Nitrogen (N) is an essential element for life and controls the magnitude of primary productivity in the ocean. In order to describe the microorganisms that catalyze N transformations in surface waters in the South Pacific Ocean, we collected high-resolution biotic and abiotic data along a 7000 km transect, from the Antarctic ice edge to the equator. The transect, conducted between late Austral autumn and early winter 2016, covered major oceanographic features such as the polar front (PF), the subtropical front (STF) and the Pacific equatorial divergence (PED). We measured N$_2$ fixation and nitrification rates and quantified the relative abundances of diazotrophs and nitrifiers in a region where few to no rate measurements are available. Even though N$_2$ fixation rates are usually below detection limits in cold environments, we were able to measure this N pathway at 7/10 stations in the cold and nutrient rich waters near the PF. This result highlights that N$_2$ fixation rates continue to be measured outside the well-known subtropical regions. The majority of the mid to high N$_2$ fixation rates (>∼20 nmol L$^{-1}$ d$^{-1}$), however, still occurred in the expected tropical and subtropical regions. High throughput sequence analyses of the dinitrogenase reductase gene (nifH) revealed that the nifH Cluster I dominated the diazotroph diversity throughout the transect. nifH gene richness did not show a latitudinal trend, nor was it significantly correlated with N$_2$ fixation rates. Nitrification rates above the mixed layer in the Southern Ocean ranged between 56 and 1440 nmol L$^{-1}$ d$^{-1}$. Our data showed a decoupling between carbon and N assimilation (NO$_3^-$ and NH$_4^+$ assimilation rates) in winter in the South Pacific Ocean. Phytoplankton community structure showed clear changes across the PF, the STF and the PED, defining clear biomes. Overall, these findings provide a better understanding of the ecosystem functionality in the South Pacific Ocean across key oceanographic biomes.

Keywords: N$_2$ fixation, nitrification, latitudinal trends, GO-SHIP, nifH marine biogeography, South Pacific Ocean
INTRODUCTION

Nitrogen (N), in all its oxidation states, is a key element for life on Earth. In marine ecosystems, N controls the magnitude of primary production, with microorganisms performing essential roles in the assimilatory and dissimilatory pathways of the N-cycle (Albright et al., 2018). These microorganisms form the base of the marine food web, and include archaea; hetero-, mixo-, and phototrophic bacteria; and phototrophic micro-eukaryotes (Falkowski, 1997).

N$_2$ fixation and nitrification are two globally important, yet relatively poorly resolved new and regenerated N input processes. Quantifying the input of new N from N$_2$ fixation, and the nitrogen budget in general, remains important and will aid in our understanding how the ocean’s productivity will be impacted by environmental change (Wang et al., 2019). Repeated N$_2$ fixation rate measurements have been conducted in the eastern and western sides of the Pacific Ocean in the northern hemisphere, including in specific oceanographic features such as eddies in the North Pacific (see e.g., Mague et al. (1974); Montoya et al. (2004); Fong et al. (2008); Raimbault and Garcia (2008), Church et al. (2009); Chen et al. (2018) and references within Shiozaki et al. (2010)). In the south eastern [e.g., Bonnet et al. (2013)] and western Pacific Ocean N$_2$ fixation has been quantified (Shiozaki et al., 2010; Knapp et al., 2018). Halm et al. (2012) sampled the southern subtropical gyre, and rates have been measured in the equatorial region [e.g., Bonnet et al. (2009)], and Shiozaki et al. (2010) provided a comparison between the western and central Pacific basins. Of greater interest to this study are the results of Fong et al. (2008); Bonnet et al. (2009), and Chen et al. (2018) which showed that mesoscale physical variability can play a significant role in modifying the abundances of diazotrophs, and that distinct spatial niches and diel patterns can be observed for Trichodesmium sp. and unicellular diazotrophs. Heterotrophic Gammaproteobacteria have been found to be widespread throughout the oligotrophic subtropical Pacific gyre and have been shown to occupy an ecological niche that overlaps with the unicellular cyanobacterial lineages A and B [UCYN-A and UCYN-B; Halm et al. (2012) and Chen et al. (2018)]. The exact ecological roles of heterotrophic Gammaproteobacteria and their contribution to N$_2$ fixation rates, however, remain elusive (Turk-Kubo et al., 2014; Benavides et al., 2018). Bonnet et al. (2017) and Chen et al. (2018) clearly showed that the abiotic factors (and currents) which reflect iron bioavailability have a significant control on the diazotroph biogeography. Yet, as the Pacific Ocean is the largest Ocean basin on Earth, the spatial and temporal controls of the input of new N from N$_2$ fixation remain poorly quantified (Shiozaki et al., 2017).

Basin wide measurements enable the quantification of changes across boundaries, as sampling region extends from one ecological province to the other. Oceanographic fronts have been shown to act as boundaries in the ocean, capable of separating and structuring microbial communities and ecosystem functions (Baltar et al., 2016; Raes et al., 2018a). Shiozaki et al. (2017), provided the first basin wide N$_2$ fixation rate measurements, conducted from the equatorial Pacific to the Bering Sea (68°N) along the 170° W meridian. The authors concluded that, although N$_2$ fixation was widespread (up to the Bering Sea) the biogeochemical controls on the numbers and activity of the different diazotrophs required further investigation. In our study we report on the distribution and activity of diazotrophs from the ice edge at 66°S until the equator along the 170°W meridional, thus complementing the basin scale transect that was visited by Shiozaki et al. (2017). Our aim was to narrow down the biotic and abiotic controls on N$_2$ fixation rates in the southern part of the Pacific Ocean.

Global data on nitrification rates above the mixed layer are sparse (Yool et al., 2007; Beman et al., 2011) and will contribute to high uncertainties when estimating the f-ratio (Dugdale and Goering, 1967; Eppley and Peterson, 1979). Neglecting or underestimating nitrification rates results in an overestimation of the f-ratio (Eppley and Peterson, 1979), and can thereby impact modeling estimates of global oceanic production. In oligotrophic systems low primary productivity rates have been associated with high nitrification activity (Clark et al., 2008). As the low nutrient ocean gyres are expanding (Polovina et al., 2008) quantifying nitrification rates merits investigation and will consequently improve our estimates of biological productivity in these oligotrophic regions (Dugdale and Goering, 1967). For completion we note that a second consequence of sparse nitrification rates are an underestimation of the levels of a potent greenhouse gas, nitrous oxide (N$_2$O), which is a by-product of the formation of hydroxylamine from ammonium (NH$_4^+$) during nitrification (Naqvi et al., 2000; Santoro et al., 2011).

Our goal for this study was to capture a snapshot of the biogeography and controls on N cycling rates and C assimilation of marine prokaryotes and eukaryotes from the ice edge (66°S) until the equator (0°S) along the 170°W meridional in the surface waters of the South Pacific Ocean. A focus of our work was to quantify N cycling rates and assess the diversity of diazotrophs and nitrifiers across ocean boundaries. Our overarching aim was to use the P15S latitudinal GO-SHIP transect as a natural laboratory to characterize trends in N$_2$ fixation and nitrification rates, quantify carbon assimilation rates, and relate changes in the δ$^{15}$N of the particulate organic matter (POM) to the turnover of biological communities and abiotic factors along the transect.

MATERIALS AND METHODS

Study Region and Water Sampling

The study region, a 7000 km transect located along the longitudinal P15S GO-SHIP line at 170°W, was surveyed at every half degree onboard the R.V. Investigator from 23 April to 29 June (late Austral autumn/early winter) 2016. The P15S GO-SHIP line is a decadal repeated transect that runs from the ice edge (66°S) to the equator (0°S; Figure 1). The expedition traversed four oceanographic provinces (Longhurst, 2010): (1) the Sub-Antarctic between 66°S and 52°S, including the Polar Front (PF; at 60°S); (2) the Subtropical front (STF) between 52°S and 40°S; (3) the South Pacific Subtropical Gyre (SPSG) between 40°S and

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1 http://www.go-ship.org/
flow-through system (intake at 6 m) for N fixation. The seawater intake on the R.V. Investigator described below). The seawater intake on the base of the mixed layer depth (MLD) for measuring at all depths for nutrient analysis; and from the surface (SFC) depth. Seawater samples were collected from the Niskin bottles rosette sampler. Full depth profiles for temperature, conductivity, temperature-depth (CTD) profiler mounted on a 36 Niskin bottle at 140 stations, using a Seabird (SBE25 plus) conductivity-temperature-salinity (CTD) profiler mounted on a 36 Niskin bottle rosette sampler. Full depth profiles for temperature, conductivity, and dissolved oxygen were collected at each station and sampling depth. Seawater samples were collected from the Niskin bottles at all depths for nutrient analysis; and from the surface (SFC) and the base of the mixed layer depth (MLD) for measuring nitrification rates and for DNA analyses (described below). For detailed information on the sensors mounted on the rosette and data availability please refer to the Supplementary Material.

Seawater samples were collected from the underway flow-through system (intake at 6 m) for N fixation rate measurements, for NO$_3^-$, and NH$_4^+$ assimilation rates, for C assimilation rates (primary productivity), for the particulate organic C and N (POC/N) and for pigment analyses (all described below). The seawater intake on the R.V. Investigator designated underway flow-through system is located on the vessel’s drop keel, which are stored out of the water when not in use. Consequently, the intake and first parts of the plumbing dry out between sampling events, which resets any biological growth at this part of the structure. Furthermore, all tubing and pipework in the underway flow-through system are flushed and consecutively flooded with fresh water at the end of each voyage. Nevertheless, we acknowledge the caveat of potential biological growth in the underway flow-through system. Another artifact associated with underway in-line sampling (for N$_2$ fixation) could be cell damage, especially in the case of *Trichodesmium* filaments. We note that we did not encounter any visual *Trichodesmium* blooms, nor did we see any trichomes or colonies on the GF/F filters. The underway flow-through system is made from Teflon which should reduce the contamination of iron supply.

**Nutrient Analysis of Seawater**

Dissolved inorganic concentrations of silicate, (Si) phosphate, (PO$_4^{3-}$), nitrate, (NO$_3^-$), nitrite, (NO$_2^-$), and ammonium (NH$_4^+$) were assayed at sea on a Bran + Luebbe AA3 HR segmented flow analyzer (detection limits, respectively, 0.2, 0.01, 0.01, 0.015, and 0.005 µmol L$^{-1}$) by the CSIRO Hydrochemistry group, following standard spectrophotometric methods (Armstrong et al., 1967; Kérouel and Aminot, 1997; Aminot et al., 2009; Hansen and Koroleff, 2009; Hydes et al., 2010). Please refer to the Supplementary Material for detailed methodology. Dissolved oxygen (O$_2$) concentrations were determined by titration following Winkler (1888).

**N$_2$ Fixation Measurements**

In total, 122 seawater samples were collected for N$_2$ fixation rate measurements. N$_2$ fixation rate measurements were performed using the modified bubble injection method (Montoya et al., 1996; Mohr et al., 2010; Klawonn et al., 2015), using 98% $^{15}$N$_2$ gas [Cambridge Isotope Laboratories, Inc., lot #NLM-363-1-LB, as recommended by Dubundo et al. (2014)]. Ten mL of 98% $^{15}$N$_2$ gas at atmospheric pressure were added to each 4.5 L incubation bottle, which were then gently rocked for 15 min on an orbital shaker at 100 rpm to enhance the dissolution of the $^{15}$N$_2$-gas bubble. This ensured consistent agitation and reduced variability of the final $^{15}$N-atom% enrichment (Klawonn et al., 2015). After shaking, the gas bubble was removed and the bottles were kept for 24 h under natural light in on-deck incubators. N$_2$ fixation measurements were terminated by filtering contents of each bottle (<10 kPa) onto a 25 mm pre-combusted (at 500°C for 24 h) Whatman® GF/F filter (nominal pore size 0.7 µm). A 12 mL subsample was collected after 24 h from each bottle and preserved in an Exetainer® vial with 100 µL saturated HgCl$_2$ solution for $^{15}$−$^{15}$N analysis. Unfortunately, samples for final $^{15}$−$^{15}$N analyses were damaged, therefore values specific to this study cannot be reported. We have, however, as the dissolution of N$_2$ gas is a physical process, used the final $^{15}$−$^{15}$N enrichment values from a voyage which we conducted in 2017 along a latitudinal gradient in the Indian Ocean spanning temperatures from 3 to 28°C and salinities ranging from 33.6 to 35.5 PSU (OSIO MD206 campaign)$^2$. Please refer to the Supplementary Material for detailed information.

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$^2$[http://dx.doi.org/10.17600/17002400](http://dx.doi.org/10.17600/17002400)
Prior to incubation, four liters of water were filtered onto pre-combusted (at 500°C for 24 h) 25 mm Whatman® GF/F filters for particulate organic N (PON). The PON samples, which were used as the initial N₀ values, were analyzed at the Isotopic Laboratory at University of California, Davis. The minimum quantifiable N₂ fixation rate was calculated as described in Montoya et al. (1996) and Gradoville et al. (2017) and varied between 0.054 and 0.472 nmol L⁻¹ d⁻¹ in the sub-Antarctic region (please refer to Supplementary Table 1). We note that we sacrificed higher replication for N₂ fixation rate measurements at the majority of stations in order to cover a greater spatial resolution (every 0.5°). In addition, we refer the reader to Supplementary Figures 2A,B which shows ¹⁵N incorporation into the PON during N₂ fixation rate measurements.

NO₃⁻, NH₄⁺ and Carbon Assimilation Rate Measurements

Incubation experiments to measure NO₃⁻, NH₄⁺, and carbon (C) assimilation rates (from hereafter primary productivity) were also collected from the clean underway flow through system. Clear, 1 L polycarbonate bottles were used to incubate seawater for 24 h in order to measure NO₃⁻, NH₄⁺, and primary productivity. Experiments were initiated by adding known concentrations of 99% K¹⁵NO₃ and 99% ¹⁵NH₄Cl to the bottles according to Knap et al. (1996). Replicates for the incubations and, thus, assimilation rate measurements, of NO₃⁻ and NH₄⁺ were sacrificed at each station in favor of a greater spatial resolution. On the other hand, all bottles with ¹⁵N-labeled tracers (¹⁵NO₃⁻, ¹⁵NH₄⁺, and ¹⁵N₂) from the NO₃⁻ and NH₄⁺ assimilation and N₂ fixation incubations, respectively) were spiked with a NaH¹³CO₃ solution (final conc. 20 µmol L⁻¹), resulting in triplicate measurements for primary productivity. Natural abundance samples for particulate organic C (POC) and PON were used as initial (t₀) values in the calculation of the N and primary productivity rates. Please refer to the Supplementary Material for the detailed methodology. Primary productivity and nitrogen assimilation data from this study are available at https://doi.pangaea.de/10.1594/PANGAEA.884052 (Raes et al., 2017) and https://doi.pangaea.de/10.1594/PANGAEA.885169 (Raes et al., 2018b).

Nitrification Measurements

Total nitrification rates (NH₄⁺ oxidation to NO₃⁻) were measured during 24 h incubations of seawater sampled from the surface and at the base of the MLD. In order to do so, clear polycarbonate bottles (0.5 L) were amended with ¹⁵NH₄⁺ at a concentration of 50 nmol L⁻¹, resulting in trace additions between 6 to 25% of ¹⁵NH₄⁺. Neutral density screens were placed around the bottles during the incubation in order to mimic light intensities at the respective depths. After 24 h 50 mL of water was filtered through a Whatman® nylon membrane filter (47 mm diameter, 0.22 µm pore size) and samples were stored at −80°C.

Prior to incubation, samples for the natural abundance of δ¹⁵N-NO₃⁻ (t₀ measurements) in each depth layer were obtained by filtering 50 mL of water through a Whatman® nylon membrane filter (47 mm diameter, 0.22 µm pore size). Technical duplicates of the δ¹⁵N-NO₃⁻ were measured using the denitrifier method (Sigman et al., 2001; McIlvin and Casciotti, 2011) on a custom gas bench (Thermo Fisher Gasbench II) coupled to a Thermo Fisher Delta V Plus at the Centre for Coastal Biogeochemistry of Southern Cross University, Bilinga, QLD, Australia. The rate of total nitrification (NH₄⁺ oxidation) was calculated following Peng et al. (2015). We were unable to measure total nitrification rates in oligotrophic waters where environmental NO₃⁻ concentrations were <0.05 µmol L⁻¹ due to the detection limit of the denitrifier method. See Supplementary Material for detailed methodology.

DNA Collection, nifH PCR Amplification, Amplicon Sequencing and Downstream Analysis

For each depth layer two L of seawater were filtered through Sterivex™ GP filters (Millipore®, Burlington, MA, United States). Cat. # SVGPL10RC; 0.2 µm pore size) with a peristaltic pump. DNA was extracted with the PowerWater Sterivex DNA Isolation Kit (Mo Bio Laboratories, United States) using a modified protocol which includes a phenol:chloroform:isoamyl alcohol extraction. DNA was then eluted in 80 µl TE buffer (Appleyard et al., 2013; see Supplementary Material for detailed information on the DNA extraction protocol).

The nifH gene which is used as a functional marker of diazotroph diversity was PCR amplified (~359-bp) from environmental DNA extracts using a nested PCR protocol (Zehr and McReynolds, 1989; Zehr and Turner, 2001). Two sets of degenerate primers were used to target the region: for the 1st PCR reaction the nifH3 reverse primer (5‘-ATRTRTTTNCGCNGCRTA-3’) and the nifH4 forward primer (5‘-TTYTAYGNARGGNGG-3’) were used. The 2nd PCR reaction was done with the nifH1 forward primer (5’-TGYGAYCCNAARGCGA-3’) and the nifH2 reverse primer (5’-ADNGCCCATCATYTCNCC-3’). Illumina adapters were incorporated with the 2nd stage PCR. PCR cycling conditions were similar to Messer et al. (2016) and detailed information is outlined in the Supplementary Material. The PCR products were purified using AMPure™ magnetic beads (Agencourt, Beckman Coulter Life Science, United States) according to the manufacturer’s instructions. Purified amplicons were then sent to the Ramaciotti Centre for Genomics (UNSW Sydney, Australia) for high throughput sequencing. The 140 nifH amplicon samples were multiplexed on one sequencing run. Nextera XT barcode incorporation, purification, library generation and sequencing using the Illumina MiSeq platform (Illumina, Inc., San Diego, United States), with 250 bp paired reads, were performed according to the manufacturer’s directions.

We used the DADA2 (version 1.4.0) pipeline implemented in R-3.6.1 to process raw sequences into amplicon sequence variants (ASVs) (Callahan et al., 2016). The parameters for the DADA2 pipeline were: truncLen = c(240,200), maxN = 0, maxEE = c(2,5), truncQ = 2, m.phix = TRUE, trimLeft = 5).

The first 5 bps were removed from the forward and reverse reads. The forward reads were trimmed to 240 bps and the reverse reads to 200 bps. The forward and reverse reads were
then truncated beyond the first instance of quality scores below 3 (“truncQ = 2”). The maximum expected error during denoising (“maxEE”) was 2 and 5 for forward and reverse reads. Denoised reads were then merged and chimeric contigs discarded using the functions “mergePairs” and “removeBimeraDenovo.”

Four L of seawater were filtered through Whatman GF/F filters (25 mm, pore size 0.7 µm) using vacuum filtration (<10 kPa). High Performance Liquid Chromatography (HPLC) was used to determine 23 different photosynthetic pigments present in the samples. HPLC of the collected samples were performed according to the methodology employed at the CSIRO laboratories in Hobart, Australia [see Hooker et al. (2012)]. The HPLC data were analyzed using diagnostic pigments of dominant phytoplankton functional guilds as well as size classes according to Hirata et al. (2008). We note that diagnostic pigment analysis has its ambiguities (e.g., fucoxanthin is a precursor for 19′-butanoyloxyfucoxanthin and 19′-hexanoyloxyfucoxanthin) but we believe our data analyses are valid. For detailed information please refer to Supplementary Material and the validation of these analyses by Uitz et al. (2006); Ras et al. (2008), Aiken et al. (2009). All pigment data are available at https://doi.pangaea.de/10.1594/PANGAEA.884052.

Richness and Statistics

*nifH* gene richness was calculated as the number of ASVs observed per sample [as per Fuhrman et al. (2008)]. Correlations between rate data, phytoplankton biomass and abiotic parameters were calculated using the Spearman coefficient *r* as a measure of rank correlation, as it assesses monotonic relationships, not purely linear relationships. Multiple error rate correction for the correlation analysis was performed using the Holm’ method (Holm, 1979). Alpha and beta diversity were plotted using the Phyloseq version 1.28.0 package (McMurdie and Holmes, 2013) in R-3.6.1 (R Core Team, 2013). Negative exponential smoothing curves with a first polynomial order were used to visualize trends and were produced using the Sigmaplot® v.14.0 software package. Polynomial regression analysis was done using the Sigmaplot® v.14.0 software package. The influence of predictor variables on *N₂* fixation rates, DIN assimilation rates and primary productivity were assessed using boosted regression trees (BRT; Elith et al. (2008), please refer to the Supplementary Material for more information). BRT was implemented in the R-3.6.1 software environment using the gbm.step function, a gaussian error structure, 10 fold cross-validation, and the following settings which resulted in models with >1200 trees in all cases; learning.rate = 0.001, tree.complexit = 10, bag.fraction = 0.5 (Elith et al., 2008). The cross-validation correlation (cv ± standard error) is a technique to evaluate how predictive the model is, and it is comparable to an *r²* value (for more information please refer to the Supplementary Material).

RESULTS

Hydrographic Conditions Above the MLD

Prominent oceanographic features encountered along the 1°S GO-SHIP transect were the PF (∼60°S), the STF (∼45°S), and the PED (∼5°S; Figure 2). The most prominent feature was the STF which divides the less saline, colder, and nutrient rich sub-Antarctic waters from the warmer and saltier subtropical waters (Figure 2). This pattern can be visualized in Figure 2, which shows strong latitudinal changes in temperature (from 11.80 to 15.62°C, Figure 2A) and salinity (from 34.6 to 35.2, Figure 2B) across the STF. The 26 and 27 kg m⁻³ pycnoclines are observed at increasingly shallower depths closer to the Equator, and their shallower location at 150 m depth at ∼5°S is a clear signal of equatorial upwelling (Figures 2A,C,D,F). Overall, along the transect, temperatures increased gradually from ∼2°C in the Southern Ocean to 30°C toward the equator (Figure 2A). Dissolved oxygen levels decreased from 350 µmol kg⁻¹ in surface waters at 66°S to 180 µmol kg⁻¹ at the equator (Figure 2C), with the lowest concentrations in the upper 500 m being found near the PED (Figure 2C).

Chemical Conditions

Changes in dissolved inorganic nutrient concentrations above the MLD were closely linked to the major oceanographic features. *NO₃⁻* concentrations generally declined from south to north, with clear changes north of the PF and north of the STF, to then increase near the PED (Figure 2D). They plummeted from values as high as 30 µmol L⁻¹ in the Southern Ocean to between 8 and <1 µmol L⁻¹ in the southern and northern boundaries of the STF, respectively, to <0.05 µmol L⁻¹ north of the STF, and then increased to 2 µmol L⁻¹ near the PED (Figure 2D). *NO₂⁻* concentrations peaked at 0.3 µmol L⁻¹ south of the STF
and decreased north until ~40°S, within the SPSG values were slightly elevated below the MLD than above it (Figure 2E). NO$_3^-$ concentrations in the PED reached 1 µmol L$^{-1}$, and a NO$_3^-$ maximum was recorded at approximately 9°S at 100 m depth, suggesting recycling of organic matter (see section “Discussion”). NO$_3^-$ concentrations were up to 2 µmol L$^{-1}$ above the MLD in the PED. PO$_4^{3-}$ concentrations decreased from 2.2 to 0.15 µmol L$^{-1}$ from the sub-Antarctic toward the STF, further declining to <0.01 µmol L$^{-1}$ north of the STF (Figure 2F). Ammonium showed a similar pattern as that of NO$_3^-$, with high surface concentrations (0.8 µmol L$^{-1}$) in the Southern Ocean which declined below the detection limit north of the STF to increase again (0.2 µmol L$^{-1}$) near the equator (Supplementary Figure 1). Silicate concentrations decreased rapidly from 75 to 0.1 µmol L$^{-1}$ as the STF was crossed northward, and remained low in tropical waters. NO$_3^-$:PO$_4^{3-}$ ratios averaged 14.24 ± 0.25 in the sub-Antarctic Zone, 9.76 ± 2.32 in the STF, 0.431 ± 0.874 in the oligotrophic SPSG, and 5.6 ± 1.4 in the PED.

**N$_2$ Fixation and Diazotrophs**

The minimum quantifiable N$_2$ fixation rates [MQRs, for description on how to calculate it please refer to Montoya et al. (1996) and Gradoville et al. (2017)] for the sub-Antarctic averaged 0.166 ± 0.138 nmol L$^{-1}$ d$^{-1}$ (Supplementary Table 1). The N$_2$ fixation rates were greater than the MQR in 7/10 of the sub-Antarctic stations (Figures 3A,B; station-specific MQRs are presented in Supplementary Table 1). North of 50°S, crossing the STF northward, N$_2$ fixation rates increased steadily from 1 to 10.5 nmol L$^{-1}$ d$^{-1}$ at 50 and 40°S, respectively (Figures 3A,B; the MQR was never greater than 1 nmol L$^{-1}$ d$^{-1}$ across the transect line). N$_2$ fixation rates ranged between 7.3 and 134 nmol L$^{-1}$ d$^{-1}$ in the SPSG (Figure 3A) and between 11 and 37.5 nmol L$^{-1}$ d$^{-1}$ north of 10°S, near the PED (Figures 3A,C).

The *nifH* gene was amplified and sequenced to assess the diversity of the diazotrophic community. *nifH* gene richness did not show a latitudinal trend (Figure 4A), nor was it significantly correlated with N$_2$ fixation rates ($r^2 = 0.009$, $p = 0.7$; Supplementary Figure 5). Overall, diazotroph diversity along the transect was dominated by Cluster I, which is the conventional Molybdenum-containing group of *nifH* sequences and encompasses proteobacteria and cyanobacteria. In the sub-Antarctic province, the community consisted predominantly of non-cyanobacterial diazotrophs such as undefined alphaproteobacteria, actinobacteria, subdivisions of the delta and epsilonproteobacteria, and unclassified diazotrophic bacteria, though relatively high abundances of cyanobacterial Oscillatoriophycideae were recorded in some stations south of 60°S. An increase in the relative abundance of...
**FIGURE 3** | (A) \(N_2\) fixation rates along the P15S GO-SHIP transect from the ice edge up to the equator. Red line is a negative exponential smoothing curve with a first polynomial order. (B) Higher resolution plot of \(N_2\) fixation rates between 66° and 40°; note Y-axis is now from zero to 2.5 nmol L\(^{-1}\) d\(^{-1}\). Horizontal filled gray bar denotes the average minimum quantifiable \(N_2\) fixation rates (calculated according to Montoya et al. (1996); Supplementary Table 1 provides the MQR at a one-degree resolution). (C) High resolution plot of \(N_2\) fixation rates between 40° and 0°; note Y-axis is now from zero to 60 nmol L\(^{-1}\) d\(^{-1}\). Gray rectangles denote oceanographic fronts (PF, Polar front; STF, Subtropical Front; Eq, Equatorial upwelling zone). Standard deviations are presented at the stations were we had duplicate and or triplicate measurements.

epsilon-proteobacteria was recorded near the STF, but the \(nif\) diversity in the area was dominated by sequences belonging to the Gammaproteobacteria. \(nif\) diversity within the SPSG was dominated by the Oscillatoriophycideae between 34.5 and 19.5°S (Figures 4B,C), and by *Trichodesmium*, *Candidatus Atelocyanobacterium*, and Gammaproteobacteria north of 19.5°S (Figure 5). Relative abundances of Gammaproteobacteria and unclassified proteobacteria increased near the PED. Cyanobacterial diazotrophs were recorded along the entire transect. A non-metric multi-dimensional scaling (nMDS) revealed significant differences between the diazotrophic communities of the sub-Antarctic and PED (ANOSIM, \(R\) value = 0.692, \(p < 0.001\); Supplementary Figure 7).

**Nitrification and Nitrifiers**

Total nitrification rates (NH\(_4^+\) oxidation and NO\(_2^-\) oxidation) above the MLD in the sub-Antarctic reached up to 1440 nmol L\(^{-1}\) d\(^{-1}\) (production of \(\sim 1.4 \mu\)mol NO\(_3^-\) L\(^{-1}\) d\(^{-1}\); Figure 6), with statistically similar values between samples taken at the surface and at the base of the MLD [Mann-Whitney, \(p = 0.510\), \(n = 16\), effect size (\(U\) value) \(= 110\)]. We were unable to quantify nitrification rates for most stations in the SPSG as NO\(_3^-\) concentrations were below the detection limit (<1 \(\mu\)mol L\(^{-1}\)) for our nitrification measurements. North of 10°S, as NO\(_3^-\) concentrations started to increase, nitrification rates reached up to 141 nmol L\(^{-1}\) d\(^{-1}\).

16S rRNA amplicon sequencing data indicated that, in the sub-Antarctic and south of the STF, the genera *Nitrospina* and *Nitrosomonas* had the highest relative abundances within the Bacteria (Domain), whereas the genera *Nitrospelagicus*, *Nitrosoarchaeum*, and *Nitrosopumilus* had the highest abundance within the Archaea (Kingdom). The highest relative abundances for all genera within the Bacteria and Archaea in the SPSG were recorded at the base of the MLD. Although all genera were also present in the PED, their relative abundance decreased north of the STF toward the equator (Figures 6B,C, and Supplementary Figure 9). Overall our data showed that the high relative abundances of nitrifying organisms (both Archaea and Bacteria) coincided with nitrification rates south of the STF.

**NO\(_3^-\), NH\(_4^+\), and C Assimilation**

NO\(_3^-\) assimilation rates in the sub-Antarctic increased steadily northward to the STF from 163.2 up to 2544 nmol L\(^{-1}\) d\(^{-1}\), despite decreasing NO\(_3^-\) concentrations above the MLD between 66°S (31 \(\mu\)mol L\(^{-1}\)) and 45°S (8 \(\mu\)mol L\(^{-1}\)). Assimilation rates
FIGURE 4 | Panel (A) shows the nifH richness along a latitudinal transect in the South Pacific Ocean (B) shows the relative abundance of diazothrophs at a Phylum level and (C) the relative abundance of diazothrophs at a Class level from the sub-Antarctic to the equator in the South Pacific Ocean. Gray rectangles denote oceanographic fronts (PF, Polar front; STF, Subtropical Front; Eq, Equatorial upwelling zone).

were low in the SPSG north of the STF, ranging between 31.2 and 318 nmol L$^{-1}$ d$^{-1}$ but increased rapidly in the PED to a maximum rate of 5688 nmol L$^{-1}$ d$^{-1}$ (Figure 7A). NH$_4^+$ assimilation rates increased from 170.4 to 1131.6 nmol L$^{-1}$ d$^{-1}$ between 66 and 44.5°S, respectively. Assimilation rates then declined to 88.8 nmol L$^{-1}$ d$^{-1}$ north of the STF and increased to 1607.6 nmol L$^{-1}$ d$^{-1}$ at 15°S and then decreased to 331.2 nmol L$^{-1}$ d$^{-1}$ toward the PED (Figure 7B). Primary productivity rates increased south to north from 127.2 nmol L$^{-1}$ d$^{-1}$ from the most southern station to 1589.4 nmol L$^{-1}$ d$^{-1}$ in the STF. We note that rates peaked at 48° and 40°S (725.1 to 1589.4 nmol L$^{-1}$ d$^{-1}$, respectively). Within the PED, productivity increased from 5°S toward the PED up to 343.9 nmol L$^{-1}$ d$^{-1}$ (Figure 7C).
The effect of enhanced N assimilation rates was tested in three different stations in the SPSG with incubation trace additions exceeding 10% of ambient nutrient concentrations. Although trace additions exceeding 10% did not significantly affect $^{15}$NO$_3$ assimilation rates ($p > 0.1$ for all three stations; see Supplementary Figure 3), they resulted in lower $^{15}$NH$_4^+$ assimilation rates ($p < 0.05$ for all three stations; see supplementary Figure 3). It should be noted that the transect was conducted in early winter and that, at the time, NH$_4^+$ assimilation rates were greater than the primary productivity rates.

**Photosynthetic Pigments and Particulate Organic Matter**

Clear changes in phytoplankton groups and size classes were recorded when crossing the PF, the STF, and the PED (Figures 8A,B). High concentrations of the pigment fucoxanthin, which is a proxy for diatoms, were recorded south of the STF whereas zeaxanthin and divinyl-chlorophyll a (a proxy for cyanobacteria) dominated in the SPSG. The southernmost sub-Antarctic (between 65°S and 57°S) phytoplankton community was dominated by diatoms, followed by haptophytes and pelagophytes. Picoeukaryotes dominated the community between the northernmost sub-Antarctic and the STF (56° and 42°S), followed by green algae and, to a lesser degree, by prymnesiophytes, dinoflagellates, and prokaryotes. A community composition shift toward cyanophytes was detected around 40°S, with a dominance by Prochlorochoccus and Synechococcus spp. in the SPSG (Supplementary Figure 4). In the northernmost PED (north of 5°S) we recorded a small presence of dinoflagellates, as well as an increase in pelagophytes, haptophytes, and green algae and a decrease in the proportion of cyanophytes (Figures 8A,B).

The $\delta^{15}$N of the PON (or $\delta$PO$_{15}$N) averaged $1.33 \pm 0.94\%$ (±SD, $n = 21$), in the sub-Antarctic, but as we crossed the STF northward, the $\delta$PO$_{15}$N increased significantly (Student’s $T$-test $p < 0.0001$) to $8.49\%$ (4.16 ± 1.99% average ± SD, $n = 18$, Figure 9A). The $\delta$PO$_{15}$N decreased within the southernmost SPSG (until ~ 24°S) to 0.49%, but it increased in its northernmost part to a maximum of 15.89% at 10°S (9.56 ± 2.08% average, $n = 28$, Figure 9A). The $\delta$PO$_{15}$N decreased within the PED to 7.3% at the equator (Figure 9A).

The PON:Σ pigment ratio can be used as an indicator for heterotrophy (Harmelin-Vivien et al., 2008), with low PON:Σ pigment ratio reflecting a high abundance of
autotrophs relative to the PON, thus a low relative biomass of heterotrophic microorganisms, and vice versa). A northward declining trend was observed for the PON:Σ pigment ratio in the sub-Antarctic, with values thereafter remaining stable in the STF (Figure 9C). The PON:Σ pigment ratio then showed an overall northward increasing trend in the SPSG, followed by a northward decreasing trend in the PED (Figure 9C). The values recorded for POC:Σ pigment ratio showed similar patterns to those of PON:Σ pigment ratio along the transect (Supplementary Figure 11). The POC:PON ratios were generally greater than the Redfield ratio (Figure 9D).
Correlations and Drivers

Fourteen predictor parameters were investigated in order to explain variations in $N_2$ fixation, DIN assimilation rates, and primary productivity using BRT models and Spearman rank correlations. Spearman rank correlations indicated significantly positive relationships between the predictor parameters latitude, temperature, and picoplankton, and the measured rates of $NH_4^+$ assimilation and $N_2$ fixation (Figure 10). Significantly negative relationships were found between both $NH_4^+$ assimilation and $N_2$ fixation rates and a suite of biotic (primary productivity, micro- and nanoplankton fractions) and abiotic factors (MLD and DIN concentrations a.o.; Figure 10). No significant correlations were found between $N_2$ fixation and DIN assimilation rates. $NO_3^-$ assimilation rates and primary productivity showed significant and positive correlations with one another and with $NO_2^-$ concentrations and the nanoplankton fraction, with primary productivity also being significantly and positively correlated with the dissolved oxygen concentrations and total chl $a$ (Figure 10).

Boosted regression Tree model outputs revealed that latitude, silicate and total chl $a$ were the main predictor variables for $N_2$ fixation rates ($cv = 0.744 \pm 0.06$; Supplementary Table 3), whereas total chl $a$, $NO_2^-$, and the nanoplankton fraction were the main predictors for $NO_3^-$ assimilation rates ($cv = 0.694 \pm 0.075$; Supplementary Table 3). Latitude, dissolved oxygen concentration and the ratio of picoplankton were the
main predictors for NH$_4^+$ assimilation rates (cv = 0.797 ± 0.036; Supplementary Table 3), whereas nanoplanckton and total chl $a$ were strong predictors for primary productivity along the transect (cv = 0.854 ± 0.041; Supplementary Table 3).

**DISCUSSION**

Our data provide insights into the spatial extent of N$_2$ fixation activity and diazotrophic community composition where few to no measurements are currently available, and expand the data set from Shiozaki et al. (2017) southward along the 170°W line into the southern Pacific Ocean. Additionally, we identified nitrification activity within a region for which sparse rate measurements have been recorded and presented the relative abundances of putative nitrifiers. Lastly, we demonstrated that both N$_2$ fixation and nitrification are active N transformation processes for which sharp gradients can be observed across oceanographic features in the South Pacific.
A Proxy for N-Cycling Pathways Using the $\delta^{15}$N of PON

The $\delta^{15}$N of PON ($\delta$PO$_{15}$N) can be used as a tracer for the sources of N and for dominant N-cycling pathways (Phillips and Gregg, 2003). We suggest that the relatively low $\delta$PO$_{15}$N levels recorded in the sub-Antarctic waters are driven by low NO$_3^-$ utilization by phytoplankton due to iron and light co-limitation at high latitudes (Boyd et al., 2000), the kinetically favorable uptake of light NO$_3^-$ and light N compounds (Sigman et al., 1999) and the recycling of organic matter (i.e., an input of low $\delta^{15}$N via NH$_4^+$, as indicated by the blue rectangle in Figure 9A). The high PON:$\Sigma$ pigment ratio in the sub-Antarctic suggests that this is a heterotrophic system in the early winter months, as seen in other systems with a high PON:$\Sigma$ pigment ratio (Harmelin-Vivien et al., 2008). Furthermore, we suggest that the increase of $\delta$PO$_{15}$N to $\sim$6‰ near the STF is driven by classic across-pycnocline mixing and by the uptake of NO$_3^-$ (as indicated in Figure 7A and by the red rectangle in Figure 9A; Liu and Kaplan (1989). The decrease in the PON:$\Sigma$ pigment ratio observed in the STF also suggests a shift toward a more autotrophic system in this area, and supports the notion of NO$_3^-$-driven productivity. In addition, we measured an increase in the nanoplanckton biomass at the STF, which showed a strong and significant correlation with primary productivity ($p < 0.05$). The BRT model outputs support these suggestions and showed that the nanoplanckton fraction alone could explain up to 62% of the primary productivity rates. We suggest that the decrease in the $\delta$PO$_{15}$N, north of the STF, can be explained by an increase in N$_2$ fixation rates [Figures 3, 9; indicated by the green rectangle; Montoya et al. (2004) and Loick et al. (2007)]. The low $\delta$PO$_{15}$N in the SPSG ($\sim$30–25°S) can also be attributed to other processes such as nitrification, food chains supported mostly by a microbial loop and viral lysis which all release low $\delta^{15}$N compounds which will later be incorporated by organisms.

**FIGURE 9** (A) Natural abundance $\delta^{15}$N from the particulate organic matter (PON) along the P15SS GO-SHIP transect in the South Pacific Ocean. Colored rectangles suggest potential biochemical pathways which may drive the variation in the $\delta^{15}$N of the POM (see a proxy for N-cycling pathways using the $\delta^{15}$N of PON in the “Discussion”). The black arrow highlights the likely role how isotopic fractionation during NO$_3^-$ assimilation generated the high $\delta^{15}$N feature at the STF. (B) Sum of all extracted pigments (µg L$^{-1}$) along the latitudinal P15S transect. (C) PON:$\Sigma$ pigment ratio along the P15SS GO-SHIP transect and (D) POC:PON ratios with green line showing the expected Redfield ratio of 6.6. Red lines are negative exponential smoothing curves with a first polynomial order. Thin black lines are fourth order polynomials and thin blue lines 95% confident intervals. Gray rectangles denote oceanographic fronts (PF, Polar front; STF, Subtropical Front; Eq, Equatorial upwelling zone).
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FIGURE 10 | Spearman correlation coefficients (r_s) plot including NO3− and NH4+ assimilation rates, primary productivity, and N2 fixation rates in the surface waters of the South Pacific Ocean (n = 85). Correlations with an adjusted p-value > 0.05 are indicated by black crosses. Mixed layer depth (MLD), Total chlorophyll a (TChl.a). Positive correlations are displayed in blue and negative correlations in red. Color intensity and the size of the squares are proportional to the correlation coefficients. Primary productivity values used in the correlation plot are averages from three replicates.

(Holmes et al., 2002). The increase in the δPO15N between 25° and 5°S (as indicated by the yellow rectangle in Figure 9A) may be explained by a longer picoplankton-based food web (Wada and Hattori, 1976), as a greater number of intermediate trophic levels results in more fractionation steps and, thereby, lead to an increase in δPO15N (refer to Raes et al. (2014) for this pattern in other oligotrophic systems). In addition, we note that the increasing trend in the δPO15N coincided with a shallowing of the NOx-cline from about ~200–250 m at 20°S to ~100 m at 10°S (Figure 2D). These data could suggest that an increase in NOx-driven productivity with a higher δ15N signature relative to N2 fixation toward the equator could also have contributed to an increase in δPO15N between 25° and 5°S. Model results from Somes et al. (2013) also predicted higher δPO15N values around 20°S in this region, which were suggested to occur due to lateral advection of high δ15N originating from the eastern tropical South Pacific (ETSP) oxygen deficient zone. We point out that the shallowing of the NOx-cline at approximately 10°S at 100 m depth coincided with a NO2− maximum. We speculate that the South Pacific gyre acts as a conveyor belt (anti-clockwise) for organic particles. The wind driven South Equatorial Current (SEC) transports thermocline waters westward across the entire South Pacific Ocean. The Southwest Pacific Ocean circulation is complex, but it is clear that the SEC when reaching the western Pacific separates into numerous jets and boundary currents that redistribute the waters from the northern branch of the South Pacific subtropical gyre to the equator and high latitudes (Ganachaud et al., 2014). We hypothesize that between 12° and 2°S and at approximately 100 m depth (density ~23–24 kg.m-3) particles accumulate in the SEC and are advected westward with the current [see also Somes et al. (2013)]. In the western Pacific the organic particles are then redistributed to the equator where they are subsequently remineralized via ammonification and nitrification, leaving a signal of high NO2−. Finally, equatorial currents and counter currents, which induce shear-driven mixing and, thus, introduce nutrients to the photic zone (Talley, 2011), resulted in an injection of NO3− across the MLD in our study (Figures 2C–F), and is likely the reason why we observed an increase in the abundance of the microplankton at the equator (Figures 2, 8, 9B; Holmes et al., 2002).

C and N Decoupling

Throughout the transect, NH4+ assimilation rates were greater than C assimilation rates, a pattern which contradicts general stoichiometric predictions based on a Redfield ratio of 106 (C) to 16 (N) (Dugdale and Goering, 1967). The lower C
assimilation rates measured in the Southern Ocean can be explained by the time of the year when this study took place – the transect was studied in early winter, during the darker winter months, when primary production is greatly reduced. Manganelli et al. (2009) noted that during the winter months, when light availability is greatly reduced, Bacteria and Archaea are the major producers of biogenic particles via heterotrophic production and chemosynthetic CO₂ fixation in the Southern Ocean. From these results we then suggest that the higher NH₄⁺ assimilation rates could possibly be explained by Bacteria and Archaea preferentially taking up organic C as a substrate during the incubations rather than the inorganic ¹³C which was supplied. Resulting in overall lower inorganic C assimilation rates compared to NH₄⁺ assimilation rates.

Additional evidence for the decoupling of C and N uptake rates and for the low autotrophic productivity rates measured in the Southern Ocean is a lower Rayleigh fractionation than that previously published for the region in summer. The relationship between NO₃⁻ assimilation and the δ¹⁵N of NO₃⁻ has been shown in several studies to conform to Rayleigh fractionation kinetics (see Sigman et al. (1999) and references within). If the conditions of Rayleigh fractionation are met, upper ocean NO₃⁻ samples should fall along a straight line that describes the δ¹⁵NO₃⁻/ln ([NO₃⁻]) relationship. The slope of this line (ε) provides an estimate of the isotope effect of NO₃⁻ uptake. Sigman et al. (1999) showed that, in the Southern Ocean, ε ranged between 4 and 6‰. When we calculated ε for our samples, we obtained values of 3.61‰ for the Southern Ocean and 2.7‰ within the STF (Supplementary Figure 10). The lower ε values (when compared to those from Sigman et al. (1999)) found in this study are suggested to be due to lower autotrophic productivity in early winter. It is worth noting that Montoya and McCarthy (1995) and Needoba and Harrison (2004) reported that the fractionation factor for NO₃⁻ uptake can vary across taxa, with generally lower ε values for flagellates than for diatoms. This suggests that the differences in the ε values from our data in comparison to those published by Sigman et al. (1999) could, in addition to the seasonality, be due to phytoplankton community composition. On the other hand, we note that our data could also infer that the low Rayleigh fractionation kinetics could be due to relatively high heterotrophic production relative to total production in early winter, and not necessarily due to low total autotrophic production and/or different phytoplankton community composition. There remains a lot of uncertainty how fraction occurs in many of these N-cycling processes hence at this stage we can only speculate.

In the tropics NH₄⁺ concentrations were below the detection limit. The addition of the ¹⁵N tracer therefore ended up being an appreciable fraction of the ambient NH₄⁺ concentrations which meant that potential assimilation rates were measured. These potential assimilation rates could then give rise to the mismatch between NH₄⁺ assimilation and primary productivity rates. The higher NH₄⁺ assimilation rates can possibly be explained by an active microbial loop in the region, which would result in a high turnover rate of NH₄⁺. This explanation is in agreement with the findings from Clark et al. (2008), who showed that low primary production rates can be associated with rapid N cycling activity in the oligotrophic regions of the central Atlantic. These authors showed that nitrification (with NH₄⁺ as the end product) could completely replace the DIN pool within 1 day, with reported turnover rates as low as 5 h.

**N₂ Fixation in the South Pacific Ocean**

Several theories about N₂ fixation in the ocean have been proposed and disproven in the last decade. This process was postulated to occur primarily in sub-tropical regions with relatively low DIN concentrations [<1 µmol L⁻¹, Breithbarth et al. (2007)], but a wide range of diazotrophic bacterial nifH sequences have been obtained from polar regions (Díez et al., 2012), including melt ponds and sea ice (Fernández-Méndez et al., 2016). Recent studies have also reported cyanobacterial N₂ fixation (via a UCYN-A and a haptophyte symbiosis) in the Western Arctic and Bering Seas, concluding that N₂ fixation is not constrained to sub-tropical waters (Shiozaki et al., 2017; Sipler et al., 2017; Harding et al., 2018). The assimilation of N₂ has a high energetic cost due to the breaking of triple N bonds. It was postulated that N₂ fixation rates would decrease when other N-compounds, which are more easily assimilated in terms of energetic costs, would be available (Falkowski, 1997). A number of studies have, however, quantified N₂ fixation rates in nutrient rich environments such as coastal upwelling regions (Sohm et al., 2011) and temperate coastal zones (Bentzon-Tilia et al., 2014). Weber and Deutsch (2014) also showed that on an ocean basin scale, iron (Fe) limitation exerts a stronger control on N₂ fixation rates than DIN concentrations. We detected low N₂ fixation rates at 7/10 stations south of 52°S (see Supplementary Table 1) and our results (high NO₃⁻, NO₂⁻, and NH₄⁺ concentrations and high NO₃⁻:PO₄³⁻ ratios in the sub-Antarctic) support the notion that N₂ fixation does not occur exclusively in warm and oligotrophic regions. Although our N₂ fixation rates are a small fraction of the total N assimilated in the Southern Ocean (<1%) when compared to the total NO₃⁻ and NH₄⁺ assimilation rates, they do highlight the broad latitudinal scale of N₂ fixation. This is the case especially when our data are considered together with that of Shiozaki et al. (2017) to reveal the bipolar scale (from 59°S to 68°N) of N₂ fixation processes along the 170°W meridional.

In this data set we present the first N₂ fixation rate measurements in the Pacific sector of the Southern Ocean, south of 50°S. In the Southern Ocean of the Indian Ocean sector González et al. (2014) measured N₂ fixation rates up to 20.1 nmol L⁻¹ d⁻¹ at low temperatures (<4°C) and high NO₃⁻ concentrations (>30 µmol L⁻¹) near the Kerguelen Islands. Although these N₂ fixation rate data are collected from a region high in dissolved Fe, the authors mentioned that the role of Fe in the N₂ fixation processes was indirect. Fe directly stimulated primary production and indirectly, through the release of dissolved organic matter, the N₂ fixation rates. The ubiquitous presence of N₂ fixation in the surface oceans, further complicates the potential to model N₂ fixation rates on a basin scale.

In our data set we note that although the predictive strength of the BRT model for N₂ fixation was strong (cv value of 0.74), its outputs showed no clear predictor variables for N₂ fixation. The BRT relationships were strongly dominated by indirect (e.g.,
non-associated) parameters such as latitude, Si, and total chl a concentration, which explained 18, 13.7, and 11.9% of the changes in N₂ fixation, respectively. Latitude itself is a proxy for a suite of physical and biogeochemical parameters (e.g., day length, temperature, nutrient and dissolved oxygen concentrations, a.o.), which highlights the complexities associated with modeling N₂ fixation rates based on biogeochemical parameters. Silicate has no known influence on the process of N₂ fixation and is present in low concentrations in environments which are N-limited and, thus, prone to N₂ fixation. Similarly, total chl a is available in high concentrations in areas which are nutrient-rich and, thus, not as prone to N₂ fixation. Although regionally high chl a concentrations have been related to high N₂ fixation rates and *Trichodesmium* abundance around New Caledonia (Shiozaki et al., 2014), chl a and Si are most likely a correlation artifact rather than true predictors of N₂ fixation across large scale ocean boundaries. We believe that our results will prove extremely useful to modellers working on N₂ fixation in the South Pacific Ocean, as in this study we reported and described clear changes in N₂ fixation rates across oceanographic boundaries.

We observed an increase in N₂ fixation rates north of the STF, when NO₃⁻ concentrations fell to <1 µmol L⁻¹. Overall, N₂ fixation rates averaged ~15 nmol L⁻¹ d⁻¹ in the SPSG, but high values (up to 130 nmol L⁻¹ d⁻¹) were also recorded. N₂ fixation rates measured in this study north of 25°S are in agreement with those presented by Bonnet et al. (2017) for the western tropical South Pacific and the equatorial Pacific, and by Caffin et al. (2018) for a longitudinal transect between 18 and 19°S along the 160°–170°W meridian line. A number of studies have, however, measured significantly lower N₂ fixation rates in similar oceanographic conditions. Shiozaki et al. (2017), for example, reported N₂ fixation rates of up to 0.360 nmol L⁻¹ d⁻¹ in the North Pacific Equatorial Counter Current Province and of up to 3.05 nmol L⁻¹ d⁻¹ in the North Pacific Tropical Gyre Province. Halim et al. (2012) and Law et al. (2012) measured N₂ fixation rates in the southwest Pacific (between 20°–45°S and 150°E–150°W and 170°W–120°W) which reached 6 nmol L⁻¹ d⁻¹ in the austal summer but bordered the detection limit (<1 nmol L⁻¹ d⁻¹) during austral winter. Bonnet et al. (2009) recorded N₂ fixation rates along the equator (from 145°W to 160°W) which ranged from <1 nmol L⁻¹ d⁻¹ to 610 nmol L⁻¹ d⁻¹. This extreme range of values highlights how variable N₂ fixation can be along the tropical and equatorial Pacific.

The diazotrophic community is responsible for a significant fraction of the primary productivity and C export in oligotrophic oceans (Karl et al., 1997). We derived potential C assimilation rates from our N₂ fixation rates by multiplying the latter by the *in situ* molar POC:PON values (rather than the average Redfield 6.6). Within our study area we estimated that N₂ fixation might have supported the assimilation of up to 2.5 nmol C L⁻¹ d⁻¹ in the sub-Antarctic, and up to 85 nmol C L⁻¹ d⁻¹ north of the STF. These derivations imply that N₂ fixation is responsible for at least one third of the C that is assimilated in the SPSG. The ratio of N₂ fixation rates: C fixation sharply increased north of the STF, N₂ was assimilated in excess to C fixation at the stations where we measured our highest N₂ fixation rates (Supplementary Figure 6). Overall, these results reinforce that N₂ fixation contributes largely to oceanic new production in oligotrophic regions.

The majority of the *nifH* sequences in surface waters along our transect in the South Pacific belonged to Cluster 1, which covers aerobic and facultative anaerobic organisms that belong to the Proteobacteria (including Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria), Cyanobacteria, Firmicutes and Actinobacteria (Zehr and McReynolds, 1989). Changes in the relative abundances of *Trichodesmium* and UCYN-A along the transect showed strong evidence that these organisms have different biogeographies, with UCYN-A dominant at mid-latitudes and *Trichodesmium* more prominent at lower latitudes (Figure 5). The presence of *Trichodesmium* DNA well below 30° S, does not, however, mean that *Trichodesmium* is actively fixing N₂. *Trichodesmium* has also been detected in subarctic regions of the Atlantic Ocean (Lipschultz and Owens, 1996), likely a reflection of its northward transport by the Gulf stream and the application of methodological improvements rather than an expanding range. The relative increase in Gammaproteobacterial *nifH* sequences near the STF and PED (Figure 4), zones in which we noted an increase in primary productivity, was an interesting finding. The ubiquitous distribution of heterotrophic diazotrophs, such as alpha- and Gammaproteobacteria, could suggest that their contribution to the input of new N via N₂ fixation is important on a basin-wide scale rather than just in the tropics. Turk-Kubo et al. (2014), on the other hand, concluded that Gammaproteobacteria did not significantly contribute to N₂ fixation rates in the eastern South Pacific. The ecological significance of the heterotrophic diazotroph groups thus remains elusive, and merits further investigation (Benavides et al., 2018). Recent research, reviewed by Zehr et al. (2017) has identified an association between the haptophyte *Braarudosphaera* and the endosymbiont Candidatus Atelocyanobacterium. Our data from the SPSG support the host-endosymbiont connection, as the highest number of Candidatus Atelocyanobacterium sequences were recorded altogether with *Braarudosphaera* sequences (Supplementary Figure 8). Shiozaki et al. (2017), who sampled the 170° W meridian in the Northern hemisphere, concluded that, although they found clear differences in the diazotrophic composition between the tropical gyre and the cold northern region, the local biogeochemical constraints on the diazotrophic community required further detailed studies. The distribution of diazotrophs along our transect was influenced by multiple factors including temperature, nutrient concentrations, and frontal zones; but we agree with Shiozaki et al. (2017) in that the local controls that drive differences in diazotrophic community composition within water masses remain elusive.

**Nitrification in the Pacific Sector of the Southern Ocean**

Nitrification can provide a source of NO₃⁻, yet rates are poorly resolved globally and often ignored as a source of regenerated N in the photic zone because the process is assumed to be inhibited by light (Peng et al., 2016). Culture studies and *in situ* rate measurements, however, demonstrated that photoinhibition of
nitrifying organisms is only partial (Qin et al., 2014). The average rates we found in the Southern Ocean (480 nmol L$^{-1}$ d$^{-1}$ and a production of $\sim$0.5 $\mu$mol NO$_3^-$ L$^{-1}$ d$^{-1}$) were higher than those measured around the same time of the year in the Indian sector of the Southern Ocean, where cumulative rates averaged between 100 and 200 $\mu$mol m$^{-2}$ d$^{-1}$ [integrated over 100 m nitrification rates between 10 and 20 nmol L$^{-1}$ d$^{-1}$ with a production of $\sim$0.01-0.2 $\mu$mol NO$_3^-$ L$^{-1}$ d$^{-1}$; (Bianchi et al., 1997)].

Overall, nitrification appears to be a very active bacterial process in the oceanic N cycle (Dore and Karl, 1996; Yool et al., 2007; Clark et al., 2008) and in the Pacific sector of the Southern Ocean in winter as seen in this data set. Our total nitrification rates could be an underestimate because the rates do not account for $^{15}$NO$_2^-$ or $^{15}$NO$_3^-$ that may have been assimilated by microbes. However, one could argue that this would be minimal given that there was a large pool of NH$_4^+$ available [which microbes should prefer as an N source (Peng et al., 2015, 2016)]. Ambient NH$_4^+$ concentrations in the water column ranged between 0.2 and 0.8 $\mu$mol L$^{-1}$. As a result, the labeled $^{15}$NH$_4^+$ additions in the nitrification experiments ranged between 6 and 25% of the total NH$_4^+$ pool. Trace additions above 10% are likely to stimulate uptake, biasing rate measurements. NH$_4^+$ regeneration on the other hand would dilute the $^{15}$N-labeled NH$_4^+$ pool, and lead to underestimation of total nitrification rates. Additional rate measurements are needed to set our high rates in a temporal context, as Ward (2005) already showed that temporal differences could be as large as 40 fold for nitrification rates. Our data suggests that the early winter months favor a heterotrophic system in the sub-Antarctic region. We speculate that the organic matter, produced in the autumn months is recycled and that the Archaea and Bacteria subsequently nitriﬁe the available NH$_4^+$.  

CONCLUSION

Our study region was a natural laboratory for resolving how hydrographic and biogeochemical boundaries influence the relative abundance and richness of microorganisms involved in the different pathways within the marine N cycle. We report herein that, as we moved along the transect and across major oceanographic boundaries, changes were observed for N$_2$ fixation and nitrification rates, microbial community composition, $\delta$PO$_{15}$N, photosynthetic pigment concentration, and carbon assimilation rates. We were able to relate our $\delta$PO$_{15}$N measurements to major N-cycling pathways across ocean boundaries, but further studies need to investigate how the isotopic fractionation of the $\delta$PO$_{15}$N and the $\delta^{15}$N of NO$_3^-$ can provide insights into the relative magnitudes of these different N-cycling processes.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the PANGEA repository which are presented in the section “Materials and Methods.” All physical and chemical data are available at the Clivar & Carbon Hydrographic Data Office (CCHDO; https://cchdo.ucsd.edu/under GO-SHIP program; voyage P15S; expocode: 096U20160426). Genomic data are available under BioProject: PRJNA385736, https://www.ncbi.nlm.nih.gov/bioproject/385736.

AUTHOR CONTRIBUTIONS

ER developed the experimental design, executed the experiment, analyzed the data, and wrote the manuscript. JK, BH, and LB analyzed the nifH, bacterial, and archaeal sequence data. S-SW analyzed the HPLC data. JR, BE, and DE analyzed and QCed the nitrification rates. AF and AW contributed to the data analysis and interpretation. All co-authors helped with the editing of 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.2020.00389/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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