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Seasonal Shifts in Diazotrophs Players: Patterns Observed Over a Two-Year Time Series in the New Caledonian Lagoon (Western Tropical South Pacific Ocean)

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Coastal and open ocean regions of the Western Tropical South Pacific ocean have been identified as a hotspot of N₂ fixation. However, the environmental factors driving the temporal variability of abundance, composition, and activity of diazotrophs are still poorly understood, especially during the winter season. To address this, we quantified N₂ fixation rates and the abundance of seven diazotroph phylotypes (UCYN-A1 symbiosis, UCYN-B, UCYN-C, *Trichodesmium*, Het-1, Het-2, and Het-3) on a monthly basis during two full years (2012 to 2014) at four stations along a coast to open ocean transect in the New Caledonian lagoon. The total *nifH* gene concentration (sum of all *nifH* gene copies) clearly decreased from the barrier reef to the shore. Apart from UCYN-B, which peaked at very high abundances (10⁶–10⁸ *nifH* gene copies L⁻¹) at two occasions at the coastal station, the UCYN-A1 symbiosis was the most abundant group at all stations, accounting for 79% of the total *nifH* gene copy counts along the transect (average abundance 4.2 ± 10.3 × 10⁴ *nifH* gene copies L⁻¹). The next most abundant groups were in order *Trichodesmium* (accounting for 14% of the total *nifH* gene copies), Het-groups (6% of the total) and UCYN-C (1% of the total). Statistical analyses reveal that the UCYN-A1 symbiosis and Het groups were associated with cold (<25°C) waters, high NO_x and PO₄³⁻, weak winds from the south (occasionally southwest), while *Trichodesmium* and UCYN-C were associated with warmer (>25°C) waters, low NO_x and PO₄³⁻ concentrations, strong and (mostly) easterly winds. Average N₂ fixation rates over the survey were 6.5 ± 6.7 nmol N L⁻¹ d⁻¹ and did not differ significantly among seasons. The year to year variability was more pronounced with average integrated rates significantly higher in the second year of the survey (162 ± 122 μmol N m⁻² d⁻¹) than the first year (66 ± 91 μmol N m⁻² d⁻¹). This dataset suggests that seasonality is less pronounced than previously thought, and that relatively high N₂ fixation rates are maintained in the New Caledonian lagoon all year long, despite seasonal changes in the diazotroph community composition.

Keywords: N₂ fixation, diazotrophs, *nifH*, abundance, New Caledonia

INTRODUCTION

Biological dinitrogen (N₂) fixation is a main source of fixed N to the oceans and is performed by a suite of specialized cyanobacteria, bacteria and archaea called “diazotrophs” (Zehr, 2011). Fixed N input fluxes on a global basis vary between 106–120 Tg N year⁻¹ (Galloway et al., 2004; Gruber, 2008; Jickells et al., 2017). The accuracy of those estimates is inevitably affected by the temporal and spatial resolution of measurements. Only few studies have addressed how the paucity of data on spatial and temporal distribution of N₂ fixation may affect global fixed N input estimates. For instance, the global database compiled in Luo et al. (2012) contains 2480 data points of volumetric seawater N₂ fixation rates from the northern hemisphere, while samples from the southern hemisphere only amount to 502. The temporal distribution of these data points also suffers from a strong seasonal bias as >65% of the N₂ fixation measurements were made during the spring-summer season. Hence, most of our recapitulative knowledge of marine N₂ fixation stems from the northern hemisphere in spring-summer conditions, largely from the sunlit layer of open ocean regions of the North Atlantic and North Pacific Oceans (Benavides and Voss, 2015; Böttjer et al., 2017).

Although the number of N₂ fixation studies has increased in recent years, temporal and spatial resolution remains coarse, as oceanographic cruises commonly sample transects with stations separated by tens of nautical miles, and typically at monthly or seasonal time scales. For instance, N₂ fixation rates varied four- to nine-fold between summer and winter at Station ALOHA in the Pacific Ocean between 2005 and 2013 (Böttjer et al., 2017). If higher diazotrophic activity during summer prevails among the available datasets it could cause an overestimation of basin-wide estimates, which illustrates the need for conducting sampling programs at finer temporal scales and throughout all seasons of the year.

Recent intensive sampling of the western tropical South Pacific (WTSP) Ocean revealed this region as a hotspot of N₂ fixation with some of the highest rates ever recorded (Messer et al., 2015; Berthelot et al., 2017; Bonnet et al., 2017). However, most of our understanding of diazotrophic activity and diversity in the WTSP derives from open ocean cruises, the majority of which took place in austral summer. Little sampling effort has been devoted to coastal environments, despite their central role in the global cycling of nutrients (Bauer et al., 2013) and increasing evidence of their role as hotspots of diazotrophic activity (Mulholland et al., 2012, 2019; Tang and Cassar, 2019). In the Australian Great Barrier Reef, Hewson et al. (2007) reported *nifH* gene sequences corresponding to usual open-ocean diazotroph phylotypes such as the UCYN-A symbiosis, UCYN-B and the diatom-diazotroph association (DDA) Het-1 (*Richelia* associated with *Rhizosolenia*). Also in the Great Barrier Reef, Messer et al. (2017) found *Trichodesmium erythraeum* to be the dominant diazotroph in their *nifH* gene libraries and measured N₂ fixation rates from 3 to 68 nmol N L⁻¹ d⁻¹.

The only other coastal N₂ fixation studies in the WTSP are those from the reef systems of New Caledonia, the second largest in the world after the Great Barrier Reef, enclosing a

24,000 km² lagoon. The New Caledonian lagoon waters are shallow (~20 m on average) and mostly oligotrophic, although inorganic nutrients are detectable year-round in the waters affected by the river and anthropogenic discharges near Noumea city (Neveux et al., 2009; Ouillon et al., 2010). Previous studies have documented high N₂ fixation rates (1.9 to 29.3 nmol N L⁻¹ d⁻¹, Garcia et al., 2007; Bonnet et al., 2016), occurrence of large *Trichodesmium* blooms (Rodier and Le Borgne, 2008; Tenório et al., 2018) and co-occurrence with other common oceanic diazotrophs such as DDAs, UCYN-A/haptophyte symbioses, *Crocospaera* (UCYN-B; Biegala and Raimbault, 2008; Turk-Kubo et al., 2015; Henke et al., 2018), and UCYN-C (Turk-Kubo et al., 2015). Most of these studies were performed during austral summer conditions when diazotrophs are considered to be most abundant and N₂ fixation rates maximal. Yet the effect of seasonal variability and nutrient inputs from the coast on the distribution and activity of these diazotrophic groups is lacking. Here we examine N₂ fixation rates and diazotroph phylotype abundances at four stations along a coast to open-ocean transect in the New Caledonian lagoon during two full years (2012 to 2014) on a monthly basis in the framework of the “Grand Observatoire de l’environnement et de la biodiversité terrestre et marine du Pacifique Sud” (GOPS). Henke et al. (2018) examined the seasonal variability and diversity of UCYN-A/haptophyte symbioses relative to environmental conditions. In the current study we sought to expand our knowledge on the broader diazotroph community and on diazotrophic activity.

MATERIALS AND METHODS

Sampling, Hydrographic and Meteorological Parameters

Between July 2012 and April 2014, seawater samples were collected monthly in the southwestern New Caledonian lagoon along a transect of four stations (L2, M09, M99, and D39; **Figure 1**) spanning from the reef barrier (Dumbea Pass) to the shore (Dumbea Bay), into which the Dumbea river flows. The sampling always started just before high tide to ensure that results would be comparable. Seawater was collected from three depths spanning the water column of the lagoon (1, 8, and 16 m at station L2, M09, D39, and 1, 3, and 6 m at station M99) using a trace metal clean Teflon pump connected to polyethylene tubing. Vertical hydrographic profiles were obtained at each station by casting a Seabird SBE19 CTD equipped with additional turbidity (Seapoint optical back scatter) and *in situ* fluorescence (Wet Lab Wetstar) sensors. Daily values of rainfall, wind direction and velocity were obtained from the Météo-France station at Faubourg Blanchot (Noumea, 22°16.30′ S–166°27.06′ E). Here we used averages of the data from the week preceding sampling in order to integrate meteorological variations.

Nutrient and Chlorophyll a Analyses

Seawater samples for nutrient analyses were collected at each depth in acid-washed scintillation vials, poisoned with HgCl₂ (10 μg L⁻¹ final concentration) and maintained in

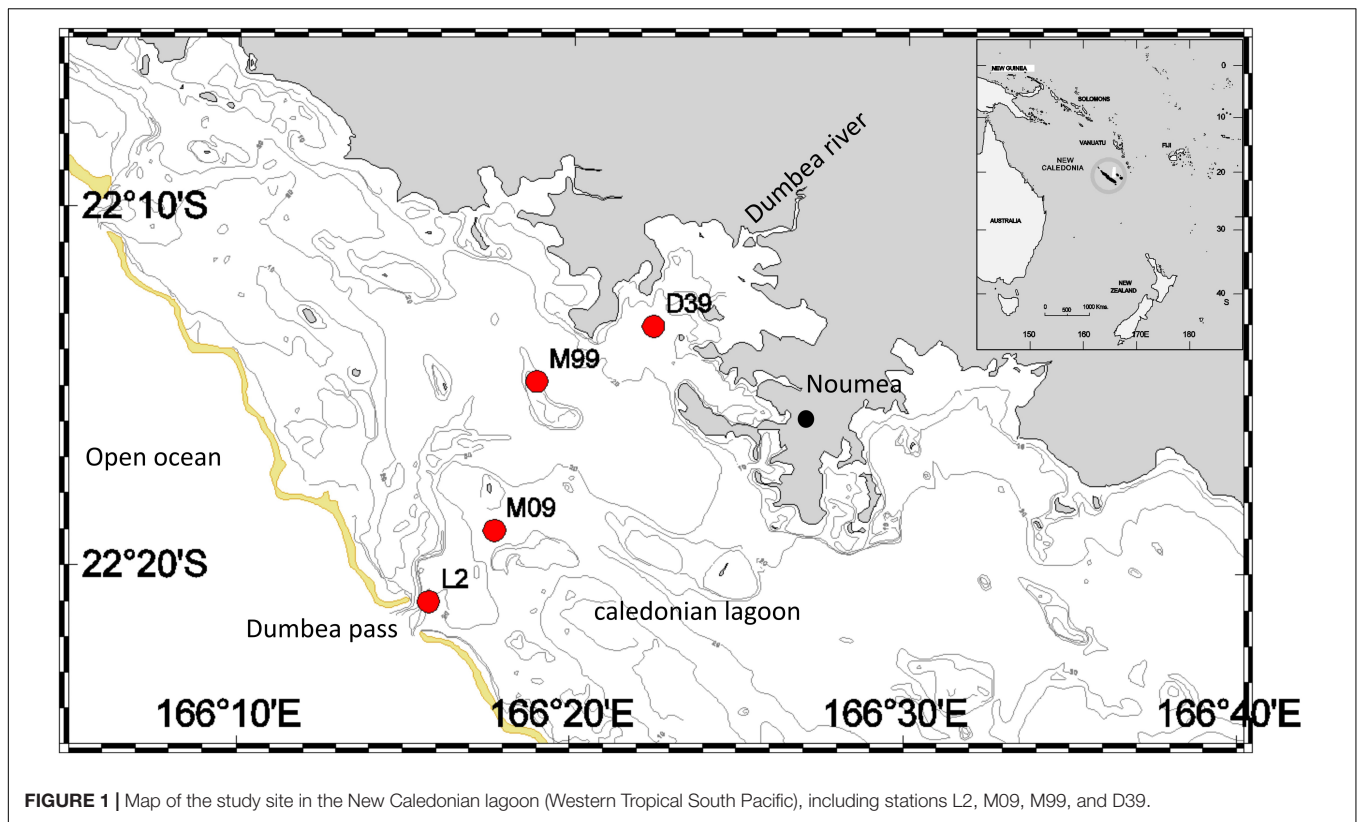


FIGURE 1 | Map of the study site in the New Caledonian lagoon (Western Tropical South Pacific), including stations L2, M09, M99, and D39.

the dark at 4°C until analysis. Soluble reactive phosphorus (PO₄³⁻; detection limit = 0.05 μmol L⁻¹) and NO_x (nitrite (NO₂⁻) + nitrate (NO₃⁻); detection limit = 0.01 μmol L⁻¹) concentrations were determined according to Aminot and Kérouel (2007). Silicate concentrations were measured according to Mullin and Riley (1955) as modified by Fanning and Pilson (1973; detection limit = 0.05 μmol L⁻¹). All assays were performed with a Bran + Luebbe AA3 autoanalyzer. Chlorophyll *a* (Chl *a*) concentrations were determined fluorometrically (Lorenzen, 1966) using a Turner Designs fluorometer (Trilogy module Chl *a* Acid-040) calibrated with pure Chl *a* standards (Sigma-Aldrich).

N₂ Fixation Rates

Seawater samples were collected at each depth in transparent 4.5 L-polycarbonate bottles closed gas-tight with septum screw caps (Nalgene). Bottles were filled to overflow and amended with 5 mL of 98.9 atom% ¹⁵N₂ (Cambridge isotopes) either by directly injecting the tracer as a bubble (Montoya et al., 1996) or as dissolved in a subset of seawater previously N₂ degassed (Mohr et al., 2010) according to the methodology fully described in Berthelot et al. (2015). A comparison between the two methods did not show any significant difference ($p > 0.05$) between N₂ fixation estimates (Supplementary Figure 1). To ensure accurate rate calculations, the ¹⁵N/¹⁴N ratio of the dissolved N₂ pool in the incubation bottles was measured regularly whatever the method used. For this purpose, after 24 h of incubation with the ¹⁵N₂ tracer, 12 mL subsamples of each bottle were moved into

Exetainers® fixed with HgCl₂ (final concentration 10 μg L⁻¹), which were stored upside down in the dark at 4°C until analyzed by membrane inlet mass spectrometry (MIMS) according to Kana et al. (1994). Lastly, time zero (not ¹⁵N-enriched) samples were collected at each monthly survey and station to determine the natural N isotopic signature (EA-IRMS) of ambient particulate N (PN). The ¹⁵N₂ gas batches used did not contain significant concentrations of other nitrogenous compounds labeled with ¹⁵N, as previously measured (Dabundo et al., 2014; Benavides et al., 2015). The minimum quantifiable rates (quantification limit, QL) were 0.035 nmol N L⁻¹ d⁻¹. Integrated rates over the water column were calculated by using the trapezoidal method.

DNA Extraction and Quantitative PCR

For DNA analyses, seawater samples were collected only from the surface (1 m) in 2.3 L polycarbonate bottles and 930–2,300 mL were filtered within 1 h through 0.2 μm Supor filters (Pall Gelman), that were stored at -80°C in bead beater tubes containing a mixture of 0.1-mm and 0.5-mm diameter glass beads (BioSpec Products, Bartlesville, OK, United States). DNA was extracted using the Qiagen Plant kit with additional steps for enhanced DNA recovery from cyanobacteria (Moisander et al., 2008) before automated on-column purification and elution using a Qiacube (Qiagen). All DNA samples were quantified by NanoDrop ND-1000 Spectrophotometry (Thermo Scientific).

The abundance of diazotrophs was determined using TaqMan® qPCR assays on either an ABI 7500 or StepOnePlus Real-Time PCR System (Applied Biosystems), with primer-probe

sets for *Trichodesmium* (Church et al., 2005), UCYN-B (Moisander et al., 2010), UCYN-C (Church et al., 2005), Het-1 (*Richelia* in *Rhizosolenia*), Het-2 (*Richelia* in *Hemiaulus*), and Het-3 (*Calothrix* in *Chaetoceros*; Foster et al., 2007), and UCYN-A1 and UCYN-A2 (Henke et al., 2018). For the purpose of this study, UCYN-A1 and UCYN-A2 have been summed, yielding to total UCYN-A abundances. Standard dilutions (10^7 – 10^1) were run in duplicate, and samples and no-template controls (NTCs) were run in triplicate. NTCs were undetectable. The efficiency of the primer-probe sets was 82–102%. Inhibition tests were carried out on all samples and each primer-probe set by adding 2 μ L of 10^5 standard to each sample. No inhibition was detected. The limit of detection (LOD) and detected but not quantifiable (DNQ) limits used were 1 and 8 gene copies per reaction, respectively, (Goebel et al., 2010). Samples that were below LOD were designated a value of 0 in the data set, whereas gene copies higher than LOD but less than DNQ were designated a conservative value of 1 *nifH* gene copy L⁻¹. Note that abundances are reported as *nifH* gene copies L⁻¹; extrapolating cell numbers from *nifH* gene copies can be difficult due to the possibility that some taxa have multiple genome copies (polyploidy; Sargent et al., 2016; White et al., 2018).

Statistical Analyses

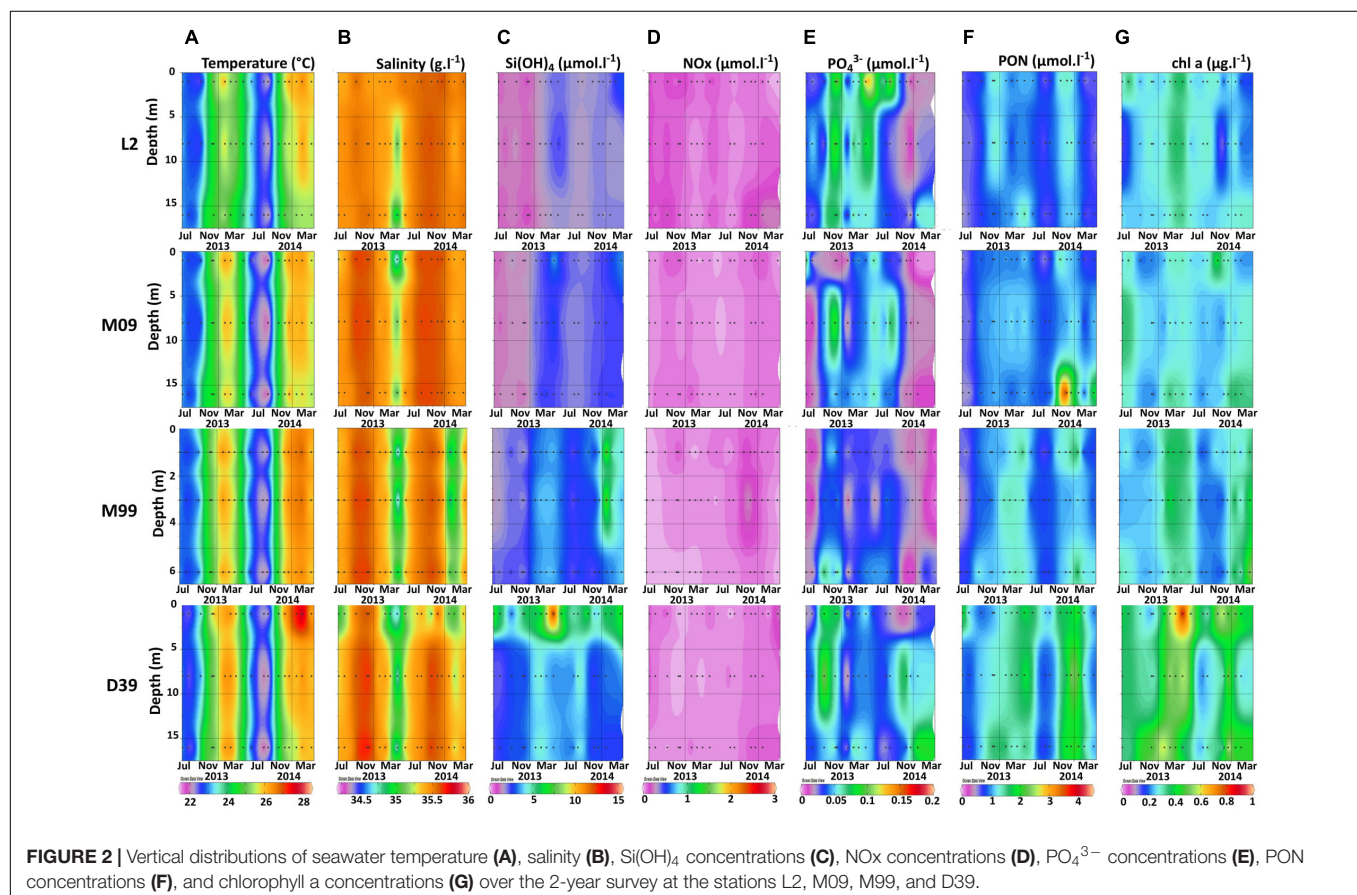
Normal distribution tests (Shapiro–Wilk) by descriptive statistics were performed on the following parameters: temperature,

salinity, Si(OH)₄, NO_x, PO₄³⁻, PON, N₂ fixation, rainfall, wind speed, wind direction and *nifH* gene copies per liter seawater for UCYN-A symbiosis, UCYN-B, UCYN-C, *Trichodesmium*, Het-1, Het-2, and Het-3. All targets (UCYN-A symbiosis, UCYN-B, UCYN-C, *Trichodesmium*, Het-1, Het-2, and Het-3) and eight environmental parameters (PO₄³⁻, Chl *a*, silicates, rainfall, wind direction, NO_x, salinity, and N₂ fixation) were not normally distributed (Shapiro–Wilk, $p < 0.05$). To investigate the relationship between diazotroph community structure and environmental variables, a redundancy analysis (RDA) was conducted using XLSTAT, version 2020.1.1. To test if the abundance of diazotrophs was different between stations and seasons, two non-parametric tests were used: the Kruskal–Wallis test and the Mann–Whitney test with 72 observations (18 per station).

RESULTS

Environmental Conditions

Seawater temperature ranged from 21.7°C to 28.1°C over the course of the survey, and was relatively homogeneous throughout the water column (**Figure 2A**). Maximum temperatures were measured between December and February, while minima were recorded between August and September, corresponding to the New Caledonian warm and cool seasons. The stations close to



the coast (D39, M99) were generally warmer than those located close to the reef barrier (M09, L2; **Figure 2A**). Salinity ranged from 34.29 to 35.72, showing three sharp decreases in August 2012, May 2013 and January 2014 (**Figure 2B**), consistent with intense rainfall (**Supplementary Table 1**). This freshening was particularly pronounced at coastal stations D39 and M99 located close to the Dumbea river (**Figure 2B**).

NO_x and PO₄³⁻ concentrations (**Figures 2D,E**) ranged from undetectable to 0.31 μmol L⁻¹ and to 0.16 μmol L⁻¹, respectively, (**Figure 2**), with no clear seasonal trend. The NO_x:PO₄³⁻ was on average 3:1 over the study period, indicative of PO₄³⁻ excess relative to NO_x. The Si(OH)₄ concentrations (range 0.8–15.0 μmol L⁻¹) gradually increased from the reef (0.84–2.92 μmol L⁻¹) to the coast (2.0–15.0 μmol L⁻¹), where they were the highest at the surface (**Figure 2C**). The Si(OH)₄:NO_x ratio was on average 45 over the study period, indicative of Si(OH)₄ excess relative to NO_x. The biomass indicators (Chl *a* and PON concentrations) were higher at coastal stations (D39 and M99) compared to reef stations (M09 and L2), and generally higher during the warm season (November–April) compared to the cold one (May–October; **Figures 2F,G**). The average weekly wind direction indicated E-SE settled trade winds (6–10 m s⁻¹) most of the year, with some sporadic S-SW episodes recorded in late winter-early summer 2012 and 2013 (**Supplementary Table 1**).

Rates of N₂ Fixation in New Caledonian Waters

N₂ fixation rates ranged from not quantifiable to 51.9 nmol N L⁻¹ d⁻¹ (mean over the survey: 6.5 ± 6.7 nmol N L⁻¹ d⁻¹). Consistent with hydrographic parameters, they were relatively homogeneous over the well-mixed water column and displayed similar temporal patterns among stations, although less variability was observed at station M99 compared to the three others (note that this station is shallower than the three others; **Figure 3A**). Generally, rates integrated over the water column (**Figure 3B**) were highest during austral summer months (October–March), but not significantly different (*p* > 0.05), and highly variable from one monthly survey to another. The year to year variability was more pronounced than the seasonal one: the average annual rates were significantly (*p* < 0.05) lower during the first year of the survey (July 2012–May 2013, 66 ± 91 μmol N m⁻² d⁻¹) than during the second year (June 2013–April 2014, 162 ± 122 μmol N m⁻² d⁻¹). When averaged over the whole survey, rates were the highest at station M09 (168 ± 153 μmol N m⁻² d⁻¹), followed by stations D39 (168 ± 122 μmol N m⁻² d⁻¹), L2 (117 ± 110 μmol N m⁻² d⁻¹), and M99 (48 ± 36 μmol N m⁻² d⁻¹), note that the depth of integration is 8 m instead of 16 m for the others).

Spatiotemporal Variability of Diazotroph Abundances

Total *nifH* counts L⁻¹ (sum of all *nifH* gene copies L⁻¹ of the five phylotypes targeted by qPCR assays) showed limited seasonal variability and were on average 2.9 ± 23 × 10⁶ *nifH* gene copies L⁻¹ along the entire survey (**Figure 4A**). UCYN-B was the most

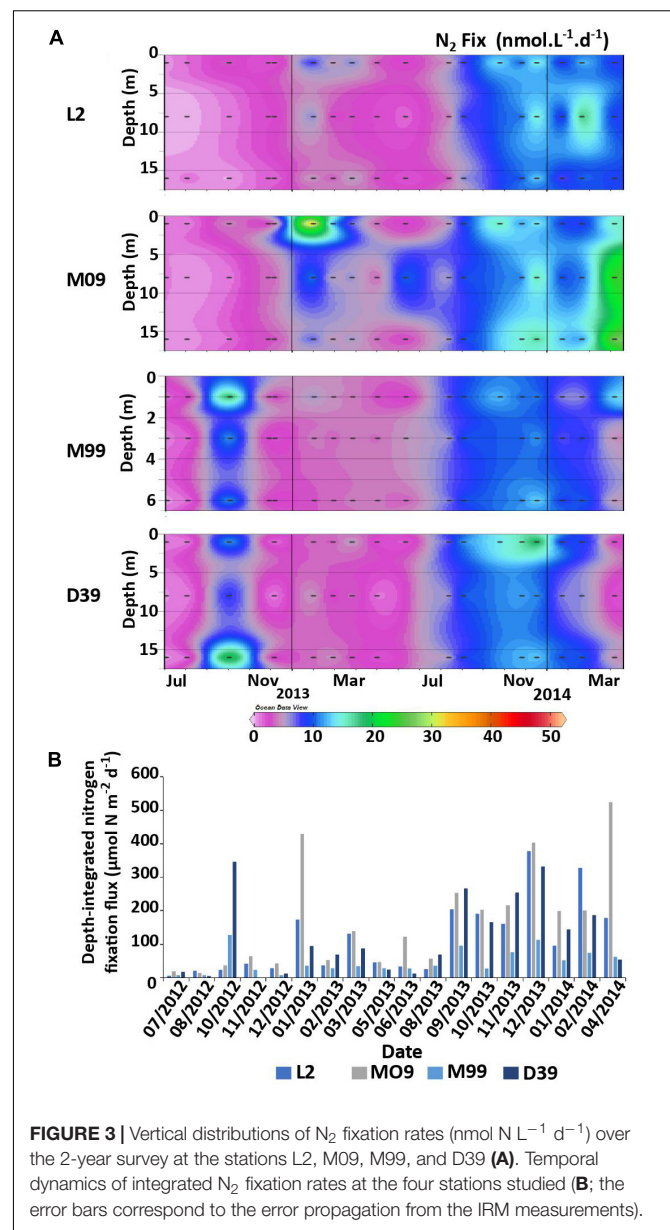


FIGURE 3 | Vertical distributions of N₂ fixation rates (nmol N L⁻¹ d⁻¹) over the 2-year survey at the stations L2, M09, M99, and D39 (**A**). Temporal dynamics of integrated N₂ fixation rates at the four stations studied (**B**); the error bars correspond to the error propagation from the IRM measurements).

abundant group, accounting for >99% of the total *nifH* gene copy counts on average over the survey all stations combined. This was due to very high abundances on two occasions at the coastal site D39 (8.4 × 10⁶ and 2.0 × 10⁸ *nifH* gene copies L⁻¹ on Feb. 28 and May 2 2013; **Figure 4B**), but abundances were otherwise sporadic (below the limit of detection in 57 out of 71 samples). Not taking into account those two peaks, the total *nifH* clearly decreased along the transect from the barrier reef (Dumbea pass) to the shore (Dumbea river; **Figure 4C**). The UCYN-A symbiosis was the most abundant group, accounting for 79% of the total *nifH* gene copy counts along the transect (average abundance 4.2 ± 10.3 × 10⁴ *nifH* gene copies L⁻¹). The next most abundant groups were *Trichodesmium* (7.7 ± 28.2 × 10³ *nifH* gene copies L⁻¹, accounting for 14% of the total *nifH* gene copies), Het-groups (3.0 ± 7.9 × 10³ *nifH* gene copies L⁻¹, 6% of the

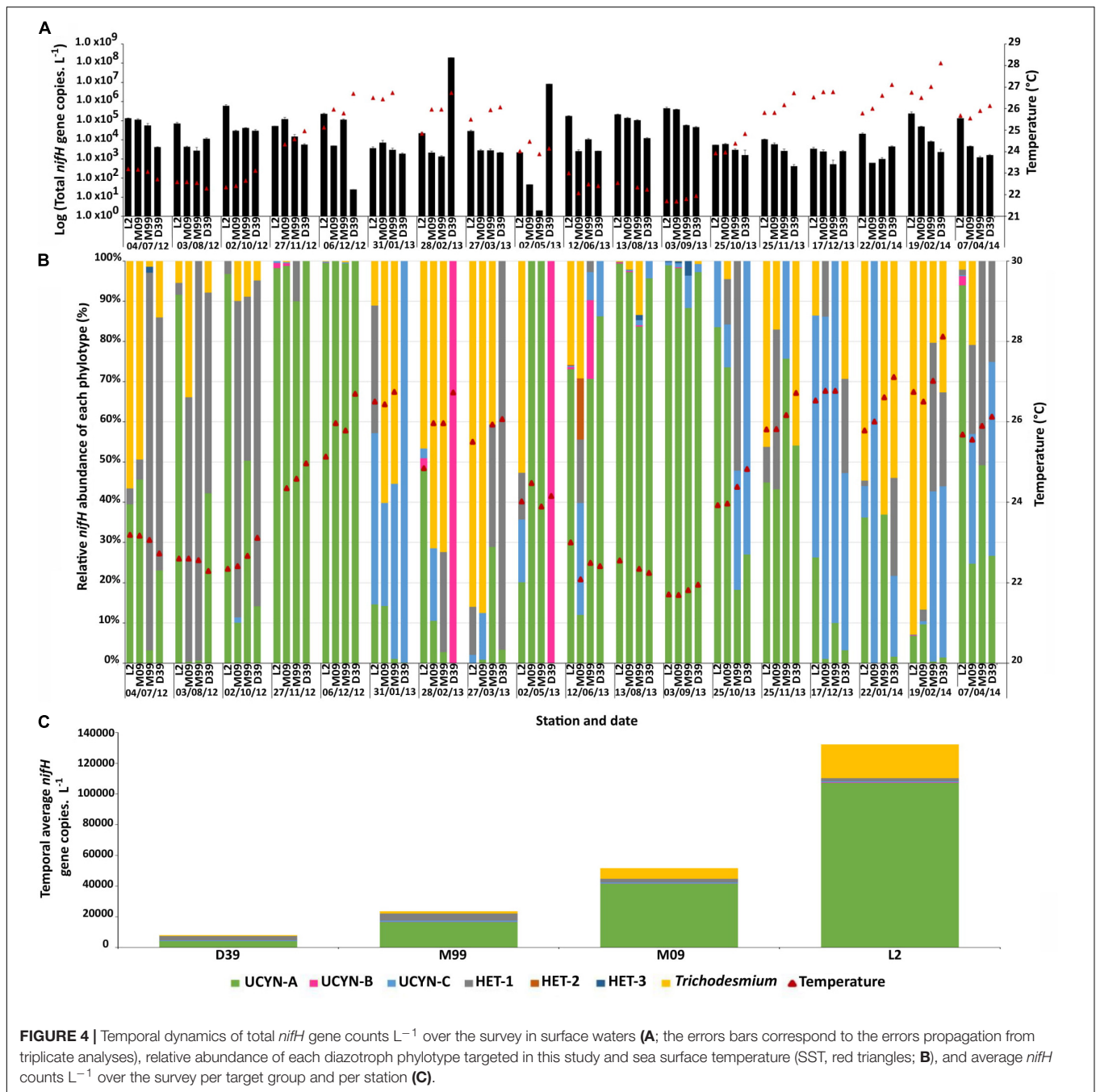


FIGURE 4 | Temporal dynamics of total *nifH* gene counts L⁻¹ over the survey in surface waters (A; the errors bars correspond to the errors propagation from triplicate analyses), relative abundance of each diazotroph phylotype targeted in this study and sea surface temperature (SST, red triangles; B), and average *nifH* counts L⁻¹ over the survey per target group and per station (C).

total; Het-1 accounted for 96% of the Het gene copies), UCYN-C ($6.8 \pm 9.7 \times 10^2$ *nifH* gene copies L⁻¹, 1% of the total *nifH* gene copies), and finally UCYN-B ($1.5 \pm 4.8 \times 10^2$ *nifH* gene copies L⁻¹, 0.3% of the total *nifH* gene copies; **Figure 4A**). The average abundance of *Trichodesmium* and UCYN-A increased significantly ($p < 0.05$) from the coast (station D39; 6.7×10^2 and 4.2×10^3 *nifH* gene copies L⁻¹, respectively) to the reef (station L2; 2.2×10^4 and 1.1×10^5 *nifH* gene copies L⁻¹, respectively). On the contrary, Het- groups were significantly ($p < 0.05$) more abundant at the two inner stations M99 and D39 compared to the reef stations.

While the total *nifH* gene count did not differ between seasons, the *nifH* gene abundance per target group showed temporal variability (**Figure 4B**). Using temperature categories (< or >25°C; Moisaner et al., 2010; Bonnet et al., 2015), the UCYN-A symbiosis and Het groups were 4.8 and 4.6 times more abundant in <25°C waters compared to >25°C. The opposite trend was observed for *Trichodesmium* and UCYN-C, which were 1.6 and 1.2 more abundant in >25°C waters.

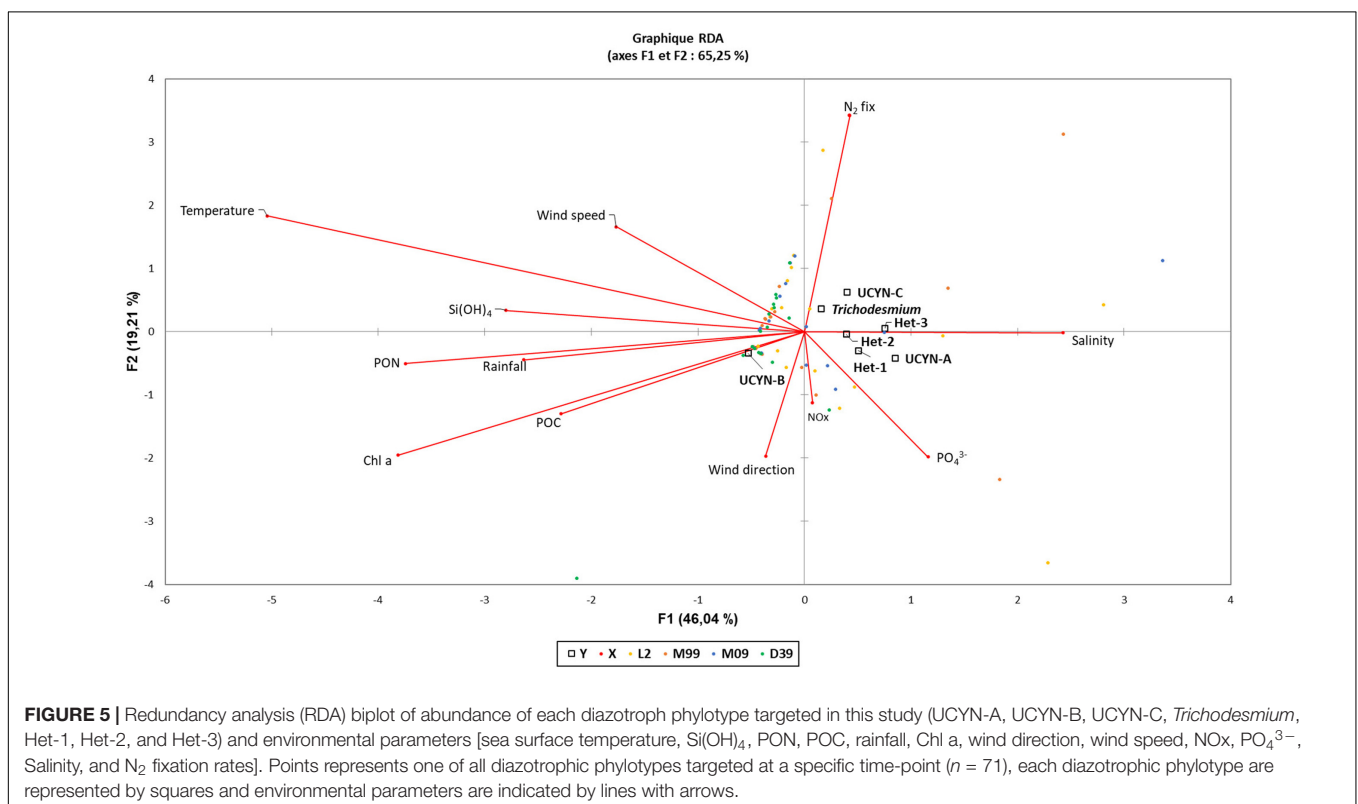
The redundancy analysis (RDA) shows that the correlation between environmental factors and diazotroph phylotypes is mainly explained by the first two axes (75% of the

total variance; **Figure 5**). The factorial axis 1 separates two phylotypes: (i) *Trichodesmium* and UCYN-C, associated with low NO_x and PO₄³⁻ concentrations, warm waters, strong and (mostly) wind direction (dominant easterly winds), and high N₂ fixation rates, (ii) UCYN-A symbiosis and Het groups, associated with higher NO_x and PO₄³⁻, colder waters, weaker winds from the south (occasionally southwest), and lower N₂ fixation rates. The factorial axis 2 separates UCYN-B from all other *nifH* phylotypes. UCYN-B are mainly associated with rainfall, desalted and warm waters characterized by high biomass (POC, PON, and Chl *a*).

DISCUSSION

Here we present a spatiotemporal study of N₂ fixation rates and diazotrophic community composition over 2 years in the New Caledonian lagoon. N₂ fixation was detected and diazotrophs were present throughout the year with generally high total mean abundances (10⁶ *nifH* gene copies L⁻¹). Inorganic nutrients are supplied to the lagoon by the Dumbea river, however, bioavailable forms are generally low (~0.05 μmol L⁻¹) in most of the lagoon waters, and NO_x are depleted relative to PO₄³⁻, making these waters favorable for N₂ fixation. The total number of diazotrophs detected by qPCR increased toward the more oligotrophic reef stations (**Figure 4C**). This pattern was mainly influenced by the UCYN-A symbiosis, which were more than two orders of magnitude more abundant at offshore stations receiving open-ocean influences (Fichez et al., 2010) compared

to inner stations. This is in accordance with previous studies reporting surface UCYN-A symbiosis abundances of 10³–10⁶ *nifH* gene copies L⁻¹ in the Coral Sea off New Caledonia (Moisander et al., 2010; Bonnet et al., 2015; Messer et al., 2015), and at the entrance of the Noumea lagoon (Turk-Kubo et al., 2015). This is also consistent with earlier reports of significant N₂ fixation and diazotroph abundances in the <3 μm size-fraction in New Caledonian waters (Garcia et al., 2007; Biegala and Raimbault, 2008). However, our results contrast with data from open ocean waters around Melanesian archipelagos, where *Trichodesmium* and UCYN-B outnumber other groups and the UCYN-A symbiosis is almost absent in surface waters and only develop deeper in the water column (Berthelot et al., 2017; Stenegren et al., 2018), at least during austral summer conditions. However, Moisander et al. (2010) found high abundances of the UCYN-A symbiosis close to New Caledonia (>10⁶ *nifH* gene copies L⁻¹), coinciding with a local upwelling system characterized by colder temperatures (~25°C) compared with surrounding waters at the same latitude (27–30°C). Bonnet et al. (2015) also reported high UCYN-A symbiosis abundances in the Coral Sea off New Caledonia but during austral winter conditions [Surface Sea Temperature (SST) ~22°C]. These studies support a niche partitioning between surface warm (>25°C) waters where *Trichodesmium* and UCYN-B thrive, and cooler (<25°C) waters preferred by the UCYN-A symbiosis. We observed the same pattern in the inner lagoon waters, where the UCYN-A symbiosis was ~5 times more abundant during the winter season (SST < 25°C), but to a lesser extent compared to open ocean waters. Indeed, the UCYN-A symbiosis



still accounted for ~60% of the diazotroph community during the summer season (SST > 25°C), which is not the case in the nearby open ocean waters (Moisander et al., 2010; Stenegren et al., 2018). The dominance of the UCYN-A symbiosis in the New Caledonian lagoon also contrasts with recent reports of *Trichodesmium* dominance and low abundances of the UCYN-A symbiosis at Great Barrier Reef and in the adjacent Coral Sea waters off Australia, especially during the winter season (Messer et al., 2015, 2017).

The UCYN-A symbiosis dominated the diazotrophic community at the four stations over both the austral summer and winter seasons (Figure 4), suggesting the lagoon waters provide favorable conditions for their growth. Henke et al. (2018) found that SST and Chl *a* concentrations were negative predictors of UCYN-A symbiosis abundance, and NO_x concentration was a positive predictor. This may be explained by the SE winds triggering an upwelling off the southwestern coast of New Caledonia (north of the Dumbea pass) that may decrease seawater temperature by 5°C in summer and 1°C in winter (Hénin and Cresswell, 2005; Ganachaud et al., 2010), and affects the lagoon SST by incoming tides. SE winds also drive cool, oligotrophic South Equatorial Current waters through the lagoon, arguing for a possible open ocean origin of the UCYN-A symbiosis detected in lagoon waters (Henke et al., 2018). The positive correlation between the UCYN-A symbiosis and S and SE winds (Henke et al., 2018) supports this hypothesis. NO_x concentrations are an order of magnitude higher in the lagoon than in open-ocean waters (Moutin et al., 2018) and Great Barrier Reef (Messer et al., 2017), which may also explain why UCYN-A thrive in the New Caledonian lagoon compared to other more oligotrophic areas of the WTSP (open-ocean waters of the Melanesian archipelagos; Bonnet et al., 2015; Stenegren et al., 2018). This is also in accordance with Mills et al. (2020) who recently reported an enhanced activity of the UCYN-A symbiosis after NO₃⁻ additions in N-limited environments. Finally, due to the inability of UCYN-A to fix inorganic carbon (Tripp et al., 2010), it is likely that the high abundance of the symbiosis in lagoon waters is sustained by photosynthates of the haptophyte host and/or labile dissolved organic matter input from the Dumbea River. Indeed, a recent study reported an increase in N₂ fixation rates and UCYN-A *nifH* gene expression in the mouth of the Dumbea River compared to waters located close to the reef (Benavides et al., 2018).

Surprisingly, *Trichodesmium* abundances were lower than those of the UCYN-A symbiosis (Figure 4). Over the past 20 years, *Trichodesmium* has been routinely studied in the New Caledonian lagoon and surrounding waters during summer conditions using satellite observations (Dupouy et al., 2000, 2011; Dupouy et al., 2018a,b) and direct field measurements (Rodier and Le Borgne, 2008; Turk-Kubo et al., 2015). These studies have revealed higher abundances in summer (~0–250 trichomes L⁻¹ and ~10⁴ *nifH* genes copies L⁻¹, respectively) than during our surveys (7.7 × 10³ *nifH* genes copies L⁻¹; note that microscope cell counts do not equal *nifH* counts; White et al., 2018). *Trichodesmium* abundances gradually increased from the coast to the open-ocean (D39 = 6.66 × 10²; M99 = 1.4 × 10³; M09 = 6.89 × 10³; and L2 = 2.19 × 10⁴ *nifH* gene copies L⁻¹),

suggesting that they could have an open-ocean origin and could be advected toward the coast with rising tide currents that flow over the reef (Jarrige et al., 1975; Rougerie, 1986). The abundance of *Trichodesmium* was higher during the summer and SST clustered positively with the abundance of *Trichodesmium*, in agreement with earlier findings (Tenório et al., 2018). This is consistent with previous studies showing that *Trichodesmium* is constrained by SST (20–30°C; Capone et al., 1997) and thrives at temperatures of 25°C or warmer (e.g., Breitbart et al., 2007; Fu et al., 2014). The distribution pattern of *Trichodesmium* determined in this study is opposite to that of the UCYN-A symbiosis, whose abundances peaked during the winter. This points toward an ecological niche partitioning between the two phylotypes, a temporal segregation that mirrors the spatial segregation observed in open-ocean waters (Moisander et al., 2010; Bonnet et al., 2015; Stenegren et al., 2018).

Heterocystous cyanobacterial symbionts (Het- groups) were present almost throughout the survey (mean 3.0 ± 7.9 × 10³ *nifH* gene copies L⁻¹; Figure 4), and were clearly dominated by Het-1 targeting *Richelia*, in line with previous lagoon (Turk-Kubo et al., 2015) and regional open-ocean studies (Stenegren et al., 2018). The abundance of Het- groups was maximum at the two inner stations (close to the Dumbea river mouth) impacted by nutrient input, and mostly in waters <25°C, consistent with previous studies reporting high Het-abundances in the vicinity of the Amazon and Mekong river plumes (e.g., Carpenter et al., 1999; Subramaniam et al., 2008; Bombar et al., 2011).

Apart from the two peaks of UCYN-B at the coastal station (see below), the unicellular groups B and C were the least abundant in lagoon waters over the 2-years survey. They did not show any clear spatial or seasonal pattern, but were present at low abundances (10² to 10³ *nifH* gene copies L⁻¹) over space and time, which is consistent with our observations during a 3-week survey performed close to the reef (Turk-Kubo et al., 2015). UCYN-B peaked on two occasions (8.4 × 10⁶ and 2.0 × 10⁸ *nifH* gene copies L⁻¹ on February 28 and May 2 2013) coinciding with high rainfall and a peak of Si(OH)₄ on May, but not in February, making a linkage questionable.

N₂ fixation rates (unquantifiable to 51.9 nmol N L⁻¹ d⁻¹; mean over the survey: 6.5 ± 6.7 nmol N L⁻¹ d⁻¹) are in line with those measured previously in the lagoon (8.0–27.7 nmol N L⁻¹ d⁻¹; Biegala and Raimbault, 2008; Berthelot et al., 2015; Bonnet et al., 2016) and open-ocean mixed layer waters around the Melanesian archipelagos (unquantifiable to 48 nmol N L⁻¹ d⁻¹, mean 8.9 ± 10.0 nmol N L⁻¹ d⁻¹, Bonnet et al., 2018), but lower than those off the tropical north-northeast coast of Australia (3 to 68 nmol N L⁻¹ d⁻¹, mean 32.0 ± 24.0 nmol N L⁻¹ d⁻¹; Messer et al., 2017). When compared with Pacific surface water rates at similar latitudes, including the Eastern tropical South Pacific (~0.9 nmol N L⁻¹ d⁻¹; Dekaezemacker et al., 2013) and the tropical South Pacific Gyre (~0.5 nmol N L⁻¹ d⁻¹; Raimbault and Garcia, 2008; Bonnet et al., 2018), the rates reported here are high. Indeed, our observations, along with those previously reported in the WTSP (Montoya et al., 2004; Bonnet et al., 2009, 2015, 2018; Messer et al., 2015, 2017; Berthelot et al., 2017), reinforce the view of the WTSP region as

a hotspot of diazotroph activity within the southern hemisphere (Bonnet et al., 2017).

N₂ fixation rates were higher (although not statistically significant) during summer, but the year to year variability was more pronounced than the seasonal variability with significantly higher N₂ fixation rates in the second year of the survey (2013; **Figure 3**), consistent with low PO₄³⁻ concentrations. This pattern was mostly dictated by offshore stations and are synchronous with higher *Trichodesmium* and UCYN-C abundances (by a factor of 2.5) at station L2 in the second year. The explanation for such an increase is unclear and may be due to a combination of: (i) higher seawater temperatures (25.44°C) in austral summer 2013 compared to 2012 (24.67°C), which may have stimulated diazotroph activity (Stal, 2009), (ii) higher occurrence of S-SW winds in 2013 that may have advected more *Trichodesmium* from the open-ocean. Notably, the ENSO index and rainfall did not differ significantly between the 2 years and are, therefore, unlikely to explain the observed differences.

With a few exceptions (Garcia et al., 2007; Bonnet et al., 2015), previous N₂ fixation studies from the New Caledonian lagoon and the WTSP were conducted over the austral summer, which is considered the main N₂ fixation season in the region (Garcia et al., 2007). Our data suggest that seasonality in the New Caledonian lagoon is less pronounced than previously thought, and that relatively high N₂ fixation rates are maintained all year long, despite seasonal changes in the dominant diazotrophs. During the 2 years of study in the New Caledonian lagoon, the composition of the diazotrophic community was variable, but provided a constant N input.

Assuming that diazotrophs fix on average $115 \pm 47 \mu\text{mol N m}^{-2} \text{d}^{-1}$ throughout the year (the average of the four stations) and considering a southwestern lagoon area of 2,066 km², planktonic N₂ fixation would introduce $0.6\text{--}1.7 \times 10^9 \text{ g N year}^{-1}$ to the lagoon. For comparison, the inorganic N inputs from the three major rivers flowing into the southwestern lagoon (Dumbea, Pirogue and La Coulée rivers) calculated from mean annual river flows from Direction of Food and Rural Veterinary Affairs (Direction des Affaires Vétérinaires Alimentaires et Rurales, DAVAR, New Caledonia) and dissolved NO_x concentrations data would introduce $0.03 \times 10^9 \text{ g N year}^{-1}$, i.e., 22–56 times less than N₂ fixation. Consequently, planktonic N₂ fixation represents a significant source of N for biological communities of the southwestern lagoon. In comparison, Charpy et al. (2007) reported mean benthic N₂ fixation rates for the southwestern lagoon of $293 \pm 96 \mu\text{mol N m}^{-2} \text{d}^{-1}$, i.e., three times higher than planktonic N₂ fixation. Collectively, these results suggest that N₂ fixation plays a critical role in sustaining the productivity of the New Caledonian waters, which features an exceptional diversity and is listed as a UNESCO World Heritage Site.

CONCLUSION

Several studies have pinpointed the importance of N₂ fixation for sustaining both planktonic (Berthelot et al., 2015) and benthic (Benavides et al., 2016) productivity during the summer season

in the New Caledonian lagoon, but here we document that high fixed N input takes place also during the cold season. Our analyses suggest that the meteorological and physicochemical characteristics of New Caledonian lagoon waters lead to the heterogeneity of diazotrophic populations both at seasonal and annual timescales. The UCYN-A symbiosis dominate the diazotrophic community, even during warmer, summer months, and likely provide a constant background of fixed N to surrounding waters, together with other UCYN groups (UCYN-B and UCYN-C). *Trichodesmium* is second most abundant group, peaking preferentially during summer months, and adding additional fixed N during the summer. However, the year to year variability was more pronounced, highlighting the need for longer time-series studies with fine temporal and geographical resolution to refine our understanding of the environmental factors regulating diazotrophs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

SB, MR, and CD conceived the study. SB, AD, and CD acquired samples and data. ES, MB, KT-K, HC, BH, SB, MR, OG, and CD analyzed samples and data. ES, MB, KT-K, BH, LR, and SB interpreted the data. ES, SB, and MB wrote the manuscript. All authors revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.581755/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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