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RESEARCH ARTICLE

High nitrogen fixation and *Braarudosphaera* presence in the North Sea

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Abstract

Nitrogen fixation, the microbial conversion of dinitrogen gas to ammonia, is a key nitrogen source and critical for sustaining marine productivity. We investigated the diversity and activity of nitrogen-fixers in surface waters of the North Sea and Norwegian coast during July 2023. We detected, for the first time, the presence of *Braarudosphaera*/UCYN-A (up to 10^4 *nifH*copies L^{-1}) and measurable nitrogen fixation rates (up to 110 nM d^{-1}) throughout the North Sea. Nitrogen-fixing organisms may be transported from the northeast Atlantic across the northern North Sea by southward current systems, via Baltic Sea inflow or be a persistent but overlooked component of the phytoplankton community. We estimate nitrogen fixation may support between 4% and 24% of summertime net primary production. Nitrogen fixation is currently neglected in numerical models of shelf seas, yet may play a critical role in sustaining shelf sea productivity in the contemporary and future ocean.

Nitrogen fixation, performed by microorganisms called "diazotrophs", converts dinitrogen gas (N_2) into ammonia. Globally, N_2 fixation balances nitrogen (N) lost by denitrification, and supports primary production and particle export (Gruber and Sarmiento 1997; Karl et al. 2012; Bopp et al. 2022). We now know that diazotroph presence and/or measurable N_2 fixation extends beyond the subtropical ocean and occurs in polar regions (Harding et al. 2018; Shiozaki et al. 2018; Shiozaki et al. 2020; von Friesen et al. 2025a,b), the mesopelagic zone (Benavides et al. 2016, 2018; Wu et al. 2024), coral reefs (Benavides et al. 2017; Lesser et al. 2018; Moynihan et al. 2022), coastal seas (Fletcher-Hoppe et al. 2023; Fulweiler et al. 2025; Mulholland et al. 2012; Rees et al. 2009; Selden et al. 2024; Tang et al. 2019; Turk-Kubo et al. 2021; von Friesen et al. 2025a) and marine sediments (Fulweiler 2023; Fulweiler et al. 2025), vastly expanding the ecological range of diazotrophs. These findings challenge long-standing assumptions regarding factors controlling N_2 fixation and highlight the need to refine our understanding of where and why N_2 fixation happens.

Diazotrophs are more diverse than previously recognized (Farnelid et al. 2011; Pierella Karlusich et al. 2021). Initially discovered in *nifH* gene surveys, a molecular marker for N_2 fixation (Zehr et al. 1998), the unicellular cyanobacterial group A or *Candidatus* Atelocyanobacterium thalassa, termed UCYN-A, is now recognized as an intracellular symbiont of marine haptophytes related to *Braarudosphaera bigelowii* (Thompson et al. 2014; Thompson et al. 2012). Based on symbiont *nifH* sequences, *B. bigelowii* has at least four major sublineages (Turk-Kubo et al. 2017) with UCYN-A1 and UCYN-A2 the most common. *B. bigelowii*/UCYN-A1 is a small ($< 2\ \mu m$) open ocean ecotype, while *B. bigelowii*/UCYN-A2 is a larger ($> 5\ \mu m$) coastal ecotype (Cabello et al. 2016; Cornejo-Castillo et al. 2019; Turk-Kubo et al. 2021). Recently, *B. bigelowii*/UCYN-A2 was reclassified as a N_2-fixing organelle or nitroplast within its host (Coale et al. 2024), but this is the only lineage that is currently in culture. Globally, *B. bigelowii*/UCYN-A is considered to be a major contributor to N_2 fixation in both open ocean and coastal ecosystems. Diverse non-cyanobacterial diazotrophs

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(NCDs), found throughout the bacterial and archaeal kingdoms, are also prevalent, and may contribute to N_2 fixation, though their activity remains poorly understood (Turk-Kubo et al. 2023a).

Seasonally stratifying shelf seas are shallow (< 200 m), nutrient-rich, highly productive conduits connecting estuaries and oceans. Primary productivity is up to five-fold higher compared to the open ocean (Simpson and Sharples 2012) and supports over 90% of the world's fish catch (Pauly et al. 2002). Diazotrophs, including *B. bigelowii*/UCYN-A2, are prevalent in coastal waters, with broad temperature and nitrate tolerances (Cabello et al. 2020; Fletcher-Hoppe et al. 2023; Mills et al. 2020; Selden et al. 2024; Tang et al. 2019; Turk-Kubo et al. 2021), but the environmental drivers and their contribution to the coastal nitrogen budget and productivity are poorly understood.

Here, we characterized diazotroph diversity, quantified *B. bigelowii*/UCYN-A sublineages, and measured surface community N_2 fixation rates in the central and northern North Sea and Norwegian coastal waters in July 2023. Our goal was to elucidate the geographic extent and activity of N_2 fixation in this temperate, seasonally stratified shelf sea region. We found *B. bigelowii*/UCYN-A (up to 10^4 *nifH* copies L^{-1}) and measurable N_2 fixation rates (~ 0.2 to 110 nM d^{-1}) throughout the North Sea. In addition to there being a potentially persistent but overlooked population of diazotrophs in the North Sea, transport of northeast Atlantic water into the North Sea, through three primary inflow gateways, alongside outflow from the Baltic Sea played a key role in the biogeography of *B. bigelowii*/UCYN-A. We believe these are the first pelagic N_2 fixation measurements and first reported occurrence of *B. bigelowii*/UCYN-A in the North Sea, expanding the biogeographical extent of N_2 fixation to an additional shelf sea ecosystem. We estimate N_2 fixation could support up to 9% of annual net primary production (NPP), but between 4% and 24% of summer NPP. Conceptual and numerical models of the North Sea, an ecologically important region of the Northwest European Shelf, currently ignore N_2 fixation, meaning we have an incomplete understanding of N supply within this shelf sea system, stunting our ability to correctly estimate net primary production now and in the future.

Materials and methods

Surface ocean hydrography and stratification

Data were collected during a northbound transit through the North Sea and Norwegian Coastal waters onboard the *RRS Discovery* (DY167) between 10th and 15th July 2023 (Fig. 1a). Surface temperature, salinity and beam transmission were measured by an SBE38, SBE45 and C-Star transmissometer respectively at 1 Hz from seawater at 5.5 m. Salinity was calibrated using bottle samples taken 4 times per day and analyzed on a Guildline Autosal 8400B salinometer. Calibrated data were averaged over 1-min intervals (*see* Data Availability statement for access to data). Water column depth was recorded by a Kongsberg EA640 echosounder.

Monthly climatologies of the average surface (< 10 m) salinity, temperature and dissolved nutrients were created from the North Sea Biogeochemical Climatology (Hinrichs et al. 2017). Monthly climatologies of the potential energy anomaly (Φ), an expression of the energy required to fully mix the water column were created from 153,062 temperature and salinity profiles (50–70°N; 8°W–12°E) obtained from the EN4.2.2 data base between 1980 and 2024 (Good et al. 2013, Supporting Information Fig. S1). Φ was derived from vertical profiles of potential density:

$$\Phi = \frac{g}{h}\int_{-h}^{0}\left(\rho_{\overline{S},\overline{T},0} - \rho_{S,T,0}\right) z\,dz$$

where ρ is the potential density at $p = 0$ calculated from conservative temperature (T°C) and absolute salinity (S g/kg) using the TEOS-10 equation of state and g is gravitational acceleration. Overbars indicate depth mean quantities. A maximum depth of $h = 200$ m was used for integration and depth averages. Individual density profile location and corresponding potential energy value was mapped onto a ¼ degree resolution grid and monthly climatology's calculated. Here we consider the water column to be mixed when $\Phi < 10\,\mathrm{J\,m^{-3}}$. Higher values of Φ reflect a more strongly stratified water column.

Chlorophyll, nutrients, and trace metals

A towed FISH and Teflon diaphragm pump (Almatec A-15) collected water at 3 m every 2 h for analysis of chlorophyll, nutrients, and trace metals. Sampling was performed in a class-1000 clean air laboratory. Chlorophyll *a* was quantified using acetone extraction and fluorometric analysis (Welschmeyer 1994). Seawater was filtered using a 0.8/0.2 μm polyethersulfone membrane (Sartobran, Sartorius) for onboard analysis of dissolved nutrients using standard colorimetric techniques (Becker et al. 2020; *see* Supporting Information Table S1 for certified reference material information). Samples were analyzed for dissolved iron (Fe) using flow injection analysis with chemiluminescence detection (Kunde et al. 2019) onboard, and dissolved manganese (Mn) and Fe were analyzed using offline preconcentration and extraction following the manifold design by Milne et al. (2010) and the extraction chemistry by Rapp et al. (2017), followed by ICP-MS detection at the University of Southampton (*see* Supporting Information Table S2 for reference material information and the Data Availability statement). Spearman's rank correlation analysis was used to determine the relationship between environmental variables, N_2 fixation rates, and the abundance of N_2-fixers at the 95% and 90% confidence interval (R Core Team 2024; *see* Supporting Information Table S3).

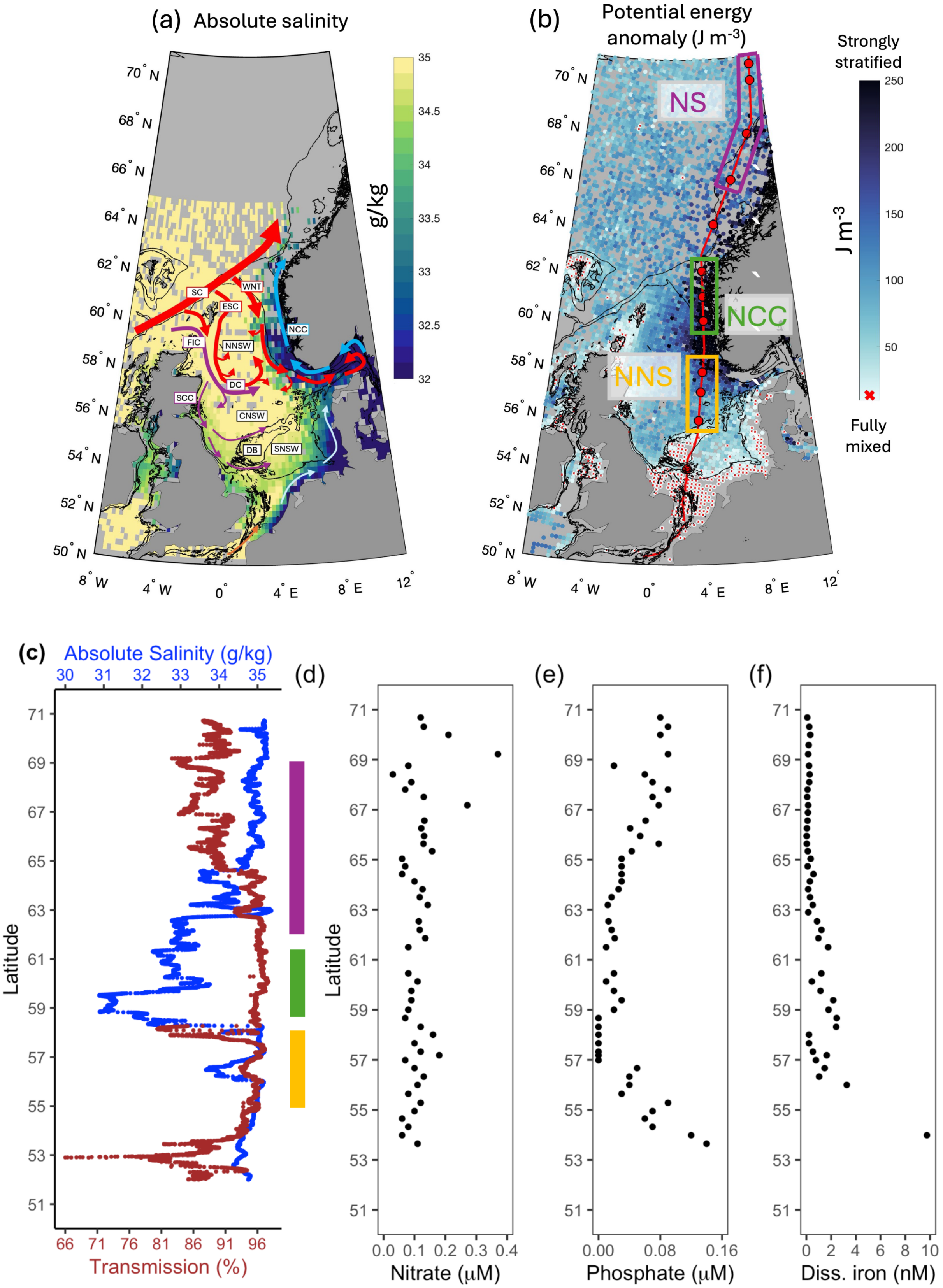

Fig. 1. Legend on next page.

Nitrogen fixation rates

Total community N_2 fixation rates were determined using the $^{15}N_2$ bubble technique (Montoya et al. 1996; White et al. 2020). Briefly, unfiltered seawater was collected from the towed-FISH into five clean, triple-rinsed 2 L polycarbonate bottles fitted with a septum cap and filled with no headspace. To three bottles, 4 mL of $^{15}N_2$ gas (Cambridge Isotope Laboratories, Lot#:1-26354) was injected via the septum cap using a gas tight syringe into each bottle. Bottles were rotated (15 min at 60 rpm) then incubated in a temperature and light controlled container on a 16 : 8 h light dark cycle. Light was set to 5 m below surface (135 μmol photon m^{-2} s^{-1}) and incubation temperature decreased from $\sim$ 17 to 12°C as we transected northwards. The fourth and fifth bottles were used to determine the $\delta^{15}N$ natural abundance of particulate nitrogen of seawater at the start and after 24-h incubation to account for assimilation of fixed nitrogen sources (White et al. 2020). A subsample of $^{15}N_2$-enriched seawater was removed from each 2 L bottle after 24 h incubation and the atom% of dissolved $^{15}N_2$ was quantified onboard using a Hiden membrane inlet mass spectrometer (MIMS). Average $^{15}N_2$ enrichments were 5.76 ± 0.1 ‰. Seawater was filtered onto pre-combusted glass fiber filters, dried in an oven at 60°C, pelletized into tin foil cups and analyzed using an EA-IRMS at the University of Liverpool Isotope Facility. N_2 fixation rates, limits of detection (LoD) and minimum quantifiable rates (MQR) were calculated according to Gradoville et al. 2017 (*see* Data Availability statement for link to calculations).

nifH gene analysis

Seawater samples were collected for diazotroph community composition analyses (*nifH* amplicon high throughput [HTS] sequencing) and abundances of targeted diazotrophic groups (digital droplet PCR) from the towed-FISH. Between 1 and 2 L of seawater was filtered onto Sterivex™ filter cartridges (0.22 μm, Merck, Rahway, NJ) via gentle peristaltic pumping. Cartridge filters were sealed, flash frozen with liquid nitrogen and stored at −80°C until extraction. DNA was extracted using the AllPrep DNA/RNA kit (Qiagen, Germantown MD), with modifications to improve cell lysis and minimize co-extraction of inhibitors, as described in detail in Varaljay et al. (2015). Quality and quantity of purified DNA was assessed using a Nanodrop (ThermoFisher Scientific, Waltham, MA).

Diazotroph community composition via *nifH* amplicon HTS

Partial *nifH* gene fragments were PCR-amplified using a nested universal *nifH* assay widely used in the marine ecosystem (nifH1-4; Zani et al. 2000; Zehr and McReynolds 1989) as described in detail in Turk-Kubo et al. 2023b. Each second-round primer (nifH1, nifH2) was modified to include a 5′ common sequence linker (Moonsamy et al. 2013) and barcoded libraries were prepared according to (Green et al. 2015) at the DNA Service Facility at the University of Illinois at Chicago. Multiplexed *nifH* amplicons were bidirectionally sequenced (2 × 300 bp) using the Illumina MiSeq platform at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign. Demultiplexed raw sequences are available under BioProject PRJNA1284977 in the Sequence Read Archive at NCBI.

Amplicons were processed following a custom *nifH* DADA2 pipeline and workflow (Morando et al. 2024) using default parameters, and the taxonomy of resulting amplicon sequence variants (ASVs) was assigned to *nifH* clusters using CART analyses (Frank et al. 2016), as well as using blastN against a custom *nifH* database derived from Genbank nr (Heller et al. 2014) and a database containing UCYN-A oligotypes (Turk-Kubo et al. 2017) and marine NCD phylotypes (Turk-Kubo et al. 2023a).

Enumerating *Braarudosphaera*/UCYN-A sublineages via ddPCR

Abundances of *B. bigelowii*/UCYN-A1 and *B. bigelowii*/UCYN-A2/A3/A4 were estimated using digital droplet PCR (ddPCR) using the assays originally developed for Taqman® quantitative PCR by Church et al. (2005) and Thompson et al. (2014), respectively (*see* Data Availability statement for link to raw data). All reaction conditions, thermocycling parameters, thresholding and calculations of the limit of detection and limit of quantitation are described in Gradoville et al. (2020). Note that the abundances determined using the *B. bigelowii*/UCYN-A2/A3/A4 assay likely represent both *B. bigelowii*/UCYN-A2 and *B. bigelowii*/UCYN-A4, both of which were

Fig. 1. (**a**) Climatological June–July–August absolute salinity (g kg^{-1}) within the top 10 m of the water column. The slope current (SC) and the main Atlantic inflow branches into the North Sea are shown in red: Fair Isle Current (FIC), East Shetland Current (ESC) and Western Norwegian Trench (WNT). Shelf and coastal water entering with the FIC and joining the Dooley Current (DC), and the Scottish Coastal Current (SCC) are shown in purple: Dogger Bank (DB); Northern North Sea Water (NNSW); Central North Sea Water (CNSW) and Southern North Sea Water (SNSW). Based on Turrell et al. (1992) and Winther and Johannessen (2006). Black bathymetry contours at 35, 50, 200 and 500 m. (**b**) July climatology of the potential energy anomaly (J m^{-3}) created from EN4 profiles of temperature and salinity between 1980 and 2024 (Supporting Information Fig. S1). Fully mixed locations indicated with red crosses. DY167 ships track (red line) and surface N_2 fixation rate sampling locations (red dots). Sampling in Northern North Sea (NNS, yellow), Norwegian Coastal Current (NCC, green) and Norwegian Sea (NS, purple) indicated within rectangles. (**c**) Latitudinal variation in absolute salinity (blue, g kg^{-1}) and transmission (red, %) during DY167 from 10th to 15th July 2023 from sensors sampling in the upper 5 m of the water column and latitudinal variation in (**d**) nitrate (μM), (**e**) phosphate (μM) and (**f**) dissolved iron (nM) from discrete samples collected every 2 h using the trace metal clean FISH, towed in the upper 3 m of the water column.

recovered via *nifH* amplicon HTS. Assumptions utilized for estimating the potential contribution of *B. bigelowii*/UCYN-A to measured N_2 fixation rates are detailed in Supporting Information Table S4.

Results and discussion

North Sea circulation and stratification

The majority of the water in the northern North Sea originates from the northeast Atlantic (Turrell et al. 1992). High-salinity northeast Atlantic water within the slope current (SC) enters the North Sea via three main inflow currents: Fair Isle Current (FIC); East Shetland Current (ESC) and the Western Norwegian Trench Inflow (WNT) (Fig. 1a, Turrell et al. 1992, Winther and Johannessen 2006). The FIC and ESC inflows are mixed with lower salinity continental shelf water and fresh riverine input carried south within the Scottish Coastal Current (SCC), forming Northern North Sea and Central North Sea waters (NNS, CNS, Fig. 1a). Eastward density driven currents (Dooley Current and a jet north of Dogger Bank) draw these waters eastward toward the Norwegian Trench (Hill et al. 2008). Atlantic origin water carried southward into the North Sea as part of the WNT inflow branch eventually meets brackish Baltic Sea outflow and is carried northwards again, ultimately forming the Norwegian Coastal Current (NCC, Fig. 1a). North of Dogger Bank, a large area of the shelf (< 200 m) seasonally stratifies for 3 months in June, July and August (Fig. 1b). From June, away from the shallower coastal areas influenced by the Scottish Coastal Current, between Dogger Bank and Shetland, the potential energy anomaly, Φ, is greater than 50 $\mathrm{J\,m^{-3}}$. It increases throughout June, July and August to >100 $\mathrm{J\,m^{-3}}$ and then begins to weaken in September. Between December and April, the whole of the Northern North Sea is well mixed, $\Phi < 10$ $\mathrm{J\,m^{-3}}$ (Fig. 1b, Supporting Information Fig. S1). In contrast, as a result of buoyant fresh water input from the Baltic, the NCC remains strongly stratified (100–300 $\mathrm{J\,m^{-3}}$) all year around (Supporting Information Fig. S1). Owing to strong tidal currents and shallow depths, Dogger Bank and the southern North Sea remain fully mixed all year, $\Phi < 10$ $\mathrm{J\,m^{-3}}$ (Supporting Information Fig. S1). Immediately south of Dogger Bank, a tidal mixing front supports an eastward jet carrying CNSW and fresh SCC water to the southern North Sea (Hill et al. 1993).

Hydrography and nutrient dynamics in July 2023

North of Dogger Bank, the absolute salinity ranged from 30.10 to 35.38 g $\mathrm{kg^{-1}}$, with the freshest waters associated with the NCC (Fig. 1c). Transmission ranged from 81% to 98% and was highest within the NCC (Fig. 1c). North of Dogger Bank, transmission was significantly negatively correlated with chlorophyll ($r = -0.69$) and particulate organic carbon (POC) ($r = -0.92$; Supporting Information Figs. S2b, S3; Supporting Information Table S3), inferring that lower transmission was indicative of higher particle loading. Nitrate was low ($\sim 0.2\ \mu$M) throughout the transect, increasing in the North Sea only (Fig. 1d). Salinity was significantly positively correlated with chlorophyll ($r = 0.40$) and phosphate ($r = 0.49$; Fig. 1e) and negatively correlated with dissolved iron ($r = 0.62$; Fig. 1f) and silicate ($r = -0.51$; Supporting Information Figs. S2c, S3; Supporting Information Table S3) indicating fresher particle poor waters were a source of dissolved iron and silicate. Transmission was positively correlated with silicate ($r = 0.47$), dissolved iron ($r = 0.60$) and manganese ($r = 0.62$; Supporting Information Fig. S2d) and negatively correlated with phosphate ($r = -0.59$) and nitrate ($r = -0.32$; Supporting Information Fig. S3; Supporting Information Table S3), indicating particle rich waters were depleted in silicate, iron and manganese but contained higher nitrate and phosphate. South of Dogger Bank, low-nitrate, permanently mixed waters were characterized by the highest particle loading (Fig. 1c) alongside the highest concentrations of phosphate and iron (Fig. 1e,f, respectively), silicate and manganese (Supporting Information Fig. S2c,d, respectively) measured along the transect.

We use these relationships alongside understanding of North Sea circulation to characterize three regions (Fig. 1b): (1) The seasonally stratified ($\Phi > 50\,\mathrm{J\,m^{-3}}$ between June and August), saline NNS and CNS waters north of Dogger Bank where saline northeast Atlantic origin inflow has been mixed with fresher riverine and continental shelf origin water. These surface waters have low particle loads and low nitrate, but elevated phosphate, iron, manganese and silicate which may have originated from coastal input and/or the shallow, well mixed region south of Dogger Bank: (2) The strongly and permanently stratified ($\Phi \gg 100\,\mathrm{J\,m^{-3}}$ year-round), fresh surface waters of the NCC with low particle loading, lower phosphate but relatively high silicate, iron and manganese, likely originating from the Baltic Sea outflow as it joins recirculating water from across the North Sea shelf (Fig. 1a): (3) Finally, the seasonally stratifying, saline waters of the Norwegian Sea (NS), which have relatively high particle loading, high nitrate and phosphate (up to 0.27 and 0.10 μM, respectively), but low iron, silicate and manganese.

Nitrogen fixation activity, community structure, and abundance of *nifH* gene

N_2 fixation rates were 1 ± 0.12 nM N $\mathrm{d^{-1}}$ at the tidal mixing front south of Dogger Bank where an eastward jet draws coastal and CNS water into the southern North Sea. In the NNS, N_2 fixation rates ranged from 17 ± 2 to 110 ± 20 nM N $\mathrm{d^{-1}}$ (Fig. 2b). Within the fresher waters of the NCC, N_2 fixation rates ranged from 36 ± 1 to 54 ± 6 nM N $\mathrm{d^{-1}}$ (Fig. 2b). In the more open Atlantic waters of the NS, beyond the core of the NCC, N_2 fixation rates decreased rapidly from 7.6 ± 0.4 to 0.4 ± 0.3 nM N $\mathrm{d^{-1}}$, then below the limits of detection at the northernmost sites (*see* Data Availability statement for link to calculations). Similar high N_2

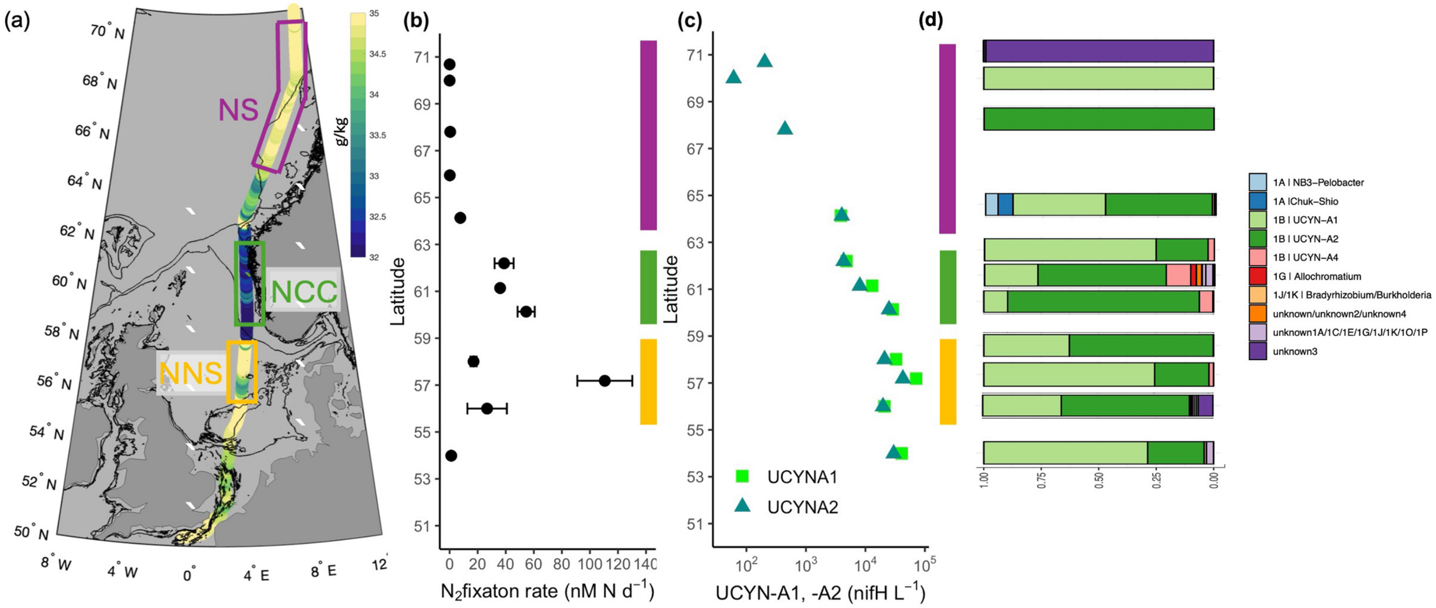

Fig. 2. Latitudinal variation in (**a**) surface ocean absolute salinity (g kg^{-1}) from 10th to 15th July 2025 (DY167) with black bathymetry contours of 35, 50, 200 and 500 m, (**b**) N_2 fixation rates (nM N d^{-1}, mean ± standard deviation, $n = 3$), (**c**) abundance of *B. bigelowii*/UCYN-A1 (squares) and *B. bigelowii*/UCYN-A2 using ddPCR (triangles, *nifH* L^{-1}), and (**d**) diversity of N_2-fixers (relative abundance). Regions are defined as Northern North Sea (NNS, yellow), Norwegian Coastal Current (NCC, green) and Norwegian Sea (NS, purple).

fixation rates have been observed in shelf sea environments including the New Jersey coast (up to 98 nM N d^{-1}; Tang et al. 2019), mid-Atlantic continental shelf (up to 50 nM N d^{-1}; Mulholland et al. 2012), mid-Atlantic shelf break (up to 100 nM N d^{-1}; Selden et al. 2024) and Southern California Current System (up to 23 nM N d^{-1}; Turk-Kubo et al. 2021).

Diazotrophc community structure, abundance, and diversity

B. bigelowii/UCYN-A was the dominant diazotroph in this shelf sea region (Fig. 2c). Based on *nifH* HTS analyses, the three sublineages, UCYN-A1, UCYN-A2, and UCYN-A4, accounted for ~ 90% of the total *nifH* pool at all sites (Fig. 2d). Abundances of *B. bigelowii*/UCYN-A ranged from 6.1×10^1 to 4.3×10^4 *nifH* copies L^{-1} for UCYN-A2/A4 and 0 to 7.2×10^4 *nifH* copies L^{-1} for UCYN-A1 (Fig. 2c). The highest abundances of *B. bigelowii*/UCYN-A2 and *B. bigelowii*/UCYN-A1 were measured in the NNS (Fig. 2c), which coincided with the highest measured N_2 fixation rates (Fig. 2b). However, extrapolated contributions of both sublineages to N_2 fixation rates in the NNS (~ 57°N) do not entirely account for the measured rates (Supporting Information Table S4), suggesting either *B. bigelowii*/UCYN-A single-cell N_2 fixation rates are higher than previously reported, or there is a contribution from other active diazotrophs. The *B. bigelowii/*UCYN-A4 sublineage was present at low relative abundances within the Baltic-influenced NCC (Fig. 2d), but single-cell N_2 fixation rates for this sublineage are currently unknown. High *B. bigelowii/*UCYN-A2 abundance (> 10^5 *nifH* L^{-1}) occur year-round in the Danish Strait with peak abundances (~ 10^7 *nifH* L^{-1}) in early autumn (Bentzon-Tilia et al. 2015). In a previous study, along the Norwegian coast during the summer and autumn of 2021, both *B. bigelowii*/UCYN-A1 and *B. bigelowii*/UCYN-A2/4 occur and actively transcribe *nifH* (a proxy for potential N_2 fixation activity; von Friesen et al. 2025a). In July 2023, north of 63°N, *B. bigelowii*/UCYN-A abundances decreased, in line with the decreasing N_2 fixation rates (Fig. 2b,c).

NCDs were also detected throughout this shelf sea region, and may possibly contribute to N_2 fixation, however, assessing their contribution to measured rates is difficult due to the lack of data linking NCD *nifH* phylotypes to active N_2 fixation (Turk-Kubo et al. 2023a). It is worth noting that among the NCD *nifH* ASVs, two phylotypes were detected in the NS that are closely related (> 97% ani) to NCDs reported previously in coastal and high latitude ecosystems (Fig. 2d). Clustering with putative aerobic delta proteobacteria (e.g., Pelobacter; *nifH* cluster 1A), NB3-Pelobacter and Chuk-Shio otu4 were originally reported in coastal sediments off the Mid Atlantic Bight (Brown and Jenkins 2014) and in pelagic samples from the Chukchi Sea (Shiozaki et al. 2018), respectively. Together these phylotypes only represent ~ 1% of the total *nifH* pool in this study, but the presence of Pelobacter-like diazotrophs has also recently been reported in the Norwegian and Barents Seas and the Canadian Arctic (von Friesen et al. 2025a; Robicheau et al. 2023), suggesting they are persistent constituents of diazotroph assemblages in cold, high latitude, coastal-influenced waters. Active *nifH* transcription of Pelobacter-like diazotrophs has been reported in coastal sediments (Brown and Jenkins 2014), but currently there is no data to support active N_2 fixation in high latitude pelagic waters, with recent surveys detecting their presence but not *nifH* transcription (von Friesen et al. 2025a). Other NCDs found at low relative abundances included *nifH* phylotypes affiliated primarily with proteobacteria (1G, 1J/K, Fig. 2d), but little can be inferred about their activity. The detection of NCDs in this study is consistent with many reports of diverse NCDs in the pelagic marine environment, but their contribution to N_2 fixation in the North Sea requires further study.

Relationship between nitrogen fixation and ocean properties

B. bigelowii/UCYN-A1 and *B. bigelowii*/UCYN-A2 abundances were significantly positively correlated ($r = 0.97$; $p = 0.0001$; Supporting Information Fig. S3; Supporting Information Table S3). N_2 fixation rates were positively correlated with *B. bigelowii*/UCYN-A2 ($r = 0.69$; $p = 0.018$) and weakly correlated to *B. bigelowii*/UCYN-A1 ($r = 0.63$; $p = 0.092$; Supporting Information Fig. S3; Supporting Information Table S3). Within the NNS, the abundance of *B. bigelowii*/UCYN-A1, typically an open ocean ecotype, was equivalent to or higher than *B. bigelowii*/UCYN-A2, the ecotype typically found in coastal waters (Fig. 2c). N_2 fixation rates were strongly positively correlated with transmission ($r = 0.59$; $p = 0.04$) and negatively correlated with phosphate ($r = -0.68$; $p = 0.02$), but were also weakly but positively correlated with dissolved iron ($r = 0.47$; $p = 0.175$) and dissolved manganese ($r = 0.731$; $p = 0.062$; Supporting Information Fig. S3; Supporting Information Table S3). Thus, the highest N_2 fixation rates and diazotroph abundance were in a low particle/high light surface ocean layer within the NNS which was deplete in phosphate but enriched in iron and manganese.

B. bigelowii/UCYN-A1 was weakly but positively correlated to salinity ($r = 0.64$; $p = 0.09$). We postulate that this open ocean ecotype was likely transported from the saline northeast Atlantic Ocean into the North Sea and advected southwards via a series of density driven current systems (Fig. 1a). In contrast, *B. bigelowii*/UCYN-A2, a coastal ecotype, may persist in the North Sea, as it does in the Baltic Sea/Danish Strait and associated outflows and coastal currents (Bentzon-Tilia et al. 2015; von Friesen et al. 2025a) and other coastal regions (Turk-Kubo et al. 2021). Previous studies show that fronts, currents and eddies enhance N_2 fixation by concentrating biomass, supplying limiting nutrients like phosphate or iron or transporting diazotrophs (Chowdhury et al. 2024; Dugenne et al. 2023; Fong et al. 2008; Hoerstmann et al. 2024; Sato et al. 2021). *B. bigelowii*/UCYN-A was likely able to grow at rates exceeding grazing losses in the relatively warm (~ 16.5°C), high-light, high dissolved iron/manganese, surface waters within the North Sea. Light plays a critical role for the *B. bigelowii*/UCYN-A2 and *B. bigelowii*/UCYN-A1, due to the light requirement of the haptophyte host (Gradoville et al. 2021; Landa et al. 2021). Also, while the relationship between iron and the *nifH* gene is well documented (Berman-Frank et al. 2001; Kustka et al. 2002), previous studies have also noted a relationship between N_2 fixation and dissolved manganese (Tang et al. 2019) which requires further investigation. North of 63°N within the NS, the decline in N_2 fixation rates and abundance of *B. bigelowii*/UCYN-A1 and *B. bigelowii*/UCYN-A2 coincided with decreasing iron (Fig. 1f) and manganese (Supporting Information Fig. S2d) and increase in chlorophyll *a* (Supporting Information Fig. S2b), indicating competition with non-N_2 fixers and/or that iron or manganese may constrain N_2 fixation.

Contribution of nitrogen fixation to the coastal ocean

Using new glider-based estimates of net primary production (NPP; Loveday et al. 2022) and assuming N_2 fixation occurs in the upper 10 m only, we estimate the daily nitrogen supplied via N_2 fixation could support between 4% and 24% of NPP in the summer, or up to 8% of annual NPP (Table 1). In the western North Atlantic ocean, N_2 fixation was estimated to support up to 50% of net community production (Tang et al. 2019). These estimates underscore the potential importance of N_2 fixation in supporting productivity in coastal regions. Furthermore, over the entire stratified northern North Sea, and assuming N_2 fixation occurs in the upper 10 m over the stratified summer period (90 d), we estimate that N_2 fixation could supply up to 0.25 ± Tg N y^{-1} (Table 1). Globally, N_2 fixation is estimated to supply 17 Tg N y^{-1} to coastal regions, equivalent to 9% of global N_2 fixation (Tang et al. 2019).

Note that the areal rates compared to NPP, alongside integrated rates over the northern North Sea, represent upper limits due to the assumptions made in scaling calculations (e.g., C : N ratios, new vs. regenerated production), heterogeneity in environmental drivers and scarcity of nitrogen fixation rates. Nevertheless, these estimates add to the growing evidence for shelf sea N_2 fixation, and its contribution to NPP.

While our observations were made in summer 2023 only in the North Sea, it remains unclear if N_2 fixation is an episodic event or a seasonal and recurrent feature of the North Sea. There is strong seasonal and interannual variability in the cross-shelf exchange between the Atlantic and North Sea, alongside on-shelf seasonality in currents and stratification (Winther and Johannessen 2006; Hjøllo et al. 2009; Holt et al. 2012; Holliday 2001; Marsh et al. 2017; Huthnance et al. 2022). Pulses of Atlantic water into the North Sea in the 1980s and 1990s were deemed responsible for the unusual occurrence of oceanic species in the North Sea (Holliday 2001), highlighting the ecosystem sensitivity to cross-shelf exchange. Alongside pulsed events, seasonality in the strength and pathways of transport processes is likely to impose monthly variations in the nutrient resources and N_2-fixers into the North Sea. There is also evidence that *B. bigelowii*/UCYN-A is responsive to seasonality in local hydrographic forcing, such as upwelling in the California

Table 1. Estimates of contribution of N_2 fixation to summer areal (m^2) NPP and annual NPP based on a minimum and maximum nitrogen fixation rates. Summertime mean NPP ± standard deviation is 368 ± 36 mg C m^{-2} d^{-1} with an annual mean NPP of 98 ± 73 g C m^{-2} y^{-1} (Loveday et al. 2022), and N_2 fixation occurs over the summertime (90 d) only (*see* Supporting Information Fig. S5). Total annual N_2 fixation in the North Sea is estimated assuming nitrogen fixation occurs over 180,000 km^2 (*see* Fig. 3).

N_2 fixation rate in the North Sea	**Min. rate: 17 nM d^{-1}**	**Max. rate: 110 nM d^{-1}**
Integrated rate (10 m), assume C : N of 6.6 : 1 (mg C m^{-2} d^{-1})	13.46	87.12
% of summer areal daily NPP	~ 4	~ 24
% of annual NPP	~ 1.2	~ 8
Total annual N_2 fixation in the North Sea (Tg N y^{-1})	0.038	0.25

Current System (Cabello et al. 2020; Turk-Kubo et al. 2021) as well as large scale climatic forcing such as El Niño (Fletcher-Hoppe et al. 2023), likely responding to changes in nutrient resources and light. We suspect N_2-fixers are a persistent but overlooked feature of the northern North Sea and Norwegian current (*see* also von Friesen et al. 2025a), with strong seasonality in the hydrography and nutrient resources controlling their abundance and activity.

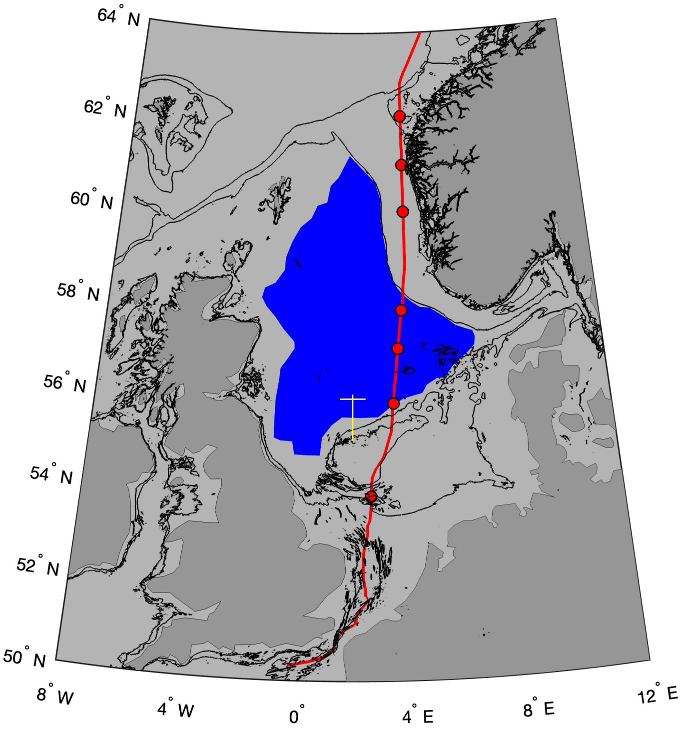

Fig. 3. Area of the Northern North Sea (180,000 km^2) in blue over which we propose that nitrogen fixation could occur based on environmental conditions. It was defined as the area that remains stratified (> 50 J m^{-3}) throughout June–July–August (Supporting Information Figs. S1, S5) with water depths > 50 and < 200 m and where the surface concentration in N < 1 μM and Si < 1 μM (Supporting Information Figs. S2, S5). Yellow cross indicated the glider tracks used to collect data to estimate NPP (Loveday et al. 2022), for comparison with daily rates of nitrogen fixation.

In conclusion, we found active and diverse N_2-fixers in the North Sea and Norwegian Sea, which adds to the accumulating evidence on the importance of N_2 fixation in shallow coastal and shelf sea environments. Strongly stratified waters of shelf seas systems are deplete in nitrate after intense spring phytoplankton growth, often leading to nitrate-limited growth and intense regenerated productivity. N_2 fixation may be a critical nitrogen source to the North Sea yet N_2 fixation is ignored in the North Sea nitrogen budget (Brion et al. 2004) or coastal ocean ecosystem models (Butenschön et al. 2016). In Earth System Models that include a representation of diazotrophy, the magnitude and spatial pattern of N_2 fixation is predicted to decline in the adjacent North Atlantic Ocean by 2100, albeit with high uncertainty (Wrightson and Tagliabue 2020). This uncertainty hampers confidence in projecting future NPP in numerical models (Bopp et al. 2022), illustrating the sensitivity of productivity to nitrogen supply from N_2 fixation. As the northwest European Shelf and surrounding northeast Atlantic warm, stratification is predicted to strengthen and the depth of winter mixing across the northeast Atlantic expected to shoal (Mathis et al. 2019; Sharples et al. 2022; Holt et al. 2022). Consequently, nutrient import onto the shelf may weaken and enhanced stratification is expected to reduce vertical fluxes of nitrate into the euphotic zone (Mathis et al. 2019). N_2 fixation has the potential to alleviate nitrate limitation, even at low but sustained rates. However, neglecting N_2 fixation in these productive shelf sea systems undermines our ability to estimate contemporary but also future productivity. In addition, Atlantic inflow may be critical for transporting open ocean diazotrophs, such as *B. bigelowii*/UCYN-A1, into the North Sea (Holliday 2001). Numerical models predict that changes in the North Atlantic and Arctic will reduce connectivity between the Atlantic and the North Sea (Holt et al. 2018), potentially reducing the prevalence of N_2-fixers in the North Sea under future ocean scenarios.

Author Contributions

Kendra Turk-Kubo: Funding acquisition, resources, formal analysis, methodology, validation, investigation, data curation, visualization, writing—original draft, writing—review and editing. Claire Mahaffey: Conceptualization, funding acquisition, project administration, investigation, resources, validation, data curation, visualization, writing—original draft, writing—review and editing. Jo Hopkins: Funding acquisition, investigation, resources, data curation, visualization, writing—original draft, writing—review and editing. Ruth Hawley: Formal analysis, methodology, validation, writing—review and editing. Louisa Norman: Formal analysis, methodology, validation. Lewis Wrightson: Formal analysis, methodology. Ben Fisher: Formal analysis, methodology. Stefanie Rynders: Formal analysis, validation, data curation. Clive Neil: Formal analysis, visualization, data curation. Maryam Ilyas: Formal analysis, validation, data curation. Maeve C. Lohan: Funding acquisition, project administration, investigation, resources, validation, writing—review and editing.

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Conflicts of Interest

None declared.

Data Availability Statement

Demultiplexed raw sequences are available under BioProject PRJNA1284977 in the Sequence Read Archive at NCBI: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1284977. Calculations and data published in this manuscript are available on the University of Liverpool Data Repository as follows: calculations for the rates of nitrogen fixation from DY167 (https://doi.org/10.17638/datacat.liverpool.ac.uk%2F3033), surface ocean hydrographic and biogeochemical properties including nutrients, iron and manganese from DY167 (https://doi.org/10.17638/datacat.liverpool.ac.uk%2F3039), and raw ddPCR abundance data for UCYN A2 and UCYN A1 from DY167 (https://doi.org/10.17638/datacat.liverpool.ac.uk%2F3040).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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