Revisiting the distribution of oceanic N₂ fixation and estimating diazotrophic contribution to marine production

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Marine N₂ fixation supports a significant portion of oceanic primary production by making N₂ bioavailable to planktonic communities, in the process influencing atmosphere-ocean carbon fluxes and our global climate. However, the geographical distribution and controlling factors of marine N₂ fixation remain elusive largely due to sparse observations. Here we present unprecedented high-resolution underway N₂ fixation estimates across over 6000 kilometers of the western North Atlantic. Unexpectedly, we find increasing N₂ fixation rates from the oligotrophic Sargasso Sea to North America coastal waters, driven primarily by cyanobacterial diazotrophs. N₂ fixation is best correlated to phosphorus availability and chlorophyll-a concentration. Globally, intense N₂ fixation activity in the coastal oceans is validated by a meta-analysis of published observations and we estimate the annual coastal N₂ fixation flux to be 16.7 Tg N. This study broadens the biogeography of N₂ fixation, highlights the interplay of regulating factors, and reveals thriving diazotrophic communities in coastal waters with potential significance to the global nitrogen and carbon cycles.
Approximately half of global primary production occurs in the oceans. In the vast expanse of the oligotrophic oceans, marine primary production is limited by nitrogen. In these regions, nitrogen (N\(_2\)) fixation by diazotrophs has been hypothesized to be an important source of new nitrogen ultimately influencing the uptake and sequestration of CO\(_2\). For instance, in the North Atlantic subtropical gyre, a significant seasonal carbon drawdown in the absence of measurable nutrients has been attributed to episodic and patchy N\(_2\) fixation events. These events are, however, difficult to capture using current methods, which rely on discrete sampling. Furthermore, most observations to date have been collected in tropical and subtropical open oceans, overlooking the potential role of coastal regions. The limited number of observations impede our ability to close regional marine nitrogen budgets, scale estimates globally, and identify factors controlling N\(_2\) fixation.

Marine N\(_2\) fixation is generally believed to be regulated by various factors including light, temperature, nutrients, and trace-metal availability. While low light, low temperature, and high biologically available nitrogen have traditionally been assumed to limit N\(_2\) fixation, recent studies have reported significant diazotrophic activities in darker, colder, and more nitrogen-rich environments, thereby broadening the putative biogeography of marine N\(_2\) fixation. The discovery of these new niches has been accompanied by increasing appreciation for the large diversity of species fixing N\(_2\), ranging from the well-known Trichodesmium and diatom-diazotroph associations (DDA) to more recently recognized unicellular cyanobacteria and non-cyanobacterial diazotrophs.

Taken together, the large uncertainty in regional and global budgets of marine N\(_2\) fixation, associated with its patchy and recently broadened biogeography, demands new tools to adequately map this important biogeochemical process. We recently deployed a new method across more than 6000 km of the North Atlantic to revisit the geographical distribution and assess the controlling factors of N\(_2\) fixation. Our near-real-time, continuous high-resolution measurements allowed us to locate hotspots of N\(_2\) fixation and adapt our sampling strategy to characterize planktonic communities and environmental properties. The contribution of N\(_2\) fixation to net community production (NCP) was simultaneously evaluated at high resolution. Our new observations reveal hotspots of marine N\(_2\) fixation along the Eastern Seaboard, and highlight the overlooked significance of N\(_2\) fixation to coastal and global nitrogen and carbon cycling.

Results and Discussion

N\(_2\) fixation distribution and controlling factors. Our survey revealed substantial variability in diel cycling behavior in surface N\(_2\) fixation rates, which ranged from less than 0.01 to nearly 15 nmol N L\(^{-1}\) h\(^{-1}\) (Fig. 1a and Supplementary Fig. 1). When integrated over 24 h, N\(_2\) fixation diel cycles, continuous estimates of daily surface N\(_2\) fixation rates (\(\leq 0.19 - 97.6\) mmol N L\(^{-1}\) d\(^{-1}\)), were in line with discrete N\(_2\) fixation rates concurrently determined by \(^{15}\)N\(_2\) incubations (\(n = 7, r = 0.97, p < 0.01\), Fig. 1b and Methods). To extrapolate surface measurements to the entire euphotic zone, we derived an empirical relationship between surface and depth-integrated N\(_2\) fixation rates (Supplementary Fig. 2). Our lower-end measurements of less than 10 mmol N m\(^{-2}\) d\(^{-1}\) are within the range of published rates near Bermuda. In contrast, high N\(_2\) fixation rates reaching 3000 mmol N m\(^{-2}\) d\(^{-1}\) near the New Jersey coast are among the top 2% of rates ever reported in the global ocean, further underscoring the high N\(_2\) fixation along the continental shelf of the eastern seaboard, and the value of high-frequency observations for identifying hotspots. The coastal region (bathymetry \(\geq -200\) m) stands in sharp contrast to open ocean areas, with depth-integrated N\(_2\) fixation rates in the coastal sectors (geometric mean of 577 mmol N m\(^{-2}\) d\(^{-1}\)) being on average an order of magnitude larger than open ocean rates (geometric mean of 85 mmol N m\(^{-2}\) d\(^{-1}\)).

Sea surface temperature did not appear to drive the spatial variability of N\(_2\) fixation in our study area (Supplementary Fig. 3 and Supplementary Fig. 4). In open ocean regions, N\(_2\) fixation rates varied substantially even when the temperature range was narrow. Moreover, high N\(_2\) fixation rates were observed at temperatures ranging from 23 °C off New Jersey to 30 °C near the Florida coast. Fixed nitrogen is known to suppress N\(_2\) fixation, but the threshold of inhibition differs among diazotrophs and can be fairly high. Observed dissolved inorganic nitrogen did not effectively regulate N\(_2\) fixation rates (Supplementary Fig. 4). We also note that a recent study shows that these coastal waters may be nitrogen limited in summer. In addition, the excess of nitrogen in subsurface waters (N\(^*\) = [NO\(_3\)] - [PO\(_4\)] \times 16), commonly used as a geochemical proxy for the distribution of N\(_2\) fixation, was not a strong predictor of overlying N\(_2\) fixation rates (Fig. 1c and Supplementary Fig. 4). This comparison should be interpreted with caution, as N\(^*\) integrates over longer spatial and temporal scales than our observations. N\(^*\) is also not well resolved in coastal waters and may be affected by other processes such as atmospheric nitrogen deposition.

In contrast, some of the N\(_2\) fixation hotspots coincided with high phosphorus concentration and excess phosphorus (P\(^*\) = [PO\(_4\)] - [NO\(_3\)]/16) at the ocean surface (Fig. 1b and Supplementary Fig. 4). Regions of N\(_2\) fixation have been hypothesized to be coupled to areas of denitrification via the upwelling of waters deficient in nitrogen relative to phosphorus. The phytoplankton bloom near the New Jersey coast, where the highest N\(_2\) fixation rates were observed, recurs almost every summer and may be associated with local upwelling. High N\(_2\) fixation rates have also been reported in other upwelling systems worldwide, including the equatorial Atlantic Ocean, the northwest African coastal upwelling, and the Benguela Upwelling System. The excess phosphorus may also result from terrestrial and/or riverine runoff. For example, N\(_2\) fixation and carbon sequestration in the tropical North Atlantic were shown to be enhanced by the Amazon River plume.

A recent study in the Eastern South Pacific suggested Fe, rather than phosphorus, may limit N\(_2\) fixation, questioning the spatial coupling between N\(_2\) fixation and denitrification. However, we did not find a strong relationship between dissolved Fe and N\(_2\) fixation rates across our study (Supplementary Fig. 4). Dissolved Fe ranged from 0.5 mmol L\(^{-1}\) near Bermuda to around 1.8 mmol L\(^{-1}\) along the Florida coast, which is higher than Fe measured in the Eastern South Pacific. Fe concentration is admittedly a poor predictor of Fe availability, as concentrations merely reflect snapshots of the complex interactions between sources and sinks. Consequently, we calculated Fe\(^*\) (Fe\(^*\) = Fe - R\(_{sp, Fe}\)PO\(_4\); where R\(_{sp, Fe}\) = 0.47 mmol Fe : 1 mol PO\(_4\)) to evaluate whether Fe potentially limits phytoplankton growth. Positive values of Fe\(^*\) across the study area indicate Fe was not limiting. The coupling between N\(_2\) fixation and N loss may be a dominant factor in regions where Fe is abundant, notably in coastal oceans. We hypothesize that, in contrast to the Eastern South Pacific, North American coastal waters support substantial N\(_2\) fixation due to high sedimentary nitrogen loss and high Fe input (e.g., from sediment and atmospheric dust deposition) (Supplementary Fig. 5). Interestingly, N\(_2\) fixation rates correlated well with dissolved manganese (Mn) concentrations. While the correlation of N\(_2\) fixation to Mn could be coincidental or symptomatic of other factors, it deserves further investigation as the physiological requirement for Mn in marine diazotrophs is poorly characterized.
Our N\textsubscript{2} fixation measurements strongly correlate to satellite estimates of chlorophyll-a concentrations ([Chl]) (Fig. 1a and Supplementary Fig. 4). This is unexpected as N\textsubscript{2} fixation is generally believed to be most significant where nitrogen is limited, such as the low biomass regions of the subtropical gyres. The low-[Chl] waters of the Sargasso Sea, typically viewed as N\textsubscript{2} fixation hotspots\textsuperscript{39}, exhibited lower N\textsubscript{2} fixation rates than those measured in the Mid-Atlantic Bight. This pattern was further supported by a meta-analysis, which showed that N\textsubscript{2} fixation is correlated to [Chl] in the global ocean (Supplementary Fig. 6). This pattern may be related to the stimulation of non-autotrophic N\textsubscript{2} fixation by organic matter in high [Chl] waters\textsuperscript{40}. However, while our field observations identify phosphorus, \( P^* \), and [Chl] as predictors of spatial variations in N\textsubscript{2} fixation in our study area, a meta-analysis of published results shows that none of the putative regulating factors of N\textsubscript{2} fixation can satisfactorily explain variations in volumetric rates globally (Supplementary Fig. 6). We posit that N\textsubscript{2} fixation is likely driven by a complex interplay of spatially-variable environmental factors, also reflecting the heterogeneity and the large diversity of marine diazotrophs and their niches.

**Fig. 1** N\textsubscript{2} fixation rates (NF) measured and calculated in August 2015 and 2016 over the western North Atlantic Ocean. a In situ hourly surface volumetric N\textsubscript{2} fixation rates determined by the continuous underwater incubation method, overlaid on chlorophyll-a concentrations measured by the MODIS satellite during the respective cruise periods (Aug 3–12 in both 2015 and 2016). Note that chlorophyll-a concentrations are shown on a logarithmic scale. b Calculated daily surface volumetric N\textsubscript{2} fixation rates overlaid on the August climatology of surface excess phosphate \( P^* \) (\( P^* = [PO_4^{3-}] - [NO_3^-]/16 \)). Nutrient data were obtained from the World Ocean Atlas 2013 version 2. Red vertical bars represent N\textsubscript{2} fixation rates determined by the discrete dissolved \(^{15}\text{N}\text{2}\) incubation method during the 2015 cruise. c Estimated depth-integrated N\textsubscript{2} fixation rates overlaid on the August climatology of subsurface excess nitrogen \( N^* \) (\( N^* = [NO_3^-] - [PO_4^{3-}] \times 16 \)).

**Distribution of diazotrophic phylotypes.** Diazotrophs and their potential hosts were identified via high-throughput quantitative 16S rRNA and 18S rRNA gene sequencing from our 2015 cruise (Methods). Although the 16S rRNA gene approach differs from the \( nifH \) method for characterizing diazotrophs in terms of specificity and coverage\textsuperscript{41}, it provides some insights into the broad distribution of diazotrophic communities. To address the cases where organisms may not be capable of N\textsubscript{2} fixation despite sharing a similar 16S rRNA gene with diazotrophs, we only searched for diazotrophs known to fix N\textsubscript{2} among our 16S rRNA gene sequences. Distinct diazotrophic communities were found to dominate in different ecological domains (Fig. 2a). Heterotrophic groups, which include...
members known to be diazotrophs, were more abundant than diazotrophic cyanobacteria in the open ocean, where \( \text{N}_2 \) fixation was relatively low. These observations are consistent with recent recognition of the widespread distribution of non-cyanobacterial diazotrophs, whose activities remain poorly constrained.

\( \text{Trichodesmium} \) peaked off South Carolina. \( \text{Richelia intracellularis} \) showed relatively high abundances in the oligotrophic open ocean and peaked in coastal waters, where its hosts—\( \text{Rhizosolenia} \) and \( \text{Hemiaulus} \)—were also found at relatively high abundances (Fig. 2b). The most striking \( \text{N}_2 \) fixation hotspot off the New Jersey coast was likely driven by a UCYN-A bloom that reached \( 2 \times 10^7 \) 16S rRNA gene copies L\(^{-1} \), which is of comparable magnitude to UCYN-A abundances (2.5–3.5 \times 10^7 L\(^{-1} \)) observed previously in the same region using the \( \text{nifH} \) method. One of UCYN-A’s hypothesized hosts, \( \text{Braarudosphaera bigelowii} \) also flourished in this region. Across all samples, the ratio of UCYN-A (16S rRNA gene):\( B. \) bigelowii (18S rRNA gene) varied from 0.1 to 35 with a median of 0.24. The two organisms co-occurred in most samples, consistent with previous studies that suggest obligate symbiosis. There is a growing interest in UCYN-A’s unusual physiological and ecological traits. Its genetic diversity, evolution, and the niches inhabited by its different lineages deserve further investigation. Meanwhile, its presence in the coastal oceans provides new opportunities to study this unique organism.

The divergent geographic distribution of different diazotrophs in our research area likely reflects their respective niches. For example, warm seawater is more favorable to \( \text{Trichodesmium} \), while UCYN-A prefers temperate environments. This difference may partly explain why \( \text{Trichodesmium} \) abundances peaked off the South Carolina coast (~30 °C) while UCYN-A dominated in regions with lower temperatures (~23 °C). Overall, the quantitative 16S rRNA and 18S rRNA gene sequencing methods revealed spatial patterns of diazotrophs and their hosts despite the assumptions of our quantitative sequencing methods, e.g., equivalent recovery efficiency for both the standard and the natural sequences in the sample (Methods).

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**Fig. 2** Absolute rRNA gene abundances of various diazotrophs and their potential hosts in surface seawater collected during the 2015 cruise. The inset map shows the locations where molecular samples were collected. The distributions of diazotrophs (a) and hosts (b) are compared with surface daily \( \text{N}_2 \) fixation rates (solid red line). Diazotrophs and hosts below detection limits at different sampling locations are not shown in the figure. “Others” in panel a includes diazotrophic proteobacteria belonging to \( \text{Bradyrhizobium} \), \( \text{Mesorhizobium} \), \( \text{Novosphingobium} \), and \( \text{Paenibacillus} \).

**Fig. 3** Distribution of Net Community Production (NCP) and contribution of \( \text{N}_2 \) fixation to NCP. a Color-coded NCP with negative values shown in grey color scale. b Distribution of the ratio of \( \text{N}_2 \) fixation-supported carbon production to NCP. Circle size in b represents the magnitude of NCP, contextualizing the large uncertainty in calculated ratios when the denominator (i.e., NCP) is small.
Variable contribution of N\textsubscript{2} fixation to new production. N\textsubscript{2} fixation has been estimated to be an important source of new nitrogen in oligotrophic waters, supporting as much as 50% of new production\textsuperscript{1,47}, yet the contribution of N\textsubscript{2} fixation to productivity in coastal regions remains relatively understudied\textsuperscript{48}. To assess the proportion of production fueled by N\textsubscript{2} fixation, we combined N\textsubscript{2} fixation observations with underway estimates of NCP based on high-frequency measurements of the dissolved O\textsubscript{2}/Ar ratio (Methods). NCP was mostly positive, with higher rates along the North American coast where high [Chl] and N\textsubscript{2} fixation rates were also observed (Fig. 3a). NCP was relatively low in the open ocean likely due to nutrient limitation. As a rough estimate, we converted N\textsubscript{2} fixation rates and NCP to their carbon equivalents using a theoretical C:N:O\textsubscript{2} stoichiometry of 106:16:138. Regional differences in stoichiometry would modify but not erase the large gradients of N\textsubscript{2} fixation and NCP observed over our cruise transects. We found that the contribution of N\textsubscript{2} fixation to NCP varied substantially over the western North Atlantic (Fig. 3b). Across large portions of the oligotrophic subtropical ocean, no more than 20% NCP was generally fueled by N\textsubscript{2} fixation (with some high excursions). Other mechanisms of nutrients supply, such as revised estimates of vertical nitrate flux\textsuperscript{49}, must therefore be invoked to support the NCP rates we observed and those that have been reported in the Sargasso Sea. In contrast, the ratio of N\textsubscript{2} fixation to NCP exceeded 50% in some regions off the Cape Hatteras and New Jersey coasts (Fig. 3b). The high contribution of N\textsubscript{2} fixation to primary production off the New Jersey coast is supported by dual-tracer \textsuperscript{15}N\textsubscript{2} and \textsuperscript{13}C incubations (Supplementary Fig. 7). Despite methodological differences between these techniques, both methods independently capture similar spatial patterns of contribution of N\textsubscript{2} fixation to biological carbon fixation (see NCP estimates in Methods). Our results highlight that N\textsubscript{2} fixation is not only high in coastal regions, but may also contribute significantly to marine production.

Updated N\textsubscript{2} fixation budget and global implications. To contextualize our findings, we performed a meta-analysis combining \textsuperscript{15}N\textsubscript{2} incubations collected during our cruises with discrete N\textsubscript{2} fixation measurements compiled from the literature, not including our underway continuous measurements (Supplementary Fig. 8). Our updated database contains over 80% more depth-integrated observations (1172 points in total) than the most up-to-date database currently available in the literature (630 points)\textsuperscript{21}. Less than 15% of observations reported in the literature were collected in coastal waters. However, these observations support our findings of high N\textsubscript{2} fixation rates in the neritic environment (Fig. 4), notably on the eastern American coast\textsuperscript{5,22}, eastern Arabian Sea\textsuperscript{50}, and estuaries of the Baltic Sea\textsuperscript{14}. The similar magnitude of depth-integrated N\textsubscript{2} fixation rates in coastal and open oceans leads to significantly higher volumetric N\textsubscript{2} fixation rates in the coastal oceans due to shallower integration depths (Fig. 4 and Methods). However, recent reports of N\textsubscript{2} fixation in the deep ocean\textsuperscript{12,13} may reverse the pattern of marginally higher depth-integrated N\textsubscript{2} fixation rates in coastal waters, if N\textsubscript{2} fixation is integrated to the aphotic zone of the open ocean.

In light of these new observations, we reassessed the budgets of marine N\textsubscript{2} fixation globally as well as separately for the neritic
and oceanic regions (Supplementary Fig. 9). Our updated geometric and arithmetic mean estimates of marine N2 fixation for the global ocean at 70.8 Tg N yr\(^{-1}\) and 196.1 Tg N yr\(^{-1}\), respectively, are slightly higher than other estimates\(^{21,51}\) (Table 1 and Supplementary Table 1). Earlier studies report coastal N2 fixation rates of 15 Tg N yr\(^{-1}\), with most activity associated with benthic diazotrophs\(^{32}\). Our updated analysis shows that a significant portion of N2 fixation is also occurring in the water column of coastal regions, contributing an additional 6.6 (geometric) or 16.7 (arithmetic) Tg N yr\(^{-1}\) to the global budget. This nitrogen input could support the equivalent of 95 Tg C yr\(^{-1}\) of primary production. These updated N2 fixation budgets have large uncertainties since they are sensitive to the extrapolation method to scale the sparse data to the global ocean. Recent studies have also identified methodological issues in historical observations, which could lead to under-\(^{52}\) or over-\(^{54}\) estimates. However, the number of observations resulting from the revised dissolved \(^{15}\)N\textsubscript{2} incubation method is currently too limited to derive robust global estimates. Nevertheless, after accounting for both aquatic and sedimentary N2 fixation, the coastal oceans may play a larger role in the nitrogen cycle than previously considered.

Using an underwater method recently developed in our lab for continuous high-resolution N2 fixation measurements, we mapped N2 fixation at unprecedented scales across the western North Atlantic, identifying hotspots of N2 fixation in the Mid-Atlantic Bight. Our study challenges the classic paradigm of N2 fixation distribution and further underscores the central role coastal regions play in the global cycling of nutrients and carbon\(^{38}\). With coastal regions being exposed to ever-increasing anthropogenic disturbances\(^{56}\), expansion of coastal monitoring efforts using high-resolution methods will be critical to evaluate ongoing biogeochemical changes and their global repercussions.

### Methods

**Underway N2 fixation rate measurements.** N2 fixation rates were estimated at high resolution using a continuous underway method of flow-through incubations for Acetylene-Reduction Assays by Cavity ring-down laser Absorption Spectroscopy (FARACAS). A description of the method is presented in Cassar et al. (2018)\(^{19}\) with a brief outline below. Nitrogenase activity in seawater is estimated based on the conventional technique of acetylene (C2H2) reduction to ethylene (C2H4)\(^{37-39}\). Instead of injecting C2H2 gas directly into the incubation bottle, C2H2 gas produced from high-purity calcium carbide (Acros Organics) is first dissolved in 0.2-μm filtered seawater that is collected from a trace-metal clean towsiphon (GeoFish) to make a C2H2–H2O tracer. The dissolved C2H2 approach was previously applied for measuring nitrogenase activity in estuarine sediments\(^{60}\). The C2H2–H2O tracer is then mixed at a constant ratio using a two-channel peristaltic pump (Masterflex) with a continuous stream of seawater supplied by the GeoFish. The mixture of C2H2–H2O tracer and seawater is continuously pumped into a 9-L flow-through incubation reactor (Chemglass) at a flow rate of ~100 mL min\(^{-1}\). The short flow-through incubation, with an e-folding residence time of 90 min (i.e. ~63%) of the seawater in the incubation reactor replaced in 90 min), minimizes the effects of C2H2 on metabolic processes and on microbial community structure\(^{61,62}\). The incubation reactor is lit by a strand of cool-light LEDs fitted with blue filters to simulate the light quality at the ocean surface. The light intensity is instantly calculated and adjusted based on the ship’s location and local time. A water jacket on the incubation reactor is flushed with a high-flow rate of continuous surface seawater to mimic the in situ sea surface temperature. Downstream of the flow-through incubation reactor, the seawater flows into a gas extraction chamber. This gas extraction chamber consists of a glass frit with medium-size pores (Chemglass) and a custom-built gas-water separation system. A flow of 35 mL min\(^{-1}\) of C2H2-free air controlled by a mass flow controller (OMEGA) continuously purges the incubated seawater, extracting ethylene out of the seawater, and carrying it to a Cavity Ring-Down laser absorption Spectrometer (CRDS, Picarro) for analyses.

This CRDS ethylene analyzer measures ethylene concentrations in real time at ppb levels with high frequency and accuracy\(^{63}\). Approximately every 3 h, the incubation reactor is bypassed to determine the background ethylene concentration in the mixture of C2H2–H2O tracer and seawater. The difference between the incubation ethylene and background ethylene concentrations represents ethylene production rates during the incubation period. Finally, ethylene production rates are converted to N2 fixation rates using a conversion factor of 4:1\(^{60,64-66}\). We acknowledge that 4:1 is a theoretical ratio with uncertainties. However, our comparison of FARACAS to the \(^{13}\)N\textsubscript{2} addition method shows good agreement when applying this conversion factor\(^{19}\). In the current configuration, the detection limit of FARACAS is 0.19 nmol N L\(^{-1}\) d\(^{-1}\) which is also comparable to the \(^{13}\)N\textsubscript{2} addition method.

**Discrete N2 fixation and primary production incubations.** For comparison to our underway survey of N2 fixation, discrete \(^{13}\)N\textsubscript{2} incubation experiments in parallel with \(^{14}\)C additions were also conducted at eight stations during the 2015 cruise using methods detailed in previous studies\(^{53,55}\). Seawater samples were collected from each station at three levels in the euphotic zone, including the surface (5 m), an intermediate depth above the Deep Chlorophyll Maximum (DCM), and the DCM. Four liters of seawater were immediately filtered onto glass microfiber filters (MFG, 0.7 μm, Sarotex) to determine natural concentrations and isotopic signatures of particulate organic carbon (POC) and particulate nitrogen (PN). For incubation experiments, 4.5-L Nalgene polycarbonate bottles were filled with natural seawater. Then, \(^{15}\)N\textsubscript{2}-enriched filtered seawater (98% \(^{15}\)N atom%, Eurisotop, batch number 23/051301) and NaH\(^{13}\)CO\textsubscript{3} solution (99%, Eurisotop) were added into the incubation bottles, reaching approximate enrichments of \(^{2}^{15}\)N atom% and 7 \(^{12}\)C atom%. Finally, 4.5-L Nalgene bottles were topped off with natural seawater from sampled depths and capped with septum-fitted screw caps. Incubations were subsequently performed for 24 h in on-deck incubators covered by blue light filters simulating light intensity at the sampled depths. Incubators were flushed with surface seawater to avoid heating due to sunlight. Finally, incubated seawater was filtered onto MFG filters, which were stored at –20 °C until further analysis on land. Filters were treated and analyzed for POC, PN, \(^{85}\)Cl\textsubscript{POC}, and \(^{85}\)N\textsubscript{PN} using an Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS; EuroVector Euro EA 3000 coupled to a Delta V Plus, Thermo Scientific) to calculate corresponding carbon uptake and N2 fixation rates. The N2 fixation rates measured by our underway method closely match the results obtained from discrete incubation experiments (n = 7, r = 0.97, p < 0.01). A more comprehensive inter-method comparison can be found in Figure 5 of Cassar et al. (2018)\(^{19}\), showing good agreement between the two methods.

**Nutrients and trace-metal analyses.** Nutrient samples were collected from a CTD rosette equipped with 24 12-L Niskin bottles. Seawater was subsampled in acid-washed 15-mL polypropylene vials and immediately preserved at –20 °C.
Nitrates + Nitrite and phosphate were analyzed on land using an Automatic Nutrients Analyzer with detection limits of 0.03 mM and 0.014 mM, respectively. For seawater metal analysis, all reagents, standards, and blanks were prepared in acid-cleaned low-density polyethylene (LDPE) or Teflon-fuorinated ethylene propylene (FEP) bottles. Bottles were cleaned following GEOTRACES protocols. Trace metal samples were collected in surface seawater (~5 m) using a towed fish (USCG) deployed along side the ship while underway. During stops, the towfish was recovered from seawater to avoid contamination. Surface seawater was pumped through Teflon tubing to a sink located in a home-made clean plastic bubble installed within the chemistry lab on the ship. There, seawater was Filtered-in-line from the Teflon tubing outlet using 0.22-μm pore-size Acropak filter cartridges (250 mL) and collected in acid-washed 60-L containers that were injected with ~20 mL of filtered seawater before final sample collection. Samples for dissolved trace metal were then acidified to pH = 2 with concentrated HCl (Ultrapur grade, Merck) under a laminar flow hood equipped with HEPA filter. Samples were then double-bagged and stored in the dark, at room temperature, until analysis. On land, all analyses were performed in cleanroom environments at the Pôle Spectrométrie Océans (Brest, France).

Seawater samples were introduced to a PFA-STE nebulizer and a cyclonic spray chamber via a SeaFastPico introduction system (Elemental Scientific Incorporated, Omaha, NE), following the protocol of Langerstrøm et al. (2015)36. High-purity grade solutions and water (Mili-Q, 18.2 MΩ cm) were used to prepare the following reagents on a daily basis. Buffers were made from 0.5 M acetic acid (Ultrapur grade, Merck) and 0.6 M ammonium hydroxide (Ultrapur grade, Merck) and was adjusted to a pH of 8.3. Elution acid was made of 1.6 M HNO3 (Ultrapur grade, Merck) in Milli-Q water and spiked with 1 μL/L in (PlasmaCAL calibration standards) to allow for drift correction. Autosalpeter and column rinsing solutions were made from 0.01 M Ultrapur (ultra pure, Merck) in Mili-Q water. Mixed element standard solution was prepared gravimetrically using high-purity standards (Fe, Mn, Cd, Co, Zn, Cu, Pb; PlasmaCAL calibration standards) in 0.8 M HNO3 (Ultrapur grade, Merck). A six-point calibration curve was prepared by standard additions to the mixed element standard solution added to our in-house standard (North Atlantic filter, 2.2 L, filtered, N87445/N48.09345/W, 40 g/L, depth, 0.15 m) and run at the beginning, the middle, and the end of each run. Final concentrations of samples and procedural blanks were calculated from normalized data. Precision was assessed through replicate samples (every tenth sample was a replicate) and accuracy was determined from analysis of consensus seawater (SAFE S1 and D2, and GSP, GSC).

Diazotrophic community structure analysis. Diazotrophic phyotypes were identified and quantified using data obtained from 16S rRNA amplicon sequencing of environmental DNA, targeting the V4 region36. Eukaryotic hosts of some diazotrophs were amplified using amplicon sequencing of the V4 region of the 18S rRNA gene. Detailed experimental protocols are described in Wang et al. (2018)41. Following PCR purification, samples were pooled at equimolar concentrations. Illumina MiSeq DNA sequencing was performed at the UCSC Genome Technology Shared Resources core facility at the Duke Center for Genomic Technologies Shared Resources core facility at the Duke Center for Genomic and Computational Biology (Durham, USA). Raw RNA sequences and metadata are available from the NCBI Sequencing Read Archive under accession number SRP221677.

We used QiIME to process and analyze our Illumina sequencing data following the pipeline described in Fadrosh et al. (2014)42,79. Taxonomy tables reporting raw counts of 16S rRNA gene and 185 rRNA gene were produced by open-reference operational taxonomic unit (OTU) picking at the 97% threshold using the Usearch 6.1 algorithm and the SILVA ribosomal RNA database version 98. The SILVA ribosomal RNA database was supplemented with the addition of full length 16S rRNA gene sequences of UCYN-A1 and UCYN-A2 (accessions: NC_013771, CP001842, JPS001000003, and JPS001000022). Absolute abundances of the 16S rRNA gene or 18S rRNA gene for each OTU were subsequently calculated based on the number of internal standards recovered. Phylotypes were called when present at 1% abundance within the mixed layer and when vertical mixing is negligible over the residence time of O2 at the ocean surface, NCP can be estimated based on the detection of biological O2 with the equations above. The NCP estimates. NCP reflects the balance between plankton community photosynthesis and respiration. An excess of photosynthesis leads to a net production of particulate and dissolved organic carbon, which can either accumulate at the ocean surface or be exported to depth. To estimate the proportion of NCP fueled by N2 fixation, we measured NCP under way using the O2/Ar method86. Oxygen concentrations in the surface ocean are influenced by biological processes, such as photosynthesis and respiration, as well as physical processes including bubble injection, temperature, and pressure changes. The biological O2 supersaturation ([O2]bio) can be calculated by removing the effects of physical processes determined from Ar supersaturation ([Ar]bio). Biological O2 supersaturation and undersaturation reflect the metabolic state of the surface ocean, suggesting autotrophic or heterotrophic conditions, respectively. Under steady-state conditions with high O2 biological and Ar, the contribution of N2 fixation to NCP can be estimated by calculating the export ratio of O2 to Ar. Our data were converted to carbon-NCP assuming a constant O2/C stoichiometry88,89. We note that our observations of NCP fueled by N2 fixation should be interpreted with caution mainly due to differences in timescales of integration. Our O2/Ar-NCP estimates integrate productivity over 3–4 days in this region, while the N2 fixation measurements reflect hourly or daily rates. We cannot rule out the possibility that high N2 fixation rates occurred during late-stages of a phytoplankton bloom when nitrogen was exhausted90 or, conversely, that release of N2 by diazotrophs relieved N limitations within this mixed layer and when vertical mixing is negligible over the residence time of O2 at the ocean surface, NCP can be estimated based on the detection of biological O2 with the equations above.

The contribution of N2 fixation to NCP measured by FARACAS and O2/Ar method shows a similar spatial pattern as the contribution of N2 fixation to primary production off the coast of New Jersey (Supplementary Fig. 7). In addition, negative NCP values accompanied by detectable N2 fixation and heterotrophic diazotrophs were observed over a large portion of the transition zone between the neritic and open ocean regions. These observations may be attributed to transient net heterotrophy, advective transport of organic matter, or vertical mixing of O2-depleted waters. Further studies within a Lagrangian framework will be required to explore the coupling between N2 fixation and the net metabolic status of marine systems.

The contribution of N2 fixation to NCP measured by FARACAS and O2/Ar method shows a similar spatial pattern as the contribution of N2 fixation to NPP measured by 15N/13C incubation. 13C-based primary production measures yield lower rates closer to NPP than NCP91. Therefore, the 15N/13C-based approach to assessing N2 fixation’s contribution to biological production should relate to our FARACAS/O2-Ar according to the following equation:

$$ N_{fix} - N_{fix} = \frac{N_{fix}}{N_{PP}} $$

Where the export ratio = NCP. In some cases, we estimate a contribution of N2 fixation to NCP of 80–100%. Should we assume an export ratio of 8.4% for the oligotrophic Sargasso Sea based on BATS estimates92, the contribution of N2 fixation to NPP implied by our approach (6–8%) is approximately in line with our discrete incubation-based estimates (Supplementary Fig. 7). Coastal environments likely exhibit higher export ratios of 0.2–0.393, which would yield a range of contribution of N2 fixation to NPP of up to 16–30%. Thus, while discrepancies exist between 15N/13C and FARACAS/O2-Ar-based approaches, the broad relationship between these quantities is as expected.
Nitrogen budget via N$_2$ fixation in the global ocean. An updated database of depth-integrated and volumetric N$_2$ fixation rates over the global ocean is presented in Supplementary Fig. 8. The complete dataset of global N$_2$ fixation is shown in the Supplementary Data 1, which includes 1172 depth-integrated and 4299 volumetric N$_2$ fixation measurements. We conducted a Welch’s t-test to evaluate whether the N$_2$ fixation rates in the coastal oceans (bathymetry $\geq$ 200 m) are significantly higher than in the open ocean. This one-tailed hypothesis was examined at the 0.01 significance level. N$_2$ fixation rates were first log-transformed since they are approximately log-normally distributed (Fig. 4a-c). Based on these analyses, the volumetric N$_2$ fixation rates at different depths and at surface are significantly larger in coastal regions than in the open ocean ($p$ < 0.01), while depth-integrated N$_2$ fixation rates appear to be similar in both systems. Nitrogen inputs through N$_2$ fixation were further evaluated for coastal and open oceans separately by scaling to the surface areas of the respective regions. The areal extents of the coastal and open oceans were calculated using ArcGIS. Land (orange), coastal (cyan), and open ocean (blue) regions were delineated using bathymetric contour lines (GEBCO One Minute Grid), with depth criteria of 0 m and $\leq$200 m as shown in Supplementary Fig. 3. Surface areas were calculated under a Cylindrical Equal Area Projection (World) with 10° latitudinal bands except at latitudes higher than 50° N/S. We calculated global budgets after removing outliers identified using a Tukey’s test. These outliers include some extremely high values in the Indian Ocean$^4$ and some extremely low rates in the eastern North Atlantic Ocean$^9$ (Supplementary Fig. 8). Global budgets including outliers were also computed but may be substantially biased and are not shown here. Geometric means were used because depth-integrated and volumetric N$_2$ fixation rates are approximately log-normally distributed (Fig. 4a-c). Flux budgets were determined by multiplying the geometric mean of N$_2$ fixation rates by the area of each latitudinal band. Uncertainties were estimated based on the propagation of errors. Regional and global N$_2$ fixation rates are presented in Table 1 and Supplementary Table 1. For comparison, we also present budgets based on the arithmetic mean of N$_2$ fixation rates. Large uncertainties are expected in high latitude regions because of the limited number of observations.

Data availability

The updated database of depth-integrated and volumetric N$_2$ fixation rates over the global ocean is provided in Supplementary Data 1. In addition, data used in this study are available from the corresponding author on reasonable request.

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**Author contributions**

N.C. designed the study. W.T. and N.C. performed the measurements and analysis of N₂ fixation and wrote the manuscript with contributions from all co-authors. S.W. performed and analyzed the NCP measurements. S.W., W.T., and S.G. performed molecular sequencing data collection and analyses. D.F.B. and F.D. performed the ¹⁵N₂ and ¹³C incubation experiments and analyses. A.G.G. H.P., G.S., and M.G. collected and conducted the trace-metal analyses.

**Additional information**

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**Competing interests:** The authors declare no competing interests.

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