Letelier, 2008). BNF has historically been considered an important process in the oligotrophic ocean gyres, but primary productivity in oligotrophic tropical coastal regions can also be N limited (Torréon et al., 2010) and such environments have the potential to play a significant role in export production in the world's oceans due to the transfer of carbon from tidal and wind-generated currents (Hyndes et al., 2014; Gattuso et al., 1998).

The New Caledonian (Noumea) coral lagoon, located off the southwestern coast of New Caledonia (South Western Pacific), is a tropical low-nutrient low-chlorophyll (LNLC) system and is bounded by one of the world's largest barrier reefs. Oligotrophic ocean water enters the lagoon from the south over the open shelf, then is driven north by the trade winds and tidal forces and exits through several deep inlets in the intertidal barrier reef that forms the western boundary of the lagoon (Ouillon et al., 2010). Primary productivity is N limited throughout the year (Torréon et al., 2010), giving microorganisms able to fix N_2 gas into bioavailable nitrogen (diazotrophs) a competitive edge over non-diazotrophic organisms. High rates of BNF during the austral summer have been reported, in both large size fractions ($> 10 \mu\text{m}$) and small size fractions ($< 10 \mu\text{m}$; Garcia et al., 2007; Biegala and Raimbault, 2008). Large blooms of the most conspicuous and well-studied diazotroph, *Trichodesmium*, have been repeatedly detected in this region using both indirect (via satellite observation; Dupouy et al., 2008, 2000) and direct measurements (Renaud et al., 2005; Masotti et al., 2007; Rodier and Le Borgne, 2008, 2010). Both free-living and organism- and particle-associated unicellular picocyanobacterial diazotrophs are also suspected to be significant contributors to BNF in the lagoon (Garcia et al., 2007; Biegala and Raimbault, 2008), yet the phyloge-

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



netic identity of these picocyanobacteria has yet to be determined. Despite evidence that diverse diazotroph communities exist in the Noumea lagoon, there is very little quantitative diazotroph distribution data from this region (Luo et al., 2012), especially for diazotrophs other than *Trichodesmium*.

5 Marine unicellular cyanobacterial diazotrophs are phylogenetically divided into three groups: the uncultivated unicellular group A (UCYN-A; Zehr et al., 2001, 2008; Tripp et al., 2010) which live in association with strains of the prymnesiophyte *Braarudosphaera bigelowii* (Thompson et al., 2014, 2012; Hagino et al., 2013); the free-living *Crocospaera* sp. (also referred to as unicellular group B or UCYN-B); and the pre-
10 sumably free-living unicellular group C (UCYN-C), which contains several cultivated cyanobacteria including *Cyanothece* sp. strain ATCC 51142 (Reddy et al., 1993) and group C TW3 (Taniuchi et al., 2012).

The marine filamentous cyanobacterial diazotrophs include the colonial, non-heterocyst-forming *Trichodesmium*, and the heterocyst-forming symbionts associated with diatoms (DDAs). DDAs form between different strains of *Richelia* sp. associated with diatoms of the genera *Rhizosolenia* (Het-1) and *Hemiaulus* (Het-2) (Villareal, 1990, 1992). Het-2 is in an obligate symbiont of *Hemiaulus*, as evidenced by its reduced genome size (Hilton et al., 2013). Although the genome of Het-1 shows evidence of
15 some genome reduction, it remains unclear whether Het-1 is also an obligate symbiont of *Rhizosolenia* (Villareal, 1992; Hilton et al., 2013). The heterocyst-forming *Calothrix* (Het-3) has long been observed living as an epiphyte with *Chaetoceros* (Carpenter and Foster, 2002) but can also grow free from its host (Foster et al., 2010), and has a non-streamlined genome (Hilton et al., 2013).

25 Although *nifH* genes are regularly recovered from diverse non-cyanobacterial diazotrophs in oligotrophic ocean waters (Halm et al., 2012; Farnelid et al., 2011; Riemann et al., 2010; Langlois et al., 2005; Hewson et al., 2007; Bird and Wyman, 2012; Bonnet et al., 2013; Fong et al., 2008; Turk-Kubo et al., 2014), their activity and relative significance to BNF remains poorly understood (Turk-Kubo et al., 2014). The most widely studied non-cyanobacterial diazotroph, γ -24774A11, is an uncultivated putative

BGD

12, 9043–9079, 2015

**Diazotroph
succession in
VAHINE mesocosms**

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



gamma proteobacteria most closely related to *Pseudomonas stutzeri* that has been hypothesized to be a potentially important contributor to overall BNF in the South Pacific (Moisander et al., 2014).

Different diazotrophs have potentially different fates in the marine environment. For example, *Trichodesmium* is rarely recovered in sediment traps (Walsby, 1992) and the transfer of fixed N from this diazotroph may be predominantly through the dissolved pool (Mulholland, 2007). In contrast, blooms of DDAs fuel an important summer export flux at the ALOHA station (Karl et al., 2012). This highlights the importance of characterizing the diazotroph community composition when performing biogeochemical studies on the fate of N₂ fixation in the ocean. Such studies are commonly performed on oceanographic cruises, where discrete samples are taken from multiple stations along a cruise track, which passes over many different water masses. This approach is of critical importance to describe the biogeographical distribution of diazotrophs with respect to environmental parameters over large oceanic provinces. However, in order to understand the how diazotroph assemblages shift in response to rapid environmental perturbations inside the same water mass, and to track the incorporation of their newly fixed N into the food web, high frequency sampling of a single water body is required, which is rarely accomplished.

We report here data from the VAHINE mesocosm experiment, detailed by (Bonnet, 2015), which was a large, multi-institute collaborative project conducted to determine which components of the food web were directly supported by newly fixed N. To answer this question, three large (50 m³) mesocosms were deployed in the Noumea lagoon to isolate a part of the water column from physical dispersion without disturbing light penetration and temperature. The same water masses were monitored for 23 days during austral summer conditions. In order to create conditions favorable for diazotrophs, the mesocosms were fertilized with dissolved inorganic phosphorus (DIP) on day 4 of the incubation. This experiment provided unique opportunities to: (1) track rapid diazotroph assemblage shifts using quantitative techniques (quantitative PCR; qPCR) for a long period of time (23 days) in a single water mass fertilized with DIP; (2) calculate in situ

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



net growth and loss rates for targeted diazotroph phylotypes in a complex community; and (3) determine the abundances of targeted diazotrophs outside the mesocosms in the Noumea lagoon during the experimental period.

2 Methods

2.1 Sampling

Three large volume mesocosms (50 m³), based on the design described in Guieu et al. (2010, 2014), were deployed at the exit of the Noumea lagoon (22°29.073 S–166°26.905 E), 28 km off the coast of New Caledonia on 13 January 2013. Detailed descriptions of the mesocosm site selection, deployment and sampling strategy are provided in (Bonnet, 2015). Large volume samples (50 L) were collected from 1, 6, and 12 m depths from each mesocosm once per day at 7 a.m. using a Teflon[®] PFA pump and PVC tubing. This daily sample was immediately transferred back to laboratories aboard the R/V *Alis*, and subsampled for a suite of stock and flux measurements. Samples for DNA analysis were immediately filtered onto 25 mm 0.2 µm Supor[®] filters (Millipore, Billarica, CA), using gentle peristaltic pumping. All filters were flash frozen in liquid nitrogen, and stored at –80 °C until shipment on dry ice from New Caledonia to the University of California, Santa Cruz.

2.2 Determining diazotroph abundances and net growth rates using the quantitative polymerase chain reaction (qPCR)

A subset of samples was processed for the quantification of diazotroph community composition. Preliminary analyses were conducted from three-depth profiles on days 19 and 20, and very little vertical stratification was observed for most of the targeted diazotrophs, with abundances for most diazotrophs measured at the same order of magnitude throughout the 15 m mesocosm (Supplement Fig. S1). *Trichodesmium* and

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Het-3 were the only exceptions to this observation. Based on these findings, further analyses were focused on samples taken from the middle of the mesocosm (6 m), collected on odd days from inside the mesocosms, and even days from outside the mesocosms. DNA was extracted using a Qiagen DNeasy Plant kit (Valencia, CA), with modifications to the protocol optimized to recover high quality DNA from cyanobacteria, including additional cell lysis steps of freeze/thaw cycles, agitation using a bead beater, as well as a proteinase K digestion (Moisander et al., 2008). The quality of DNA extracts was evaluated using a NanoDrop (Thermo Scientific, Waltham, MA), and concentrations were determined using a PicoGreen[®] dsDNA Quantitation Kit (Molecular Probes, Eugene, OR, USA), according to manufacturer's guidelines.

Nine diazotrophic phylotypes were quantified in samples from the mesocosms and the Noumea lagoon, using quantitative PCR (qPCR), using Taqman[®] assays widely used in the marine environment. This study targeted representatives from known major marine diazotroph lineages: two unicellular diazotrophic symbionts of different *Braarudosphaera bigelowii* strains, UCYN-A1 (Church et al., 2005a), UCYN-A2 (Thompson et al., 2014); two free-living unicellular diazotroph cyanobacterial phylotypes UCYN-B (*Crocospaera* sp.; Moisander et al., 2010), and UCYN-C (Foster et al., 2007); filamentous, colonial, non-heterocyst forming *Trichodesmium* spp. (Church et al., 2005a); three diatom-diazotroph associations (DDAs), *Richelia* associated with *Rhizosolenia* (Het-1; Church et al., 2005b), *Richelia* associated with *Hemiaulus* (Het-2; Foster et al., 2007), and *Calothrix* associated with *Chaetoceros* (Het-3; Foster et al., 2007); and a widespread γ -proteobacterial phylotype γ -24774A11 (Moisander et al., 2008).

Recombinant plasmids with the targeted organism *nifH* fragment were used as qPCR standards, and each 96 well plate was run with a serial dilution (10^0 – 10^7 *nifH* copies reaction⁻¹) of the appropriate standard. All qPCR reactions were set up with 1 μ L of DNA extract in a 15 μ L volume with the following reagents and final concentrations: 1X Taqman[®] Master Mix (Applied Biosystems, Carlsbad, CA, USA), 0.4 μ M each forward and reverse primers, and 0.2 μ M probe (5'-FAM and 3'-TAMRA labeled). Thermocycle parameters were as described in Goebel et al. (2010) for all assays, with the exception

BGD

12, 9043–9079, 2015

**Diazotroph
succession in
VAHINE mesocosms**

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



of using a 64 °C annealing temperature for UCYN-A2. The qPCR reaction efficiencies were as follows: UCYN-A1 – 96.7 ± 2.8 %; UCYN-A2 – 94.9 ± 3.4 %; UCYN-B – 101.3 ± 2.2 %; UCYN-C – 95.4 ± 2.9 %; γ -24774A11 – 96.2 ± 3.2 %; *Trichodesmium* – 93.6 ± 1.4 %; Het-1 – 96.1 ± 4.7 %; Het-2 – 98.3 ± 2.0 %; and Het-3 – 97.5 ± 0.9 %. Based on the differences in sample volumes, the limits of detection (LOD) and quantification (LOQ) for all qPCR assays ranged between 11–56 *nifH* copies L⁻¹, and 87–444 *nifH* copies L⁻¹, respectively. Samples were determined to be “detected, not quantified” (DNQ) when calculated abundances were greater than the LOD, but less than the LOQ. Abundances are reported as *nifH* copies L⁻¹, rather than cells L⁻¹ because there is currently little information about the number of *nifH* copies per genome in these diazotroph targets.

Growth and loss rates were calculated for individual diazotrophs inside the mesocosms when abundances were higher than the LOQ on two consecutive sampling days, as described in Moisander et al. (2012), using the following formula: $k = 2.303 \cdot (\log_{10}(N_{t_2} - N_{t_1})) / (t_2 - t_1)$, where N_x = abundance at time x . This assumes that the organisms were growing exponentially during the experiment, which cannot be easily verified in field populations. These rates implicitly include grazing, mortality and viral lysis, and thus are net growth and loss rates.

2.3 PCR amplification of *Cyanothece*-like organisms

Partial *nifH* gene fragments (233 base pairs) from *Cyanothece*-like organisms were PCR amplified using primers designed as part of this study to broadly target the UCYN-C group, including cultivated *Cyanothece* spp., *Gleoeocapsa* spp., the cyanobionts of diatoms *E. turgida*, and *R. gibba*, as well as many closely related uncultivated *Cyanothece*-like ecotypes. The oligonucleotide primers *Cyanothece_nifH_F* (5'-CTT AGC AGC AGA ACG GGG AA-3') and *Cyanothece_nifH_R* (5'-GCA TTG CGA AAC CAC CAC AA-3') were designed using NCBI's Primer BLAST, screened in silico for cross reactivity to non-target *nifH* phylotypes in a curated *nifH* ARB database (Heller et al., 2014) and synthesized by Sigma Oligos (St. Louis, MO, USA).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Duplicate PCR reactions were carried out in 20 μ L volumes with 1X Platinum[®] Taq PCR buffer (Invitrogen, Carlsbad, CA), 3.0 mM MgCl₂, 400 μ M dNTP mix, 0.2 μ M of each forward and reverse primers, 1 U Platinum[®] Taq polymerase (Invitrogen), and 2 μ L of DNA extract. Thermocycle parameters were as follows: the initial denaturation step at 94 °C for 5 min was followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s and a final elongation step at 72 °C for 10 min. PCR amplicons were cleaned using the QiaQuick Gel Extraction Kit (Qiagen), and sequenced directly using Sanger technology at the UC Berkeley DNA Sequencing Center using *Cyanothece_nifH_R* to prime the sequencing reaction.

Raw sequences were processed using Sequencher 5.2.4 (Gene Codes Corporation, Ann Arbor, MI) and phylogenetic analyses were conducted in ARB (Ludwig et al., 2004), using a curated database of all *nifH* sequences available in Genbank (Heller et al., 2014) and in MEGA 6.06 (Tamura et al., 2011).

2.4 Statistical analyses

Pearson correlation coefficients were calculated for each paired variable, after transformation to assure normality, and samples collected from the incubations and the Noumea lagoon were treated independently. Supplement Tables S1 and S2 detail linear associations and transformations required for each variable inside and outside the mesocosms, respectively. Diazotroph abundance and physico-chemical environmental data was analyzed in R (Team, 2012). Biplot from principal component analysis was used to examine the temporal and spatial variation in environmental parameters. Correspondence analysis of the abundance data in each mesocosm separately was performed with the *ca* package (Nenadic and Greenacre, 2007). Biplots of sampling days (rows) and organisms (columns) were compared to examine reproducibility across mesocosms. Least squares regression models were calculated to fit and predict the effect of temperature, salinity, and PO₄ on diazotroph abundance.

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



3 Results and discussion

3.1 Dominant diazotrophs in VAHINE mesocosms experiment shift from *Richelia* to *Cyanothece*-like ecotypes

During the VAHINE mesocosm experiment, three periods could be roughly defined based on the identity of the most abundant diazotroph and biogeochemical parameters (described in detail in Berthelot et al., 2015). A diazotroph community that is similar to the Noumea lagoon field community and low DIP concentrations characterized the period prior to the DIP spike (hereafter referred to as P0). The second period, days 5–14 (hereafter referred to as P1), was characterized by a diazotroph assemblage dominated by the heterocystous cyanobacteria *Richelia* associated with *Rhizosolenia* (Het-1), high DIP availability, as well as moderate N₂ fixation rates ($9.8 \pm 4.0 \text{ nmol L}^{-1} \text{ d}^{-1}$). In the second half of the experiment, days 15–23 (hereafter referred to as P2), the diazotroph assemblage shifted to be dominated by UCYN-C, and was characterized by low DIP availability, and high N₂ fixation rates ($27.7 \pm 8.6 \text{ nmol L}^{-1} \text{ d}^{-1}$).

The diazotroph assemblage in the Noumea lagoon on the day that the mesocosm experiment was initiated was composed primarily of Het-1 ($3.1 \times 10^4 \text{ nifH copies L}^{-1}$), and *Richelia* associated with *Hemiaulus* (Het-2; $1.2 \times 10^4 \text{ nifH copies L}^{-1}$), as well as the *B. bigelowii*-associated UCYN-A2 ($1.5 \times 10^4 \text{ nifH copies L}^{-1}$) and UCYN-A1 ($5.6 \times 10^3 \text{ nifH copies L}^{-1}$). Together these four phylotypes accounted for 95% of the total *nifH* pool quantified in day 1 samplings (Fig. 2e, Supplement Table S4). *Trichodesmium* and the uncultivated unicellular cyanobacterial group C (UCYN-C) were minor components of the diazotrophic community at $2.8 \times 10^3 \text{ nifH copies L}^{-1}$ (4%) and $7.8 \times 10^2 \text{ nifH copies L}^{-1}$ (1%), respectively. γ -24774A11, a heterotrophic diazotroph with widespread occurrence, and *Calothrix* associated with *Chaetoceros* (Het-3) were present but at abundances too low to quantify (DNQ), and no *Crocospaera* sp. (UCYN-B) could be detected (Figs. 1 and 2e, Supplement Tables S3 and S4).

During P0, evidence of shifts from the original Noumea lagoon diazotroph assemblage could be observed in all three mesocosms (Fig. 2b–e). In M1, M2 and M3, Het-1

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



remained the most abundant diazotroph, and the relative proportions of Het-2 and UCYN-A1 were similar to those measured outside the mesocosms. However, UCYN-A2 abundances decreased and *Trichodesmium* abundances increased over this three-day period with respect to their abundances in the lagoon. These changes were most pronounced in M3. As in the lagoon, UCYN-C and γ -24774A11 were present at low abundances (ca. 10^2 *nifH* copies L⁻¹) in the day 3 sampling.

Diazotrophic assemblages within each mesocosm remained relatively consistent throughout the first thirteen days of the experiment, with Het-1 the most abundant diazotroph (Fig. 2b–d). However, a significant shift in the diazotroph assemblage began about halfway through the 23 day experiment, evidenced by the increasing abundances of UCYN-C and Het-3, and decreasing abundances of Het-1 and UCYN-A1 (Figs. 2b–d and 3). Increasing UCYN-C abundances were first seen in M1 (day 11), then M2 (day 13), followed by M3 (day 15), which reflected the time in each mesocosm when the DIP turnover time dropped below 1 d⁻¹, signaling DIP limitation (Berthelot et al., 2015; Moutin et al., 2005). In all three mesocosms, which acted as biological replicates, UCYN-C abundances were not only strongly correlated with the decreasing DIP concentrations ($p = 0.004$, $r = -0.51$), but also increasing temperatures in the mesocosms ($p = 0.000$, $r = +0.86$) and could be weakly correlated with decreasing Het-1 ($p = 0.002$, $r = -0.54$), Het-2 ($p = 0.01$, $r = -0.37$) and UCYN-A1 ($p = 0.000$, $r = -0.64$) abundances. A similar correlation between UCYN-C abundance and temperature was observed outside in the Noumea Lagoon (although UCYN-C abundances in the lagoon remained low; see Sect. 3.4), and this data suggests that temperatures below 25.6 °C are not optimal temperatures for growth of this group. This is consistent with the warmer temperatures required for growth for the UCYN-C isolate TW3 (Taniuchi et al., 2012), and the occurrence of the UCYN-C group in low latitude, warm waters (> 28 °C) in the Tropical North Atlantic (Langlois et al., 2008). However, temperature alone cannot explain the dominance of UCYN-C in these mesocosms, as similar temperature increases were recorded in the lagoon without a corresponding increase in UCYN-C abundances, and other diazotrophs also can grow optimally at these tem-

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

peratures (e.g. *Crocospaera* sp., Webb et al., 2009 and *Trichodesmium* sp., Breitbarth et al., 2007).

Het-3 was undetected in all mesocosms until day 7, and then remained at low abundances (usually below quantitation limits) until day fifteen of the incubation. After day 15, Het-3 abundances were of similar order of magnitude to the Het-3 population in the Noumea Lagoon (ca. 10^2 – 10^3 *nifH* copies L⁻¹). Increases in Het-3 abundances after day 15 could be strongly correlated to increases in temperature ($p = 0.000$, $r = +0.82$) as well as salinity ($p = 0.000$, $r = +0.78$), total chl a ($p = 0.000$, $r = +0.71$), and UCYN-C abundances ($p = 0.000$, $r = +0.77$). Het-3 abundances could also be weakly correlated to increases in bulk N₂ fixation rates ($p = 0.01$, $r = +0.49$), and decreases in PO₄ concentrations ($p = 0.003$, $r = -0.54$), UCYN-A1 ($p = 0.000$, $r = -0.64$) and Het-1 ($p = 0.03$, $r = -0.38$) abundances. Het-3 abundances never accounted for more than 2% of the total diazotroph community, however their distribution throughout the water column (Supplement Fig. S1) and their recovery in the sediment traps (Bonnet, 2015) suggests the rapid sinking of these organisms may play an important role in supplying fixed N to sediments in this shallow coastal system.

Both UCYN-A2 and UCYN-A1 abundances peaked early in the mesocosm incubations, at days 5 and 7, respectively. UCYN-A2 abundances were not strongly correlated to any environmental factors, and were only weakly correlated to bulk N₂ fixation rates ($p = 0.008$, $r = +0.49$) and N₂ fixation rates associated with the < 10 μm size fraction ($p = 0.045$, $r = +0.38$). However, decreasing UCYN-A1 abundances could be strongly correlated to temperature increases ($p = 0.000$, $r = -0.75$), and decreasing PO₄ concentrations ($p = 0.000$, $r = +0.69$). This implies that the *B. bigelowii*/UCYN-A1 association benefitted either directly or indirectly from the day 4 introduction of PO₄ in the early part of the experiment. There is evidence that UCYN-A1 *nifH* transcription (a proxy for active N₂ fixation) is limited by the availability of inorganic P (Turk-Kubo et al., 2012). UCYN-A1 is unable to directly utilize components of the dissolved organic phosphate (DOP) pool, such as phosphoesters and phosphonates (Tripp et al., 2010), but nothing is known about the capability of the *B. bigelowii* host to utilize DOP substrates.

Diazotroph
succession in
VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



The correlation between UCYN-A1 abundances (thus assumed correlation between the symbiosis as a whole) and inorganic P concentrations in the VAHINE mesocosms provides further evidence that inorganic P may be the preferred P source for the *B. bigelowii* host.

Multiple regression models underscore the importance of a small number of environmental factors in the abundances of three of the diazotrophs targeted, both inside and outside the mesocosms. The log abundances of UCYN-A1, UCYN-C and Het-3 in all three mesocosms and in the lagoon can be modeled well using only the temperature, salinity, and PO₄ data, with R^2 values between 0.77–0.85, and R^2 -cv between 0.60–0.76 (Table 1), when the sample location (M1–M3 and outside) are included as a variable. Although the three-predictor model provides the highest quality fit to the data, the goodness-of-prediction values (R^2 -cv) are comparable between models that use all three predictors vs. models using just T alone in the case of UCYN-A1 and UCYN-C, and just PO₄ alone for all three diazotrophs (Table 1).

These results from the linear regression model imply that the most important environmental factor best correlated with the dramatic increase in UCYN-C and Het-3 abundances was the decreasing concentration of PO₄. There are several potential explanations for this observation. Considering that UCYN-C maintained low population abundances in the Noumea Lagoon during the time of the mesocosm experiment, it follows that UCYN-C may have benefitted either indirectly from the DIP fertilization, or directly from a physical aspect of the mesocosms themselves. A biofilm had accumulated on the sides of the mesocosm bags, and although this was not sampled for molecular analysis, it is possible that the UCYN-C ecotype was a component of this biofilm community, thus dependent on a physical environment not representative of water column conditions. It also may be possible that the inverse correlation between UCYN-C abundances and DIP concentrations may be a result of UCYN-C being able to outcompete other diazotrophs for organic P substrates, under low DIP conditions. *Cyanothece* sp. strains PCC 8801 and 8802 have genes used in phosphonate metabolism (Bandyopadhyay et al., 2011), which strongly implies that some strains are able to use this

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



organic P substrate to meet cellular P requirements. The strain most closely related to the UCYN-C ecotype, *Cyanothece* sp. CCY0100, also has genes for phosphonate metabolism and transport (JGI website). It is evident that some component of the microbial community in the mesocosms was utilizing DOP, as DOP stocks decreased in the second half of the experiment (Berthelot et al., 2015), however, UCYN-C abundances could not be correlated to this drawdown (data not shown). *Trichodesmium erythraeum* IMS101 and *Calothrix rhizosoleniae* SC01 (Het-3) are the only two other diazotrophs targeted in this study known to possess the metabolic capability to utilize phosphonates (Dyhrman et al., 2006; Hilton et al., 2013). *Richelia* associated with *Hemiaulus* (Het-2) do not have any genes for the metabolism of phosphonates (Hilton et al., 2013), but it remains unclear whether the symbiont of *Rhizosolenia* (Het-1) is able to use phosphonates, as four genes related to phosphonate metabolism were identified in the genome (Hilton, 2014). However, as with UCYN-C, no correlation between DOP concentration and *Trichodesmium*, Het-1 or Het-3 abundances were seen (data not shown).

3.2 Phylogenetic identity of diazotroph targeted with UCYN-C qPCR assay

The UCYN-C qPCR assay used in this study was designed to target a *nifH* sequence type recovered from Amazon-influenced waters in the Tropical North Pacific (Foster et al., 2007). In addition to uncultivated sequences of marine origin, this assay also targets cultivated members of the UCYN-C group including *Cyanothece* sp. strains AT51142, AT51472, CCY 0110, as well as some freshwater cyanobacterial symbionts of diatoms (Fig. 4). Due to the importance of this group in the mesocosm experiment, and the uncertainty about exactly which organism(s) were targeted with the qPCR assay, PCR amplification of a partial *nifH* fragment was used to characterize the identity of the *Cyanothece*-like organisms. Two closely related sequence types were recovered from 6 m samples on days 15 and 20, represented by 60341CB and 60343CB (Fig. 4), which shared 95 % nucleotide similarity. The *Cyanothece*-like sequences recovered from the mesocosms were most closely related to *Cyanothece* sp. CCY0100 (92–93 % nucleotide identity; Fig. 4), which was isolated from coastal waters in Chwaka

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Bay, Zanzibar. VAHINE mesocosm ecotypes shared only 90–91 % nucleotide identity to the sequences used to design the UCYN-C primer of Foster et al. (2007), and the UCYN-C isolate TW3 (Taniuchi et al., 2012). Thus the ecotypes that reached such high abundances in the mesocosms were more closely related to an ecotype reported in a coastal environment than those recovered from open water regimes.

3.3 In situ net growth rates

The VAHINE mesocosm experiment provided a rare opportunity to repeatedly sample the same water mass for an extended period of time, thus the ability to empirically determine in situ net growth or loss rates for individual diazotroph phylotypes, based on the change in *nifH* gene copies L⁻¹ between sampling days. Growth (and loss) rates are critical input parameters for mathematical models of oceanic N budgets. Culture-based rates or estimates are often employed because there have been so few measurements of in situ rates from natural populations of diazotrophs, and some species such as UCYN-A remain uncultivated. However, due to the lack of competition and grazing in culture, these rates may be overestimated compared to in situ rates.

Surprisingly, maximum net growth rates for each diazotroph phylotype were among the highest reported for oceanic diazotrophic organisms (even when considering culture-based growth rates), yet showed substantial variability between mesocosms, both in terms of the absolute rates and the patterns of growth and loss across time (Table 2). For example, maximum growth rates for UCYN-A2 ranged between 0.52 and 1.71 d⁻¹, but occurred early in the experiment in M3 (day 5), in the middle of the experiment in M2 (day 11) and at the end of the experiment in M1 (day 20). The two phylotypes with consistent timing of maximum growth rates across mesocosms were UCYN-C and Het-3. UCYN-C had the highest maximum growth rates of all phylotypes, which ranged between 1.23–2.16 d⁻¹ and occurred within a four-day period (day 11–15) in all mesocosms. Het-3, which was virtually absent for the first half of the experiment in all three mesocosms, had maximum net growth rates between 0.57–1.09 day⁻¹, that occurred within a five day period (day 15–20) in all mesocosms.

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Diazotroph
succession in
VAHINE mesocosms**K. A. Turk-Kubo et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Moisander et al. (2012) reported maximum net growth rates from nutrient amendment experiments conducted in the South Western Pacific close to New Caledonia for UCYN-A1 (0.19 d^{-1}), UCYN-B (0.61 d^{-1}) and γ -24774A11 (0.52 d^{-1}). The maximum net growth rates calculated for these phylotypes during the VAHINE project were considerably higher, at 0.73 , 1.38 , and 1.07 d^{-1} for UCYN-A1, UCYN-B and γ -24774A11, respectively. These results are unexpected considering that the rates determined by Moisander et al. (2012) were from a series of nutrient amendment incubations in 4.5 L bottles, where presumably favorable conditions for the growth of diazotrophs were present.

The maximum net growth rates determined in situ for UCYN-A2 and UCYN-C were among the highest measured at 1.71 , and 2.16 d^{-1} , respectively, and represent the only reported growth rates for these uncultivated diazotrophs. Very little is known about the newly described uncultivated *B. bigelowii*/UCYN-A2 association, but the difference between UCYN-A1 and UCYN-A2 net growth rates and their patterns within the same mesocosm indicate that the growth rates of each association are likely dependent upon different environmental variables. It also seems plausible, due to the fact that *B. bigelowii* host of UCYN-A2 is thought to be much larger than that of UCYN-A1 (Thompson et al., 2014), that they are grazed by different protists association experiences less grazing pressure.

Experimental growth rates as high as 1.92 d^{-1} have been reported for batch cultures of *Cyanothece* sp. ATCC 51142 (Vu et al., 2012), and Taniuchi et al. (2012) measured rates up to 0.85 d^{-1} in the presumably oligotrophic UCYN-C TW3 isolated from the Kuroshio Current. Thus, rates calculated for UCYN-C in the VAHINE mesocosms experiment are similar in magnitude. However, such high rates in culture are a direct result of the lack of competition with other organisms for nutrients important for growth and the lack of grazing pressure. Despite high growth rates in culture, TW3 is found at low abundances in the Kuroshio Current, presumably due to these factors (Taniuchi et al., 2012). Although the factors behind such high growth rates for UCYN-C in the

mesocosms are not clear, it is possible that UCYN-C was free from significant grazing in these experiments.

Maximum net growth rates for the filamentous diazotrophs were also generally higher than previously reported growth rates. *Trichodesmium* sp. maximum net growth rates for were as high as 1.46 ± 0.05 (M1) and $1.55 \pm 0.02 \text{ d}^{-1}$ (M3), and microscopic analyses indicated (data not shown) that both *T. erythraeum* and *T. thiebautii* were present in the mesocosms, the former being the dominant *Trichodesmium* species. These calculated rates are much higher than the specific growth rate previously reported for cultures of *T. erythraeum* ($0.29 \pm 0.04 \text{ d}^{-1}$; Hutchins et al., 2007) and net growth rates for *T. thiebautii* populations in the Noumea lagoon ($0.11\text{--}0.38 \text{ d}^{-1}$; Rodier and Le Borgne, 2008). Maximum growth rates for the DDAs Het-1, Het-2, and Het-3 were 1.28, 2.24, and 1.09 d^{-1} , respectively. Although these are the first reported growth rates for these DDAs determined using in situ cellular abundances, Foster et al. (2011) determined maximum net growth rates to be much lower when using nanoSIMS-based techniques to quantify ^{15}N incorporation in biomass, which is to be expected if the DDAs are using other N sources (eg. dissolved organic N and/or recycled N) in addition to fixing N (Bonnet, 2015).

For many diazotrophs, the pattern of net growth and loss rates indicated very dynamic populations that were specific to each mesocosm. Most diazotrophs experienced multiple transitions between net growth and net loss within a single mesocosm throughout the 23 day incubation (Table 2). For example, in M1, net growth rates for *Trichodesmium* were observed approximately every other sampling period (on days 9, 13, 15, 19, and 23), and in some intervening periods experienced loss rates similar in magnitude to growth rates (e.g. day 7 and 11). In contrast, net growth occurred on days 7, 11, 13, 20, and 23 in M3, and one of the largest net loss rates was measured at day 9 ($-2.16 \pm 0.18 \text{ d}^{-1}$). The dynamic nature of *Trichodesmium* sp. growth and loss rates in each mesocosm most likely results from a combination of grazing pressure and viral lysis. If multiple *Trichodesmium* ecotypes existed within the mesocosms, it is also possible that these dynamic growth rates are not representative of a single ecotype

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

growing and dying, but the dynamics of multiple ecotypes unresolved using the qPCR assay.

3.4 Symbiotic *Richelia* and UCYN-A ecotypes dominated diazotrophic community structure in the Noumea lagoon during the VAHINE mesocosms experiment

The VAHINE mesocosm experiment provided an additional opportunity to characterize the diazotroph community structure in the Noumea lagoon using quantitative techniques. As described above, the diazotroph community at the onset of the VAHINE mesocosms experiment was dominated by Het-1, Het-2, UCYN-A2 and UCYN-A1 (see Sect. 3.1). Het-1 remained the most abundant diazotroph for most of the sampling days (3.1×10^4 – 5.5×10^5 *nifH* copies L⁻¹; Fig. 1) during the period of study, accounting for between 39–92 % of the total diazotroph community (Fig. 2e, Supplement Tables S3 and S4). Het-2 abundances were consistently lower than Het-1, and variable throughout the period of study, ranging between 4.0×10^3 – 1.6×10^4 *nifH* copies L⁻¹, and reaching peak abundances on day 10 and again on day 18 (Fig. 1, Supplement Table S3). Bonnet (2015) discusses the BNF rates in detail, however, it should be noted that in the lagoon only Het-2 abundances had significantly strong positive correlation with bulk N₂ fixation rates ($p = 0.01$, $r = +0.78$).

Although *Richelia* living in association with *Rhizosolenia* (Het-1) and *Hemiaulus* (Het-2) are known to be widely distributed and potentially significant N₂-fixers in oligotrophic oceans (Carpenter et al., 1999; Subramaniam et al., 2008; Karl et al., 2012), reports of their presence in coastal waters in the Southwest Pacific are rare, and previous studies during the austral summer in the Noumea Lagoon reported very low abundances of heterocystous symbionts (Rodier and Le Borgne, 2010; Biegala and Raimbault, 2008). This is the first report on quantitative abundances of *Richelia* in the Noumea lagoon, which are comparable to abundances reported from the Tropical North Atlantic (Foster et al., 2007; Goebel et al., 2010) and the North Pacific Subtropical Gyre (Church et al., 2008; Foster and Zehr, 2006), where the DDAs are thought to have a significant impact

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



on C sequestration due to their productivity and rapid sinking (Subramaniam et al., 2008; Karl et al., 2012; Villareal et al., 2012), and may play a role in supporting the benthic community (Houlbreque and Ferrière-Pagès, 2009).

UCYN-A2 was the second most abundant member of the diazotrophic community in the lagoon for the first 10 days of the experiment (Fig. 2e), and was present at abundances as high as 1.1×10^5 *nifH* copies L⁻¹ (day 6; Fig. 1, Supplement Table S3). UCYN-A2 abundances declined steadily throughout the period of the 23 day experiment ($p = 0.04$, $r = -0.63$), and had a significantly strong negative correlation to sea temperature ($p = 0.003$, $r = -0.82$) and salinity ($p = 0.03$, $r = -0.89$), both of which were increasing in Noumea lagoon throughout the VAHINE deployment (Bonnet, 2015). Surprisingly, UCYN-A1 abundances were consistently lower than those of UCYN-A2, ranging between 2.8×10^3 – 6.4×10^4 *nifH* copies L⁻¹ with peaks in abundance at day 6 and again at day 22 (Fig. 1, Supplement Table S3). Interestingly, UCYN-A1 abundances had significant positive correlation to total chl *a* in the > 10 µm size fraction ($p = 0.03$, $r = +0.72$). The significant inverse relationship between UCYN-A2 abundances and water temperatures in the Noumea lagoon, suggests that the *B. bigelowii*/UCYN-A2 association may thrive at lower temperatures than other diazotrophs (e.g. *Trichodesmium*), similar to the *B. bigelowii*/UCYN-A1 association (Moisander et al., 2010).

The coexistence of the *B. bigelowii*/UCYN-A1 and *B. bigelowii*/UCYN-A2 ecotypes in the Noumea Lagoon, both present at reasonably high abundances, indicates that the ecological niches of these cryptic symbioses overlap. Very little information currently exists about the ratio of UCYN-A1 to UCYN-A2 in tropical oligotroph oceanic regimes, but UCYN-A1 is typically found at higher relative abundances (as inferred from clone-library based studies; Thompson et al., 2014) in oligotrophic regions. The Noumea Lagoon is the first location where the co-occurrence of each ecotype has been verified using quantitative techniques, but it must be noted that the difference in abundances may be a result of the number of UCYN-A2 cells associated with each *B. bigelowii* host, which has been reported to be as high as 11 : 1 (Thompson et al., 2014).

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Trichodesmium spp. have been routinely observed in the Noumea Lagoon using satellite observations (e.g. Dupouy et al., 2000, 2008) and direct field measurements (Renaud et al., 2005; Masotti et al., 2007; Rodier and Le Borgne, 2010, 2008). Conspicuous blooms that occur in the austral spring and summer months have been associated with warm sea surface temperatures ($> 26^{\circ}\text{C}$) and recent NO_3 and soluble reactive P (SRP) enrichments in the lagoon (Rodier and Le Borgne, 2008). Although *Trichodesmium* was present at relatively low abundances during the first 8 days of the experiment (3.4×10^2 – 6.5×10^3 *nifH* copies L^{-1} ; Fig. 1, Supplement Table S3), abundances increased steadily throughout the experiment ($p = 0.01$, $r = +0.73$). During sampling days 14 and 16, *Trichodesmium* abundances accounted for ca. 15% of the total diazotroph population, and on the final sampling day (day 22) *Trichodesmium* reached peak abundances of 1.4×10^5 *nifH* copies L^{-1} (42% of the entire diazotrophic community signal; Fig. 2e). The only environmental parameter with which changes in *Trichodesmium* abundances could be strongly correlated was NH_4^+ ($p = 0.02$, $r = +0.81$).

The UCYN-C group comprised a minor part of the Noumea lagoon diazotroph community, and was detected at low abundances (3.2×10^2 – 4.8×10^3 *nifH* copies L^{-1} ; Fig. 1; Supplement Table S3) that never accounted for greater than 2% of the total diazotrophic community. However, UCYN-C abundances did show a linear increase over the duration of the experiment ($p = 0.01$, $r = +0.74$), and significant positive correlations to changes in salinity ($p = 0.02$, $r = +0.80$), and NH_4 ($p = 0.03$, $r = +0.76$). The unicellular UCYN-C group was first described when *nifH* sequences with 90% nucleotide identity to the cultivated *Cyanothece* sp. PCC 51142 were recovered from surface waters in the Tropical North Atlantic (Langlois et al., 2005; Foster et al., 2007). This phylotype has rarely been quantified in the oligotrophic ocean, and when present, abundances are low (Foster et al., 2007; Langlois et al., 2008; Needoba et al., 2007; Goebel et al., 2010; Ratten et al., 2014), therefore very little is known about its distribution or importance. The qPCR assay used in this study (Foster et al., 2007) amplifies not only the uncultivated Atlantic phylotype, but also many cultivated *Cyanothece*- and

Gloeotheca-like isolates, which are expected to be present in an environment like the Noumea Lagoon, as *Cyanotheca*- and *Gloeotheca*-like organisms have been reported in shallow marine sediments (Hunter et al., 2006; Bauer et al., 2008) and intertidal sands (Reddy et al., 1993; Ohki et al., 2008).

5 γ -24774A11 was also consistently detected at low abundances (ca. 10^2 – 10^3 *nifH* copies L⁻¹; Fig. 1; Supplement Table S3), which increased throughout the duration of the experiment ($p = 0.03$, $r = +0.65$). Despite being the most widely studied marine heterotrophic diazotroph, very little is known about this phylotype. Recent studies suggest that this γ -proteobacterial diazotroph is likely to be free-living and may be able to
10 sustain in a broad range of environmental conditions, as evidenced by low but uniform abundances throughout the photic zone in the South Pacific (Moisander et al., 2014).

The epiphytic *Chaetoceros* symbiont, *Calothrix* (Het-3), was also consistently detected at low abundances (ca. 10^2 – 10^3 *nifH* copies L⁻¹; Fig. 1; Supplement Table S3). *Chaetoceros* can often be found as part of the neritic diatom assemblage, therefore this
15 association is generally observed in coastal or coastal transition zone regions (Gómez et al., 2005). This is the first report of Het-3 in the coastal oligotrophic waters surrounding New Caledonia.

Crocospaera sp. (UCYN-B), previously reported to be members of the unicellular diazotroph community in the Noumea Lagoon (Biegala and Raimbault, 2008), were not
20 initially detected, but were present at low abundances that increased over the 23 day period ($p = 0.04$, $r = +0.63$) to abundances as high as 2.7×10^3 *nifH* copies L⁻¹, and had a significant strong correlation with total chl *a* ($p = 0.02$, $r = +0.81$).

4 Conclusions

25 The VAHINE mesocosm experiment was conducted to trace the incorporation of N₂ fixed by diazotrophs into the food web (Bonnet, 2015). Despite lack of sampling replication within each mesocosm, consistent changes in the relative abundances of four diazotrophic phylotypes were reproducibly seen in all three mesocosms, although the

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



5 timing of the increases were specific to an individual mesocosm. UCYN-C and Het-3 increased in abundances over time, and UCYN-A1 and Het-1 decreased in abundance over time in all three mesocosms. The experimental conditions selected for the dominance of UCYN-C during P2, an ecotype never before quantified at high abundances
10 in the marine water column, which enabled the calculation of growth rates of this uncultivated ecotype, and provided insight into its dynamics with respect to environmental parameters. Although the data strongly suggests that the drawdown of DIP provided an environment favorable for high UCYN-C growth rates, further studies are required to better understand the environmental conditions that stimulated this bloom, and whether
15 such blooms are seen in the Noumea Lagoon itself.

The experimental set up provided a rare opportunity to calculate in situ net growth rates for natural populations of diazotrophs, including the uncultivated UCYN-A. This study provided the first growth rates for the UCYN-C phylotype and for UCYN-A2, both of which were surprisingly high, implying not only favorable conditions, but also a lack
20 of grazing pressure. Maximum net growth rates were high for all diazotroph ecotypes, but most also experienced intermittent periods of growth and loss within the 23 day experiment, which was also an unexpected finding. Along with net rates recently reported by Moisander et al. (2012), we anticipate that this data will be important for future modeling efforts.

25 The analysis of the diazotroph assemblage outside the mesocosms represents the first quantitative data on diazotroph populations in the Noumea Lagoon. Not previously considered to be a significant diazotrophs in this region, DDAs must now be considered a potentially important contributor to BNF in these waters, especially in the austral summer. Although the presence of UCYN-A in the lagoon has long been suspected due to the relative importance of daytime nitrogen fixation in the small size fraction (Biegala and Raimbault, 2008), we report the first quantitative data on UCYN-A abundances in the Noumea lagoon. Furthermore, the co-occurrence of two UCYN- A ecotypes revealed in this study, provides important insight into the overlap in environmental niches for these two ecotypes.

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Author contributions. S. Bonnet designed and executed the experiments, and S. Bonnet and A. Desnues sampled the experiments for molecular analyses. M. E. Hogan extracted DNA samples, and K. A. Turk-Kubo conducted all qPCR and PCR analysis, and analyzed the data. I. E. Frank performed statistical analyses. K. A. Turk-Kubo and J. P. Zehr prepared the manuscript with input from all co-authors.

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References

- Bandyopadhyay, A., Elvitigala, T., Welsh, E., Stöckel, J., Liberton, M., Min, H., Sherman, L. A., and Pakrasi, H. B.: Novel metabolic attributes of the genus *Cyanothece*, comprising a group of unicellular nitrogen-fixing cyanobacteria, *MBio*, 2, e00214–00211, 2011.
- Bauer, K., Díez, B., Lugomela, C., Seppälä, S., Borg, A. J., and Bergman, B.: Variability in benthic diazotrophy and cyanobacterial diversity in a tropical intertidal lagoon, *FEMS Microbiol. Ecol.*, 63, 205–221, 2008.
- Berthelot, H., Moutin, T., L'Helguen, S., Leblanc, K., Hélias, S., Grosso, O., Leblond, N., Charrière, B., and Bonnet, S.: N_2 fixation and DON as significant N sources for primary and export productions during the VAHINE mesocosms experiment (New Caledonia lagoon), *Biogeosciences*, in preparation, 2015.

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Diazotroph
succession in
VAHINE mesocosms**K. A. Turk-Kubo et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Biegala, I. C. and Raimbault, P.: High abundance of diazotrophic picocyanobacteria (< 3 μm) in a Southwest Pacific coral lagoon, *Aquat. Microb. Ecol.*, 51, 45–53, doi:10.3354/ame01185, 2008.

Bird, C. and Wyman, M.: Transcriptionally active heterotrophic diazotrophs are widespread in the upper water column of the Arabian Sea, *FEMS Microbiol. Ecol.*, 84, 189–200, doi:10.1111/1574-6941.12049, 2012.

Bonnet, S.: Introduction to the project VAHINE: Variability of vertical and trophic transfer of fixed N_2 in the south west Pacific, *Biogeosciences*, in preparation, 2015.

Bonnet, S., Dekaezemaeker, J., Turk-Kubo, K. A., Moutin, T., Hamersley, R. M., Grosso, O., Zehr, J. P., and Capone, D. G.: Aphotic N_2 fixation in the eastern tropical South Pacific Ocean, *PLoS ONE*, 8, e81265, doi:10.1371/journal.pone.0081265, 2013.

Breitbarth, E., Oschlies, A., and LaRoche, J.: Physiological constraints on the global distribution of *Trichodesmium* – effect of temperature on diazotrophy, *Biogeosciences*, 4, 53–61, doi:10.5194/bg-4-53-2007, 2007.

Carpenter, E. J. and Foster, R. A.: Marine cyanobacterial symbioses, in: *Cyanobacteria in Symbiosis*, edited by: Rai, A. N., Bergman, B., and Rasmussen, U., Kluwer Academic Publishers, Dordrecht, the Netherlands, 11–17, 2002.

Carpenter, E. J., Montoya, J. P., Burns, J., Mulholland, M. R., Subramaniam, A., and Capone, D. G.: Extensive bloom of a N_2 -fixing diatom/cyanobacterial association in the tropical Atlantic Ocean, *Mar. Ecol.-Prog. Ser.*, 185, 273–283, 1999.

Church, M., Jenkins, B., Karl, D., and Zehr, J.: Vertical distributions of nitrogen-fixing phenotypes at Stn ALOHA in the oligotrophic North Pacific, *Aquat. Microb. Ecol.*, 38, 3–14, doi:10.3354/ame038003, 2005a.

Church, M., Short, C., Jenkins, B., Karl, D., and Zehr, J.: Temporal patterns of nitrogenase gene (*nifH*) expression in the oligotrophic North Pacific Ocean, *Appl. Environ. Microb.*, 71, 5362–5370, 2005b.

Church, M. J., Bjorkman, K. M., Karl, D. M., Saito, M. A., and Zehr, J. P.: Regional distributions of nitrogen-fixing bacteria in the Pacific Ocean, *Limnol. Oceanogr.*, 53, 63–77, 2008.

Dupouy, C., Neveux, J., Subramaniam, A., Mulholland, M. R., Montoya, J. P., Campbell, L., Carpenter, E. J., and Capone, D. G.: Satellite captures *Trichodesmium* blooms in the south-western tropical Pacific, *EOS T. Am. Geophys. Un.*, 81, 13–16, 2000.

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

[Title Page](#)

[Abstract](#)

[Introduction](#)

[Conclusions](#)

[References](#)

[Tables](#)

[Figures](#)



[Back](#)

[Close](#)

[Full Screen / Esc](#)

[Printer-friendly Version](#)

[Interactive Discussion](#)



Dupouy, C., Benielli-Gary, D., Dandonneau, Y., Neveux, J., Dirberg, G., and Westberry, T.: On the feasibility of detecting *Trichodesmium* blooms with SeaWiFS in the South Western Tropical Pacific, Proc. SPIE, 715010, doi:10.1117/12.806295, 2008.

Dyhrman, S. T., Chappell, P. D., Haley, S. T., Moffett, J. W., Orchard, E. D., Waterbury, J. B., and Webb, E. A.: Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*, Nature, 439, 68–71, 2006.

Farnelid, H., Andersson, A. F., Bertilsson, S., Al-Soud, W. A., Hansen, L. H., Sorensen, S., Steward, G. F., Hagstrom, A., and Riemann, L.: Nitrogenase gene amplicons from global marine surface waters are dominated by genes of non-cyanobacteria, PLoS ONE, 6, e19223, doi:10.1371/journal.pone.0019223, 2011.

Fong, A. A., Karl, D., Lukas, R., Letelier, R. M., Zehr, J. P., and Church, M. J.: Nitrogen fixation in an anticyclonic eddy in the oligotrophic North Pacific Ocean, ISME J., 2, 663–676, 2008.

Foster, R. A. and Zehr, J. P.: Characterization of diatom–cyanobacteria symbioses on the basis of *nifH*, *hetR* and 16S rRNA sequences, Environ. Microbiol., 8, 1913–1925, doi:10.1111/j.1462-2920.2006.01068.x, 2006.

Foster, R. A., Subramaniam, A., Mahaffey, C., Carpenter, E. J., Capone, D. G., and Zehr, J. P.: Influence of the Amazon River plume on distributions of free-living and symbiotic cyanobacteria in the western tropical north Atlantic Ocean, Limnol. Oceanogr., 52, 517–532, 2007.

Foster, R. A., Goebel, N. L., and Zehr, J. P.: Isolation of *Calothrix rhizosoleniae* (CYANOBACTERIA) strain SC01 from *Chaetoceros* (BACILLARIOPHYTA) spp. diatoms of the subtropical north Pacific Ocean, J. Phycol., 46, 1028–1037, doi:10.1111/j.1529-8817.2010.00885.x, 2010.

Foster, R. A., Kuypers, M. M. M., Vagner, T., Paerl, R. W., Musat, N., and Zehr, J. P.: Nitrogen fixation and transfer in open ocean diatom–cyanobacterial symbioses, ISME J., 5, 1484–1493, doi:10.1038/ismej.2011.26, 2011.

Garcia, N., Raimbault, P., and Sandroni, V.: Seasonal nitrogen fixation and primary production in the Southwest Pacific: nanoplankton diazotrophy and transfer of nitrogen to picoplankton organisms, Mar. Ecol.-Prog. Ser., 343, 25–33, 2007.

Gattuso, J.-P., Frankignoulle, M., and Wollast, R.: Carbon and carbonate metabolism in coastal aquatic ecosystems, Annu. Rev. Ecol. Syst., 405–434, doi:10.1146/annurev.ecolsys.29.1.405, 1998.

Goebel, N. L., Turk, K. A., Achilles, K. M., Paerl, R. W., Hewson, I., Morrison, A. E., Montoya, J. P., Edwards, C. A., and Zehr, J. P.: Abundance and distribution of major groups

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



of diazotrophic cyanobacteria and their potential contribution to N_2 fixation in the tropical Atlantic Ocean, *Environ. Microbiol.*, 12, 3272–3289, doi:10.1111/j.1462-2920.2010.02303.x, 2010.

Gómez, F., Furuya, K., and Takeda, S.: Distribution of the cyanobacterium *Richelia intracellu-*
laris as an epiphyte of the diatom *Chaetoceros compressus* in the western Pacific Ocean, *J.*
Plankton Res., 27, 323–330, 2005.

Gruber, N. and Sarmiento, J. L.: Global patterns of marine nitrogen fixation and denitrification, *Global Biogeochem. Cy.*, 11, 235–266, 1997.

Hagino, K., Onuma, R., Kawachi, M., and Horiguchi, T.: Discovery of an endosymbiotic nitrogen-
 fixing cyanobacterium UCYN-A in *Braarudosphaera bigelowii* (Prymnesiophyceae), *PLoS*
One, 8, e81749, doi:10.1371/journal.pone.0081749, 2013.

Halm, H., Lam, P., Ferdelman, T. G., Lavik, G., Dittmar, T., LaRoche, J., D'Hondt, S., and
 Kuypers, M. M.: Heterotrophic organisms dominate nitrogen fixation in the South Pacific
 Gyre, *ISME J.*, 6, 1238–1249, doi:10.1038/ismej.2011.182, 2012.

Heller, P., Tripp, H. J., Turk-Kubo, K., and Zehr, J. P.: ARBitrator: a software pipeline for on-
 demand retrieval of auto-curated *nifH* sequences from GenBank, *Bioinformatics*, 30, 2883–
 2890, doi:10.1093/bioinformatics/btu417, 2014.

Hewson, I., Moisaner, P. H., Achilles, K. M., Carlson, C. A., Jenkins, B. D., Mondragon, E. A.,
 Morrison, A. E., and Zehr, J. P.: Characteristics of diazotrophs in surface to abyssopelagic
 waters of the Sargasso Sea, *Aquat. Microb. Ecol.*, 46, 15–30, 2007.

Hilton, J.: Ecology and evolution of diatom-associated cyanobacteria through genetic analyses,
 PhD, Ocean Sciences Department, University of California, Santa Cruz, Santa Cruz, 2014.

Hilton, J. A., Foster, R. A., Tripp, H. J., Carter, B. J., Zehr, J. P., and Villareal, T. A.: Genomic
 deletions disrupt nitrogen metabolism pathways of a cyanobacterial diatom symbiont, *Nature*
Communications, 4, 1767, doi:10.1038/ncomms2748, 2013.

Houlbreque, F. and Ferrière-Pagès, C.: Heterotrophy in tropical scleractinian corals, *Biol. Rev.*,
 84, 1–17, doi:10.1111/j.1469-185X.2008.00058.x, 2009.

Hunter, E. M., Mills, H. J., and Kostka, J. E.: Microbial community diversity associated with
 carbon and nitrogen cycling in permeable shelf sediments, *Appl. Environ. Microb.*, 72, 5689–
 5701, 2006.

Hutchins, D. A., Fu, F. X., Zhang, Y., Warner, M. E., Feng, Y., Portune, K., Bernhardt, P. W.,
 and Mulholland, M. R.: CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)

[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)


rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry, *Limnol. Oceanogr.*, 52, 1293–1304, 2007.

Hyndes, G. A., Nagelkerken, I., McLeod, R. J., Connolly, R. M., Lavery, P. S., and Vanderkilt, M. A.: Mechanisms and ecological role of carbon transfer within coastal seascapes, *Biol. Rev.*, 89, 232–254, 2014.

Karl, D., Letelier, R., Tupas, L., Dore, J., Christian, J., and Hebel, D.: The role of nitrogen fixation in biogeochemical cycling in the subtropical North Pacific Ocean, *Nature*, 388, 533–538, 1997.

Karl, D. M. and Letelier, R. M.: Nitrogen fixation-enhanced carbon sequestration in low nitrate, low chlorophyll seascapes, *Mar. Ecol.-Prog. Ser.*, 364, 257–268, doi:10.3354/meps07547, 2008.

Karl, D. M., Church, M. J., Dore, J. E., Letelier, R. M., and Mahaffey, C.: Predictable and efficient carbon sequestration in the North Pacific Ocean supported by symbiotic nitrogen fixation, *P. Natl. Acad. Sci. USA*, 109, 1842–1849, doi:10.1073/pnas.1120312109, 2012.

Langlois, R. J., LaRoche, J., and Raab, P. A.: Diazotrophic diversity and distribution in the tropical and subtropical Atlantic Ocean, *Appl. Environ. Microb.*, 71, 7910–7919, 2005.

Langlois, R. J., Hummer, D., and LaRoche, J.: Abundances and distributions of the dominant *nifH* phylotypes in the Northern Atlantic Ocean, *Appl. Environ. Microb.*, 74, 1922–1931, 2008.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Förster, W., Brettske, I., Gerber, S., Ginhart, A. W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lüßmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., and Schleifer, K. H.: ARB: a software environment for sequence data, *Nucleic Acids Res.*, 32, 1363–1371, 2004.

Luo, Y.-W., Doney, S. C., Anderson, L. A., Benavides, M., Berman-Frank, I., Bode, A., Bonnet, S., Boström, K. H., Böttjer, D., Capone, D. G., Carpenter, E. J., Chen, Y. L., Church, M. J., Dore, J. E., Falcón, L. I., Fernández, A., Foster, R. A., Furuya, K., Gómez, F., Gundersen, K., Hynes, A. M., Karl, D. M., Kitajima, S., Langlois, R. J., LaRoche, J., Letelier, R. M., Marañón, E., McGillicuddy Jr., D. J., Moisaner, P. H., Moore, C. M., Mouriño-Carballido, B., Mulholland, M. R., Needoba, J. A., Orcutt, K. M., Poulton, A. J., Rahav, E., Raimbault, P., Rees, A. P., Riemann, L., Shiozaki, T., Subramaniam, A., Tyrrell, T., Turk-Kubo, K. A., Varela, M., Villareal, T. A., Webb, E. A., White, A. E., Wu, J., and Zehr, J. P.: Database of

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)

[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)


diazotrophs in global ocean: abundance, biomass and nitrogen fixation rates, *Earth Syst. Sci. Data*, 4, 47–73, doi:10.5194/essd-4-47-2012, 2012.

Masotti, I., Ruiz-Pino, D., and Le Bouteiller, A.: Photosynthetic characteristics of *Trichodesmium* in the southwest Pacific Ocean: importance and significance, *Mar. Ecol.-Prog. Ser.*, 338, 47–59, 2007.

Moisander, P. H., Beinart, R. A., Voss, M., and Zehr, J. P.: Diversity and abundance of diazotrophic microorganisms in the South China Sea during intermonsoon, *ISME J.*, 2, 954–967, 2008.

Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., Montoya, J. P., and Zehr, J. P.: Unicellular cyanobacterial distributions broaden the oceanic N₂ fixation domain, *Science*, 327, 1512–1514, doi:10.1126/science.1185468, 2010.

Moisander, P. H., Zhang, R., Boyle, E. A., Hewson, I., Montoya, J. P., and Zehr, J. P.: Analogous nutrient limitations in unicellular diazotrophs and *Prochlorococcus* in the South Pacific Ocean, *ISME J.*, 6, 733–744, 2012.

Moisander, P. H., Serros, T., Paerl, R. W., Beinart, R. A., and Zehr, J. P.: Gammaproteobacterial diazotrophs and *nifH* gene expression in surface waters of the South Pacific Ocean, *ISME J.*, 8, 1962–1973, doi:10.1038/ismej.2014.49, 2014.

Moutin, T., Van Den Broeck, N., Beker, B., Dupouy, C., Rimmelin, P., and Le Bouteiller, A.: Phosphate availability controls *Trichodesmium* spp. biomass in the SW Pacific Ocean, *Mar. Ecol.-Prog. Ser.*, 297, 15–21, 2005.

Mulholland, M. R.: The fate of nitrogen fixed by diazotrophs in the ocean, *Biogeosciences*, 4, 37–51, doi:10.5194/bg-4-37-2007, 2007.

Needoba, J. A., Foster, R. A., Sakamoto, C., Zehr, J. P., and Johnson, K. S.: Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean, *Limnol. Oceanogr.*, 52, 1317–1327, 2007.

Nenadic, O. and Greenacre, M.: Correspondence Analysis in R, with Two-and Three-Dimensional Graphics: The ca Package, *J. Stat. Softw.*, volume 20, issue 3, 1-13. 2007.

Ohki, K., Kamiya, M., Honda, D., Kumazawa, S., and Ho, K. K.: Morphological and phylogenetic studies on unicellular diazotrophic cyanobacteria (cyanophytes) isolated from the coastal waters around singapore, *J. Phycol.*, 44, 142–151, 2008.

Ouillon, S., Douillet, P., Lefebvre, J.-P., Le Gendre, R., Jouon, A., Bonneton, P., Fernandez, J.-M., Chevillon, C., Magand, O., and Lefèvre, J.: Circulation and suspended sediment transport

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)

[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)


in a coral reef lagoon: the south-west lagoon of New Caledonia, Mar. Pollut. Bull., 61, 269–296, 2010.

Ratten, J., LaRoche, J., Desai, D. K., Shelley, R., Landing, W. M., Boyle, E. A., Cutter, G. A., and Langlois, R.: Source of iron and phosphate affect the distribution of diazotrophs in the North Atlantic, Deep-Sea Res. Pt. II, 116, 332–341, doi:10.1016/j.dsr2.2014.11.012, 2014.

R Core Team: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, http://www.R-project.org/ (last access: 24 November 2014), 2013.

Reddy, K., Haskell, J., Sherman, D., and Sherman, L.: Unicellular, aerobic nitrogen-fixing cyanobacteria of the genus *Cyanothece*, J. Bacteriol., 175, 1284–1292, 1993.

Renaud, F., Pringault, O., and Rochelle-Newall, E.: Effects of the colonial cyanobacterium *Trichodesmium* spp. on bacterial activity, Aquat. Microb. Ecol., 41, 261–270, 2005.

Riemann, L., Farnelid, H., and Steward, G. F.: Nitrogenase genes in non-cyanobacterial plankton: prevalence, diversity and regulation in marine waters, Aquat. Microb. Ecol., 61, 225–237, doi:10.3354/Ame01431, 2010.

Rodier, M. and Le Borgne, R.: Population dynamics and environmental conditions affecting *Trichodesmium* spp. (filamentous cyanobacteria) blooms in the south–west lagoon of New Caledonia, J. Exp. Mar. Biol. Ecol., 358, 20–32, 2008.

Rodier, M. and Le Borgne, R.: Population and trophic dynamics of *Trichodesmium thiebautii* in the SE lagoon of New Caledonia. Comparison with *T. erythraeum* in the SW lagoon, Mar. Pollut. Bull., 61, 349–359, 2010.

Subramaniam, A., Yager, P. L., Carpenter, E. J., Mahaffey, C., Bjorkman, K., Cooley, S., Kustka, A. B., Montoya, J. P., Sanudo-Wilhelmy, S. A., Shipe, R., and Capone, D. G.: Amazon River enhances diazotrophy and carbon sequestration in the tropical North Atlantic Ocean, P. Natl. Acad. Sci. USA, 105, 10460–10465, doi:10.1073/pnas.0710279105, 2008.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S.: MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol., 28, 2731–2739, doi:10.1093/molbev/msr121, 2011.

Taniuchi, Y., Chen, Y.-I. L., Chen, H.-Y., Tsai, M.-L., and Ohki, K.: Isolation and characterization of the unicellular diazotrophic cyanobacterium Group C TW3 from the tropical western Pacific Ocean, Environ. Microbiol., 14, 641–654, doi:10.1111/j.1462-2920.2011.02606.x, 2012.

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)

[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)


Thompson, A., Carter, B. J., Turk-Kubo, K., Malfatti, F., Azam, F., and Zehr, J. P.: Genetic diversity of the unicellular nitrogen-fixing cyanobacteria UCYN-A and its prymnesiophyte host, *Environ. Microbiol.*, 16, 3238–3249, doi:10.1111/1462-2920.12490, 2014.

Thompson, A. W., Foster, R. A., Krupke, A., Carter, B. J., Musat, N., Vaultot, D., Kuypers, M. M. M., and Zehr, J. P.: Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga, *Science*, 337, 1546–1550, doi:10.1126/science.1222700, 2012.

Torréton, J.-P., Rochelle-Newall, E., Pringault, O., Jacquet, S., Faure, V., and Briand, E.: Variability of primary and bacterial production in a coral reef lagoon (New Caledonia), *Mar. Pollut. Bull.*, 61, 335–348, 2010.

Tripp, H. J., Bench, S. R., Turk, K. A., Foster, R. A., Desany, B. A., Niazi, F., Affourtit, J. P., and Zehr, J. P.: Metabolic streamlining in an open ocean nitrogen-fixing cyanobacterium, *Nature*, 464, 90–94, doi:10.1038/nature08786, 2010a.

Turk-Kubo, K. A., Achilles, K. M., Serros, T. R., Ochiai, M., Montoya, J. P., and Zehr, J. P.: Nitrogenase (*nifH*) gene expression in diazotrophic cyanobacteria in the Tropical North Atlantic in response to nutrient amendments, *Frontiers in Aquatic Microbiology*, 3, 386, doi:10.3389/fmicb.2012.00386, 2012.

Turk-Kubo, K. A., Karamchandani, M., Capone, D. G., and Zehr, J. P.: The paradox of marine heterotrophic nitrogen fixation: abundances of heterotrophic diazotrophs do not account for nitrogen fixation rates in the Eastern Tropical South Pacific, *Environ. Microbiol.*, 16, 3095–3114, doi:10.1111/1462-2920.12346, 2014.

Villareal, T. A.: Laboratory culture and preliminary characterization of the nitrogen-fixing *Rhizosolenia-Richelia* symbiosis, *Mar. Ecol.*, 11, 117–132, 1990.

Villareal, T. A.: Marine nitrogen-fixing diatom – cyanobacteria symbioses, in: *Marine Pelagic Cyanobacteria: Trichodesmium and other Diazotrophs*, edited by: Carpenter, E. J., Capone, D. G., and Rueter, J. G., Series C: Mathematical and Physical Sciences, Kluwer Academic Publishers, the Netherlands, 163–175, 1992.

Villareal, T. A., Brown, C. G., Brzezinski, M. A., Krause, J. W., and Wilson, C.: Summer diatom blooms in the North Pacific subtropical gyre: 2008–2009, *PLoS ONE*, 7, e33109, doi:10.1371/journal.pone.0033109, 2012.

Vu, T. T., Stolyar, S. M., Pinchuk, G. E., Hill, E. A., Kucek, L. A., Brown, R. N., Lipton, M. S., Osterman, A., Fredrickson, J. K., and Konopka, A. E.: Genome-scale modeling of light-driven reductant partitioning and carbon fluxes in diazotrophic unicellular cyanobacterium *Cyanoth-*

ece sp. ATCC 51142, Plos. Comput. Biol., 8, e1002460, doi:10.1371/journal.pcbi.1002460, 2012.

Walsby, A.: The gas vesicles and buoyancy of *Trichodesmium*, in: Marine Pelagic Cyanobacteria: *Trichodesmium* and Other Diazotrophs, Springer, Science and Business Media, Heidelberg, the Netherlands, 141–161, 1992.

Webb, E. A., Ehrenreich, I. M., Brown, S. L., Valois, F. W., and Waterbury, J. B.: Phenotypic and genotypic characterization of multiple strains of the diazotrophic cyanobacterium, *Crocosphaera watsonii*, isolated from the open ocean, Environ. Microbiol., 11, 338–348, 2009.

Zehr, J. P., Waterbury, J. B., Turner, P. J., Montoya, J. P., Omoregie, E., Steward, G. F., Hansen, A., and Karl, D. M.: Unicellular cyanobacteria fix N₂ in the subtropical North Pacific Ocean, Nature, 412, 635–638, 2001.

Zehr, J. P., Bench, S. R., Carter, B. J., Hewson, I., Niazi, F., Shi, T., Tripp, H. J., and Affourtit, J. P.: Globally distributed uncultivated oceanic N₂-fixing cyanobacteria lack oxygenic photosystem II, Science, 322, 1110–1112, 2008.

BGD

12, 9043–9079, 2015

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



**Diazotroph
succession in
VAHINE mesocosms**

K. A. Turk-Kubo et al.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Table 1. Results from multiple regression models predicting diazotroph abundances from environmental parameters. Abundances from both the Noumea lagoon and the VAHINE mesocosms experiments are included in the linear regression models. R^2 -cv is the cross-validated fit of the model, and when similar to the R^2 value, indicates a high predictive ability for future datasets.

	Temp., Sal. and PO ₄		Temp. and PO ₄		Temp.		PO ₄	
	R^2	R^2 -cv	R^2	R^2 -cv	R^2	R^2 -cv	R^2	R^2 -cv
UCYN-A1	0.85	0.76	0.84	0.76	0.70	0.59	0.73	0.63
UCYN-	0.84	0.72	0.79	0.68	0.71	0.60	0.75	0.66
Het-3	0.77	0.60	0.70	0.57	0.55	0.39	0.70	0.60

Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

Table 2. Diazotroph net growth and mortality rates (d^{-1} during VAHINE mesocosms experiment. Rates in bold are the maximum rates measured in each mesocosm. “–” denotes periods where no rates could be calculated due to missing data, and “na” denotes missing rates due to abundances being DNQ or UD. Standard error (\pm) reported for each growth rate is derived from qPCR measurements for replicate amplifications.

	time	Net growth rate/net mortality rate (d^{-1})								
		UCYN-A1	UCYN-A2	UCYN-	UCYN-UCYN-	g-24774A1	<i>Tricho.</i>	Het-1	Het-2	Het-3
M1	5	0.54 ± 0.05	0.26 ± 0.04	na	-0.04 ± 0.53	0.27 ± 0.13	0.15 ± 0.04	0.07 ± 0.05	-0.55 ± 0.18	na
	7	0.08 ± 0.07	-0.45 ± 0.04	na	na	-0.08 ± 0.11	-0.94 ± 0.05	0.03 ± 0.03	0.3 ± 0.17	na
	9	-0.81 ± 0.07	-0.48 ± 0.04	-0.41 ± 0.16	na	-0.24 ± 0.06	1.46 ± 0.05	0.34 ± 0.04	0.55 ± 0.03	na
	11	-0.47 ± 0.01	-0.02 ± 0.11	-0.65 ± 0.16	0.46 ± 0.23	0.19 ± 0.03	-1.12 ± 0.03	-0.15 ± 0.09	0.01 ± 0.04	na
	13	0.73 ± 0.04	-0.16 ± 0.15	0.85 ± 0.03	1.23 ± 0.07	-0.15 ± 0.22	0.39 ± 0.03	-0.23 ± 0.09	-0.21 ± 0.03	na
	15	-0.61 ± 0.04	0.18 ± 0.11	na	0.52 ± 0.03	0.27 ± 0.22	0.16 ± 0.04	-1.39 ± 0.04	-0.41 ± 0.02	0.21 ± 0.1
	18	-0.23 ± 0.08	-0.03 ± 0.08	na	0.06 ± 0.02	0.2 ± 0.02	-0.62 ± 0.08	1.7 ± 0.01	-0.03 ± 0.02	0.15 ± 0.16
	19	0.55 ± 0.08	0.31 ± 0.08	0.1 ± 0.11	0.29 ± 0.02	0.03	0.71 ± 0.08	0.7 ± 0.02	0.49 ± 0	-0.13 ± 0.22
	20	0.6 ± 0.05	0.77 ± 0.04	0.65 ± 0.13	-0.24 ± 0.07	0.2 ± 0.03	0.01 ± 0.01	-0.35 ± 0.02	-0.37 ± 0.02	0.57 ± 0.16
	23	0.47 ± 0.04	0.02 ± 0.06	0.55 ± 0.08	0.22 ± 0.07	-0.8 ± 0.07	0.74 ± 0.02	0.06 ± 0.01	0.01 ± 0.06	-0.1 ± 0.04
M2	5	–	–	–	–	–	–	–	–	–
	7	na	-1.63 ± 0.22	na	na	na	na	na	na	na
	9	-0.06 ± 0.03	0.52 ± 0.26	-0.36 ± 0.13	0.51 ± 0.24	-0.91 ± 0.04	0.03 ± 0.01	-0.53 ± 0.01	-0.06 ± 0.01	na
	11	-0.15 ± 0.03	0.29 ± 0.14	na	0.58 ± 0.24	0.31 ± 0.09	0.04 ± 0.02	0.56 ± 0.07	0.1 ± 0.011	na
	13	-0.47 ± 0.01	0.38 ± 0.1	na	0.79 ± 0.14	0.18 ± 0.09	-0.5 ± 0.06	-0.26 ± 0.07	0.44 ± 0.03	na
	15	-0.5 ± 0.07	0.33 ± 0.11	0.18 ± 0.07	2.16 ± 0.07	0.43 ± 0.06	0.22 ± 0.05	-1.29 ± 0.03	-0.63 ± 0.03	na
	18	-0.08 ± 0.11	na	0.3 ± 0.17	-0.27 ± 0.03	0.04 ± 0.07	0.14 ± 0.04	0.35 ± 0.02	-0.04 ± 0.01	0.76 ± 0.15
	19	–	–	–	–	–	–	–	–	–
	20	na	-0.46 ± 0.14	na	na	na	na	na	na	na
	23	0.43 ± 0.05	na	0.36 ± 0.07	0.05 ± 0.09	1.07 ± 0.07	0.09 ± 0.04	0.00 ± 0.07	0.04 ± 0.09	0.04 ± 0.09
M3	5	0.13 ± 0.02	1.71 ± 0.32	0.7 ± 0.48	0.96 ± 0.43	na	0.17 ± 0.03	0.64 ± 0.03	-0.35 ± 0.04	na
	7	0.29 ± 0.02	-1.64 ± 0.12	-0.42 ± 0.15	-0.38 ± 0.09	-0.39 ± 0.09	0.45 ± 0.06	-0.08 ± 0.05	0.54 ± 0.04	na
	9	-0.38 ± 0.03	-0.03 ± 0.1	-0.14 ± 0.3	0.13	0.29 ± 0.12	-2.16 ± 0.18	-0.63 ± 0.05	-0.72 ± 0.03	na
	11	-0.4 ± 0.03	0.18 ± 0.05	0.68 ± 0.27	0.1 ± 0.15	0.06 ± 0.14	1.2 ± 0.18	1.17 ± 0.06	0.84 ± 0.03	na
	13	-0.43 ± 0.07	0.16 ± 0.1	na	-0.02 ± 0.09	0.5 ± 0.09	0.8 ± 0.05	0.06 ± 0.06	-0.03 ± 0.03	na
	15	-0.48 ± 0.11	0.42 ± 0.11	na	1.91 ± 0.02	-0.95 ± 0.1	-0.4 ± 0.01	-1.16 ± 0.03	-0.66 ± 0.05	na
	18	-0.22 ± 0.21	-0.03 ± 0.07	0.21 ± 0.01	0.2 ± 0.04	-0.56 ± 0.11	-0.35 ± 0.01	-0.48 ± 0.01	-0.69 ± 0.07	0.06 ± 0.05
	19	-0.3 ± 0.19	-0.36 ± 0.14	-0.99 ± 0.06	-0.44 ± 0.06	1.03 ± 0.1	0.05 ± 0.01	-0.16 ± 0.11	2.24 ± 0.06	1.09 ± 0.06
	20	0.55 ± 0.12	1.25 ± 0.13	1.38 ± 0.06	0.28 ± 0.07	na	1.55 ± 0.02	1.28 ± 0.11	0.45 ± 0.07	-1.12 ± 0.05
	23	0.45 ± 0.14	-0.04 ± 0.08	0.07 ± 0.02	-0.02 ± 0.06	na	0.72 ± 0.03	-0.36 ± 0.02	-0.14 ± 0.08	0.77 ± 0.11

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

⏪

⏩

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Diazotroph
succession in
VAHINE mesocosms

K. A. Turk-Kubo et al.

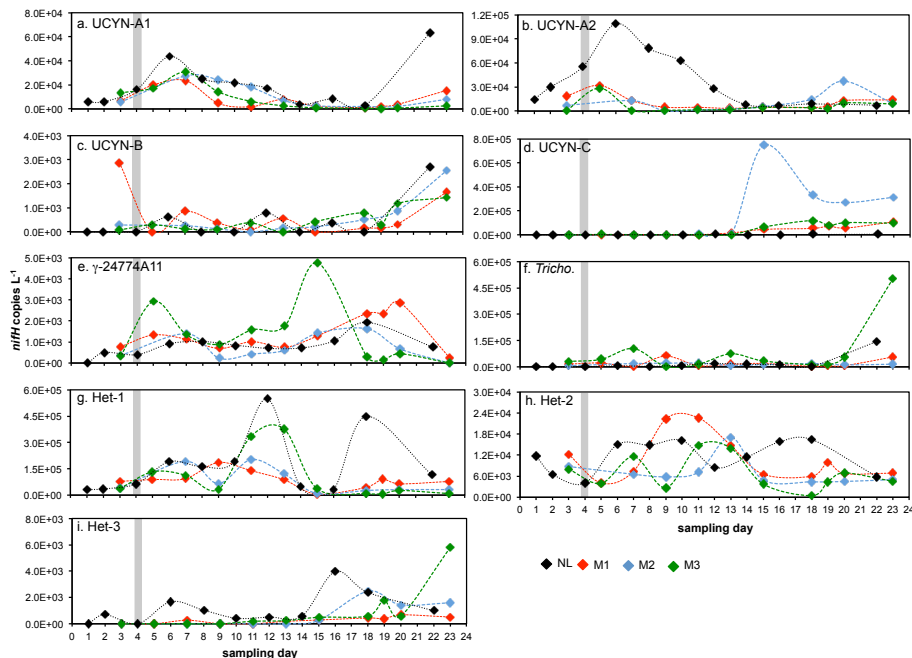


Figure 1. Abundances of diazotroph phylotypes at 6 m depth during the VAHINE mesocosm experiment and in the Noumea lagoon (NL) during the experimental period. Abundances were determined for each mesocosm (M1–M3) and the Noumea Lagoon (NL) using qPCR targeting the *nifH* gene sequences specific to each phylotype, and are reported as *nifH* copies L^{-1} . A grey line indicates the DIP spike on day 4. Note the different scales on the y axis for each phylotype.

[Title Page](#)[Abstract](#)[Introduction](#)[Conclusions](#)[References](#)[Tables](#)[Figures](#)[Back](#)[Close](#)[Full Screen / Esc](#)[Printer-friendly Version](#)[Interactive Discussion](#)

Diazotroph
succession in
VAHINE mesocosms

K. A. Turk-Kubo et al.

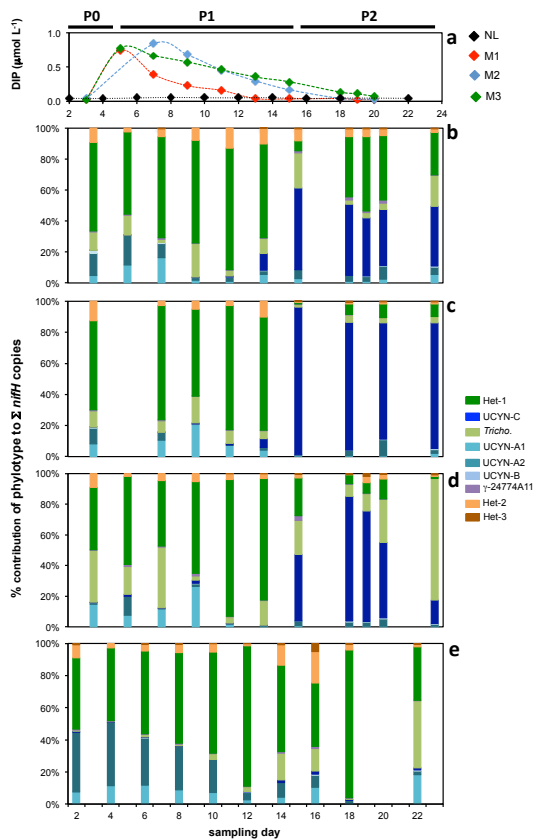


Figure 2. Diazotroph community composition at 6 m depth during the VAHINE mesocosm experiment and in the Noumea lagoon (NL) during the experimental period. DIP concentrations (a) decreased steadily following the spike at day 4 (data from in Bonnet et al., 2015). *nifH*-based abundances were summed for each sampling day to determine the percent contribution to the total diazotroph community from each phylotype in M1 (b), M2 (c), M3 (d) and the NL (e).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Diazotroph succession in VAHINE mesocosms

K. A. Turk-Kubo et al.

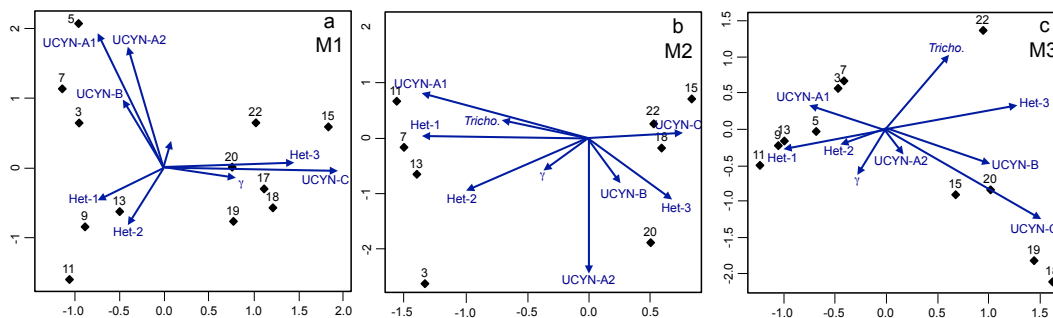


Figure 3. Correspondence analysis biplot of diazotroph abundances for each mesocosm. The horizontal axis is representative of time, evidenced by the progression of time points projected onto the x axis. Variances covered by the two axes are 61% + 18% in M1, 88% + 5% in M2, and 56% + 30% in M3.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



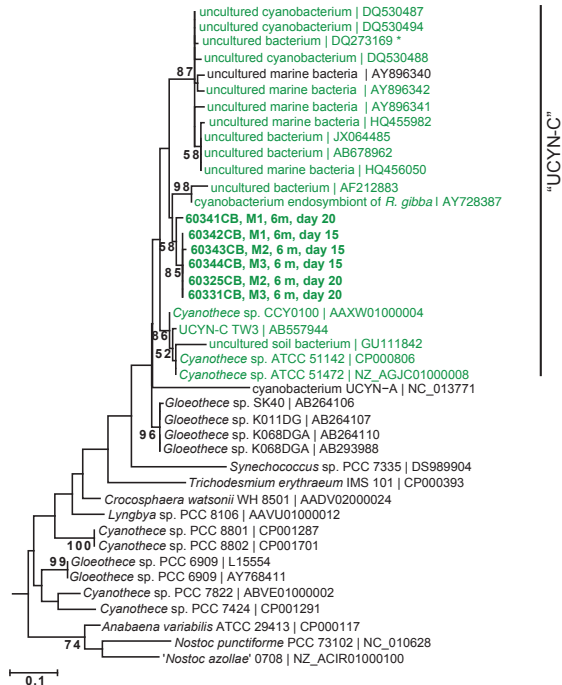


Figure 4. Maximum likelihood tree of *Cyanothecae*-like diazotrophs based on partial *nifH* nucleotide sequences. Sequences that are targeted by the UCYN-C qPCR assay (Foster et al., 2007) with no greater than 2 mismatches in each primer and probe are green, and the original sequence used for sequence design is marked with an asterisk (*). *Cyanothecae*-like sequences recovered from the VAHINE mesocosms are bold. Bootstrapping was conducted using 1000 replicate trees, and nodes with values > 50 are displayed. Branch lengths were inferred using the Tamura-Nei model, and the scale bar indicates the number of substitutions per site.

Diazotroph
succession in
VAHINE mesocosms

K. A. Turk-Kubo et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures



Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

