De novo amino acid synthesis and turnover during N2 fixation

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Abstract

Cyanobacteria are the main autotrophs and N2-fixing (diazotrophic) organisms in large parts of the oligotrophic global ocean, where generally all heterotrophic production depends on their activity. Amino acids (AAs) from cyanobacteria are essential macronutrients for these heterotrophic food webs, yet little is known about the de novo synthesis of AAs during N2 fixation. Through a combination of bulk and amino acid nitrogen (AAN) specific analyses of field based N2 fixation experiments, we demonstrate that the de novo synthesis of 13 AAs accounted for the majority of bulk N2 fixation rates at four stations in the central Baltic Sea in July 2015. Slow AA turnover times of 87 ± 14 d coincided with low phosphate concentrations and high cell-carbon biomasses of unicellular cyanobacteria. Very fast turnover times of 17 ± 3 d coincided with high phosphate concentrations and undecayed Nodularia spumigena cells, but unexpectedly also with phosphate depletion and decayed N. spumigena cells. In a decayed bloom, volumetric N2 fixation rates into AAN provided a much better estimate of the net incorporation of N2 into biomass than fixation into bulk nitrogen that rather reflected gross N2 fixation. In an undecayed bloom, the turnover times of 13 AAs can be predicted from a single bulk N2 fixation rate. This is the first direct evidence that the very late, decayed stage of a cyanobacteria bloom can be a flashpoint of very fast AA turnover during N2 fixation with hitherto uncharacterized consequences for heterotrophic food webs and diazotroph N inputs to the global ocean.

Highlights

1. Cyanobacteria composition and bloom stage can be critical factors for amino acid (AA) turnover.
2. In a decaying bloom, amino acid nitrogen based volumetric rates reflected net N2 fixation.
3. Bulk nitrogen based volumetric rates in a decaying bloom reflected gross N2 fixation.
4. In undecayed blooms, AA turnover can be predicted from single bulk N2 fixation rates.
5. Decaying cyanobacteria blooms can be flashpoints of AA turnover during N2 fixation.

N2 fixation provides more than 40% of the nitrogen demand for the net primary production in the global ocean (Deutsch et al. 2007). This makes nitrogen originating from N2 fixation (diazotroph N) the single most important natural source of nitrogen for marine biological production, yet there are strong indications that inputs estimated from direct measurements of N2 fixation experiments in the field underestimate diazotroph N inputs (Voss et al. 2013). This has been related to the sparsity of measurements and to an extreme variability of N2 fixation rates in the field (Capone et al. 2005; Voss et al. 2013), which can at times reach volumetric rates of up to 60 nmol L⁻¹ h⁻¹ while the majority of volumetric N2 fixation rates are < 1.0 nmol L⁻¹ h⁻¹ (Montoya et al. 2004; Luo et al. 2012; Loick-Wilde et al. 2016). It is unclear how frequent and under which circumstances extremely high N2 fixation rates occur in the field (Voss et al. 2013).

In contrast to other marine primary producers like diatoms and dinoflagellates, cyanobacteria are often regarded as a dead-end for the transfer of organic matter into higher trophic levels (reviewed by Carpenter and Capone 2008). The resistance of cyanobacteria against zooplankton grazing is usually attributed to their toxicity, large size, and poor nutritional quality (Paerl 1988; Sellner 1997). The prevailing view is that cyanobacteria blooms have to decay or be lysed by viruses before their diazotrophic nitrogen is released as ammonium or dissolved organic nitrogen (DON; primarily as amino acids.
which can be incorporated into the microbial food web and then transferred into higher trophic levels like zooplankton or fish (summarized by Wannicke et al. 2009). However, direct exudation of AAs by cyanobacteria also occurs and interestingly DON exudation was found to relate positively to volumetric total (or bulk) N2 fixation rates in laboratory and field studies (Mulholland and Bernhardt 2005 and references therein). It is currently unresolved if volumetric bulk N2 fixation rates may allow for a first order estimation of the availability of diazotroph AAs for the ambient food webs.

Rates of N2 fixation have been measured in marine systems since the early 1960s and bulk N2 fixation measurements are now a routine component of biogeochemical research. Pioneering studies used gas chromatography mass spectrometry and later elemental analyzers coupled to isotope ratio mass spectrometers (EA-IRMS) to estimate total nitrogen fixation rates from short term 15N2 incubations with minimum disruption of the system (Montoya et al. 1996). Although there is no doubt that our understanding of diazotroph nitrogen inputs to food webs and ecosystems has been greatly advanced by the use of bulk N2 fixation measurements, one major limitation is that these experiments cannot address which AAs are synthesized during N2 fixation and how the turnover times of individual AA pools may change along with bulk N2 fixation rates.

With the development of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) techniques, it is now possible to measure compound-specific stable isotope ratios on very small samples (Macko et al. 1997; Meier-Augenstein 1999). One of the targets of
GC-C-IRMS is isotopic analysis of individual AAs. With this approach, fascinating details of diet and consumer relationships (Steffan et al. 2015), marine organic nitrogen cycling (McCarthy et al. 2013), and changes of nitrogen sources on geological time scales (Sherwood et al. 2011) have been revealed. Biogeochemical studies using GC-C-IRMS to examine N isotope values of individual AAs for the estimation of amino acid nitrogen (AAN) synthesis and turnover during N₂ fixation have not yet (to the best of our knowledge) been published.

In this paper, we examine the de novo synthesis of 13 AAs during bulk N₂ fixation using a combination of bulk and AA-specific nitrogen stable isotopes. Our primary research objective was to quantify the synthesis rates and turnover times of the majority of proteinogenic AAs during N₂ fixation, and to relate them to bulk N₂ fixation rates. Our ship-based experiments explicitly focused on the changes in individual de novo AAN synthesis rates and related turnover times in relation to bulk N₂ fixation rates by cyanobacteria from the central Baltic Sea in July 2015. Comparisons among rates from four stations provided examples from a wide range of bulk N₂ fixation rates and contrasting bloom situations in the central Baltic Sea. The cyanobacteria bloom at the easternmost station was in a very late, decaying stage, while the blooms at the three westernmost stations were at earlier, active stages of growth.

Material and methods

Samples were collected at the Baltic Sea Monitoring Stations (Sta.) 109, 213, 259, and 271 in the central Baltic Sea on RV Meteor between 25 July 2015 and 31 July 2015 (Fig. 1). Hydrographic variables were measured using a Seabird 911 conductivity, temperature, depth (CTD) equipped with sensors for conductivity, temperature, pressure, oxygen, and fluorescence. Inorganic nutrient concentrations were measured after Rhode and Nehring (1979) and after Grasshoff et al. (1983). The detection limit and precision are 0.02 µM for phosphate, 0.05 µM for ammonium, 0.05 µM for NO₃, and 0.02 µM for NO₂. Particulate organic carbon (POC) and nitrogen (PON) concentrations, as well as dissolved organic carbon (DOC) and DON concentrations were determined for standard depths according to HELCOM (2009) and Sugimura and Suzuki (1988).

Chlorophyll a (Chl a) and phytoplankton samples were collected from the mixed layer (ML) or below (mixed sample from 1 m, 5 m, and 10 m, and one discrete sample from 20 m depth) and analyzed following HELCOM (2015) protocols. Only intact and pigmented cells were included in the phytoplankton cell-counts and cell-carbon estimations.

N₂ fixation rates into PON and into 13 AAs were measured according to Montoya et al. (1996) with modifications (Fig. 2) using ¹⁵N₂ gas from Cambridge (98% ¹⁵N₂, Lot 1–16727, which was uncontaminated by other ¹⁵N-dissolved inorganic nitrogen species according to Dabundo et al. 2014). We carried out triplicate incubations in 4.6 L Nalgene bottles using water from the surface (Sta. 109, 213), from 10 m depth (Sta. 213, 259, 271), and from 20 m depth (Sta. 109). We note that our estimates of N₂ fixation may be conservative due to the time required for equilibration of ¹⁵N₂ gas with the incubation medium (Dabundo et al. 2014). Our experimental bottles were incubated under simulated in situ conditions on deck using neutral density screening and running surface seawater for cooling for 12 h, 24 h, and 48 h at Sta. 271, and for 24 h at Sta. 109, 213, and 259. Incubations were then terminated by vacuum filtration (200 mbar) of 500 mL samples onto pre-combusted glass fiber filters (GFF, approximately 0.7 µm pore size, 25 mm) for the estimation of bulk N₂ fixation rates. For N₂ fixation into the AAN of 13 AAs, the residual 4100 mL of
each sample was filtered onto preweighted Nuclepore filters (0.8 μm pore size, 45 mm) using low pressure filtration (10–15 psi), then shock frozen in liquid nitrogen before storage at −20°C at sea and during transport. Nuclepore and GFF filter samples were subsequently freeze dried for 48 h and samples on Nuclepore filters were weighed.

Elemental concentrations (total PON and POC), total hydrolysable amino acid (THAA) concentrations, and bulk and AA specific nitrogen stable isotope abundances of POM and experimental samples were measured by EA-IRMS, GC-MS, and GC-C-IRMS following standard protocols as in Loick et al. (2007) and Hofmann et al. (2003) with the modifications described by Veuger et al. (2005). EA-IRMS analyses quantified and determined the total organic nitrogen and carbon concentrations (pmol L⁻¹) from 13 individual AAs in nmol L⁻¹. THAA concentrations (pmol L⁻¹) were determined by GC-MS and GC-C-IRMS analyses quantified the concentrations and nitrogen isotope composition of isopropyl-TFA-derivatives of 13 AAs including glutamic acid + glutamine (Glu + Gln), aspartic acid + asparagine (Asp + Asn), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), threonine (Thr), serine (Ser), valine (Val), phenylalanine (Phe), alanine (Ala), proline (Pro), glycine (Gly), and lysine (Lys). During the initial hydrolysis, glutamine and asparagine are converted to glutamic and aspartic acids, respectively, so our measurements of the concentrations and nitrogen isotope composition of glutamic and aspartic acids include contributions from glutamine and asparagine and we will refer to these mixtures as Glu + Gln and Asp + Asn, respectively. Details of the EA-IRMS, GC-MS, and GC-C-IRMS analyses are given in the Supporting Information Section S1.

This study allowed us to calculate the flux of ¹⁵N-tracers from ¹⁵N₂ into PON and into AAN of phytoplankton samples dominated by diazotrophic cyanobacteria, primarily by *Nodularia spumigena*. The incorporation of nitrogen (N) from N₂ into the PON and the total hydrolysable amino acid nitrogen (THAA) and individual hydrolysable AAN pools of phytoplankton can be expressed as a specific rate V (h⁻¹) or as a volumetric rate ρ based on either the PON concentration (nmol L⁻¹ h⁻¹), total THAA concentration (sum of AAN from 13 individual AAs in nmol L⁻¹ h⁻¹), or individual AAN concentrations (pmol L⁻¹ h⁻¹) of the samples (Fig. 2). Specific rates and volumetric rates of ¹⁵N fixation into PON (also termed “bulk” rates) were calculated after Montoya et al. (1996). For the calculation of specific rates (V) into the nonessential AAs Glu + Gln, Asp + Asn, Ala, Gly, Ser, and Pro, as well as into the essential AAs Lys, Leu, Ile, Val, Thr, Tyr (semi-essential), and Phe (Guillaune 1997), we modified the mass balance approach of Montoya et al. (1996) as follows:

\[ V \left[ T^{-1} \right] = \frac{1}{\Delta t} \times \left( \frac{A_{AANt} - A_{AAN0}}{A_{N2} - A_{AAN0}} \right) \]  

(1)

where Δt is the duration of the incubation, \( A_{N2} \) is the ¹⁵N enrichment of the N₂ available for fixation, \( A_{AAN0} \) the ¹⁵N enrichment of amino acid N at the start of the experiment, \( A_{AANt} \) is the ¹⁵N enrichment of AAN at timepoint tx. The inverse of V provides an estimate of the turnover time for AAN solely on the basis of the input due to N₂ fixation and is a measure of how long it takes to turn over the pool of an AA given that input, which makes it a good measure of how dynamic a pool of AAs is. Note that for specific rate calculations into THAAN, the molar-weighted sum of individual ¹³⁵N values from the 13 AAs was used as estimates of the initial and final ¹⁵N enrichment of each AA (McCarthy et al. 2013).

The volumetric rate of N₂ fixation (ρ) into AAN is simply:

\[ \rho (N L^{-1} T^{-1}) = V \times [A_{AANt}] \]  

(2)

where \([A_{AANt}]\) is the concentration of AAN in the sample at timepoint tx. Note that for volumetric rate calculations into the THAAN pool, the sum of AAN from the 13 individual AAs was used. Specific and volumetric rates of N₂ fixation into AAN and THAAN were calculated for all samples and all time points.

We analyzed the community structure and similarity of the species/taxon-specific cell-carbon biomasses to identify differences in the phytoplankton communities between stations. The similarities were analyzed by means of SIMPER (similarity percentages) and the community structure by means of cluster analysis. Both, the SIMPER and the cluster analysis were based on the Bray-Curtis distance between the cell-carbon biomasses of the individual microplankton species/taxa from individual samples (0–10 m and 20 m) at each station. All community analyses were analyzed using PRIMER-6 Software (Primer-E, UK).

**Results**

**Environmental conditions in the central Baltic Sea in July 2015**

In July 2015, the ML in the central Baltic Sea was between 18 m and 28 m deep with a sea surface temperature around 16°C (Fig. 3) and a decrease in sea surface salinity from west to north-east from 7.9 (Sta. 109) to 6.8 (Sta. 271). At the three western stations (Sta. 109, 213, and 259), low yet detectable ML concentrations of phosphate >0.20 μmol L⁻¹ occurred with a local maximum of 0.76 μmol L⁻¹ at 10 m depth at the westernmost Sta. 109 (Fig. 3A–C). In contrast, nitrate plus nitrite was at the detection limit at the easternmost Sta. 271 (Fig. 3D). Nitrate plus nitrite (around 0.30 μmol L⁻¹) as well as ammonium (around 0.30 μmol L⁻¹) concentrations were low yet detectable in the ML at all stations (Fig. 3).

Fluorescence in the Chl a range was maximal and in a similar range in the surface MLs at all stations (Fig. 3), which was confirmed by discrete Chl a measurements (M. Nausch pers. comm.). In contrast, POC and PON concentrations in the surface ML were higher at Sta. 271 (POC: 39 ± 1 μmol L⁻¹; PON: 5.2 ± 0.2 μmol L⁻¹) compared to the other three stations (POC: 28 ± 3 μmol L⁻¹; PON 3.6 ± 0.5 μmol L⁻¹, Fig. 4A,B).

Based on the DOC and DON profiles, the average DOC concentration in the surface waters at all stations was...
DON concentrations in the ML were around 16 $\mu$mol L$^{-1}$ at Sta. 109, 259, and 271, and had a maximum of 19.8 $\mu$mol L$^{-1}$ at Sta. 213 (Fig. 4C,D).

The cluster analysis of the phytoplankton cell-carbon biomass distribution revealed that the phytoplankton community structure in the ML and at 20 m was similar by 57% at

Fig. 3. Temperature (°C), salinity, nutrients ($\mu$mol L$^{-1}$), and relative Chl $a$ profiles at four central Baltic Sea stations (A) 109, (B) 213, (C) 259, (D) 271 in July 2015.

Fig. 4. (A) POC, (B) PON, (C) DOC, and (D) DON profiles (all in $\mu$mol L$^{-1}$) at four central Baltic Sea stations in July 2015.

$316 \pm 7 \; \mu$mol L$^{-1}$. DON concentrations in the ML were around 16 $\mu$mol L$^{-1}$ at Sta. 109, 259, and 271, and had a maximum of 19.8 $\mu$mol L$^{-1}$ at Sta. 213 (Fig. 4C,D).
Sta. 213, 259, and 271 (Supporting Information Table 1 and Supporting Information Fig. S1). The phytoplankton community in the upper 10 m at Sta. 109 had a lower similarity of 50% with the communities at Sta. 213, 259, and 271 mainly due to the relatively higher cell-carbon biomass of the diazotroph cyanobacterium *Aphanizomenon* sp. At Sta. 109 at 20 m depth, the similarity was only 40% with the phytoplankton cell-carbon biomass distribution at the other sites mainly due to the low cell-carbon biomass of cyanobacteria in general (e.g., *N. spumigena* was absent), and due to a high cell-carbon biomass of the dinoflagellate *Dinophysis norvegica*.

Surface-water biomass (0–10 m) was dominated by diazotrophic cyanobacteria cell-carbon at all stations (51%±4% of total cell-carbon), with a maximum of 46.7 μg L⁻¹ at Sta. 109 and an average of 31.7 ± 1.6 μg L⁻¹ at Sta. 213, 259, and 271. At 20 m depth, cyanobacteria cell-carbon biomass was high at Sta. 213, 259, and 271 (44%±2% of total cell-carbon), while at Sta. 109 cyanobacteria biomass was low (8% of total cell-carbon).

At Sta. 109, *N. spumigena* and *Aphanizomenon* sp. co-dominated the surface cyanobacteria community (45% and 46% of the cyanobacteria-specific cell-carbon, respectively). At Sta. 259 and 271, *N. spumigena* dominated the cyanobacteria community by 53%±5% and *Aphanizomenon* sp. contributed 12%±7% of the cyanobacteria-specific cell-carbon in the upper 10 m. At Sta. 213, *N. spumigena* and *Aphanizomenon* sp. biomasses were lower with 22% and 15% of the cyanobacteria-specific cell-carbon, respectively. At this station, the unicellular, diazotrophic cyanobacteria *Aphanocapsa* sp. and *Aphanothece* sp. together accounted for 40% of the cyanobacteria-specific cell-carbon whereas their biomasses were much lower at the other stations (12%±7% of cyanobacteria-specific cell-carbon).

An additional important, qualitative difference between the stations was that at the three western stations (Sta. 109, 213, and 259) the *N. spumigena* cells in surface waters (0–10 m) appeared largely intact and healthy (Fig. 5A–C,E–G) and were associated with higher overall cell-carbon mole% POC (Supporting Information Fig. S2). In contrast, samples from the easternmost Sta. 271 included many empty, pale, and broken *N. spumigena* cells within the colonies, many with epiphytes like the diatom *Nitzschia paleacea* attached to them (Fig. 5D,H), and were associated with lower cell-carbon mole% POC (Supporting Information Fig. S2).

**AA synthesis and turnover during N₂ fixation**

Consistent with the POM profiles at the four stations (Fig. 4A,B), the POM samples used for the ¹⁵N₂ fixation experiments showed higher PON (Fig. 6A) and POC (not shown) concentrations at Sta. 271 compared to the other three stations. The molar ratios of the individual AAs to THAA were similar in all samples (Supporting Information Fig. S3A), whereas the absolute concentrations of AAs were variable among replicates and stations (Supporting Information Fig. S3B). Highest concentrations were always found for Asp and Asn, and lowest for Tyr (Supporting Information Fig. S3B). Importantly, the THAAN mole% PON values were significantly lower (Student’s *t*-test, *p* < 0.001) at Sta. 271 (29 ± 4 mole% PON) compared to the other three stations where THAAN made up the majority of PON (64 ± 14 mole% PON, Fig. 6B).

The development of the bulk PON, individual AAN, and averaged THAAN δ¹⁵N values from all experiments and time points are shown in the Supporting Information Table 2.
15N2 fixation substantially increased the bulk d15N PON values in the samples from all stations above the natural d15N PON value range of 1.6–3.6 at time t0 to a maximum d15N PON value of 1061±306% after 48 h during the extended incubations at Sta. 271 (Fig. 7A, Supporting Information Table 2). The bulk PON d15N and individual AA d15N values revealed that Glu+Gln and Asp+Asn were the most enriched in 15N (primary AAs, Fig. 7). The pool of bulk PON compounds as well as most individual AAs showed lower 15N-enrichments compared to the primary AAs in most experiments (Fig. 7). In a few instances, the d15N value of PON was as high as the d15N values in the primary AAs pools, which was most prominently visible in a sample from Sta. 271 after 48 h of incubation (Fig. 7A). For the most part, Ile was almost as enriched in 15N as the primary AAs, while bulk PON, Gly, and Lys were considerably less enriched in 15N-label relative to Glu+Gln and Asp+Asn. These trends are clearly indicated by regressions of individual AA labeling as a function of the labeling of Glu+Gln. According to McCarthy et al. (2013), the slopes of these regressions
of $^{15}$N$_2$ incorporation into AAs were found in the ML either 0.0003 h$^{-1}$. The analytical error associated with the specific rate measurements was 0.03 h$^{-1}$.

Table 1. Specific rates $V$ (mean ± SD × 10$^{-3}$ h$^{-1}$) of $^{15}$N$_2$ incorporation into PON, THAAN (averaged from 13 AAs), and into nitrogen of 13 individual amino acids (AAN) during N$_2$ fixation experiments in the central Baltic Sea in July 2015. Note that there were no replicates for Sta. 109 at 20 m and only two for Sta. 259 at 10 m. The analytical error associated with the specific rate measurements was 0.03 × 10$^{-3}$ h$^{-1}$.

| Station Depth (m) | 109 | 213 | 259 | 271 |
|------------------|-----|-----|-----|-----|
|                   | 3.5 | 20  | 3.5 | 10  | 10  | 10  | 10  | 10  |
| Inc. time (h)     | 24  | 12  | 24  | 48  |     | 24  | 12  |     |     |
| Bulk PON ($\times$ 10$^{-3}$ h$^{-1}$) | 1.8 ± 0.1 | 0.3 | 0.5 ± 0.1 | 0.8 ± 0.2 |     | 0.7 ± 0.1 | 2.2 ± 0.5 | 1.2 ± 0.3 | 1.1 ± 0.3 |
| THAAN ($\times$ 10$^{-3}$ h$^{-1}$) | 2.3 ± 0.2 | 0.2 ± 0.3 | 0.6 ± 0.1 | 0.8 ± 0.2 |     | 1.1 ± 0.1 | 2.9 ± 0.3 | 1.6 ± 0.2 | 1.3 ± 0.2 |
| AAN ($\times$ 10$^{-3}$ h$^{-1}$) |     |     |     |     |     |     |     |     |     |
| Glu + Gln         | 2.9 ± 0.2 | 0.4 | 0.7 ± 0.2 | 1.1 ± 0.2 | 1.4 ± 0.0 | 3.8 ± 0.5 | 2.1 ± 0.2 | 1.6 ± 0.1 |
| Asp + Asn         | 2.9 ± 0.2 | 0.4 | 0.7 ± 0.1 | 1.0 ± 0.2 | 1.3 ± 0.0 | 3.5 ± 0.3 | 1.8 ± 0.2 | 1.5 ± 0.1 |
| Ile               | 2.6 ± 0.1 | 0.4 | 0.7 ± 0.2 | 1.1 ± 0.2 | 1.3 ± 0.0 | 3.0 ± 0.4 | 1.8 ± 0.2 | 1.5 ± 0.1 |
| Leu               | 2.4 ± 0.2 | 0.4 | 0.6 ± 0.1 | 0.9 ± 0.2 | 1.1 ± 0.0 | 2.9 ± 0.4 | 1.7 ± 0.2 | 1.4 ± 0.1 |
| Tyr               | 2.2 ± 0.2 | 0.3 | 0.6 ± 0.1 | 0.8 ± 0.2 | 1.0 ± 0.0 | 2.6 ± 0.4 | 1.6 ± 0.2 | 1.3 ± 0.1 |
| Thr               | 2.1 ± 0.2 | 0.3 | 0.5 ± 0.1 | 0.8 ± 0.2 | 1.0 ± 0.0 | 2.6 ± 0.3 | 1.6 ± 0.2 | 1.3 ± 0.1 |
| Ser               | 2.2 ± 0.2 | 0.3 | 0.5 ± 0.1 | 0.8 ± 0.2 | 0.9 ± 0.0 | 2.5 ± 0.4 | 1.5 ± 0.2 | 1.3 ± 0.1 |
| Val               | 2.2 ± 0.2 | 0.3 | 0.6 ± 0.1 | 0.8 ± 0.2 | 1.1 ± 0.0 | 2.5 ± 0.3 | 1.5 ± 0.2 | 1.3 ± 0.1 |
| Phe               | 2.1 ± 0.1 | 0.3 | 0.5 ± 0.1 | 0.7 ± 0.2 | 0.9 ± 0.0 | 2.4 ± 0.3 | 1.5 ± 0.2 | 1.3 ± 0.1 |
| Ala               | 2.1 ± 0.2 | 0.3 | 0.5 ± 0.1 | 0.8 ± 0.2 | 0.9 ± 0.0 | 2.4 ± 0.3 | 1.5 ± 0.2 | 1.2 ± 0.1 |
| Pro               | 2.0 ± 0.1 | 0.3 | 0.5 ± 0.1 | 0.6 ± 0.1 | 0.8 ± 0.0 | 2.4 ± 0.2 | 1.4 ± 0.1 | 1.2 ± 0.1 |
| Gly               | 2.0 ± 0.2 | 0.3 | 0.4 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.0 | 2.2 ± 0.3 | 1.3 ± 0.2 | 1.1 ± 0.1 |
| Lys               | 1.8 ± 0.1 | 0.3 | 0.4 ± 0.1 | 0.7 ± 0.1 | 0.8 ± 0.0 | 2.1 ± 0.3 | 1.2 ± 0.2 | 1.0 ± 0.1 |

Ala, alanine; Asp + Asn, aspartic acid + asparagine; Glu + Gln, glutamic acid + glutamine; Gly, glycine; Ile, isoleucine; Inc. time, incubation time; Leu, leucine; Lys, lysine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.

indicate how much N in the product (AA) came from the precursor (Glu + Gln or additionally ammonium in case of Asn, Riccardi et al. 1989), while $c$ are the Y-intercept values that are interpreted as total fractionation coefficients. Accordingly, if all N in the product originated from the precursor, then a slope of 1 would be expected, while the Y-intercept represents an estimate of the $\delta^{15}$N fractionation between Glu + Gln and each AA produced from Glu + Gln (McCarthy et al. 2013). We found a slope of 0.89 for Ile and slopes of 0.70 and 0.63 for Gly and Lys, respectively, while the lowest slope of 0.50 was found for bulk PON (Fig. 7 and Supporting Information Table 2). The average slope from all AAs was 0.8 (Supporting Information Table 3), which is a typical value for cyanobacteria (McCarthy et al. 2013).

The specific rates of $^{15}$N$_2$ incorporation into the individual AA pools ranged over an order of magnitude from 0.0003 h$^{-1}$ to 0.0029 h$^{-1}$ (Table 1). The highest specific rates of $^{15}$N$_2$ incorporation into AAs were found in the ML either at the westernmost Sta. 109, or at the easternmost Sta. 271 after 12 h of incubation (Table 1). At both sites, filamentous cyanobacteria dominated the diazotroph cell-carbon biomass. The lowest specific rates of $^{15}$N$_2$ incorporation into AAs was found either at a depth with low diazotroph cell-carbon biomass (in 20 m depth at Sta. 109) or where unicellular diazotrophs largely contributed to the cell-carbon biomass of cyanobacteria (in 3.5 m depth at Sta. 213).

The specific rates and turnover times from the time-series incubations at Sta. 271 revealed that $^{15}$N$_2$ incorporation slowed down with time (Tables 1, 2). Interestingly, the relative specific rates and turnover times of the individual AAs were not affected by this and were similar throughout the study area including Sta. 271 (Tables 1, 2). Generally, $^{15}$N was incorporated fastest into Glu + Gln, Asp + Asn, and Ile, while the slowest incorporation of $^{15}$N was into Pro, Gly, and Lys (Table 1). Intermediate rates were found for Leu, Tyr, Thr, Ser, Val, Phe, and Ala (Table 1). This resulted in faster turnover times of the Glu + Gln, Asp + Asn, and Ile pools compared to those of Gly, and Lys (Table 2, Supporting Information Fig. S4) and altogether encompassed a large range of THAAN turnover times of 15±2 d to 122 d (Table 2). This pattern was also reflected in the slopes of the linear regression of the individual AA turnover times vs. the turnover time of Glu + Gln. The AAs with faster turnover times (i.e., Asp + Asn and Ile) had slopes close to 1, whereas AAs with much slower relative turnover times had slopes > 1 (e.g., 1.58 for Gly and 1.56 for Lys; Table 3 and Supporting Information Fig. S4). In consequence, the Glu + Gln pool was turned over approximately 60% faster than the Lys pool (based on the turnover times in Table 2).

The volumetric N$_2$ fixation rates into individual AAs largely followed the AAN concentrations of individual AAN with highest relative rates for Asp + Asn, Glu + Gln, Ala, Lys,
**Table 2.** Turnover times (mean ± SD in days) of bulk PON and of 13 AAs during N2 fixation experiments in the central Baltic Sea in July 2015. Abbreviations as in Table 1. Note that there were no parallels for Sta. 109 at 20 m and only two for Sta. 259 at 10 m.

| Station | 109  | 213  | 259  |
|---------|------|------|------|
| Inc. time (h) | 3.5  | 20   | 3.5  |
| Bulk PON (d) | 23 ± 1 | 146  | 58 ± 14 |
| THAAN (d) | 18 ± 1 | 122  | 52 ± 10 |

| Station | 109  | 213  | 259  |
|---------|------|------|------|
| Depth (m) | 10   | 10   | 10   |
| Inc. time (h) | 24   | 12   | 24   |
| Bulk PON (d) | 87 ± 14 | 58 ± 14 | 58 ± 7 |
| THAAN (d) | 78 ± 18 | 52 ± 10 | 37 ± 4 |

**Table 3.** Linear regression results for individual and total hydrolysable amino acid (THAAN) turnover times as a function of the turnover time of Glu + Gln from eight N2 fixation experiments at four stations in the central Baltic Sea in July 2015 (Supporting Information Fig. S4). Regression statistics for turnover times of individual AA vs. turnover time of Glu + Gln across all N2 fixation experiments from all stations. All regression relationships tested were statistically significant (Prob > F < 0.05). The slope indicates how fast N in the product (AA) turns over relative to Glu + Gln, while c (the Y-intercept value) is interpreted as the total fractionation coefficient. Accordingly, if all N in a given AA has the same turnover time as Glu + Gln, then a slope of 1 would be expected, while a slower turnover time would produce a slope of > 1. See text for more details. Abbreviations as in Table 1.

| Glu + Gln vs. | r² | Slope | c  | Prob > F |
|---------------|----|-------|----|----------|
| Asp + Asn     | 0.9969 | 0.98 | 2.73 | <3.7E-20 |
| Ile           | 0.9995 | 0.93 | 3.95 | <8.7E-19 |
| Leu           | 0.9986 | 1.15 | 1.85 | <3.1E-21 |
| Tyr           | 0.9982 | 1.22 | 3.42 | <5.6E-22 |
| Thr           | 0.9973 | 1.33 | 1.46 | <1.7E-19 |
| Ser           | 0.9988 | 1.36 | -0.05 | <4.9E-20 |
| Val           | 0.9990 | 1.22 | 2.57 | <2.8E-19 |
| Phe           | 0.9985 | 1.44 | 0.03 | <8.6E-19 |
| Ala           | 0.9971 | 1.34 | 2.87 | <5.6E-20 |
| Pro           | 0.9953 | 1.49 | 2.18 | <1.3E-18 |
| Gly           | 0.9968 | 1.58 | 1.86 | <2.9E-20 |
| Lys           | 0.9995 | 1.56 | 2.54 | <4.5E-19 |
| THAAN (average) | 0.9980 ± 0.0012 | 1.30 ± 0.2 | — | — |

and Gly, intermediate relative rates for Leu, Val, Ile, Thr, and Ser, and very low relative rates for Phe, Pro, and Tyr (Table 4).

The THAA and POM experienced relatively consistent labeling of 15N as indicated by the near 1:1 ratio of THAAN and PON δ15N signatures (Fig. 8A), the specific rates of 15N2 incorporation (Fig. 8B), and N pool turnover times (Fig. 8C). However, we also noticed a small but consistent departure from this relationship at higher N2 fixation rates, with higher labeling of THAAN relative to PON (Fig. 8A) and
higher specific rates of $^{15}$N incorporation into AAs than into PON (Fig. 8B).

The close relationship between the $^{15}$N$_2$ incorporation into PON and into THAAN was also reflected in a nearly 1 : 1 relationship between the volumetric rates of $^{15}$N$_2$ incorporation into PON and into THAAN at the three westernmost stations (Fig. 8D). Notably, at the easternmost Sta. 271, a large offset from the 1 : 1 ratio (Fig. 8D) was associated with a low THAAN mole% PON share at Sta. 271 (Fig. 6B) and a volumetric N$_2$ fixation rate into THAAN that was 63% ± 2% lower than the volumetric N$_2$ fixation rate into PON (Table 4).

**Discussion**

AAs from de novo synthesis during N$_2$ fixation are essential macronutrients for food webs in large parts of the oligotrophic global ocean (Mulholland et al. 2006). Despite their critical role for these heterotrophic food webs (Suttle et al. 1991; Capone et al. 1994), their synthesis rates and turnover times during N$_2$ fixation have hitherto remained elusive.

Ammonium is the first product of N$_2$ fixation, and there are only three AAs—glutamic acid (Glu), glutamine (Gln), and asparagine (Asn)—that receive their nitrogen directly from diazotroph ammonium during their synthesis (Riccardi et al. 1989). All other AAs and nitrogenous compounds in a cell receive their nitrogen from these three AAs (e.g., Asp receives its N from Glu and Asn, Riccardi et al. 1989). Thus, it was not surprising that the pools of Glu + Gln and Asp + Asn were the most highly labeled in all experiments, but it also implies that the duration of the experiments was short relative to the turnover time of N in AAs (Table 2). The other AAs as well as the numerous, unidentified nitrogenous compounds that comprise the bulk PON pool had lower enrichments in $^{15}$N compared to these primary Asp + Asn and Glu + Gln pools, but in relatively constant ratios (Fig. 7, Supporting Information Table 3). The transfer of $^{15}$N into bulk PON was more variable than $^{15}$N movement into the individual AA pools, as indicated by the higher scatter of $^{15}$N PON values (Fig. 7A). In some cases (e.g., at Sta. 271 after 48 h of $^{15}$N$_2$ fixation), the high $\delta^{15}$N of the PON seemed to directly reflect the high $\delta^{15}$N values of the primary AA pools (Fig. 7A), which suggests that the system was at or near isotopic equilibrium with extensive transfer of $^{15}$N to other nitrogen pools as discussed in more detail below. However, the low slope of 0.50 of the linear regression between the bulk $\delta^{15}$N values vs. the $\delta^{15}$N values of the Glu + Gln pools (Fig. 7A, Supporting Information Table 3) suggests that at least 50% of the PON still was of nondiazotrophic or detrital origin.

The synthesis of AAs is highly regulated in cyanobacteria (Riccardi et al. 1989), which can explain the similar relative synthesis rates and turnover times of individual AA pools during different $^{15}$N$_2$ fixation intensities. Consequently, the

Table 4. Volumetric rates $\rho$ (mean ± SD) of $^{15}$N incorporation during N$_2$ fixation into bulk PON, into the sum of nitrogen from 13 AAs (THAAN), and into the individual AAN from $^{15}$N$_2$ fixation experiments in the central Baltic Sea in July 2015. AA and other abbreviations as in Table 1. At Sta. 271, bulk rates (in italics) reflect gross rather than net N$_2$ fixation (see text for details). Note that there were no replicates for Sta. 109 at 20 m and only two for Sta. 259 at 10 m.

| Station Depth (m) | 109 | 213 | 259 | 271 |
|------------------|-----|-----|-----|-----|
| Inc. time (h)    | 24  | 24  | 24  | 24  |
| Bulk PON (nmol L$^{-1}$ h$^{-1}$) | 8.5 ± 0.3 | 1.6 ± 0.3 | 2.9 ± 0.3 | 2.7 ± 0.4 |
| THAAN (nmol L$^{-1}$ h$^{-1}$)     | 7.0 ± 1.9 | 1.2 ± 0.2 | 2.7 ± 0.4 | 3.6 ± 0.7 |
| AAN (nmol L$^{-1}$ h$^{-1}$)       |        |      |      |      |
| Asp + Asn        | 1481 ± 372 | 224 ± 48 | 520 ± 166 | 740 ± 116 |
| Glu + Gln        | 819 ± 223 | 140 ± 22 | 337 ± 101 | 542 ± 71  |
| Ala              | 813 ± 195 | 164 ± 22 | 231 ± 36  | 427 ± 108 |
| Lys              | 633 ± 183 | 99 ± 19  | 337 ± 23  | 243 ± 47  |
| Gly              | 595 ± 158 | 110 ± 13 | 182 ± 68  | 297 ± 58  |
| Leu              | 581 ± 155 | 100 ± 19 | 135 ± 40  | 277 ± 68  |
| Val              | 477 ± 129 | 68 ± 35  | 110 ± 74  | 216 ± 50  |
| Ile              | 396 ± 115 | 57 ± 32  | 115 ± 34  | 175 ± 35  |
| Thr              | 345 ± 97  | 47 ± 19  | 106 ± 33  | 171 ± 32  |
| Ser              | 267 ± 78  | 39 ± 10  | 112 ± 37  | 104 ± 15  |
| Phe              | 233 ± 64  | 36 ± 8   | 75 ± 37   | 103 ± 20  |
| Pro              | 194 ± 50  | 36 ± 6   | 72 ± 27   | 80 ± 19   |
| Tyr              | 114 ± 57  | 15 ± 4   | 36 ± 15   | 31 ± 5    |

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AA synthesis and turnover during N$_2$ fixation
synthesis of the 13 proteinogenic AAs largely explained total organic nitrogen synthesis during N2 fixation (Fig. 8) despite somewhat different diazotroph communities at the four stations. Any deviation from the 1 : 1 relationship between bulk and THAAN $\delta^{15}$N values, specific rates $V$ of $^{15}$N2 incorporation (mean ± SD $10^3$ h$^{-1}$), turnover times (d), and volumetric rates $\rho$ of $^{15}$N2 incorporation (mean ± SD in nmol L$^{-1}$ h$^{-1}$) in particulate organic matter including mainly intact (Sta. 109, 213, 259) or decayed (Sta. 271) N. spumigena cells from the central Baltic Sea stations in July 2015. The dashed lines indicate 1 : 1 relationships. The precision of the natural abundance measurements is given in permil and was 0.2‰ for bulk and 1.0‰ for AAN. The precision for the enriched samples is given in percent and was better than 1% for the labeled measurements.

Fig. 8. Particulate THAAN vs. bulk PON for (A) $\delta^{15}$N values (mean ± SD ‰ vs. N2, THAAN values as molar-weighted sum of 13 AAs), (B) specific rates $V$ of $^{15}$N2 incorporation (mean ± SD $10^3$ h$^{-1}$), (C) turnover times (d), and (D) volumetric rates $\rho$ of $^{15}$N2 incorporation (mean ± SD in nmol L$^{-1}$ h$^{-1}$) in particulate organic matter including mainly intact (Sta. 109, 213, 259) or decayed (Sta. 271) N. spumigena cells from the central Baltic Sea stations in July 2015. The dashed lines indicate 1 : 1 relationships. The precision of the natural abundance measurements is given in permil and was 0.2‰ for bulk and 1.0‰ for AAN. The precision for the enriched samples is given in percent and was better than 1% for the labeled measurements.

The differences in the magnitude of the observed protein turnover times (Table 2) might reflect differences in the cyanobacterial community composition between the stations. Fastest AA turnover during N2 fixation was found at stations where filamentous cyanobacteria like N. spumigena and Aphanizomenon sp. dominated or co-dominated the cyanobacteria community. Slowest turnover was found either where cyanobacteria cell-carbon biomass was low or where unicellular diazotrophs formed the majority of the cyanobacterial cell-carbon biomass. Tentatively, yet statistically not significant due to large standard deviations, low turnover times were also reflected in lower AA concentrations (Supporting Information Fig. S3B). Species-specific differences in AA turnover times during N2 fixation remain to be tested in more detail, but may be particularly important when comparing N2 fixation from different ecosystems like river plumes, oceanic, or mesopelagic waters.

An unexpected outcome of this study was the very low contribution of THAAN to PON at Sta. 271 (< 30 mole%, Fig. 6B), which confirmed the visibly degraded state of the N. spumigena community (Fig. 5D, H). PON dominated by undecayed phytoplankton cells typically contains 50%
to >90% AAN, while an AA content below ∼40% is a common indicator of a highly degraded state of marine particulate organic material (summarized by Cowie and Hedges 1994). N. spumigena cells, like Trichodesmium cells, can accumulate and decay in surface waters due to their gas vesicles (Capone et al. 1997; Ploug 2008), meaning that PON from a decaying N. spumigena bloom is thus comprised of biomass from intact cells as well as of detritus from dead cells. This combination is otherwise predominantly seen in sinking particles at greater depths of the water column (Wakeham and Lee 1988) or in marine sediments (Cowie and Hedges 1994; Lomstein et al. 2012). The higher share of dead or decaying N. spumigena cells at Sta. 271 was also reflected in higher PON and POC concentrations relative to Chl a and to cell-carbon concentrations in comparison to the other three stations. In consequence, the volumetric bulk N2 fixation rates into PON differed significantly from the volumetric rates of N2 fixation into THAAN at Sta. 271 (Fig. 8D). The mismatch between N2 fixation into PON at Sta. 271 (e.g., 15.7 ± 4.3 nmol L\(^{-1}\) h\(^{-1}\) at \(t_{12}\)) and into THAAN (e.g., 6.1 ± 1.3 nmol L\(^{-1}\) h\(^{-1}\) at \(t_{12}\), Table 4) presents a clear contrast to the other stations, where THAAN represented the majority of PON in the blooms of undecayed cyanobacteria cells and where volumetric rates of fixation into PON and into THAAN were very similar (Fig. 8D; Table 4). Given the state of the bloom at Sta. 271, with high detrital concentrations and many moribund and dead cells, this mismatch may reflect a decoupling between N2 fixation and biomass synthesis, with potentially significant rates of release of fixed N (namely as \(^{15}\)N-ammonium) by the metabolically active cells. We note that the volumetric rates of N2 fixation into PON and into THAAN become more similar with increasing incubation time, suggesting that exuded N was being assimilated and incorporated into AAs, most likely by non-diazotrophic organisms. Further, the near isotopic equilibrium between the \(^{15}\)N values of PON and THAAN at Sta. 271 (Fig. 7A) reflected the conversion of \(^{15}\)N into other compounds like nucleic acids, chlorophyll, or non-proteinogeneous AAs in a decaying cyanobacteria bloom with a highly patchy distribution (Fig. 9). Altogether, this means that despite the onset of degradation, N turnover must have been faster in the decaying cyanobacteria bloom than in the healthy blooms. Further investigations are needed to determine whether partially damaged cells need to increase N2 fixation for AA synthesis in order to compensate for cell leakage. Our findings more generally suggest that in systems where a significant portion of the POM is degraded (e.g., deeper in the water column, in marine sediments, or in decaying cyanobacteria blooms) volumetric N2 fixation rates into THAAN provide a much better estimate of the net incorporation of N2 into biomass than volumetric N2 fixation rates into PON, which instead may provide an estimate of the overall (gross) rate of N2 fixation in the system.
the world ocean of $<100 \mu\text{mol m}^{-2} \text{d}^{-1}$ (reviewed by Montoya et al. 2004). Very high volumetric bulk N$_2$ fixation rates of $>6 \text{ nmol L}^{-1} \text{h}^{-1}$ as found during our study at Sta. 109 and 271 (Table 4) have been sporadically observed both in the Baltic Sea (reviewed by Wasmund et al. 2001) as well as in the tropical ocean off Australia where the most extreme marine rate of $60 \text{ nmol L}^{-1} \text{h}^{-1}$ has been found (Montoya et al. 2004). These numbers provide important context for our N$_2$ fixation rates demonstrating that they were very high rates in a global context.

N$_2$ fixation in the Baltic Sea is mainly regulated by phosphate availability (Elmgren and Larsson 2001), and the very high phosphate loads in the Baltic Sea can explain the comparatively high N$_2$ fixation rates and short turnover times in a global context (Conley et al. 2009). Interestingly, the fastest turnover times of the individual AA pools of 11–23 d during N$_2$ fixation (Table 2) were found either in the ML at the westernmost Sta. 109 where the local phosphate maximum occurred (Fig. 3A) or at the phosphate-depleted, easternmost Sta. 271 (Fig. 3D). At both stations, the fast AA turnover times were similar to the fast turnover times of 10–18 d reported for exuded DON originating from ammonium and nitrate uptake in oceanic and coastal waters (Bronk et al. 1994). High fixation rates have commonly been associated with the exponential growth phase of a cyanobacteria bloom (Huber 1986; Lehtimaki et al. 1997), which may have been the case at the three westernmost stations. These stations had measurable phosphate concentrations in the ML (Fig. 3) and cyanobacteria cells that generally appeared healthy (Fig. 5) and undecayed according to THAAN mole% PON shares $>40\%$ (Fig. 6B), all of which indicate early cyanobacteria bloom stages. In contrast, at the easternmost Sta. 271, phosphate was depleted and most N. spumigena cells were heavily decayed, as only single cells in the colonies appeared pigmented and healthy (Fig. 5). The very high specific (Table 1) and THAAN-based volumetric (Table 4) N$_2$ fixation rates at Sta. 271 in a phosphate-depleted water column during a decaying N. spumigena bloom were surprising, as decaying blooms have more commonly been associated with low rates of N$_2$ fixation (Huber 1986; Lehtimaki et al. 1997). These high rates at Sta. 271 must have been generated by the remaining healthy cells, which were most likely fueled by cytosol components including potentially limiting phosphate-rich compounds released by the lysing cyanobacteria (Nausch and Nausch 2006). This shows that the very late phase of an already heavily decaying cyanobacteria bloom can be a flashpoint of N$_2$ fixation and AA turnover and can cause an extension of N$_2$ fixation in the bloom life cycle by boosting N$_2$ fixation rates and possibly diazotroph ammonium and DON release rates before the bloom collapses. At this stage of a bloom, volumetric N$_2$ fixation rates based on $^{15}$N incorporation into AAs provide a better estimate of the net rate of biomass production than rates based on $^{15}$N incorporation into bulk PON, which likely include at least some exuded N and are therefore closer to gross rates of N$_2$ fixation.

The release of organic compounds from cyanobacteria cells at Sta. 271 is consistent with the decrease we observed...
Table 5. Power function ($\ell(x) = ax^n$) regression results for the turnover times of 13 AAs and THAAN as a function of the volumetric rates of N₂ fixation into bulk PON $r$ from particulate organic matter samples during five N₂ fixation experiments with undecayed cyanobacteria at three stations in the central Baltic Sea in July 2015. See also Fig. 10 and text for more details. Regression statistics for volumetric N₂ fixation rates vs. the turnover times of individual AA and THAAN from the five experiments. All regression relationships tested were statistically significant (Prob $> F < 0.05$).

|       | $r^2$ | $a$  | $n$  | Prob $> F$ |
|-------|------|-----|-----|------------|
| Glu + Gln       | 0.9848 | 83 | -0.828 | <8.0E-4 |
| Asp + Asn       | 0.9783 | 89 | -0.846 | <1.4E-3 |
| Ile           | 0.9861 | 82 | -0.769 | <7.0E-4 |
| Leu           | 0.9869 | 101 | -0.818 | <6.4E-4 |
| Tyr           | 0.9904 | 109 | -0.807 | <4.0E-4 |
| Thr           | 0.9805 | 117 | -0.831 | <9.7E-5 |
| Ser           | 0.9954 | 114 | -0.834 | <9.5E-5 |
| Val           | 0.9836 | 107 | -0.814 | <1.2E-4 |
| Phe           | 0.9908 | 128 | -0.870 | <2.8E-5 |
| Ala           | 0.9853 | 122 | -0.836 | <6.3E-5 |
| Pro           | 0.9839 | 136 | -0.854 | <1.3E-5 |
| Gly           | 0.9904 | 140 | -0.855 | <8.5E-5 |
| Lys           | 0.9891 | 133 | -0.812 | <3.1E-5 |
| THAAN         | 0.9885 | 112 | -0.831 | <5.3E-4 |

in specific and volumetric N₂ fixation rates with increasing incubation time during our N₂ fixation experiment at Sta. 271. Similar observations have been made during other dense (e.g., >40–80 $\mu$mol POC L$^{-1}$) cyanobacteria blooms in the Baltic Sea and have been attributed to the release or degradation of organic nitrogen previously produced via nitrogen fixation (Wasmund et al. 2001). We cannot rule out that this was also occurring at the other three stations, where cyanobacteria-dominated biomass was also high in the MLs (e.g., POC concentrations of 28 ± 3 $\mu$mol). The enhanced ammonium concentrations at all four stations tentatively suggest that this may have been the case, although enhanced ammonium concentrations typically have been associated with remineralization processes during the decay of a cyanobacteria bloom in the Baltic Sea (Wasmund 1997 and references therein). However, healthy and intact cyanobacteria can exude as much as 50–90% of their recently fixed N₂ as ammonium and DON as shown for Trichodesmium spp. (summarized by Mulholland et al. 2006), Aphanizomenon spp. (Adam et al. 2016), and N. spumigena (Ploug et al. 2011). The trigger for this release is largely unclear but may be related to the inter-cell transfer of nitrogen and AAs (Mulholland and Capone 1999) or to the release of nitrogenous compounds during grazing of cyanobacteria cells by some specialists (Mulholland et al. 2006). We cannot resolve the mechanism(s) leading to the enhanced ammonium concentrations in the MLs; however, they probably originated directly or indirectly from N₂ fixation given that the nitracline depths were below the MLs at all stations.

The concentration of DON was enhanced in the ML of all stations, with clearly elevated concentrations at Sta. 213 compared to the other three stations (Fig. 4D). The most striking difference between Sta. 213 and the other three stations in our data set were the substantially higher cell-carbon biomasses of the non-heterocystous cyanobacteria Aphanocapsa sp. and Aphanathece sp. at Sta. 213. Coccoid, non-heterocystous diazotrophs < 10 µm in diameter contributed between 26% and 76% of total N₂ fixation in the central Baltic Sea in summer (Wasmund et al. 2001). Yet, it is unknown if coccoid cyanobacteria are more often directly grazed or if they exude more nitrogen during N₂ fixation than filamentous cyanobacteria, or whether these processes could help to explain the higher DON concentrations in the ML at Sta. 213. Future studies that combine AA synthesis and exudation experiments are needed to shed more light on the coupling between these processes during N₂ fixation.

Finally, our study shows that in undecayed cyanobacteria blooms the close relationships between AA and bulk nitrogen synthesis rates during N₂ fixation can be used to predict the turnover times of total and individual hydrolysable AAs from a single volumetric bulk N₂ fixation rate. In contrast, in decayed cyanobacteria blooms (and likely also at other sites with high detrital biomass), the turnover times of AAs need to be measured directly due to bulk volumetric rates reflecting gross rather than net N₂ fixation. In the undecayed blooms at the three westernmost stations, the relationship between AA turnover times and volumetric bulk N₂ fixation rates were best fitted by inverse power functions (Fig. 10; Table 5). The relative error associated with these first order estimations can be derived from the standard deviations of the turnover times of the AAs at Sta. 109, 213, and 259 shown in Table 2. Accordingly, the empirically estimated relative error averaged 13% ± 10% for all AAs from these three stations. This shows that a very high volumetric net N₂ fixation rate of 6 nmol L$^{-1}$ h$^{-1}$ is sufficient to generate diazotrophic AA turnover times of 19–31 d (Table 5). Presuming coupled AA synthesis and release rates during N₂ fixation, the consequence for the heterotrophic food web is that the supply of essential AAs may be sustained by diazotrophic cyanobacteria during flashpoints of de novo AA synthesis and turnover during N₂ fixation.

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Conflict of Interest
None declared.

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