Role of marine nitrogen-fixing organisms in the formation of atmospheric reactive nitrogen

（大気反応性窒素の放出生成に対する海洋窒素固定生物の役割）

DISSERTATION

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Abstract

Marine atmospheric reactive nitrogen emitted from the sea surface is an important factor that affects air quality and climate over the ocean via influencing atmospheric photochemical field, particle formation, and cloud formation. Among atmospheric reactive nitrogen, water-soluble organic nitrogen (WSON) and ammonia/ammonium affect the particle formation, water-solubility, acidity, and light-absorbing properties of aerosol particles. First, size-segregated aerosol and surface seawater (SSW) samples were simultaneously collected over the subtropical North Pacific to investigate the origin of aerosol WSON in the marine atmosphere. The fine-mode WSON concentration at 200–240ºE along 23ºN defined as the eastern North Pacific (ENP) was significantly higher than that at 135–200ºE, defined as the western North Pacific (WNP). Analysis of the stable carbon isotope ratio of water-soluble organic carbon (WSOC) ($\delta^{13}C_{WSOC}$) together with backward trajectory indicated that most of the observed WSON in the fine particles in the ENP originated from the ocean surface. Positive relations were found among nitrogen fixation rate, dissolved organic nitrogen (DON) in SSW, and the WSON concentrations. The result suggests that reactive nitrogen (DON and ammonium), produced and exuded by nitrogen-fixing microorganisms in SSW, contributed to the formation of WSON aerosols.

To demonstrate the contribution of nitrogen-fixing microorganisms to the formation of atmospheric reactive nitrogen, including WSON, a laboratory incubation experiment was

conducted, using *Trichodesmium erythraeum* IMS101 (hereafter referred to as *Trichodesmium*), which is one of the most representative nitrogen-fixing microorganisms. During the incubation period of approximately two months, the growth and decline of the nitrogen-fixing microorganisms were observed. During the exponential phase, the DN and DOC concentrations in seawater increased with increasing the chlorophyll-*a* concentration, suggesting that DN and DOC were exuded from *Trichodesmium* during that phase. Throughout the experiment, ammonium and ammonia were the most abundant reactive nitrogen (avg. ~70%) in both particle and gas phases, followed by WSON (avg. ~20%). During the decline and death phases, the concentrations of atmospheric ammonia and gas-phase basic WSON significantly increased, suggesting the decomposition of DN and dead *Trichodesmium* cells and/or photochemical reactions in seawater, followed by the production of low-molecular-weight, more volatile, basic DN that was more easily transferred from seawater to the atmosphere.

These results demonstrated sea-to-air emissions of WSON and ammonium/ammonia associated with the growth and decline of *Trichodesmium* for the first time. The basic-gas reactive nitrogen components significantly released from seawater can affect the acidity of the atmosphere, and their reactions with acidic gas species can promote the particle formation. This in turn leads to an increase in the particle number concentration, which can further affect the number of cloud particles and thus impact on climate over the ocean.

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List of publications

Dobashi, T., Miyazaki Y., Tachibana, E., Takahashi K., Horii, S., Hashihama, F., Yasui-Tamura, S., Iwamoto, Y., Wong, S. K., and Hamasaki, K.: Marine nitrogen fixation as a possible source of atmospheric water-soluble organic nitrogen aerosols in the subtropical North Pacific, *Biogeosciences*, 20, 439–449, https://doi.org/10.5194/bg-20-439-2023, 2023.

Chapter 1. General Introduction

1.1 Marine atmospheric aerosols and their impact on climate

The ocean covers about 70% of the Earth's surface and acts as a major source of atmospheric aerosols. Marine atmospheric aerosol is one of the key factors that control climate change, as they directly and indirectly (via the formation of cloud droplets) reflect and absorb sunlight, influencing the radiation budget [IPCC, 2021]. Cloud formation from marine atmospheric aerosols also affects amounts and processes of precipitation [Rosenfeld et al., 2019]. In the open ocean, marine atmospheric aerosols potentially impact the amount and reflectivity of low-level clouds [Garrett et al., 2004].

The most extensively studied marine atmospheric aerosols are sulfate and sea-salt particles, which are the main components of marine aerosols. Sulfate particles in the marine atmosphere are produced by the oxidation of dimethyl sulfide (DMS), which originates from the decomposition of dimethylsulphoniopropionate (DMSP) produced by marine organisms, particularly phytoplankton. Sulfate has been recognized as one of the most important components of marine aerosols as it can act as cloud condensation nuclei (CCN) to form cloud droplets [Weber et al., 1997].

On the other hand, studies in recent ~20 years have led to an improved understanding of marine organic aerosols (OAs) originating from the ocean surface. The production of marine OAs is associated with marine microbial activities, such as phytoplankton productivity, at the sea surface [O'Dowd et al., 2004]. Because organic matter in marine

aerosols can both promote and suppress cloud formation depending on their chemical components and particle mixing state [Miyazaki et al., 2020], it is important to understand the origin, formation process, amounts, and chemical/physical characteristics of OAs. There are two major formation processes of marine OAs: direct (primary) emissions as particles and secondary formation from gas-phase precursors such as volatile organic compounds (VOCs). Primary OA (POA) is emitted from the sea surface to the atmosphere as sea spray via bubble bursting and breaking waves. Previous studies reported that monosaccharides, amino acids, and proteins are present in marine POA and can serve as a biomarker for marine biological activity [Kuznetsova et al., 2005; Hawkins and Russell, 2010; Rastelli et al., 2017; Schiffer et al., 2018; Miyazaki et al., 2018; Zeppenfeld et al., 2021]. Marine secondary OA (SOA) is produced via the oxidation of volatile organic compounds (VOCs) emitted by phytoplankton and bacteria [Halsey et al., 2017; Davie-Martin et al., 2020; Fox et al., 2020; Moore et al., 2020; Croft et al., 2021; Zheng et al., 2021], as well as photochemical reactions in the sea surface microlayer (SML) [Bernard et al., 2016; Brüggemann et al., 2018]. However, there is still large uncertainty in our understanding of the amount, chemical composition, and formation processes associated with marine biota and marine atmospheric aerosols, particularly SOA.

1.2 Atmospheric water-soluble organic nitrogen (WSON)

Atmospheric water-soluble organic nitrogen (WSON) has the potential to affect the

water solubility, light absorption, and acidity of aerosol particles, as well as the global biogeochemical cycling of nitrogen [Mohr et al., 2013; van Pinxteren et al., 2017; Facchini et al., 2008; Nehir and Kosak, 2018]. Some organic compounds with chemical structures containing nitrogen, such as imidazoles, can absorb light [Noziere et al. 2009], affecting the light-absorbing properties of aerosols and their ambient temperatures. In addition, some organic nitrogen in aerosols has both acid and base functional groups (e.g., nitro- and amino groups [Zhang et al., 2011]), which may affect the acidity of aerosol particles.

Most previous studies have traditionally focused on the atmospheric deposition of reactive nitrogen onto the ocean surface. This reactive nitrogen includes nitrate and ammonia, which are available for marine microorganisms. Duce et al. [2012] used a global chemical transport model to calculate the amount of atmospheric deposition of reactive nitrogen from terrestrial and marine origins into the ocean. They showed that atmospheric deposition of anthropogenic nitrogen resulted in up to 3% of annual oceanic new production, which emphasized the potential importance of the impact of nitrogen deposition on the ocean nitrogen cycle. In spite of many studies on inorganic nitrogen in terms of atmospheric deposition, the amount and origin of WSON in the marine atmosphere have little been understood.

Miyazaki et al. [2011] suggested a possible oceanic source of aerosol WSON for the first time based on the shipboard measurements in the western North Pacific. Altieri et al.

[2016] showed that WSON in marine aerosol was strongly correlated with surface ocean primary productivity and wind speed, suggesting a marine biogenic source of aerosol WSON in the western North Atlantic Ocean. Their study also indicated that the high concentrations of WSON aerosols were attributed to primary marine sources. These previous studies require our understanding of specific biogenic sources of WSON in the marine atmosphere associated with biota in seawater.

1.3 Marine atmospheric NH_4^+ and NH_3

Gaseous ammonia (NH_3) plays a key role in atmospheric chemical processes (and following deposition to the ocean) in the biogeochemical cycles. NH_3 reacts rapidly with atmospherically-formed sulfuric and nitric acids to form fine particles existing as ammonium (NH_4^+). Therefore, NH_3 and NH_4^+ are important factors that control the acidity of aerosol particles. Poulot et al. [2015] used global ocean biogeochemical models estimating the global oceanic emission of atmospheric NH_3 to show that the ocean source dominates relative to terrestrial sources over the Equatorial Pacific. They suggested evidence for a missing source of atmospheric NH_3 over the Equatorial Pacific that could be due to the photolysis of organic nitrogen (ON) at the ocean surface or in the atmosphere. Zhang and Anastasio [2003] reported the formation of NH_4^+ from the photolysis of amino acids in the ocean. However, the oceanic sources of atmospheric NH_4^+ and NH_3 have not been well understood, as well as WSON.

1.4 Marine nitrogen-fixing organisms as a possible origin of atmospheric reactive nitrogen

Based on the background described in the previous sections, this thesis focuses on oceanic nitrogen-fixation processes as a possible new source of atmospheric reactive nitrogen over the ocean. Nitrogen fixation is a biological process that converts inert nitrogen (N_2), which is metabolically useless to all but a few microorganisms, to reactive nitrogen (NH_4^+, dissolved ON (DON)). **Figure 1.1** shows the spatial distribution of nitrate concentration at the sea surface [World Ocean Atlas. 2018], while **Figure 1.2** shows the estimated nitrogen-fixation rates in the subtropical and tropical Pacific and western Atlantic [Gruber et al., 2016]. Over oligotrophic ocean areas, such as the subtropical and tropical Pacific, where the nitrate concentrations are deficient (**Figure 1.1**), nitrogen fixation rates are generally higher than in other regions (**Figure 1.2**).

A previous laboratory incubation experiment using nitrogen-fixing organisms showed that the production of DON was associated with the growth of nitrogen-fixing organisms [Berthelot et al., 2015]. In addition, Bonnet et al. [2016] estimated the amount of reactive nitrogen released by nitrogen-fixing organisms in seawater. *Trichodesmium* is a representative nitrogen-fixing organism, which is ubiquitously distributed in the subtropical ocean [Zehr and Capone. 2020]. Nitrogen fixation by *Trichodesmium* accounts for more than ~50 % of the total nitrogen fixation in the world ocean [Mahaffey

et al., 2005; Westberry et al., 2006]. However, the contribution of the nitrogen fixation process to the atmospheric emissions of reactive nitrogen has not been studied so far.

1.5 Objectives of the study

The objective of this thesis is to elucidate the role of marine nitrogen-fixing microorganisms on the atmospheric emission and formation of reactive nitrogen (i.e., WSON and NH_3/ NH_4^+). So far, the influence of specific marine microorganisms on the sea-to-air emissions of reactive nitrogen has not been investigated. This thesis shows results from the shipboard measurements of aerosol WSON and biological/chemical parameters of surface seawater observed in the subtropical Pacific (Chapter 2). Then Chapter 3 presents the results of a laboratory incubation experiment using the representative nitrogen-fixing organism, *Trichodesmium erythraeum*, to observe resulting biological/chemical parameters in artificial seawater and discuss the atmospheric emissions of reactive nitrogen associated with the nitrogen-fixation process. General conclusions are presented in Chapter 4.

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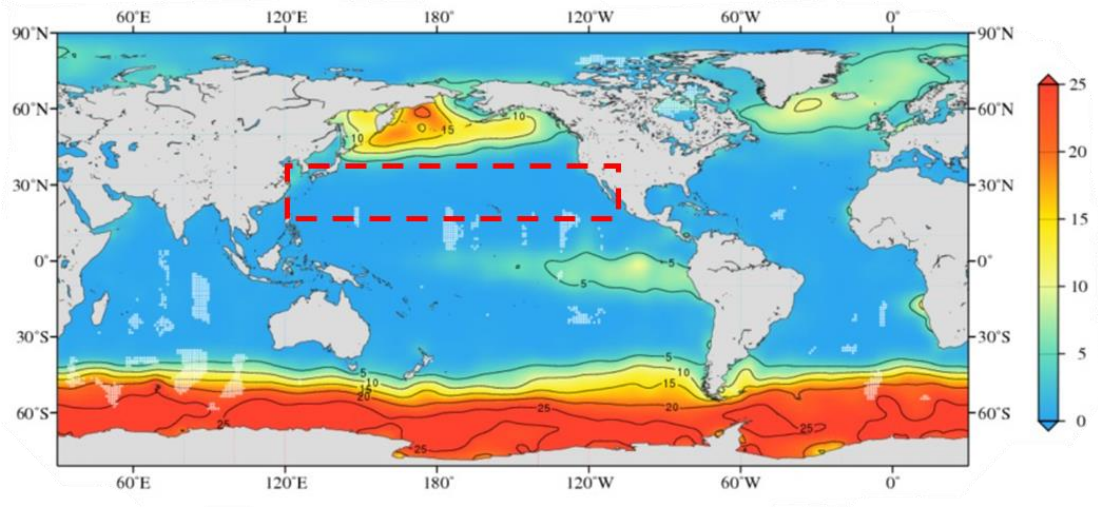

Figure 1.1 Nitrate concentration at the sea surface (μmol kg^{-1}) [World Ocean Atlas, 2018]. The original figure was slightly modified: the dashed rectangle in red indicates the oceanic nitrate depletion region of the tropical/subtropical North Pacific, where this thesis focuses on the nitrogen fixation process.

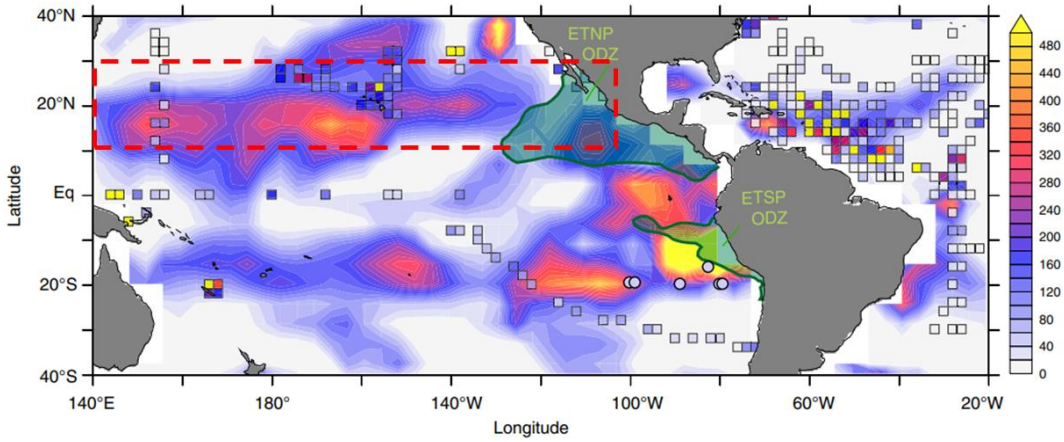

Figure 1.2 Estimated (color contours) and observed (squares) values of nitrogen-fixation rate (μmol m^{-2} d^{-1}) in the subtropical and tropical Pacific and western Atlantic [Gruber et al., 2016]. Estimated values were calculated from surface nutrients through an inverse modeling approach [Deutsch et al., 2007]. The original figure was slightly modified: the dashed rectangle in red indicates the oceanic region of the tropical/subtropical North Pacific with high nitrogen-fixing rates, focusing on the thesis.

Chapter 2. Origin of Water-soluble Organic Nitrogen in Marine Atmospheric Aerosols in the Subtropical North Pacific

2.1 Introduction

There is substantial uncertainty in our understanding of the linkage between the formation of aerosol WSON and marine microbial activity at the sea surface. Among the major groups of marine phytoplankton, cyanobacteria are the dominant primary producers in subtropical gyres [Rousseaux et al., 2012; Ottesen et al., 2014], which occupy one-third of Earth's surface. In particular, N_2-fixing microorganisms, including cyanobacteria, are widely distributed in the subtropical North Pacific [Cheung et al., 2020; Zehr et al., 2020], which is a primarily oligotrophic area with a weak vertical supply of nitrate from below the euphotic layer [Shiozaki et al., 2017; Yamaguchi et al., 2021]. In this oceanic region, nitrogen fixation by marine microorganisms, such as *Trichodesmium*, a genus of filamentous cyanobacteria, is expected to be a possible source of reactive nitrogen in the atmosphere [Frischkorn et al., 2018]. However, the effects of N_2-fixing microorganisms on the sea-to-air emissions of reactive nitrogen species, including WSON, have not yet been investigated.

This chapter aimed to elucidate the origin and formation process of WSON aerosols

in the oligotrophic subtropical ocean, particularly their linkage with N_2-fixing microorganisms in surface seawater. WSON and other reactive nitrogen species were measured in size-segregated aerosols collected onboard a research vessel sailing over the subtropical North Pacific along the longitudinal transect in the summer of 2017. The discussion includes comparisons of the observed nitrogen aerosols with dissolved organic nitrogen (DON) concentrations and nitrogen fixation rates in SSW to investigate possible sources of WSON over the oceanic region.

2.2 Experimental

2.2.1 Aerosol sampling

Ambient aerosol sampling was conducted from 12 August to 5 October 2017 on board the research vessel *Hakuho Maru* [Hashihama et al., 2020; Yamaguchi et al., 2021]. Sampling was carried out during cruise KH–17–4 from Vancouver to Tokyo via Honolulu in the subtropical North Pacific (**Figure 2.1**). The aerosol samples were collected using a high-volume air sampler (HVAS; Model 120SL, Kimoto Electric, Osaka, Japan) located on the deck above the bridge of the ship. A cascade impactor (CI; Model TE-234, Tisch Environmental, Cleves, OH, USA) was attached to the HVAS to collect size-segregated particles at a flow rate of ~1130 L min^{-1}, without temperature or humidity control. In the present study, we used analytical results obtained from the one bottom stage and four upper stages of the impactor, which collected particles with aerodynamic diameter (D_p) <

0.95 μm every 12 or 24 h and D_p > 0.95 μm every 96 h, respectively. Aerosol particles collected at the bottom and upper stages were referred to as fine and coarse particles, respectively. The atmospheric residence time of fine particles is typically ~1 week, where as that of coarse particles is generally much shorter (e.g., ~hours to days). To avoid possible contamination from ship exhaust during aerosol sampling, the sampling pump of the HVAS was shut off when the relative wind direction was out of ±60 degrees to the bow and/or when the relative wind speed was low (<5 m s^{-1}).

The aerosol samples were collected on quartz fiber filters (25 cm × 20 cm), which were precombusted at 450 °C for 6 h to remove any contaminants. Each collected filter was stored in a glass jar with a Teflon-lined screwed cap at −20°C to limit chemical reactions on the filter and losses of volatile compounds. In this study, aerosol samples collected with a total air volume < 100 m^3 were not used. In total, 51 and 9 samples are presented for the fine and coarse particles, respectively. These numbers accounted for 88% and 82% of the total numbers of fine and coarse aerosol samples collected, respectively.

2.2.2 Surface seawater (SSW) sampling and chlorophyll *a* (Chl-*a*) analysis

SSW samples were collected overboard at the depth of 0 m using an acid-cleaned bucket during each aerosol-sampling duration. The average temperature of SSW at each sampling point was 26.1 ± 2.1°C. In total, analytical results of 12 SSW samples obtained

along 23°N were shown in the present study. The SSW samples for Chl-*a* analysis were filtered using 25-mm Whatman GF/F filters (GE Healthcare, Buckinghamshire, UK). Chl-*a* concentrations were further measured fluorometrically using 10-AU™ field and laboratory fluorometers (Turner Designs, Sunnyvale, CA) after extraction with N′, N′-dimethylformamide [Suzuki and Ishimaru,1990].

2.2.3 Chemical analysis of water-soluble aerosols and elemental carbon

To measure the concentrations of water-soluble organic carbon (WSOC) and water-soluble total nitrogen (WSTN) in the aerosol filter samples, a filter cut of 19.63 cm^2 was extracted with 15 mL of ultrapure water under ultrasonication and filtered using a disk filter (Millex-GV, 0.22 μm, Millipore, Billerica, MA, USA). The concentrations of WSOC and WSTN were determined using a total organic carbon (TOC) analyzer with a TN unit (Model TOC-L_{CHP}+TNM-L, SHIMADZU). The sample was first injected into a combustion tube in the analyzer, which is filled with an oxidation catalyst (fiber platinum on quartz) and heated to 720°C to derive nitrogen monoxide under a constant flow of ultra-pure air. The nitrogen content was determined by measuring nitrogen monoxide with an ozone chemiluminescence detector [Miyazaki et al., 2011]. Additionally, another cut of the filter (7.07 cm^2) was extracted with 10 mL of ultrapure water to measure the concentrations of Na^+, NH_4^+, NO_3^-, NO_2^-, and MSA. The same syringe filter type as described above was used, before the extract was injected into an ion chromatograph

(Model 761 compact IC; Metrohm). Here the WSON concentration was defined as the difference between the WSTN and inorganic nitrogen (NH_4^+, NO_3^-, NO_2^-) concentrations. As an anthropogenic tracer, mass concentrations of elemental carbon (EC) were measured using a Sunset lab carbon analyzer. A filter punch of 1.54 cm^2 was used for the analysis of EC.

2.2.4 Stable carbon isotopic characterization of water-soluble organic aerosols

To determine the $\delta^{13}C$ of WSOC ($\delta^{13}C_{WSOC}$) in the collected aerosols, a portion of the filter (9.08 cm^2) was extracted with 20 mL of ultrapure water. The extracted samples were then concentrated via rotary evaporation, and 40 μL of each sample was transferred to be absorbed onto 15 mg of precombusted Chromosorb in a precleaned tin cup. The $^{13}C_{WSOC}$ was then measured using an elemental analyzer (Flash EA 1112)/continuous flow carrier gas system (ConFlo)-isotope ratio mass spectrometer (Delta V, Thermo Finnigan) to determine the $\delta^{13}C$ of WSOC ($\delta^{13}C_{WSOC}$) [Miyazaki et al., 2012]. The ^{13}C data were reported relative to an established reference of carbon Vienna PeeDee Belemnite (VPDB).

2.2.5 Estimation of nitrogen-fixation rate

Primary production and nitrogen fixation rates were determined using methods previously reported [Shiozaki et al., 2017], which followed Hama et al. [1983] and Mohr

et al. [2010], respectively. The SSW samples were collected using an acid-cleaned bucket. The SSW samples to estimate the initial ^{15}N and ^{13}C enrichment of POM were filtered immediately onto precombusted GF/F filters. For incubation ^{13}C-labeled sodium bicarbonate (99 atom% ^{13}C; Cambridge Isotope Laboratories, Inc.) was added to each duplicate bottle at a final concentration of 200 μmol L^{-1}. The 110 mL of $^{15}N_2$-enriched SSW, in which 1.1 ml of $^{15}N_2$ gas (>99 atom% ^{15}N, Shoko Science) was dissolved using a Sterapore membrane unit (20M1500A: Mitsubishi Rayon Co., Ltd.), was added to each bottle. The samples were then incubated for 24 h using an on-deck incubator with surface SSW running continuously. The incubation was terminated by gentle vacuum filtration of the SSW samples through precombusted GF/F filters. The filters were then frozen at −20°C until the post-measurement on the ground. The filter samples were dried at 50°C in an oven and were exposed to hydrogen chloride (HCl) fumes for 2 h to remove inorganic carbon, followed by being dried at 50°C again. The N and C contents and their stable isotope ratios were then determined using a DELTA V advantage mass spectrometer (Thermo Electron, USA) connected to an elemental analyzer. nitrogen fixation activity was regarded to be significant when the atom% of ^{15}N for each incubated bottle was higher than that for the initial sample by 0.00146 atom% [Montoya et al., 1996]. Primary production and nitrogen fixation rates were determined twice at each sampling station.

2.2.6 Determination of dissolved inorganic and organic nitrogen

Surface seawater samples for the determination of dissolved inorganic and organic nitrogen were collected at 10-m depth using a conductivity–temperature–depth (CTD) system (Sea-Bird Electronics) equipped with acid-cleaned Niskin-X bottles (General Oceanics) [Hashihama et al., 2020]. Total dissolved nitrogen (TDN) was measured by a persulfate oxidation method with a gas-segmented continuous flow analyzer [Yasui-Tamura et al., 2020]. TDN sample was converted to NO_3^- by oxidation and analyzed by colorimetric method. Concentrations of NH_4^+ and $NO_3^- + NO_2^-$ in seawater were determined by using sensitive liquid waveguide spectrophotometry [Hashihama et al., 2009; Hashihama et al., 2015]. DON concentration was determined as the difference between the TDN and dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_3^- + NO_2^-$) concentrations in surface seawater.

2.3 Results and Discussion

2.3.1 Longitudinal distributions of WSON

Figures 2.2a and **2.2b** show the longitudinal distribution of the concentrations of WSON in fine particles ($WSON_F$) with particle diameter (D_P) < 0.95 μm and in coarse particles ($WSON_C$) with D_P > 0.95 μm from samples collected at 23ºN in the subtropical North Pacific (**Figure 2.1**). The average concentrations of $WSON_F$ and $WSON_C$ were 6.1±6.2 ngN m^{-3} and 22±21 ngN m^{-3}, respectively, during the cruise measurement. The sum of these values is within range of the WSON concentrations in total suspended

particles (TSP) collected over the western North Pacific [3.0–35 ngN m^{-3}; Duce et al., 2008]. Here, along the cruise track at 23ºN, the oceanic region at 200–240ºE is defined as the eastern North Pacific (ENP), whereas the region at 135–200ºE is defined as the western North Pacific (WNP). This classification corresponds to the two legs of the cruise: the first leg was from Vancouver to Honolulu, whereas the second one was from Honolulu to Tokyo. Concentrations of WSON in both the fine and coarse particles showed a distinct longitudinal gradient, with substantially higher concentrations in ENP. The average concentrations of $WSON_F$ and $WSON_C$ in ENP were approximately 3–6 times as large as those in the WNP (**Table 2.1**).

The longitudinal distributions of the mass concentrations of inorganic nitrogen (NH_4^+, NO_3^-, and NO_2^-) generally showed similar patterns to those of WSON (**Figure 2.3**). The sum of the mass concentrations of the inorganic nitrogen in ENP was approximately twice as large as those in WNP. Overall, NH_4^+-N was the dominant component of water-soluble total nitrogen (WSTN) (~78%) in the fine-mode aerosols, followed by WSON (16%) (**Figure 2.2c**). In contrast, NO_3^--N was the most abundant WSTN component of the coarse-mode aerosols, which accounted for 83% of the WSTN mass on average. Notably, the mass fraction of WSON in ENP was significant, accounting for up to ~50% (average: 19±15%) and ~11% of the WSTN in the fine- and coarse-particle masses, respectively (**Figures 2.2c** and **2.2d**). This result indicates the importance of the abundance of WSON, particularly in the fine particles in ENP.

Figure 2.2e presents the concentrations of Chl-*a* in surface seawater (SSW) samples obtained during the cruise, as well as the average concentrations of Chl-*a* during August–September 2017 derived from the MODIS-Aqua ocean color data (https://neo.sci.gsfc.nasa.gov/view.php?datasetId=MY1DMM_CHLORA&year=2017). The two different measurements of Chl-*a* generally showed similar longitudinal distributions, which resemble the longitudinal trend of the WSON concentrations. Moreover, 5-day back trajectories, calculated by HYSPLIT (https://www.ready.noaa.gov/HYSPLIT_traj.php), showed that the sampled air masses were transported primarily over the oceanic regions in the Pacific (**Figure 2.1**). These results suggest that most of the observed aerosols were transported within the marine boundary layer with less influence from terrestrial sources prior to sampling. This is supported by the concentrations of nss-Ca^{2+} and nss-K^{+} as well as nss-SO_4^{2-}/methanesulfonic acid (MSA) ratios which did not show significant differences between WNP and ENP (**Table 2.1**). Although the trajectory suggested that the observed air masses were influenced by other oceanic regions, they were diluted during the transported and were likely more influenced by the sampling areas nearby. The overall results suggested that the observed aerosols, particularly those in ENP, were largely influenced by marine sources associated with phytoplankton.

2.3.2 Isotopic characterization of aerosol organic carbon and formation processes of WSON

Previously, a method using stable carbon isotope ratios ($\delta^{13}C$) of aerosol organic carbon was successfully used to determine the contributions of marine and terrestrial sources to organic aerosols in the marine atmosphere [Cachier et al., 1986; Miyazaki et al., 2016]. The $\delta^{13}C$ of water-soluble organic carbon (WSOC; $\delta^{13}C_{WSOC}$) as a function of the $WSON_F$ concentrations is shown in **Figure 2.4**. The $\delta^{13}C_{WSOC}$ value was only available for $WSOC_F$ in this study. The average $\delta^{13}C_{WSOC}$ observed in this study was −22.8±1.7‰, with 71% of the data (12 out of 17 data points) in the range of typical marine origin [between −24‰ and −18‰; Miyazaki et al., 2016], while that of terrestrial origin is between −27‰ and −25‰ [Cachier et al., 1986; Dasari et al., 2019]. This isotopic characterization supports that most of the organic carbon fractions of the observed aerosols were of marine origin.

To elucidate the possible formation processes of the observed WSON, we used several tracers of marine origins. **Figures 2.5a** and **2.5b** show scatter plots between WSON and Na^+ concentrations. The concentrations of Na^+, used as a tracer of marine primary emissions, did not show any significant positive correlations with those of $WSON_F$ (R^2 = 0.04) or $WSON_C$ (R^2 = 0.03) in the study area. Specifically, the R^2 value between $WSON_F$ and Na^+_F was 0.003 in ENP, while $WSON_F$ and Na^+_F concentrations even showed a negative correlation (R^2 = 0.49) in WNP (**Figure 2.5a**). The correlation cannot be statistically discussed for the coarse mode separately in ENP and WNP due to the limited number of data points. Nevertheless, the WSON concentration was higher in the ENP

than in the WNP for the same Na^+ concentration level in the coarse particles (**Figure 2.5b**), suggesting that WSON was more abundant relative to Na^+ in coarse particles in ENP. Moreover, concentrations of glucose, a molecular tracer of marine primary aerosols [Miyazaki et al., 2018], were below the lower detection limit for most of the samples during the cruise. These results suggest insignificant contributions of direct emissions from the sea surface to the observed WSON.

The above results also imply that secondary formation was likely the dominant process underlying the formation of WSON observed in this study. The process includes accommodation of secondary WSON onto sea salt particles. **Figures 2.5c** and **2.5d** present scatter plots between WSON and MSA concentrations in each particle size category. MSA has been widely used as a tracer of marine SOA because it is an oxidation product of dimethyl sulfate (DMS). MSA is either produced by gas-phase MSA directly scavenged by aerosols or rapidly produced in the aqueous phase from scavenged dimethylsulfoxide (DMSO) and methanesulfinic acid (MSIA) [Zhu et al., 2006]. The concentrations of WSON did not show any significant correlations with those of MSA in each size range regardless of the oceanic region. The insignificant correlation suggests that the origin of the observed WSON aerosols differed from that of DMS or that the formation pathways of WSON were different from the oxidation processes of DMS. It is also possible that dependence of temperature and/or OH levels in the subtropics on the relative yields of MSA to sulfate in the oxidation of DMS [e.g., Mungall et al., 2018]

might also partly affect the insignificant correlations between WSON and MSA. In fact, no relations were found between sulfate, known as an oxidation product of DMS, and WSON concentrations ($R^2 < 0.02$) in fine particles (data not shown). On the other hand, sulfate and WSON concentrations in coarse particles showed some positive relationships, although the number of data is limited. This may reflect overlapping processes of biogenic sulfate and WSON on a longer timescale (i.e., several days of sampling of coarse particles) even though the exact origins are different.

2.3.3 Distributions of nitrogen fixation rate, dissolved organic nitrogen, and aerosol WSON

To further explore the origin and possible formation process of the observed WSON aerosols associated with phytoplankton, we focused on nitrogen fixation in SSW as a possible source of atmospheric reactive nitrogen. nitrogen fixation is the biological conversion of N_2 to NH_4^+ or dissolved ON (DON), which represents the main external source of bioavailable nitrogen in marine environments. A significant fraction of fixed N_2 can be directly released by N_2-fixing microorganisms as dissolved inorganic nitrogen (NH_4^+, NO_3^-, and NO_2^-) and DON in the ocean. Data obtained from Station ALOHA in the subtropical North Pacific have shown enrichment of nitrogen pools (i.e., NH_4^+, NO_3^-, DON) within *Trichodesmium* blooms [Karl et al., 1992; Letelier and Karl, 1994]. It has been shown that majority of the recently fixed N_2 is released directly as DON during the growth of *Trichodesmium*, primarily as dissolved free amino acids [Capone et al., 1994;

Glibert and Bronk, 1994]. Luo et al. [2014] estimated that, on average, spatially integrated nitrogen fixation over the Pacific accounted for ~50% of that in the global ocean, pointing to the importance of the Pacific in terms of this process.

In the subtropical North Pacific, previously measured nitrogen isotope ratios ($\delta^{15}N$) in particulate organic matter (POM) from seawater suggested that POM was significantly affected by nitrogen supplied from N_2-fixing microorganisms [Horii et al., 2018]. Indeed, in the current study, the average $\delta^{15}N$ of POM in the SSW samples was −0.3±1.0 ‰ (data not shown), which is within the range of $\delta^{15}N$ values of diazotrophic cyanobacteria typically ranging from −2 ‰ to 0 ‰ [Horii et al., 2018]. This indicates that the organic matter in SSW collected in the study region is derived from nitrogen supplied by N_2-fixing microorganisms such as cyanobacteria.

Figure 2.6 shows the latitudinal distributions of the nitrogen fixation rate and concentrations of DON and TDN in the SSW samples compared with those of aerosol WSON concentrations in each size category. The average nitrogen fixation rate in the SSW samples collected in ENP was 38.6±13.3 ngN L^{-1} d^{-1}, which was significantly higher than that in the WNP (11.5±10.6 ngN L^{-1} d^{-1}). Phosphate concentrations in SSW showed similar longitudinal gradient, which is likely due to the P demand of microorganisms in this oceanic region [Hashihama et al., 2020]. The longitudinal gradient of nitrogen fixation rate depends on the distribution of phosphate, whose concentration was reported to be higher in the ENP than in the WNP. The observed range of the nitrogen

fixation rate in SSW is similar to those reported for the same oceanic region in previous studies [Montoya et al., 2004; Bonnet et al., 2009; Yamaguchi et al., 2019]. Moreover, the longitudinal gradient of the nitrogen fixation rate is similar to that along 23ºN in the tropical Pacific, as shown by previous field measurements and model simulations [Luo et al., 2014; Dutheil et al., 2018; Wang et al., 2019; Shiozaki et al., 2018; Hashihama et al., 2020]. The higher nitrogen fixation rate in ENP compared to that in WNP could be attributed to the greater abundance of N_2-fixing microorganisms such as *Trichodesmium* and a symbiotic unicellular cyanobacterium (UCYN-A) measured by quantitative polymerase chain reaction (qPCR) of *nifH*. In particular, the number of the *nifH* copy of UCYN-A in ENP was three orders of magnitude larger than that in WNP (data not shown).

The longitudinal distributions of DON clearly showed that DON concentrations in SSW of ENP (4.77±0.53 μM) were larger than those of WNP (4.03±0.47 μM) (**Figure 2.6c**). Furthermore, nitrogen fixation rate and DON concentrations in SSW showed a positive correlation with R^2 of 0.63, where DON concentration accounted for >98% of TDN concentration, which showed the dominance of DON in TDN during the cruise. These results suggest that the majority of DON in SSW were released in association with the recently fixed N_2 during the growth of N_2-fixing microorganisms. It is noted that the nitrogen fixation rate was calculated for particulate nitrogen in this study, whereas Konno et al. [2010] suggested that nitrogen fixation rate of the filtrate fraction (i.e., similar to DON) accounted for on average 50% of the total nitrogen fixation rates in the western

North Pacific. That study points to possible underestimation of total nitrogen fixation rate discussed in this study.

The WSON concentrations in both fine and coarse particles showed positive correlations with the nitrogen fixation rates in the SSW samples (**Figures 2.6** and **2.7**), with R^2 values of 0.27 ($p < 0.05$) and 0.60 ($p < 0.05$) in the fine and coarse modes, respectively. Additionally, concentrations of $WSON_{F+C}$ and DON were positively correlated ($R^2 = 0.36$, $p < 0.05$). Meanwhile, the R^2 values for the WSON concentrations and primary productivity in SSW were 0.08 ($p = 0.24$) and 0.58 ($p < 0.05$) in the fine and coarse modes, respectively (**Figures 2.8** and **2.9**), which were lower than those for WSON and nitrogen fixation rate. The positive relation between the WSON mass concentrations and nitrogen fixation rate together with the $\delta^{15}N$ value of POM in SSW suggests that reactive nitrogen produced by N_2-fixing microorganisms in SSW significantly contributed to the formation of WSON aerosols.

2.3.4 Discussion on nitrogen fixation as a possible source of WSON in marine aerosols

Previous laboratory experiments also showed that DON and NH_4^+ are released in seawater through nitrogen fixation by microorganisms known as diazotrophs [Wannicke et al., 2009; Berthelot et al., 2015]. Here, we discuss mass-based N:C ratios in seawater and atmospheric aerosols. The average WSTN:WSOC ratio in all the size ranges of the observed aerosols was 1.70±0.94, while those in ENP and WNP were 1.61±0.99 and

1.87±0.82, respectively. It is noted that there may be bias in the WSTN:WSOC ratios, particularly in WNP, because the WSOC concentrations were below the detection limit in many samples. Yvon-Durocher et al. [2015] reported that the N:C ratios of marine algal assemblages over the subtropical Pacific ranged between 0.10 and 0.13, while Wannicke et al. [2009] obtained N:C ratios of *Trichodesmium* (0.21±0.02) through a laboratory experiment. Furthermore, Berthelot et al. [2015] reported that the ratio of dissolved nitrogen (DN) to DOC released during nitrogen fixation was 0.07±0.48. The currently observed WSTN:WSOC ratio in the aerosols was much higher than the N:C ratios of microbes and the DN:DOC ratios in seawater affected by nitrogen fixation. The higher WSTN:WSOC ratios in aerosols relative to the typical DN:DOC ratios in seawater suggest that nitrogen-containing aerosols are preferentially produced relative to organic carbon in the atmosphere.

It is possible to argue that anthropogenic sources might contribute to the observed WSTN, including WSON and NH_4^+, as well as WSTN:WSOC ratios in aerosols shown above. Concentrations of EC as an anthropogenic tracer were below the lower detection limit (~0.1 μgC m^{-3}) in all the aerosol samples, where they did not show any statistically significant differences between WNP and ENP (**Figure 2.6**). This result suggests that the effects of anthropogenic sources on the observed aerosols in ENP were likely small. This is consistent with the stable carbon isotope analysis, which suggested that most of the observed aerosols were of marine origin rather than terrestrial sources, including

anthropogenic origin. Although measurement data of gas species were not available in this study, it is unlikely that only gas-phase precursors were of anthropogenic origin, explaining the secondary formation of WSON, although most of the observed aerosols originated from the ocean surface.

Previous studies showed that oceanic regions at low latitudes, including the subtropics, act as a source of NH_3 [the net flux of NH_3 is out of the ocean to the atmosphere; Jickells et al., 2003; Johnson et al., 2008]. Paulot et al. [2015] used two global ocean biogeochemical models to show evidence for a missing source of atmospheric ammonia (NH_3 and NH_4^+) over the equatorial Pacific that was attributable to photolysis of marine ON at the ocean surface or in the atmosphere. In fact, DON was the dominant component of TDN (>98%) in surface seawater, the longitudinal distribution of which was similar to that of WSON in our study (**Figure 2.6**). Furthermore, the observed NH_4^+ concentration levels in our study (ave. 40-50 ng m^{-3}) agree well with those predicted by Paulot et al. [2015] in the same oceanic region. Indeed, NH_4^+ was the dominant component of the aerosol reactive nitrogen in the fine particles (**Figure 2.2c**) in this study, whereas the correlations between NH_4^+ concentrations and nitrogen fixation rates were insignificant ($R^2 = 0.06$). This insignificant correlation is partially attributable to the phase partitioning of ammonia into the gas phase in the subtropical region. A possible source of NO_3^- is not necessarily anthropogenic in the equatorial Pacific, while the contribution of an oceanic source in the open ocean cannot be ruled out. Several recent field studies suggested

oceanic sources of aerosol NO_3^- in the eastern equatorial Pacific with evidence of extremely low values of $\delta^{15}N$ of NO_3^- [e.g., lower than −5‰; Kamezaki et al., 2019; Carter et al. 2021]. In this oceanic region, alkyl nitrates are suggested to contribute to nitrate production, where relatively high concentrations of alkyl nitrates have been observed over the equatorial Pacific. The low concentrations of EC and stable carbon isotope ratios in aerosols, together with the higher concentrations of DON in ENP, partly supported the oceanic source of aerosol NO_3^- in this oceanic region, although $\delta^{15}N$ of NO_3^- was not available in this study. To summarize, relatively high concentrations of inorganic nitrogen as well as WSON in ENP relative to WNP were also partly attributable to more active nitrogen fixation in ENP.

The secondary formation processes of WSON include emissions of gas-phase ON from the ocean and/or marine VOCs reacting with NH_3 [Paulot et al., 2015; Altieri et al., 2021]. Specifically, aliphatic amines by gas-to-particle conversion are one candidate for WSON species observed in this study, as those amines of marine origin were observed in gas and aerosol phases in tropical and/or subtropical open oceans [e.g., Miyazaki et al., 2010; van Pinxteren et al., 2019]. Although the exact mechanism of the WSON formation is not apparent in this study, the current results of the shipboard measurements suggest that nitrogen fixation in SSW could partly explain one of the missing sources of atmospheric WSON and ammonia indicated by previous modeling studies.

To summarize, the current result suggests that N_2-fixing microorganisms in the SSW

likely contributed to the formation of aerosol WSON and possibly to other reactive nitrogen species, such as NH_3/NH_4^+, in the oceanic region of this study. Further field studies are required to elucidate the effect of nitrogen fixation in surface seawater on the emission of atmospheric reactive nitrogen in different oceanic regions. This includes, for example, simultaneous measurements of gas and particle phases of organic and inorganic nitrogen species together with measurements of biological and chemical parameters relevant to N_2-fixing microorganisms in surface seawater by shipboard observations. Additional laboratory studies are needed to evaluate the factors controlling the atmospheric emissions of reactive nitrogen associated with nitrogen fixation in surface seawater. Jiang et al. [2018] predicted that global ocean warming in the future would result in large increases in growth and nitrogen fixation by *Trichodesmium*. Consequently, the formation process and the amount of atmospheric WSON associated with nitrogen fixation in surface seawater are expected to change, which should be important from the viewpoints of the climate effect of marine atmospheric aerosols and of the air-sea exchange of nitrogen.

2.4 Conclusions

This chapter investigated the origin and formation process of WSON aerosols in the oligotrophic subtropical North Pacific in terms of their linkage with N_2-fixing microorganisms in SSW based on the cruise measurements. The average concentration of

WSON in fine-mode aerosols along 23ºN in the eastern North Pacific (7.5±6.6 ngN m^{-3}) was much higher than that in the western North Pacific (2.4±1.9 ngN m^{-3}) during the research cruise. The stable carbon isotope ratio of WSOC, together with backward trajectories, indicated that most of the observed WSON in the fine particles in the eastern North Pacific originated from the ocean surface. Relations of the concentrations of WSON with those of Na^+ and MSA imply that secondary formation, which differed from the oxidation processes of DMS, was likely the dominant process underlying the formation of WSON observed in this study. Instead, significant positive correlations were found among nitrogen fixation rate, DON concentrations in SSW, and aerosol WSON concentrations. Meanwhile, the EC concentrations in all the samples were below the lower detection limit and did not show any statistically significant differences between WNP and ENP, suggesting that the effects of anthropogenic sources on the observed aerosols were likely small in this study. The overall results suggest that reactive nitrogen, such as dissolved organic nitrogen and ammonium, produced and exuded by nitrogen-fixing microorganisms in SSW, likely contributed to the formation of WSON aerosols over the oceanic region. This study provides new implications for the role of marine microbial activity in the formation of reactive nitrogen aerosols at the ocean surface.

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Table 2.1 Average concentrations of nitrogen species and stable carbon isotope ratios in the fine (D_p < 0.95 μm) and coarse (D_p > 0.95 μm) aerosol particles collected along 23ºN in each oceanic region.

	The western North Pacific (WNP, 135–200ºE)		The eastern North Pacific (ENP, 200–240ºE)		All (135–240ºE)	
	Fine	Coarse	Fine	Coarse	Fine	Coarse
WSON (ngN m^{-3})	2.4±1.9	7.9±6.0	7.5±6.6	49.0±12.6	6.1±6.2	21.6±21.3
NH_4^+ (ngN	42.6±15.8	3.6±3.2	35.5±22.2	17.5±8.3	37.4±20.9	8.3±8.5
NO_3^- (ngN m^{-3})	0.5±0.4	154.6±65.8	1.8±1.8	373.2±91.1	1.5±1.7	227.5±127.6
NO_2^- (ngN m^{-3})	0.4±0.2	32.9±54.0	1.4±1.4	9.4±9.4	1.1±1.3	25.0±45.8
nss-Ca^{2+} (ng m^{-3})	1.6±1.5	166.4±81.5	0.4±1.1	211.8±4.3	0.7±1.3	181.5±69.9
nss-K^+ (ng m^{-3})	5.2±4.8	120.5±60.7	3.9±3.0	141.8±9.9	4.2±3.6	127.6±50.9
nss-SO_4^{2-} /MSA	5.5±3.6	11.4±3.5	4.7±7.2	19.5±10.6	4.9±6.4	14.1±7.8
$\delta^{13}C_{WSOC}$ (‰)	−23.9±1.4	NA	−22.1±1.6	NA	−22.8±1.7	NA

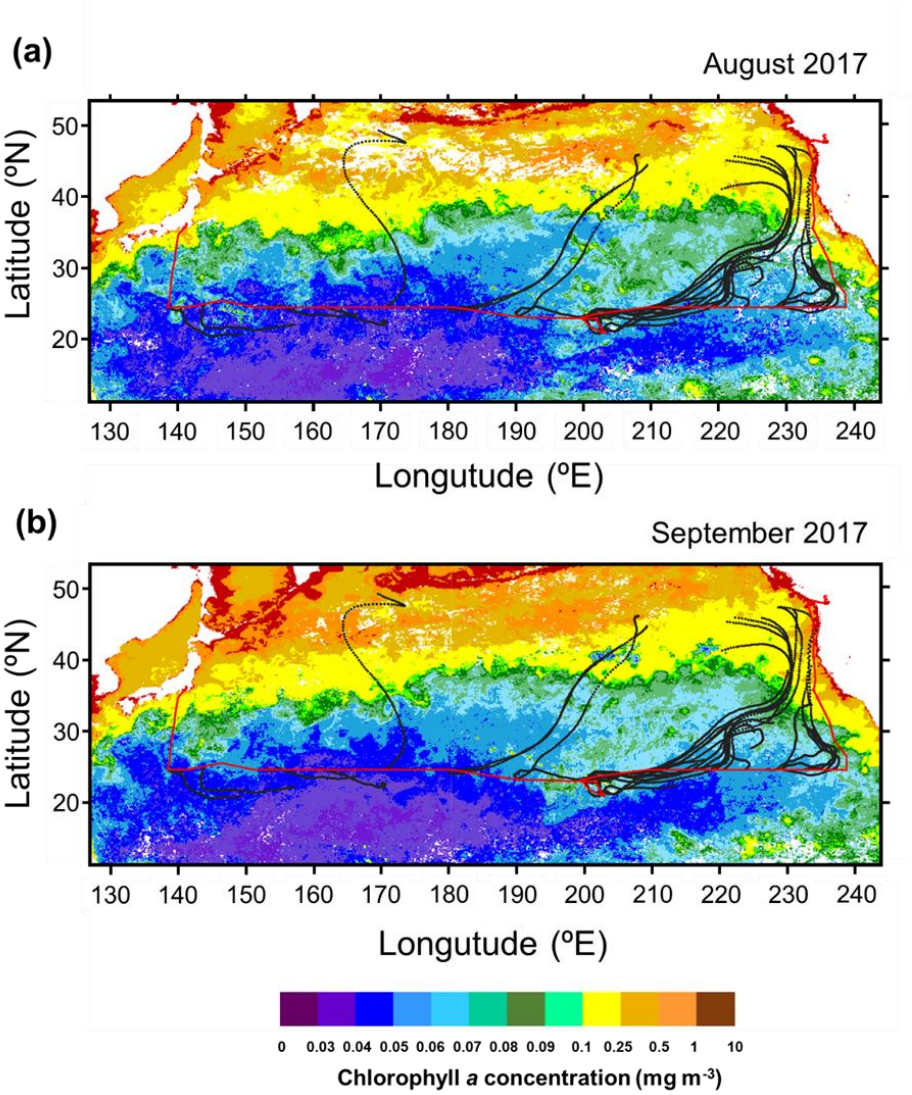

Figure 2.1 *R/V Hakuho Maru* cruise track in the subtropical North Pacific between 12 August and 5 October 2017 (red), together with typical 5-day back trajectories (black). Also shown are monthly averaged concentrations of Chl-*a* for (a)

August and (b) September 2017 derived from the MODIS-Aqua (https://neo.sci.gsfc.nasa.gov/view.php?datasetId=MY1DMM_CHLORA&year=2017). The area in which data are missing is shown in white.

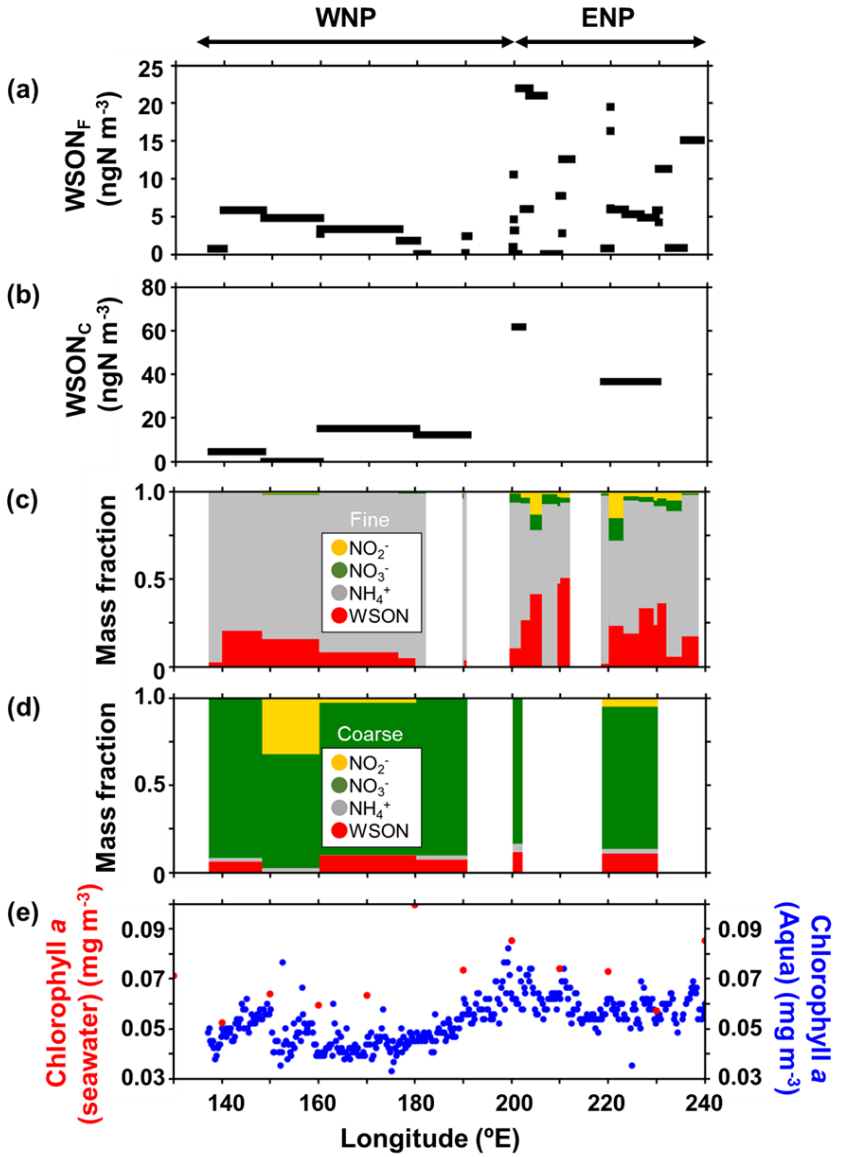

Figure 2.2 Longitudinal distributions of each parameter of the atmospheric aerosols and surface seawater along 23ºN; the mass concentrations of WSON in (a) fine particles ($WSON_F$) and (b) coarse particles ($WSON_C$); the chemical mass fractions of nitrogen species in (c) $WSON_F$ and (d) $WSON_C$; (e) Chl-*a* concentrations in the surface seawater samples during the cruise (red) and the average Chl-*a* concentrations from August–September 2017, as measured by

MODIS-Aqua (blue). WNP and ENP denote the oceanic regions of the western North Pacific and eastern North Pacific, respectively, defined in this study.

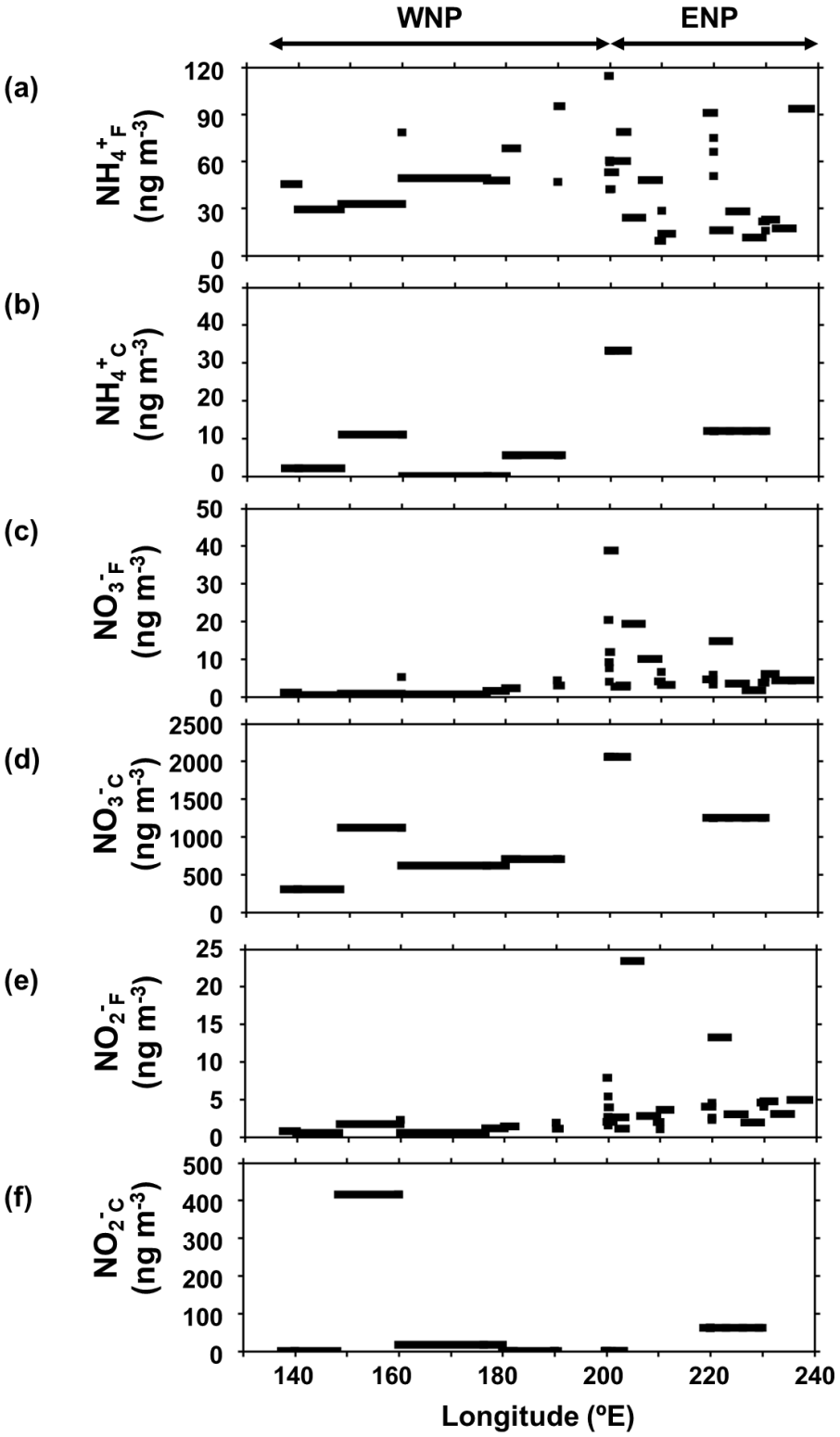

Figure 2.3 Longitudinal distributions of mass concentrations of (a) $NH_4^+{}_F$, (b) $NH_4^+{}_C$, (c) $NO_3^-{}_F$, (d) $NO_3^-{}_C$, (e) $NO_2^-{}_F$, and (f) $NO_2^-{}_C$ along 23ºN.

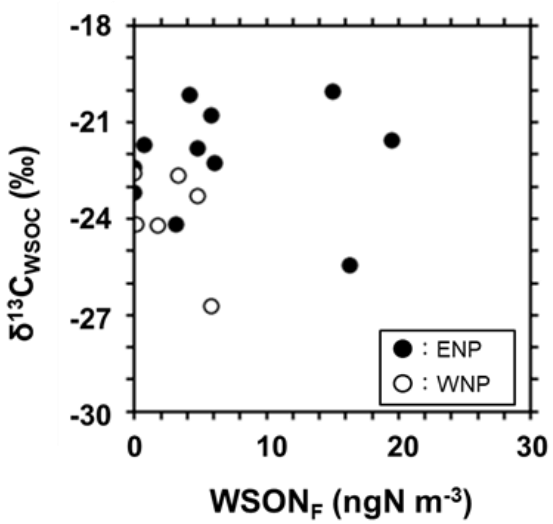

Figure 2.4 $\delta^{13}C$ of WSOC ($\delta^{13}C_{WSOC}$) as a function of the concentrations of $WSON_F$. Solid and open circles indicate the data of the eastern North Pacific (ENP; 200–240ºE) and the western North Pacific (WNP; 135–200ºE), respectively.

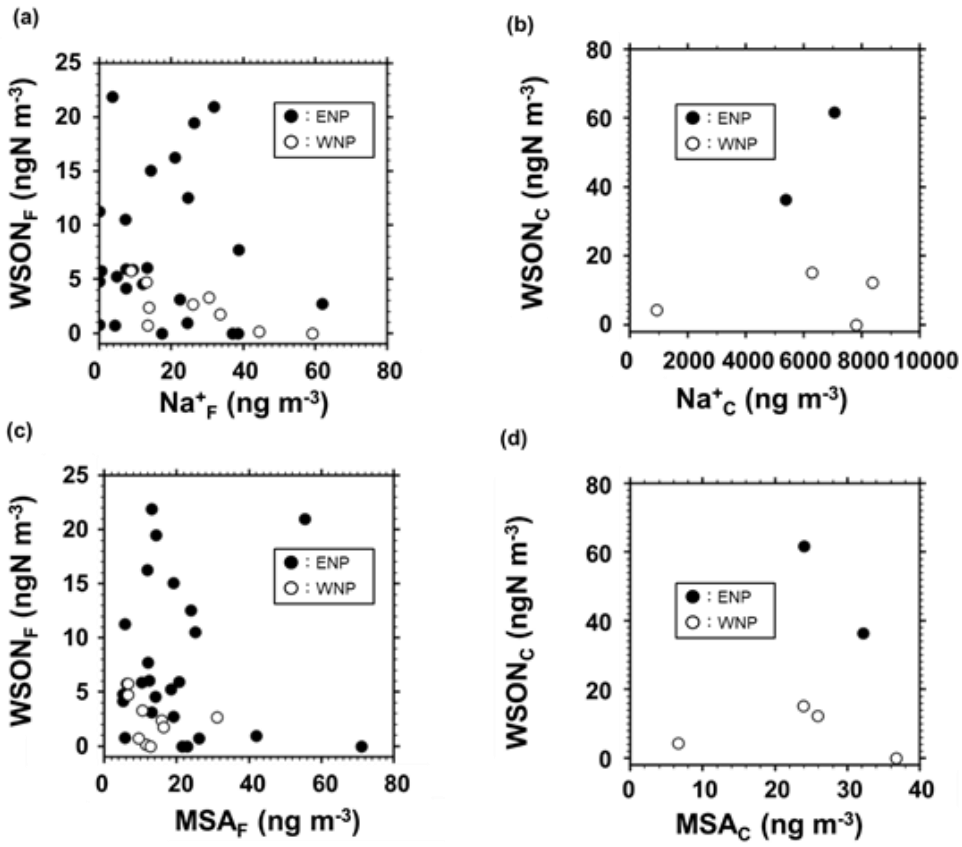

Figure 2.5 Concentrations of WSON as functions of those of sodium (a, b) and MSA (c, d), where (a) and (c) are for the fine particles and (b) and (d) are for the coarse particles. Solid and open circles represent the data of the eastern North Pacific (ENP) and western North Pacific (WNP), respectively.

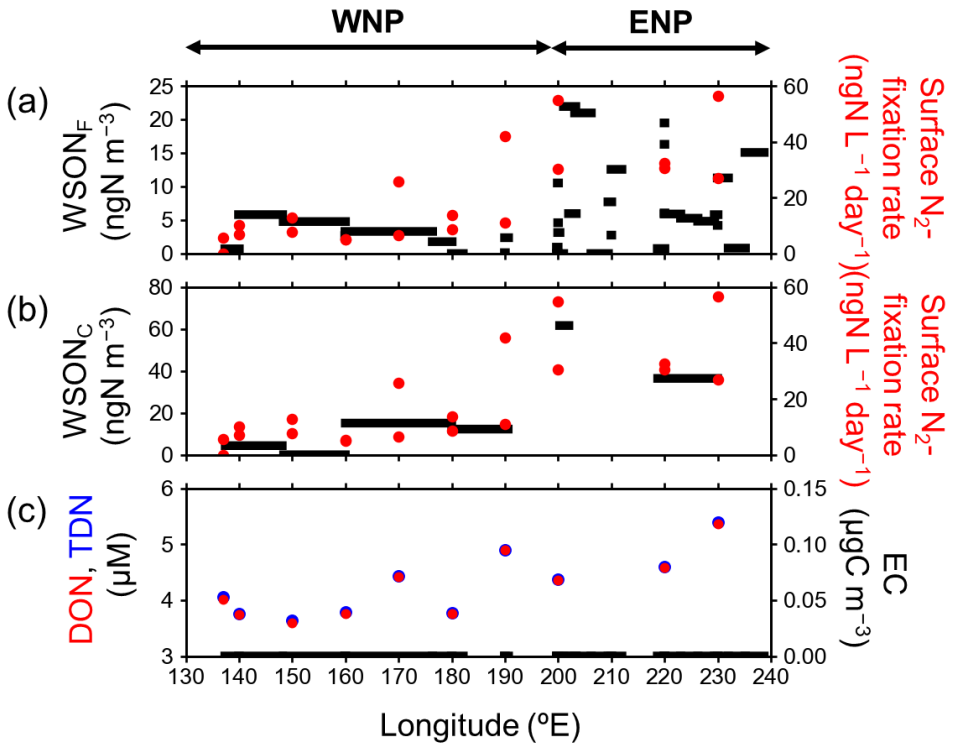

Figure 2.6 Longitudinal distributions of mass concentrations of (a) $WSON_F$ (black) and (b) $WSON_C$ (black), together with nitrogen fixation rate in the SSW samples (solid red circle), and (c) DON (solid red circle) and TDN (solid blue circle) concentrations in surface seawater and aerosol EC concentrations (black) during the entire cruise.

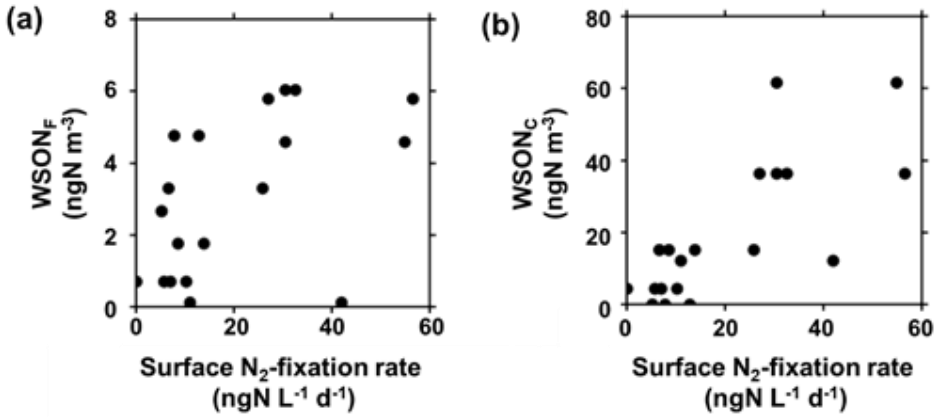

Figure 2.7 Mass concentrations of (a) $WSON_F$ and (b) $WSON_C$ as a function of nitrogen fixation rate in SSW. The data of nitrogen fixation rate in SSW were merged into the duration of each corresponding aerosol sampling. For each one aerosol sample, one or more corresponding measurement data of nitrogen fixation rate in SSW were obtained, so that the number of the data points in the panels is more than that of the aerosol samples.

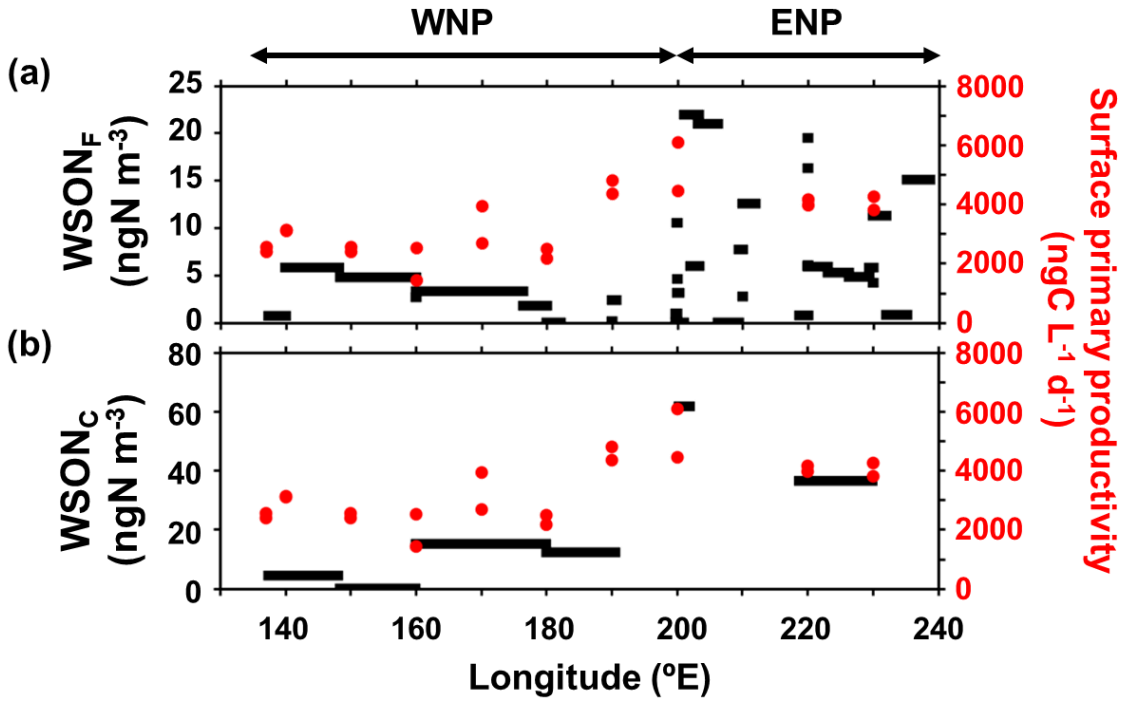

Figure 2.8 Longitudinal distributions of mass concentrations of (a) $WSON_F$ and (b) $WSON_C$, together with primary productivity in the SSW samples during the entire cruise.

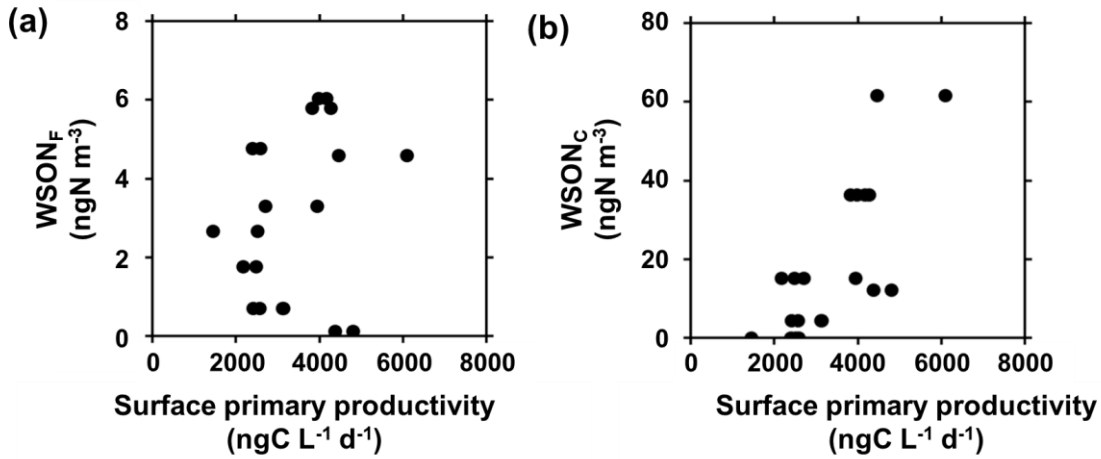

Figure 2.9 The mass concentrations of (a) $WSON_F$ and (b) $WSON_C$ as a function of primary productivity in SSW.

Chapter 3. Effects of Marine Nitrogen Fixation on Atmospheric Emissions of Reactive Nitrogen Revealed by a Laboratory Incubation Experiment

3.1 Introduction

This chapter shows the results of an incubation experiment in a laboratory to prove that atmospheric reactive nitrogen, including WSON, is indeed emitted from seawater associated with marine nitrogen fixation, as suggested in Chapter 2. Previous laboratory studies on sea-to-air emissions of biochemical and microbiological components have mainly focused on organic matter in sea spray aerosols and the gas phase associated with microbes. For example, Rastelli et al. [2017] simulated the emission of airborne sea sprays through bubble-bursting experiments using the seawater of the North-Eastern Atlantic Ocean offshore Ireland. They showed that the submicrometer aerosol of primary emissions was highly enriched in organic matter such as lipids, proteins, and carbohydrates compared with seawater. Their results suggested that enhanced biological activity and the release of lipid compounds at the sea surface can significantly increase the carbon content of the emitted marine aerosols. Bernard et al. [2016] focused on abiotic processes emitted as gases from the sea-surface microlayer (SML) through photochemical reactions, followed by particle formations in the atmosphere. They used a multiphase

atmospheric simulation chamber to show that not only marine secondary organic aerosols (SOA) derived from gases released by microorganisms but also the process of becoming SOA by photochemical reactions in the SML is important.

To the author's knowledge, no experiments have been conducted so far to incubate nitrogen-fixing microorganisms with a simultaneous atmospheric observation of gases and particles emitted from seawater. Based on the results shown in Chapter 2, this chapter aims to elucidate the contribution of nitrogen-fixing microorganisms to the formation of atmospheric reactive nitrogen, including WSON, by a laboratory incubation experiment. In this study, *Trichodesmium* was chosen as a representative of nitrogen-fixing microorganisms for the experiment. *Trichodesmium* is widely distributed in the tropical and subtropical oceans [Zehr and Capone., 2020]. *Trichodesmium* contributes to ~50% (or more) of the total amount of nitrogen fixed by nitrogen fixation in the global ocean [Mahaffey et al., 2005; Westberry et al., 2006]. *Trichodesmium* was incubated in artificial seawater with continuous atmospheric samplings of particulate and gas-phase reactive nitrogen together with seawater sampling once a day.

3.2 Experimental method

3.2.1 Incubation of *Trichodesmium* with artificial seawater and atmospheric sampling system

Figure 3.1 shows an experimental system set up for incubating nitrogen-fixing

cyanobacterium *Trichodesmium* followed by collecting atmospheric and seawater samples. In this experiment, *Trichodesmium erythraeum* IMS101 (hereafter described as *Trichodesmium*) was incubated in an acrylic tank containing approximately 20 L of artificial seawater under conditions of controlled temperature and light intensity by using an incubator and fluorescent lamps (FL40S.BRN plantluks, TOSHIBA Corp.) with the visible wavelength range. The light condition also enables photochemical reaction in the atmosphere and seawater. Atmospheric samples were collected continuously for 24 hours, while seawater samples were collected once per ~24 hours. Details of each sampling are given in the following sections. The incoming air from outside the tank was filtered with an air filter (Disposable Adsorption Units, Balston) that can absorb gases (e.g., ethylene, propylene, and ammonia) to reduce any contamination that entered the tank.

3.2.2 Incubation condition

In this experiment, artificial-seawater-based YBC-II [Chen et al. 1996], free of reactive nitrogen, was used as a culture medium for the incubation of *Trichodesmium*. Incubation was conducted in this medium at 25ºC with a daily cycle of light intensity: 195 μmol photon m^{-2} s^{-1} for 12 hours and another 12 hours in the dark. Temperature and light intensity were set to make optimal conditions for the growth of *Trichodesmium* based on the previous incubation experiment [Boatman et al., 2017].

3.2.3 Atmospheric sampling

Atmospheric samples were collected at a flow rate of 20 L min^{-1} with a NILU impactor (Tokyo Dylec. Corp.). The flow rate was controlled by a volumetric flow meter with the integrated sampling volume monitored. The NILU impactor consists of four impaction stages. The first top stage collected particles with particle diameter (D_p) larger than 2.5 μm, the sample of which was not used in this study, because the larger particles could not be collected by the impactor in the experimental setup. For the second stage, particles with D_p < 2.5 μm was collected on quartz fiber filters with an effective diameter of 47 mm). The third and fourth stages collected acidic and basic gases onto a cellulose filter impregnated with 2% sodium carbonate ($NaCO_3$) and 2% phosphoric acid, respectively. Before the sampling, the cellulose filter was stored at −20 ºC in a freezer to prevent drying of the impregnation.

Prior to the incubation of *Trichodesmium*, atmospheric samples were collected under conditions with no *Trichodesmium* in seawater. These filter samples were used as blank field filters to determine the concentrations of each chemical parameter. That is, the concentration of the field blank was subtracted from that of the samples collected during the incubation of *Trichodesmium*.

3.2.4 Artificial seawater sampling

Artificial seawater was collected once per 24 hours. For *in vivo* fluorescence intensity

measurements, 1 ml of seawater was collected every day during the incubation. For the measurement of chlorophyll *a* (Chl-*a*) concentration, 50 ml × 2 seawater samples were collected eight times throughout the experiment. In addition, eighteen samples of 1 ml seawater were collected to measure bacteria concentration during the period. All of these seawater samples were collected using a peristaltic pump.

3.2.5 Chemical analysis of atmospheric samples

To measure the mass concentrations of water-soluble organic carbon (WSOC) and water-soluble total nitrogen (WSTN) in the filter samples, a filter cut of 6.28 cm^2 was extracted with 15 mL of ultrapure water under ultrasonication and filtered using a disc filter (Millex-GV, 0.22 μm, Millipore, Billerica, MA, USA). The concentrations of WSOC and WSTN were determined using a total organic carbon (TOC) analyzer with a TN unit (Model TOC-L_{CHP}+TNM-L, SHIMADZU).

Another cut of the filter (6.28 cm^2) was extracted with 7 or 50 mL of ultrapure water to measure the concentrations of NH_4^+, NO_3^-, and NO_2^-. The same syringe filter type as described above was used, before the extract was injected into an ion chromatograph (Model 761 compact IC; Metrohm). The WSON concentration was defined as the difference between the WSTN and inorganic nitrogen (NH_4^+, NO_3^-, and NO_2^-) concentrations.

3.2.6 Measurements of chemical and biological parameters of artificial seawater

To measure the concentrations of dissolved organic carbon (DOC) and dissolved nitrogen (DN) in the seawater samples, 7.5 ml of the sample was filtered using a disc filter (Millex-GV, 0.22 μm, Millipore, Billerica, MA, USA). The filtered seawater sample was diluted with 7.5 ml of ultrapure water. A total of 15 ml sample was used for the determination of the DOC and DN concentrations by using a total organic carbon (TOC) analyzer with a TN unit (Model TOC-L_{CHP}+TNM-L, SHIMADZU).

In vivo fluorescence intensity in 1-ml seawater samples was measured by Qubit ® 2.0 Fluorometer (Thermo Fisher SCIENTIFIC) every day of the experiment. Phytoplankton pigment (Chl-*a* concentration) was measured using ultra-high performance liquid chromatography (Shimazu UHPLC Nexera X2) [Suzuki et al., 2015]. The measurement data showed a linear relationship between the *in vivo* fluorescence intensity and Chl-*a* concentration. Chl-*a* concentration for all the samples was then calculated with a linear regression line from the *in vivo* fluorescence intensity. To measure bacteria concentration, another 1-ml seawater sample was put into a cryovial containing 20 μL of 10% PFA (paraformaldehyde), which was stored at −80 ºC until the analysis. Bacteria concentration was then measured using a flow cytometer (BD FACSCantoTMII).

3.3 Results and discussion

3.3.1 Definition of growth phases of *Trichodesmium*

Figure 3.3 shows the observed temporal variations in the concentrations of Chl-*a* and bacteria in seawater during an incubation period of about 2 months. Here, Day 0 is defined as the date when *Trichodesmiumn* was put into the culture medium to start the monitoring. Chl-*a* concentration started to increase after Day 20, reaching the maximum concentration of 106.4 mg m^{-3} on Day 30. Then the concentration showed a decrease until Day 48.

To define each period of the growth and decline stages in *Trichodesmiumn*, a growth rate (GR) of *Trichodesmiumn* was calculated using the following equation:

$$\mathrm{GR} = \frac{\log\left(C_{(t)}\right) - \log\left(C_{(t_0)}\right)}{t - t_0} \times 1000$$

where $C_{(t)}$ and $C_{(t_0)}$ denote concentrations of Chl-*a* at a start time (t_0) and end time (t) of each seawater sampling, respectively. **Figure 3.4** presents the temporal changes in the growth rate with the 5-day running-mean values (**Table 3.1**). First, a period when the Chl-*a* concentrations was below 2.0 mg m^{-3} (Days 0–21) was defined as the lag phase. The exponential phase was set when GR was greater than 1.5 (Days 21–29). The stationary phase was defined as a period when GR was between −6 and 1.5 (Days 29–44). The decline phase was defined as the period when GR was between −10 and − 6 (Days 44–48), whereas the death phase was defined as the period when GR was greater than −6 and after the decline phase (Days 48–53). The resulting temporal variation in Chl-*a*

concentrations classified into each period is shown in **Figure 3.5**. In the following sections, atmospheric and seawater parameters are discussed based on these classified growing stages.

3.3.2 Temporal variation of DN and DOC in seawater

Figure 3.6 shows temporal changes in concentrations of DN and DOC, the DN/DOC ratio, together with those of Chl-*a* concentrations. The average values of the parameters obtained during each phase are presented in **Figure 3.7**. The DN and DOC concentrations showed a simultaneous increase with Chl-*a* concentration during the exponential phase. This simultaneous increase suggests that DN and DOC were released from *Trichodesmium* associated with its increasing numbers and activities, such as primary production and nitrogen fixation. A similar result was obtained by Mulholland et al. [2004], who showed that nitrogen fixation rates increased associated with the growth of *Trichodesmium*, followed by an increase in DON and NH_4^+ concentration in seawater by a laboratory incubation experiment. DN concentrations continued to increase even during the stationary and decline phases when Chl-*a* concentrations continuously decreased. This may be due to the reduction of the amount of DN consumed/recycled by *Trichodesmium* during the stationary and decline phases. It is also possible that the production of DN was enhanced due to increased decomposition by bacteria. Indeed, this is partly supported by a significant increase in the cell concentrations of bacteria during

these three phases (**Figure 3.6c**).

It is noted that the decrease in DOC concentration was more evident than DN during the death phase. This implies that DOC was more consumed by bacteria and/or decomposed to be emitted into the atmosphere relative to DN during that phase. It should also be noted that the DN/DOC ratio showed a continuous increase from the exponential to death phases. This result clearly showed that DN was more preferentially produced relative to DOC in terms of the net amount from the exponential to death phases. Note that the accumulation of DN and DOC, particularly during the decline and death phases, might contribute to the increase in those concentrations, which was different from the actual condition in the ocean where dynamical mixing and consumption of DN and DOC by other microorganisms occur. Nevertheless, the experimental result showed that DN and DOC were released associated with nitrogen fixation during the exponential and stationary phases.

3.3.3 Temporal variation of atmospheric reactive nitrogen

Figure 3.8 shows the time series of WSON concentrations in the particle and gas phases, together with the concentrations of seawater parameters throughout the experiment. The average concentrations of each parameter during each growth phase are summarized in **Figures 3.9a** and **3.10a**. The mass concentration of WSON in the particle phase showed an increase during the exponential phase with a maximum value of 34.2

ngN m^{-3} (**Figure 3.8a**), while it decreased during the decline and death phases. Concentrations of gas-phase acidic WSON increased during the exponential and stationary phases and decreased during the decline and death phases. This temporal trend is similar to that of Chl-*a*. The result suggests that acidic compounds of DN, which were exudates in nitrogen fixation by *Trichodesmium* in seawater, were emitted into the atmosphere as gas-phase acidic WSON. On the other hand, the gas-phase basic WSON showed extremely high concentrations during the decline and death phases, with averages of 2.5±2.0 μgN m^{-3} and 2.7±3.2 μgN m^{-3}, respectively. The maximum concentration of gas-phase basic WSON was as high as ~8.0 μgN m^{-3} observed during the death phase. Although specific compounds of gas-phase basic WSON were not measured in this study, some semi-volatile WSON compounds, such as alkylamines, are one of the candidates.

Figure 3.11 shows temporal variations in the NH_4^+ and NH_3 concentrations during the experiment. The average concentrations during each growth phase are also summarized in **Figures 3.9b** and **3.10b**. The concentration of particle-phase NH_4^+ showed an increase during the exponential phase, with its peak during the stationary phase. The concentration of NH_4^+ decreased during the decline and death phases. In contrast, NH_3 concentrations showed extremely high concentrations during the decline and death phases, with a maximum concentration of ~19 μg m^{-3}. This temporal trend is similar to that of gas-phase basic WSON. The simultaneous increase in the concentrations of gas-phase basic WSON and NH_3 during the decline and death phases suggest the decomposition of DN and dead

cells of *Trichodesmium* by photochemical reactions and/or bacteria in the culture medium resulted in the increased atmospheric emissions of such basic reactive nitrogen.

Figure 3.12 shows temporal variations of NO_3^- and NO_2^- in the particle phase and HNO_3 and HONO in the gas phase. The average concentrations during each growth phase are summarized in **Figures 3.9c, 3.9d,** and **3.10c**. Concentrations of particulate NO_3^- showed a slight increase associated with the growth of *Trichodesmium*. The concentrations of gas-phase HNO_3 and HONO increased during the exponential and stationary phases, while these levels were much lower than those of WSON and NH_4^+/NH_3. The increase in the concentrations of nitrate and nitrite was likely produced by the nitrification of DN in the culture medium, resulting in the formation of gas-phase HNO_3 and HONO.

3.3.4 Gas-particle phase partitioning and budget of reactive nitrogen species associated with the growth of *Trichodesmium*

Figure 3.13 shows the mass fraction of particle-, acidic gas-, and basic gas-phases of each reactive nitrogen species in each growth phase. For WSON, the mass fraction of gas-phase basic WSON increased from stationary to death phases. For NH_4^+/NH_3, gas-phase (NH_3) dominated during the entire period. With regard to NO_3^-/HNO_3, the fraction of particle phase increased as the growth stage proceeded, the trend of which was similar to that of NO_2^-/HONO. Since NH_3 and basic WSON are neutralizing agents of HNO_3 to

form particles (e.g., NH_4NO_3), this trend was explained by gas-to-particle shift/formation of nitrate and nitrite due to the increase in the emission of NH_3 and basic WSON from the stationary to death phases.

Figure 3.14 shows the mass fraction of each reactive nitrogen species in the particle and gas phases. In the particle phase, NH_4^+ was the most dominant component, which accounted for 62% of the total reactive nitrogen, followed by WSON (33%) throughout the growth stage of *Trichodesmium*. In the acidic gas phase, WSON was the most dominant reactive nitrogen, which accounted for 68% on average. In the basic gas phase, NH_3 was the most dominant (89%), followed by WSON. **Figure 3.15** shows the mass fraction of each gas-phase reactive nitrogen to the total. As is expected from **Figure 3.14**, NH_3 was the most dominant component (86%), whereas the mass fraction of gas-phase basic WSON increased during the decline and death phases. This means that the relative contribution of sea-to-air emissions of gas-phase basic WSON became larger during those phases.

3.3.5 Nitrogen-to-carbon ratios of the parameters in the atmospheric and seawater samples

In this section, N/C ratios of atmospheric and seawater parameters are discussed. **Figure 3.16** shows the time series of WSOC concentrations in the particle and gas phases, together with the concentrations of seawater parameters throughout the experiment. In

general, mass concentrations of both acidic and basic WSOC in the gas phase increased during the exponential and stationary phases and decreased during the decline and death phases. This temporal trend is similar to that of DOC and Chl-*a* concentration. The result suggests that acidic and basic compounds of DOC, which were also like exudates by nitrogen fixation by *Trichodesmium* in seawater, were emitted into the atmosphere as gas- and particle-phase WSOC.

Figure 3.17 shows the average values of the WSTN/WSOC ratio (the sum of the total mass of particle and gas phases) in the atmospheric samples and DN/DOC ratio in the seawater samples in each growth phase. For reference, the C/N ratios of those data are shown in **Table 3.2.** Throughout the experiment, the N/C ratios in the atmospheric samples were larger than those in the seawater samples. Specifically, the N/C ratios in the atmospheric samples during the exponential and stationary phases (both 0.83) were 6-12 times higher than those in seawater (0.07 and 0.13 during the exponential and stationary phases, respectively). These results suggest preferential sea-to-air emissions of reactive nitrogen relative to organic carbon associated with the growth of *Trichodesmium*. This may be due to more volatile characteristics of DN relative to DOC released by *Trichodesmium* as well as the following decomposition of those components. During the decline and death phases, the WSTN/WSOC ratios in the atmosphere increased significantly relative to the increase in the DN/DOC ratio in seawater. This result suggests the consumption of DOC by bacteria and decomposition of DN and dead cells of

Trichodesmium by photochemical reactions and/or bacteria followed by sea-to-air emissions of DN relative to those of DOC during those phases.

3.3.6 Sea-to-air emission flux of reactive nitrogen

To quantitatively evaluate atmospheric reactive nitrogen produced associated with the growth of *Trichodesmium*, the sea-to-air emission flux of WSTN (F_{WSTN}) was calculated using the following equation:

$$F_{WSTN} = \frac{(\mathrm{C} - C_b) \times V}{S \times \Delta t}$$

where C denotes the concentration of WSTN (ngN m^{-3}), C_b denotes the background concentration of WSTN (ngN m^{-3}) in the experimental system, V denotes sampled air volume (m^3), S denotes the area of sea surface (m^2) of the experimental tank, and Δt denotes sampling duration (day). It should be noted that the experimental conditions of this system differ from those of the actual ocean-atmospheric environment. Hence, the following ambient conditions are assumed in this calculation: low atmospheric pressure, low wind speed with no waves, and active cyanobacterial bloom at the ocean surface.

Figure 3.18 shows the average sea-to-air flux of the total WSTN in each growth phase of *Trichodesmium* during the experiment. The fluxes during the decline and death phases were substantially higher than those during the exponential and stationary phases, which

reflected the significant difference in the mass concentration of WSTN in the two periods. The average flux was 2.9±2.7 (mgN m^{-2} day^{-1}), which is substantially larger than the NH_4^+ flux (0.06–0.27 mgN m^{-2} day^{-1}) estimated for the subtropical North Pacific by global biogeochemical model [Poulot et al., 2015]. Because the difference in the estimated flux may be partly due to the extremely high concentrations of Chl-*a* (i.e., the amount of *Trichodesmium*) in the current experimental system, the flux per unit mass of Chl-*a* was calculated for direct comparison. (**Table 3.3**). The result showed that the flux during the stationary phase estimated in this study accounted for only ~1% of that estimated by Poulot et al. [2015], whereas the flux during the decline phase accounted for up to ~40% of the flux estimated for the subtropical North Pacific. Despite the uncertainty in the flux calculation and experimental conditions, the result suggests that DN released associated with nitrogen fixation can partly explain sea-to-air emissions of water-soluble nitrogen, including WSON and ammonia.

3.4 Conclusion

To investigate the contribution of nitrogen-fixing organisms to the formation of atmospheric reactive nitrogen, the non-heterocystous, diazotrophic cyanobacterium *Trichodesmium erythraeum* (CCMP1985), as a representative nitrogen-fixing organism, was incubated in artificial seawater under controlled light and temperature in a laboratory. Simultaneously, continuous atmospheric sampling of particulate and gas-phase reactive

nitrogen was conducted with discrete seawater collection over 55 days. The concentrations of DN and DOC in seawater showed a simultaneous increase with Chl-*a* concentration during the exponential phase, suggesting that DN and DOC were released by *Trichodesmium* associated with its nitrogen fixation. DN concentrations continued to increase even from the stationary phase to the death phase, which was likely due to a reduction in the amount of DN consumed or recycled by *Trichodesmium* during those phases. Furthermore, the continuous increase of the DN/DOC ratio throughout the experiment indicates larger net production of DN relative to that of DOC in seawater during the growth and decline phases of *Trichodesmium*.

NH_4^+/NH_3 was the most abundant reactive nitrogen species, followed by WSON in the particle and gas phases throughout the entire period. On average, NH_4^+ and WSON accounted for 62% and 33% of the reactive nitrogen species, respectively, measured in the particle phase. Meanwhile, in the gas phase, NH_3 and WSON accounted for 86% and 13% of the reactive nitrogen, respectively. In particular, basic WSON was found to be the second most abundant reactive nitrogen species. NH_4^+, particle-phase WSON, and gas-phase acidic WSON showed increased concentrations during the exponential and stationary phases. On the other hand, it was found for the first time that concentrations of NH_3 and basic WSON in the gas phase showed a significant increase during the decline and death phases of *Trichodesmium*. The substantial increase in the atmospheric emissions of NH_3 and basic WSON was likely due to the considerable decomposition of

DN by bacteria and/or photochemical reactions in seawater during those phases. This is followed by producing low-molecular-weight, more volatile, basic DN that was more easily transferred from seawater to the atmosphere.

The overall experiments demonstrated sea-to-air emissions of NH_4^+/NH_3 and WSON associated with the growth and decline of *Trichodesmium*. Furthermore, the results suggested that gas-phase organic nitrogen mainly consisted of highly volatile, basic components whose emission significantly increased with an increase in heterotrophic bacteria during the decline and death phases of *Trichodesmium*. Our results suggest, for the first time, that the source of ammonia/ammonium and WSON associated with nitrogen fixation are crucial for changing atmospheric acidity and particle formation over the ocean, as they all have the nature of alkalinity in the atmosphere.

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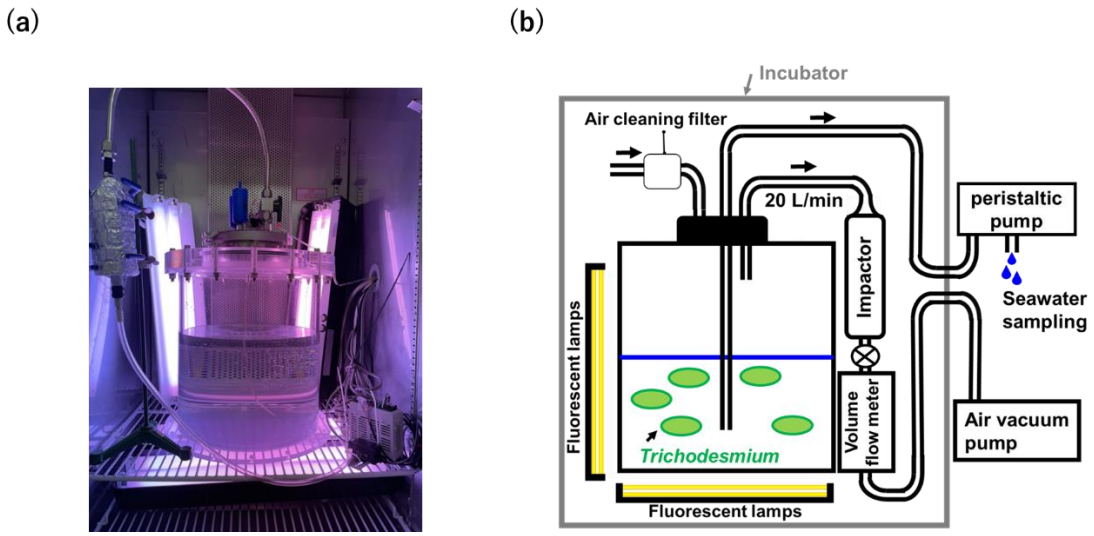


Figure 3.1 An experimental system setup for the incubation of *Trichodesmium* in artificial seawater and collection of atmospheric and seawater samples ((a) picture and (b) schematic diagram of the system).

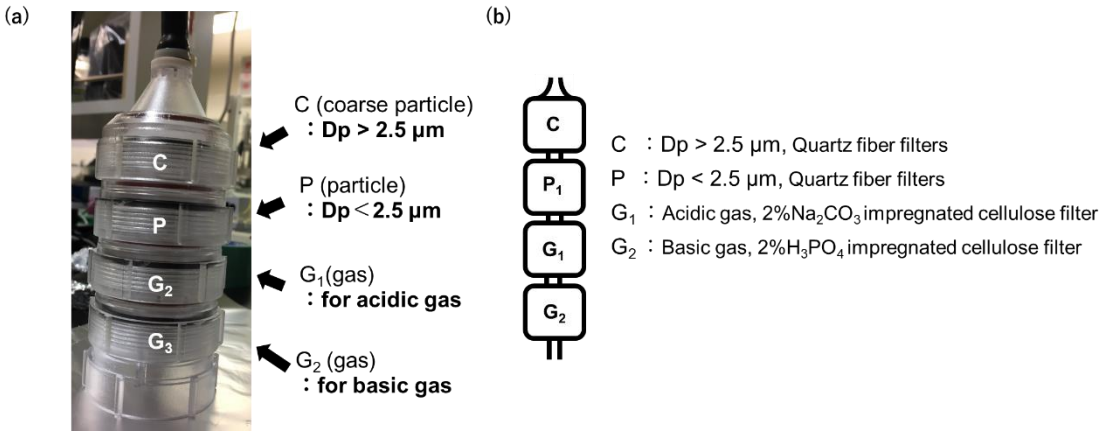


Figure 3.2 NILU impactor for collection of atmospheric particle and gas-phase samples.

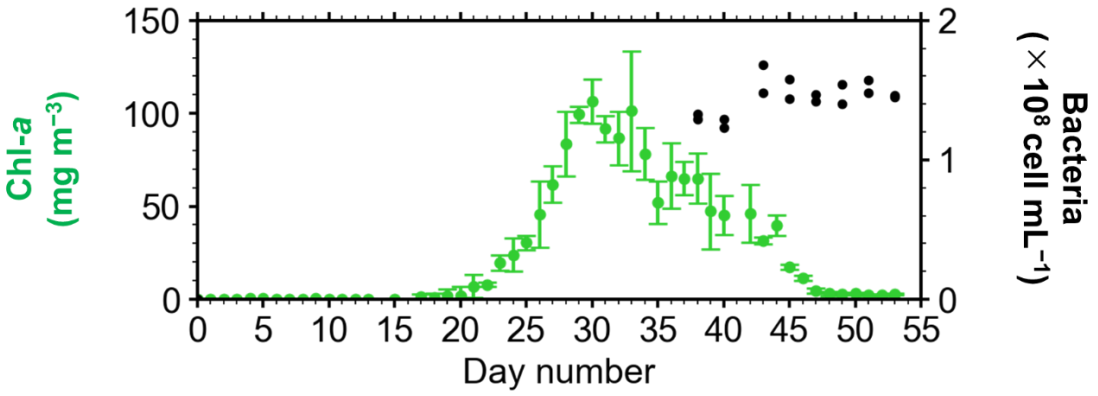


Figure 3.3 Temporal variations in the concentrations of Chl-*a* associated with the incubation of *Trichodesmium* and bacteria in artificial seawater.

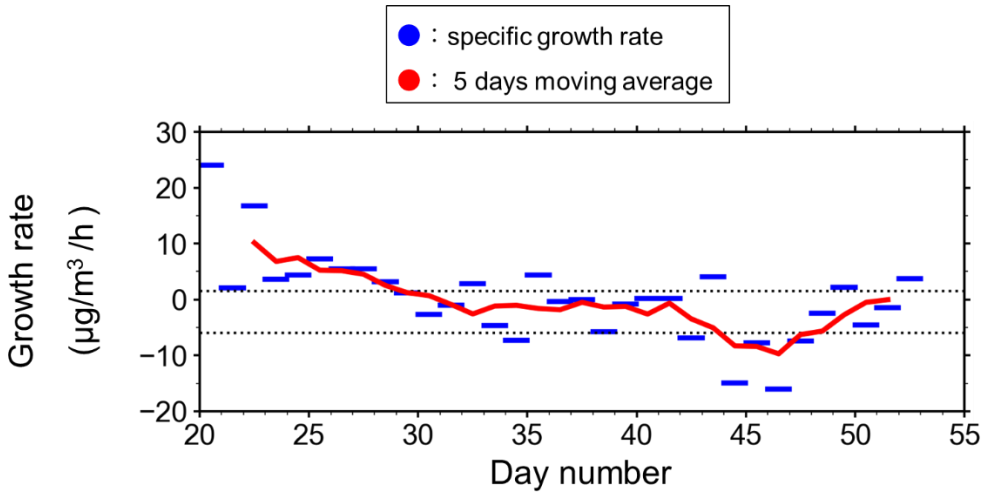


Figure 3.4 Temporal variation of the growth rate of *Trichodesmium* for individual seawater samples together with 5-day running-mean values.

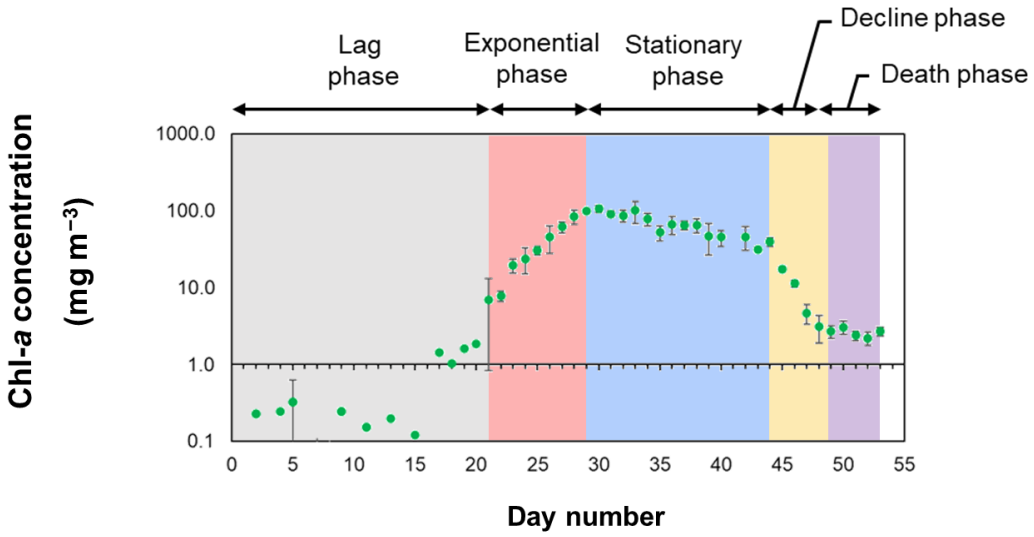


Figure 3.5 A schematic of each growth phase of *Trichodesmium* defined by its growth rate, together with the temporal variation of Chl-*a* concentration.

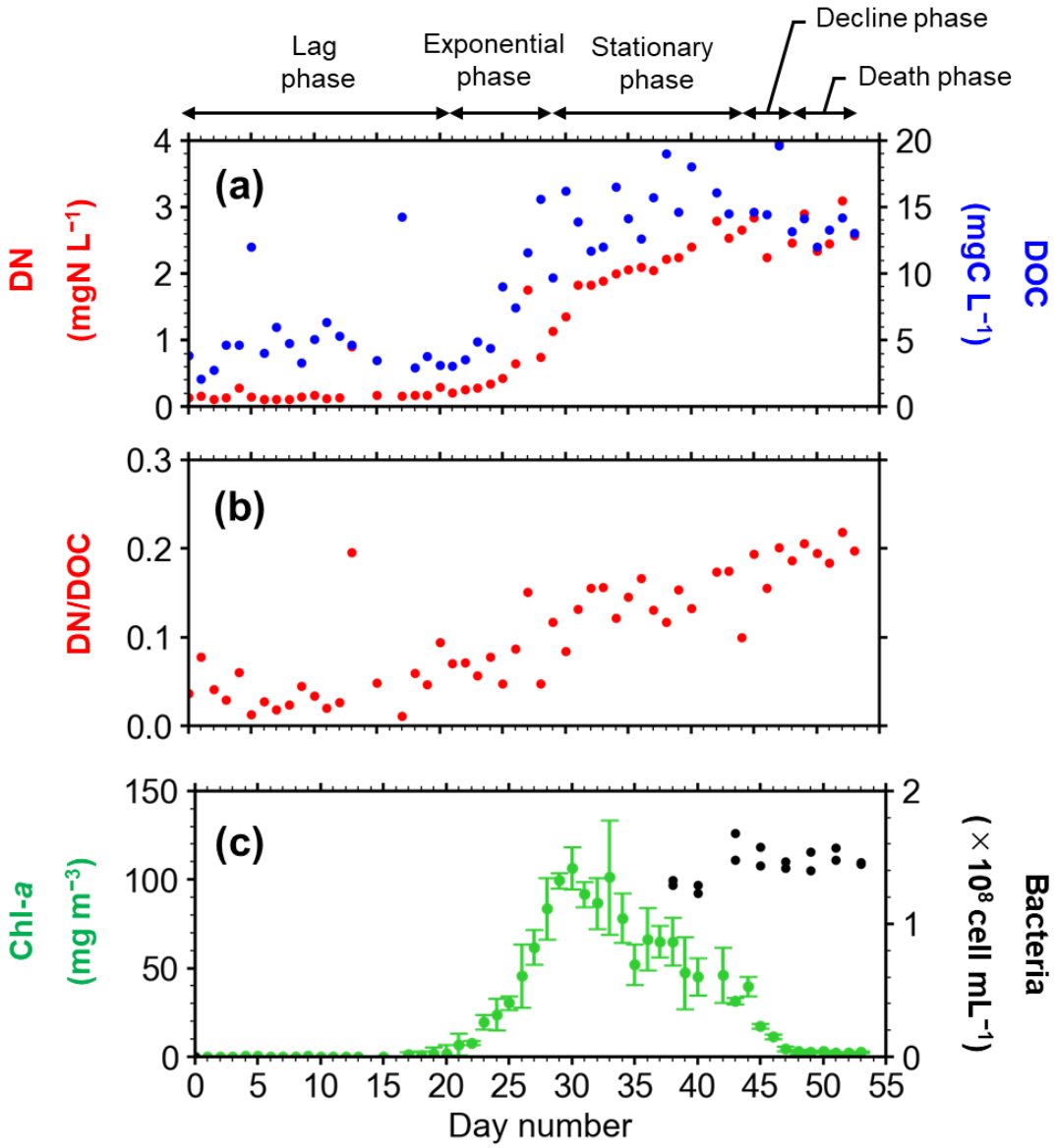


Figure 3.6 Temporal variation in (a) DN and DOC concentrations, (b) DN/DOC ratios, and (c) Chl-*a* and bacteria concentrations in artificial seawater during the experiment.

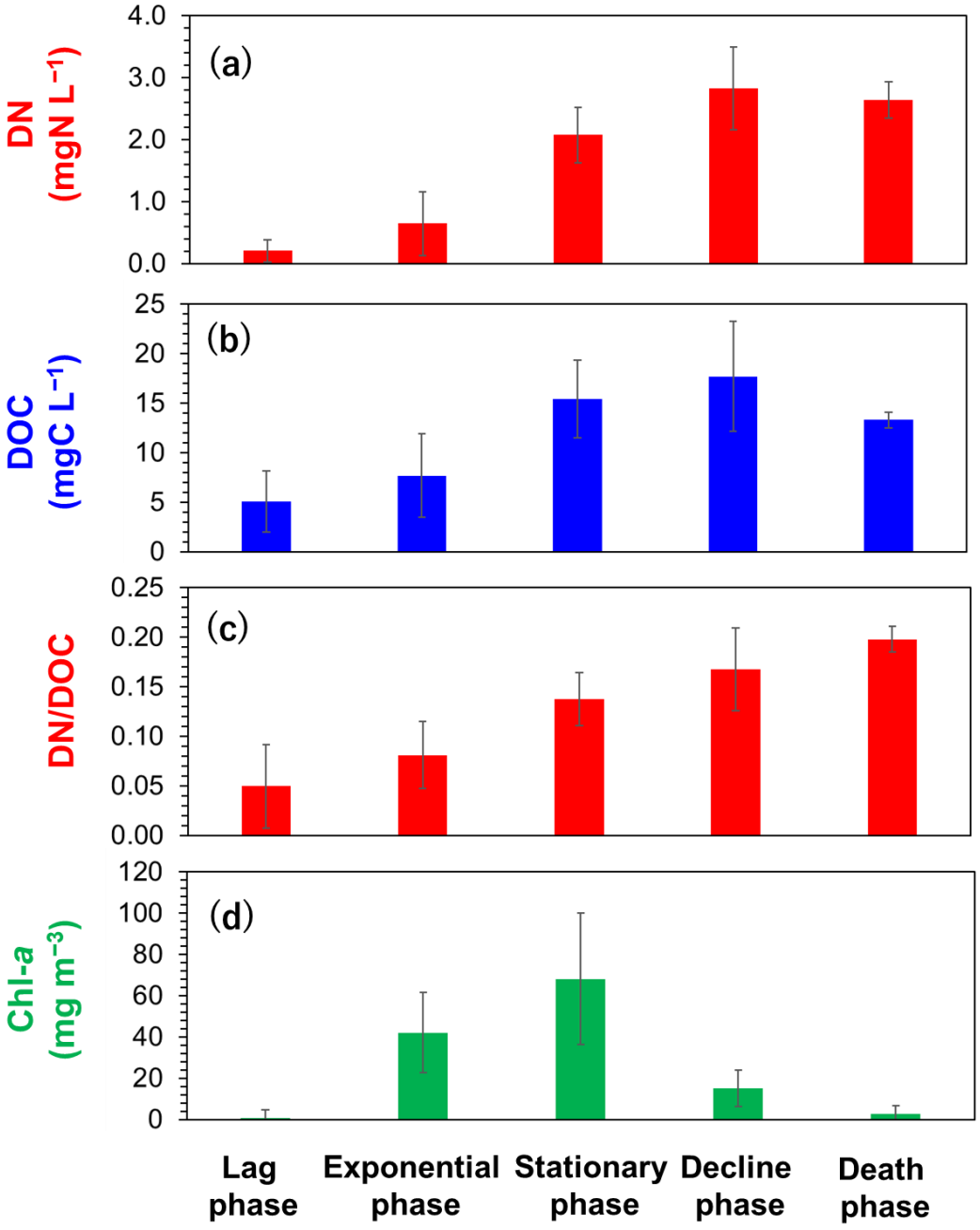


Figure 3.7 Average concentrations of (a) DN, (b) DOC, (c) DN/DOC, and (d) Chl-*a* during each growth phase of *Trichodesmium*.

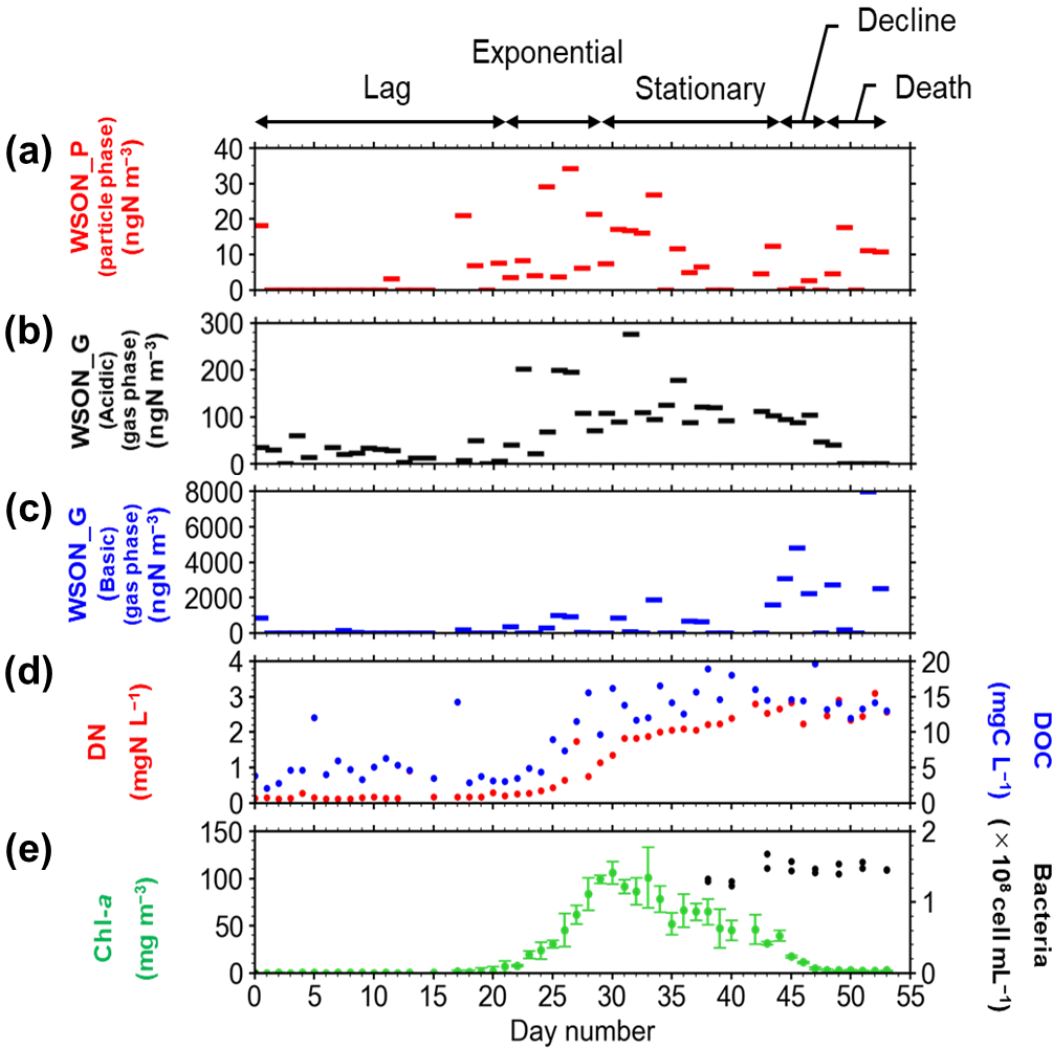


Figure 3.8 Temporal variation in the concentrations of (a) WSON (particle phase), (b) WSON (acidic gas phase), (c) WSON (basic gas phase) in atmospheric samples, and concentrations of (d) DN and DOC, and (e) Chl-*a* and bacteria in seawater samples.

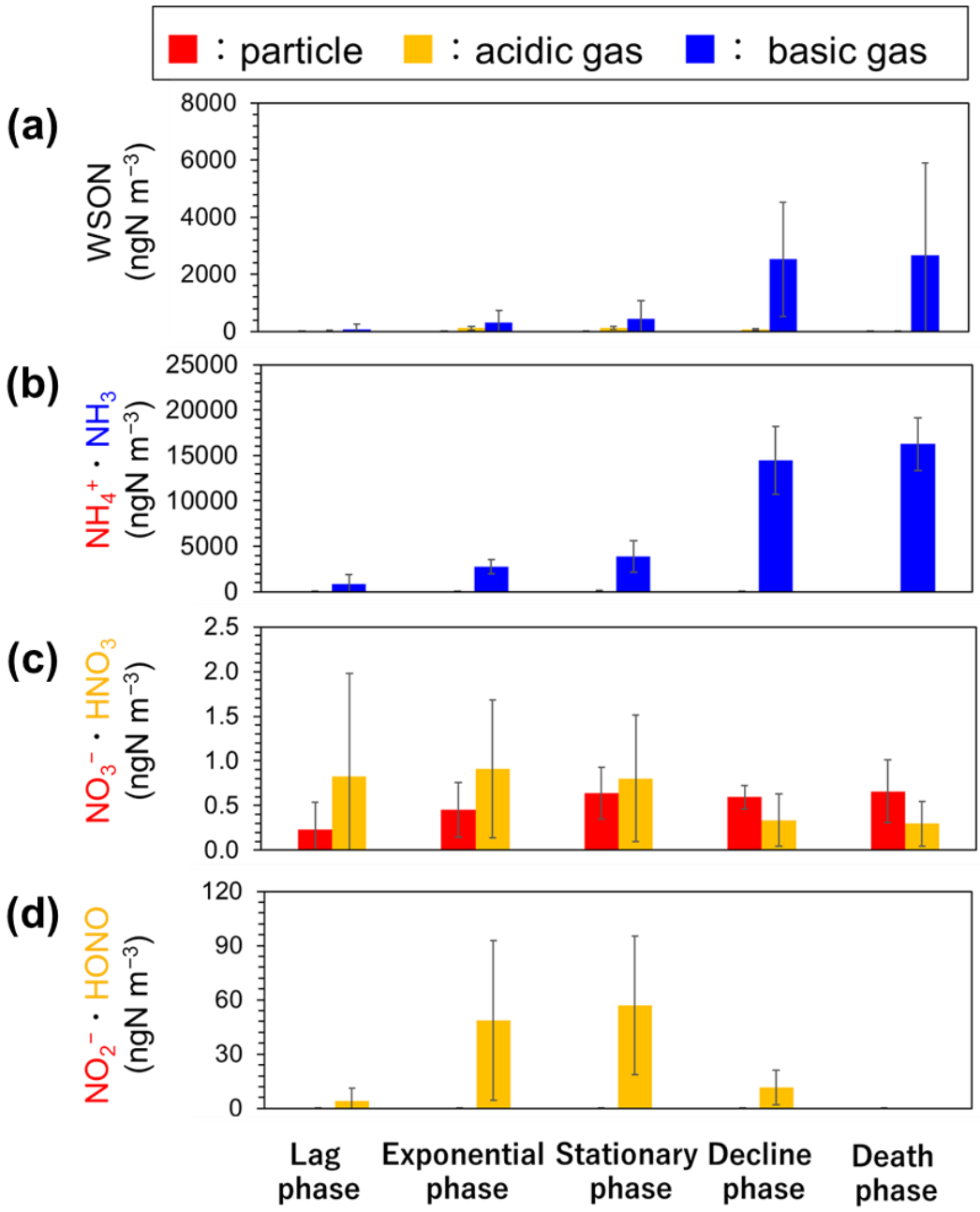


Figure 3.9 Average concentrations of (a) WSON, (b) NH_4^+, NH_3, (c) NO_3^-, HNO_3, (d) NO_2^-, and HONO in each growth phase of *Trichodesmium*.

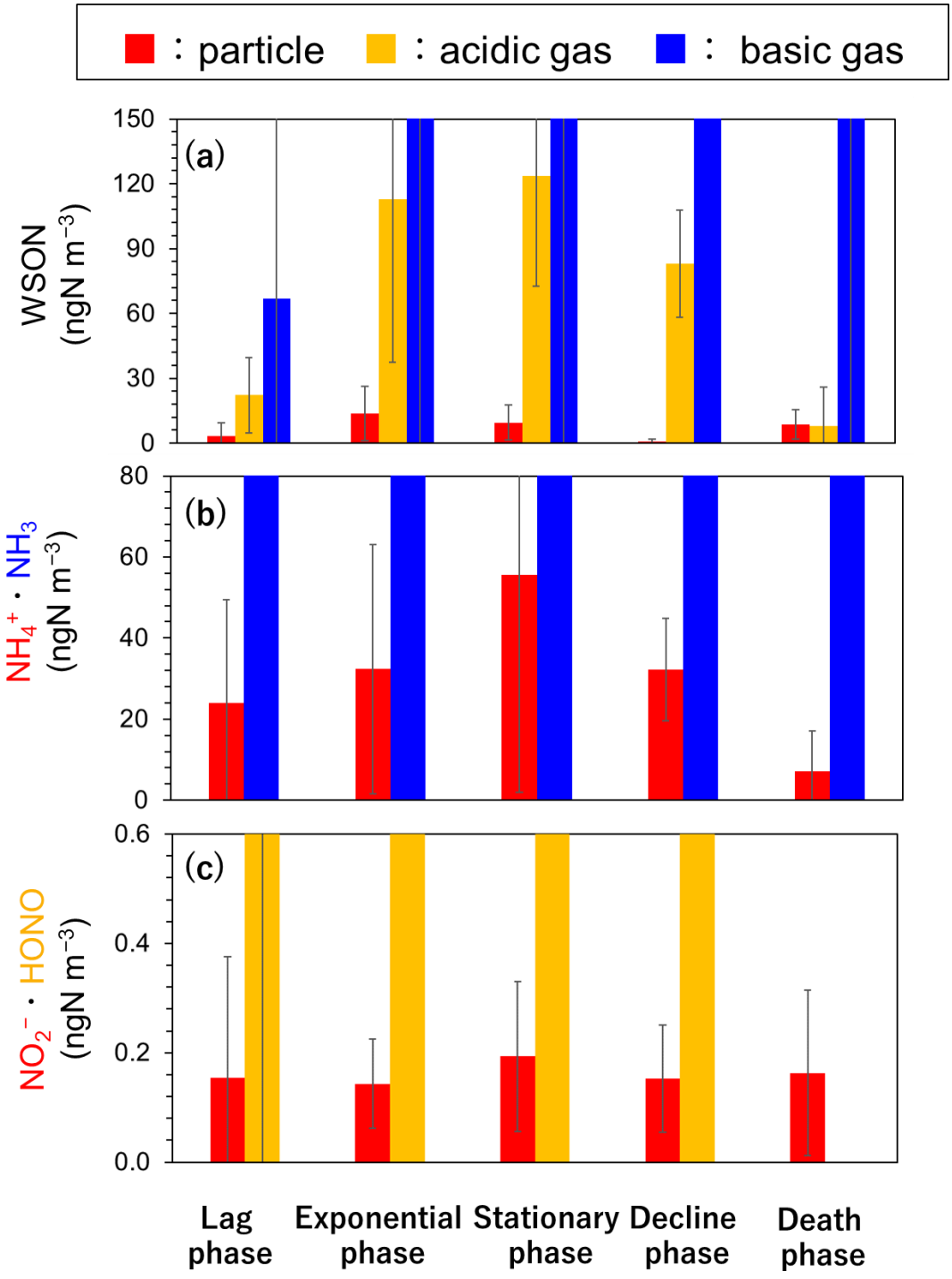


Figure 3.10 Same as Figure 3.9 except for NO_3^- and HNO_3. The scales of the y-axis are different from those in Figure 3.9.

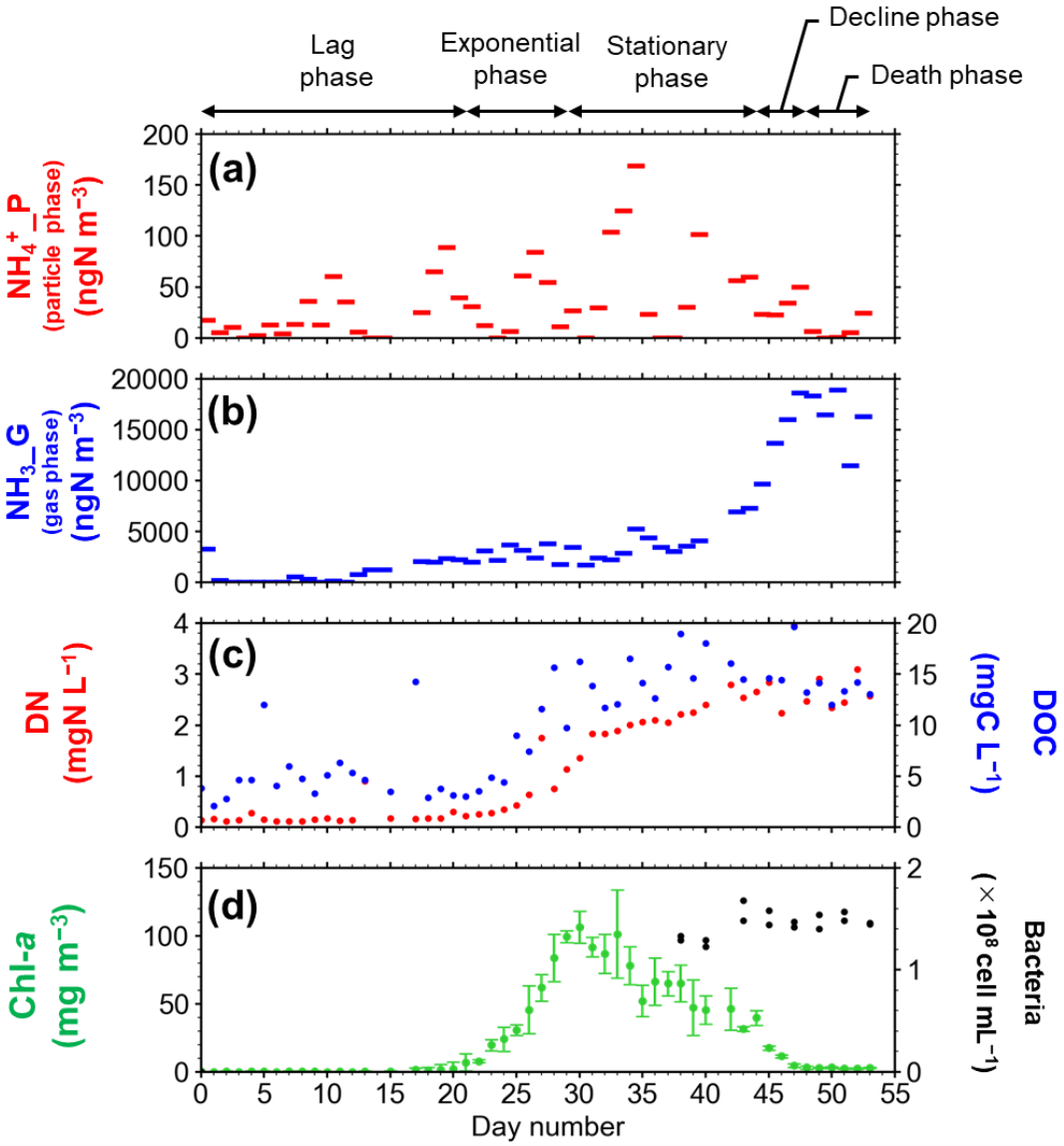


Figure 3.11 Temporal variation of atmospheric concentrations of (a) NH_4^+ (particle phase) and (b) NH_3 (gas phase), and seawater concentrations of (c) DN, DOC, (d) Chl-*a*, and bacteria.

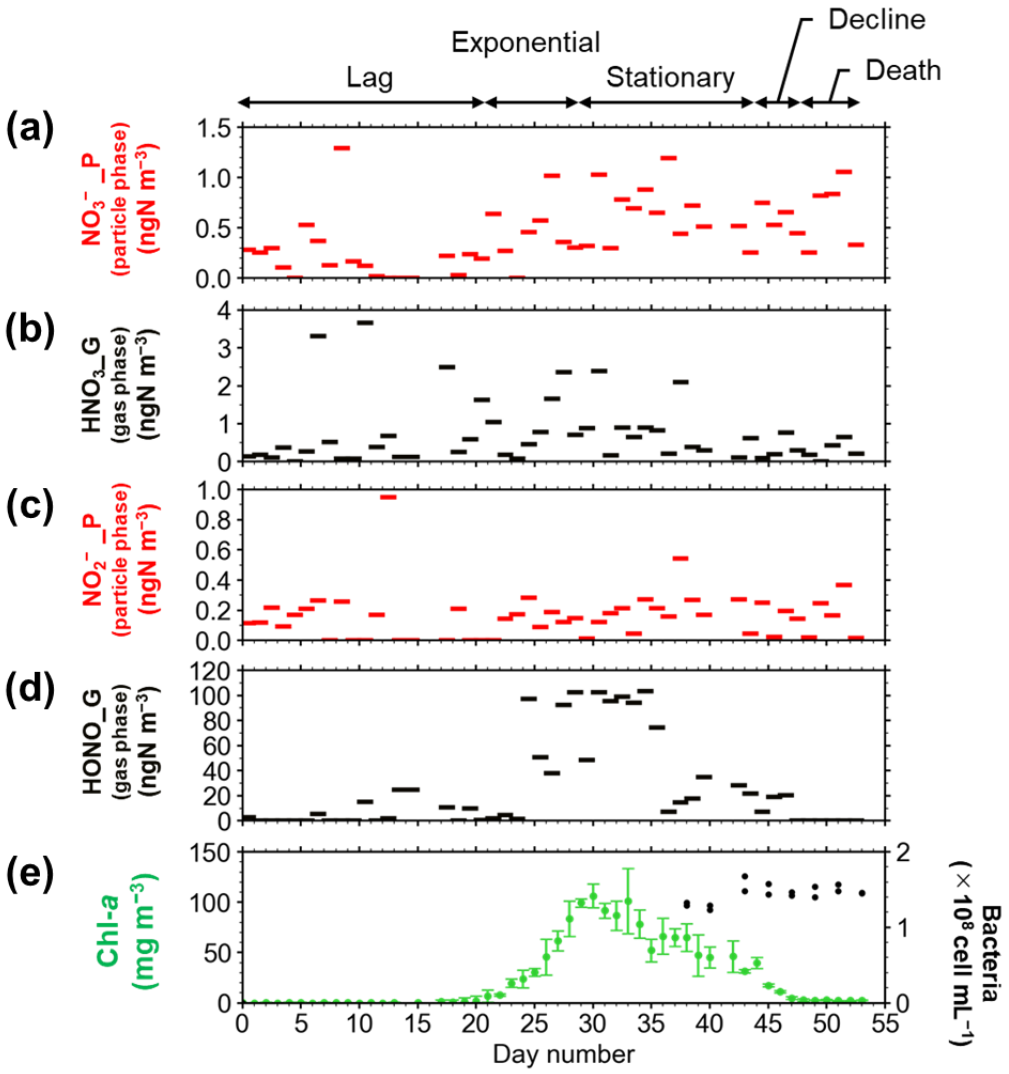


Figure 3.12 Temporal variation of (a) NO_3^- (particle phase), (b) HNO_3 (gas phase), (c) NO_2^- (particle phase), (d) HONO (gas phase), and (e) Chl-*a* and bacteria.

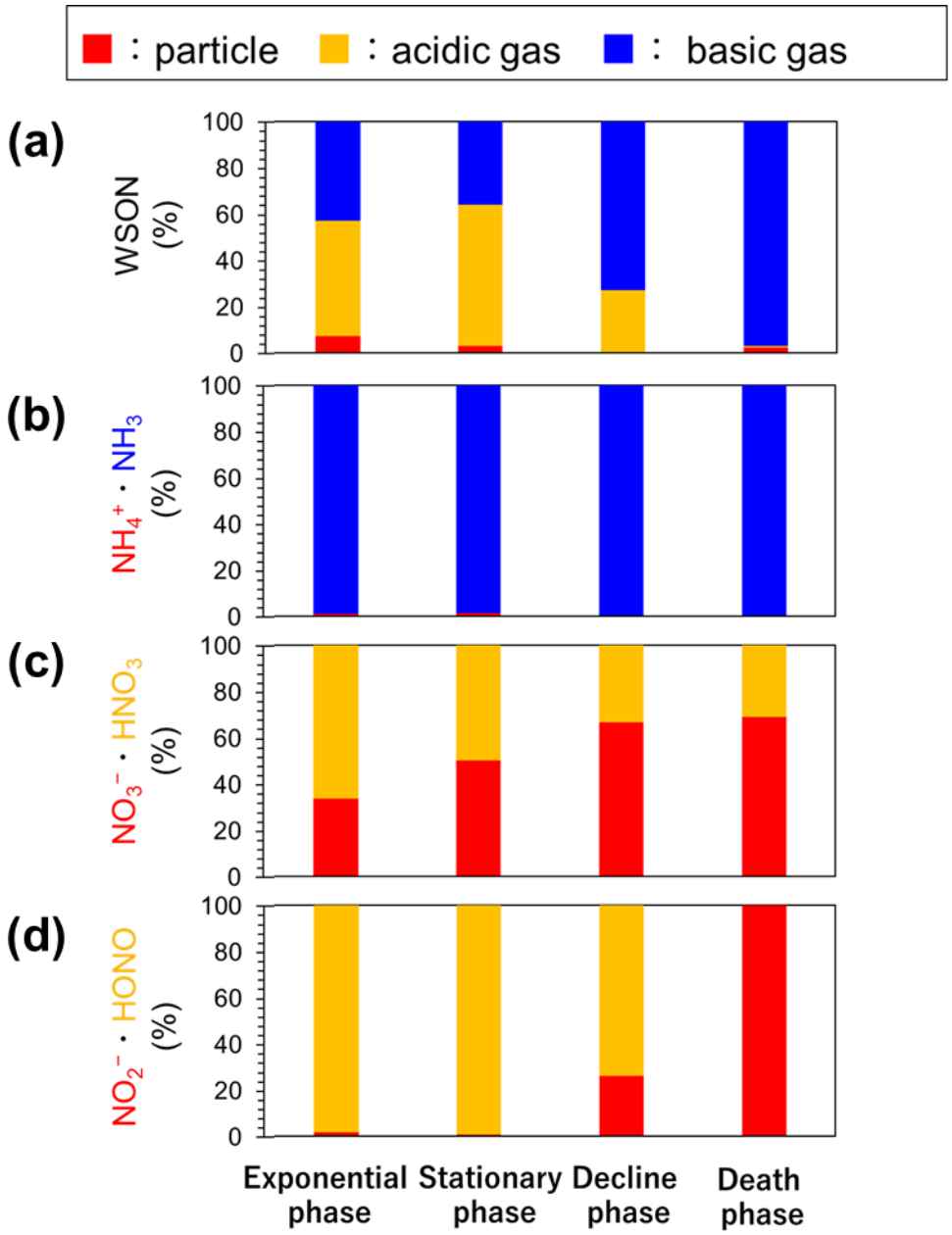


Figure 3.13 Average mass fractions of particle and gas phases of (a) WSON, (b) NH_4^+/NH_3, (c) NO_3^-/HNO_3, (d) $NO_2^-/HONO$ in each growth phase of *Trichodesmium*.

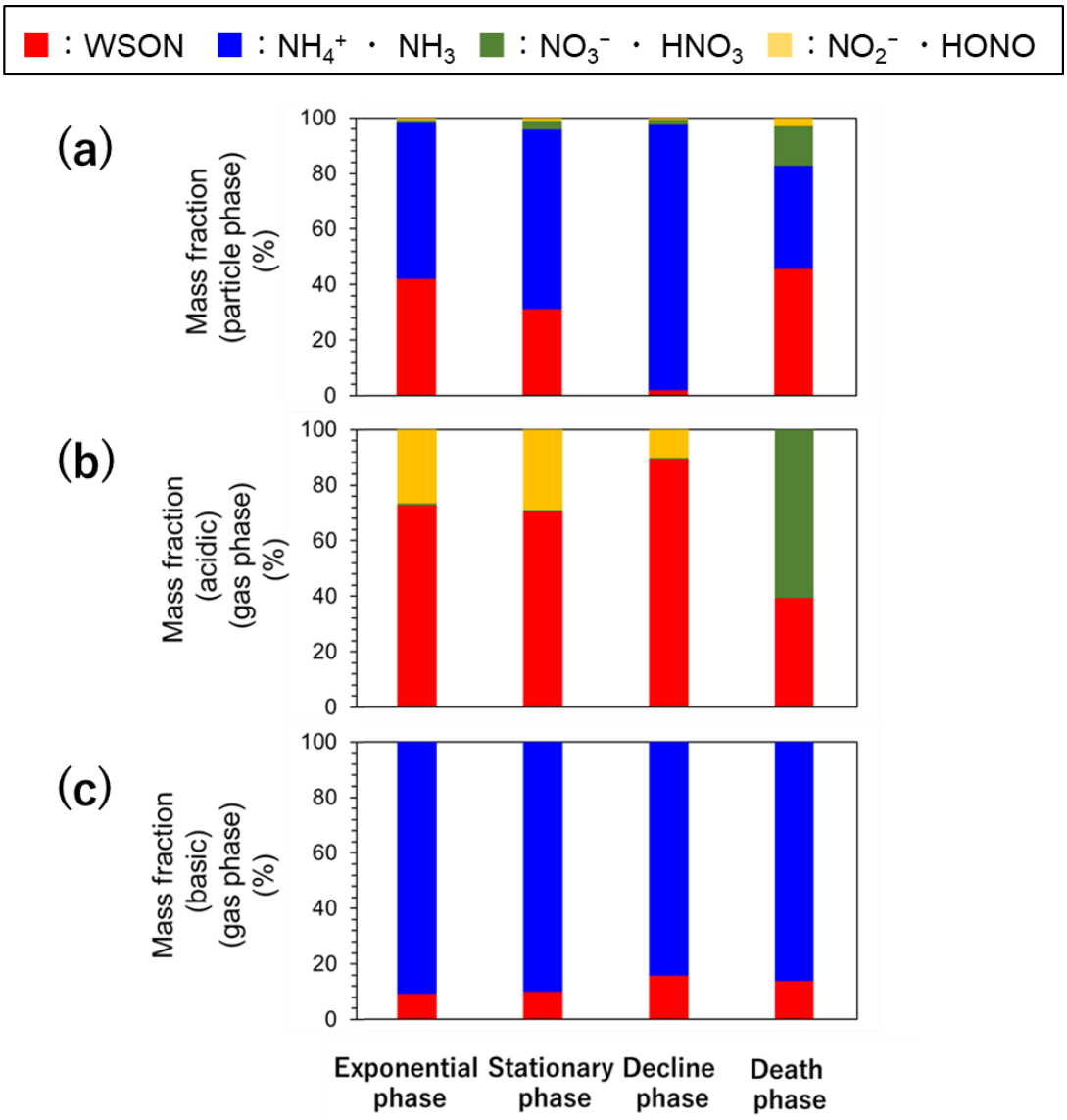


Figure 3.14 The average chemical mass fractions of reactive nitrogen species in the (a) particle phase, (b) acidic gas phase, and (c) basic gas phase in each growth phase of *Trichodesmium*.

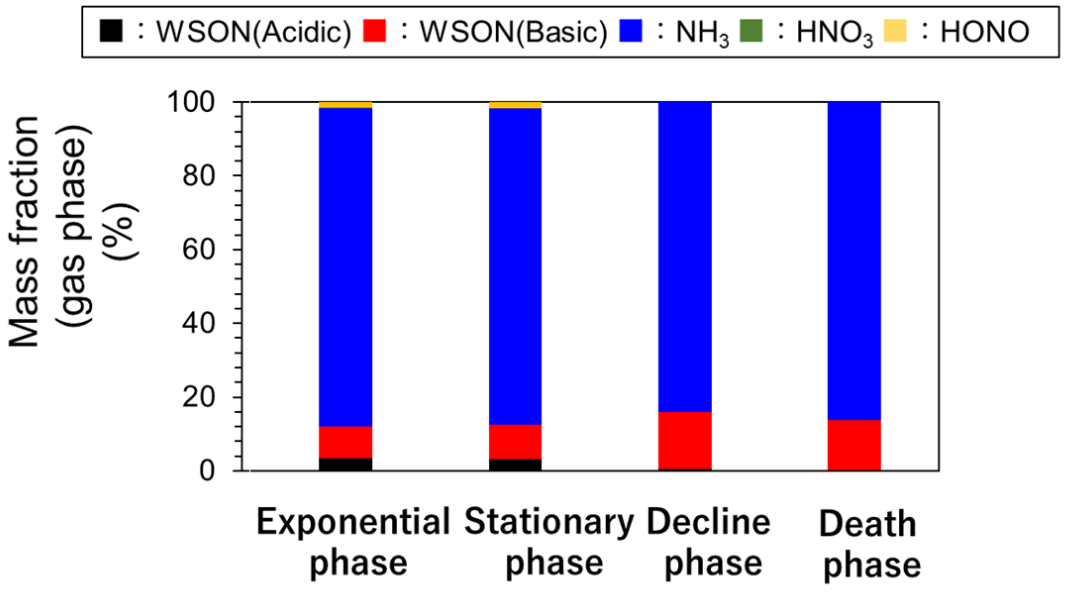


Figure 3.15 The average chemical mass fractions of reactive nitrogen species in the gas phase in each growth phase of *Trichodesmium*.

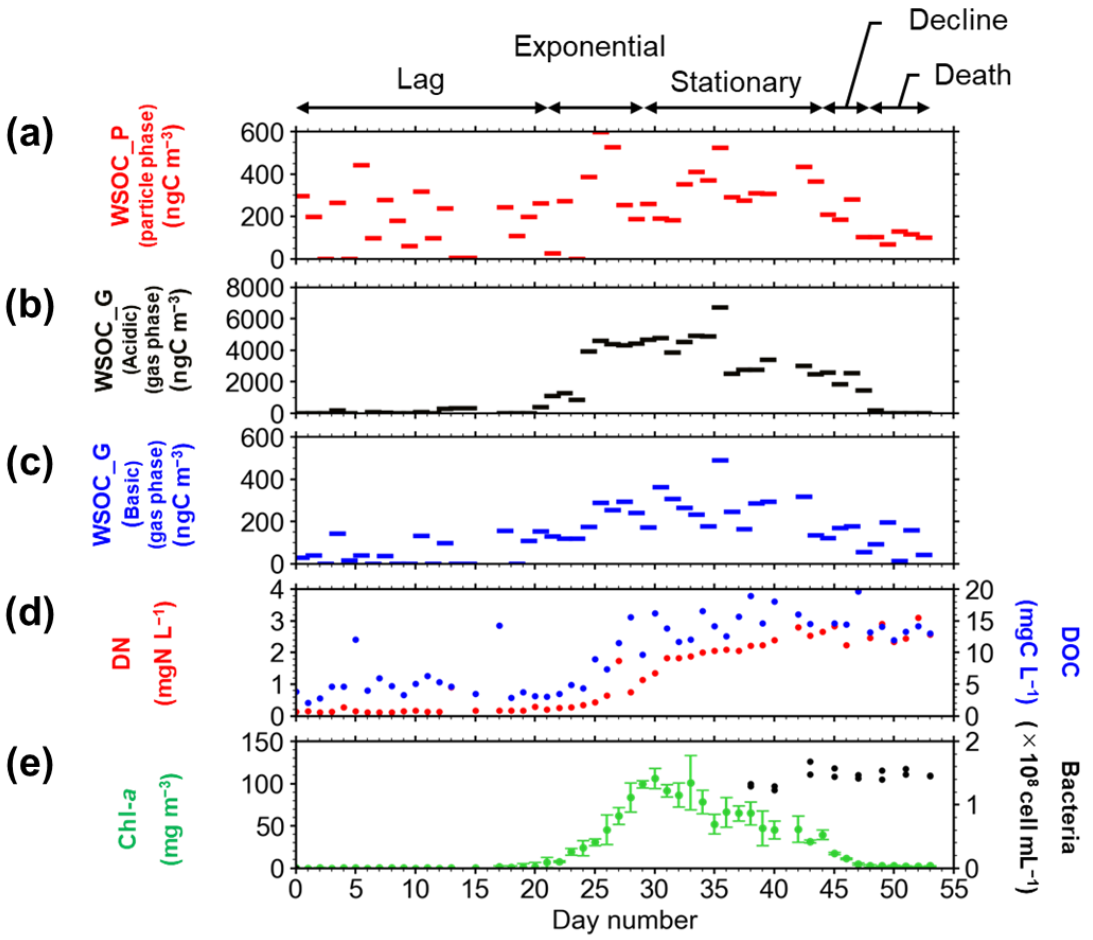


Figure 3.16 Temporal variation of concentrations of WSOC in (a) the particle phase, (b) the acidic gas phase, and (c) the basic gas phase. Also shown are concentrations of (d) DN, DOC, (e) Chl-*a* and bacteria in seawater.

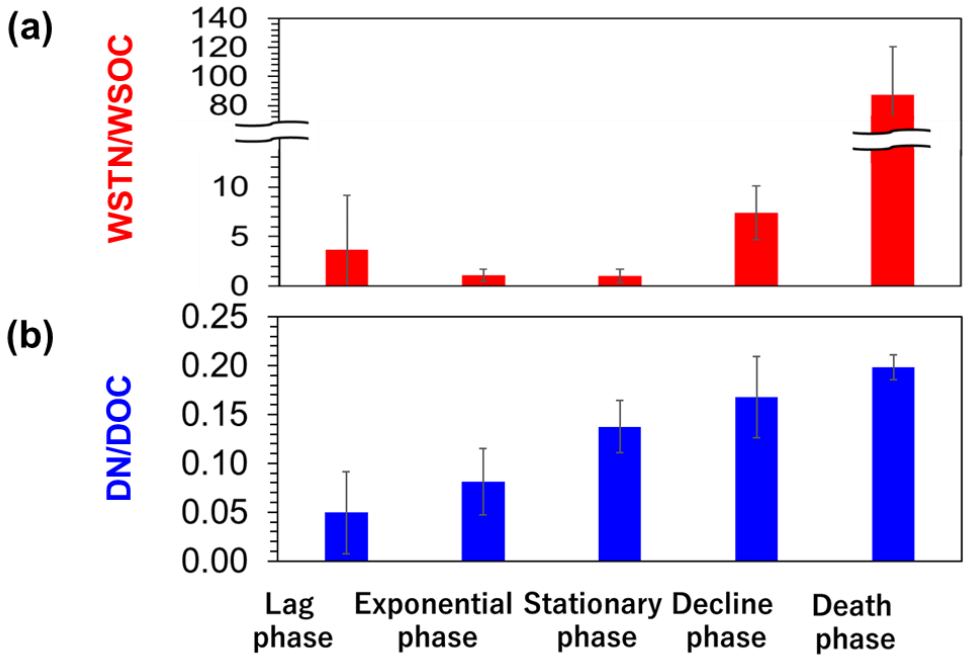


Figure 3.17 Average values of (a) WSTN/WSOC in the atmospheric samples and (b) DN/DOC in the seawater in each growth phase.

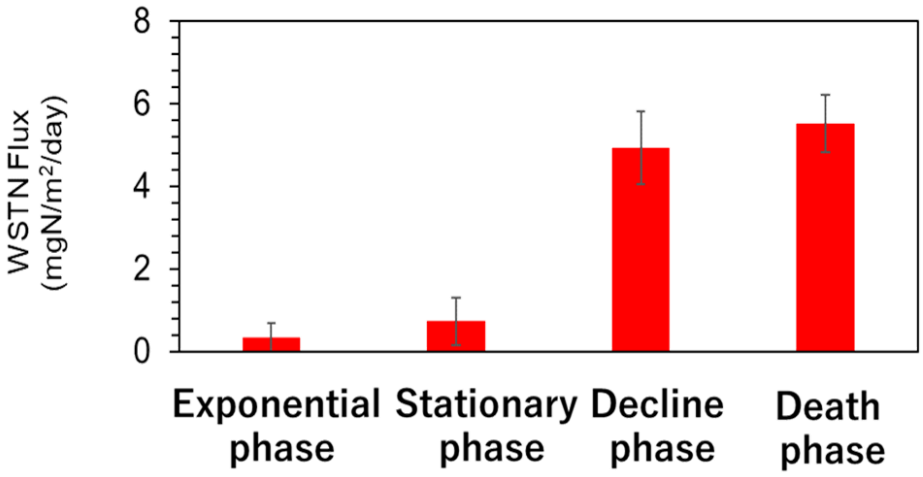


Figure 3.18 Average values of the sea-to-air flux of WSTN in each growth phase.

Table. 3.1 Each growth stage defined by 5-day running-mean values of the growth rate (GR; μg m^{-3} h^{-1}) of *Trichodesmium*.

Growth stage	GR range	day number
Lag phase	--- ([Chl-*a*] < 2.0 mg m^{-3})	0–21
Exponential phase	1.5 ≦ GR	21–29
Stationary phase	−6 ≦ GR ≦ 1.5	29–44
Decline phase	−10 ≦ GR ≦ −6	44–48
Death phase	−6 ≦ GR	48–53

Table. 3.2 C/N ratio in the atmospheric and seawater samples.

	Lag	**Exponential**	**Stationary**	**Decline**	**Death**
WSOC /WSTN	2.1±3.0	1.2±0.8	1.2±0.5	0.2±0.1	$1.2\times10^{-2}\pm0.4\times10^{-2}$
DOC/DN	32.4±22.3	14.1±5.0	7.6±1.7	6.4±2.1	5.1±0.3

Table. 3.3 Sea-to-air emission flux of WSTN per Chl-*a* mass.

		Sea-to-air flux (μgN/m^2/day/[Chl-*a* (mg/m^3)])
Exponential and Stationary phases	**This study**	**7.68 ～10.7**
Decline phase	**This study**	**～330**
NH_3/NH_4^+ in the subtropical North Pacific	**Paulot et al. (2015)**	**770–3,830**

Chapter 4. General Conclusions

This study investigated the role of marine nitrogen-fixing organisms in the formation of atmospheric reactive nitrogen based on ship-board observations and laboratory incubation experiments. The ship-board observations were conducted in the oligotrophic subtropical North Pacific from August to October 2017 with simultaneous collection of atmospheric aerosol and surface seawater samples to elucidate the origin of WSON in marine atmospheric aerosols. The average concentration of WSON in fine-mode aerosols along 23ºN in the eastern North Pacific (7.5±6.6 ngN m^{-3}) was substantially higher than that in the western North Pacific (2.4±1.9 ngN m^{-3}) during the research cruise.

The stable carbon isotope ratio of WSOC, together with backward trajectories, indicated that most of the observed WSON in the fine particles in the eastern North Pacific originated from the ocean surface. Relations of the concentrations of WSON with several tracers imply that secondary formation, which differed from the oxidation processes of DMS, was likely the dominant process underlying the formation of WSON observed in this study. Instead, significant positive correlations were found among nitrogen fixation rate, DON concentrations in SSW, and aerosol WSON concentrations. Meanwhile, the undetectable concentrations of EC in aerosols suggest that effects of anthropogenic sources on the observed aerosols were likely small in this study. The overall results suggest that reactive nitrogen (i.e., dissolved organic nitrogen and ammonium) produced

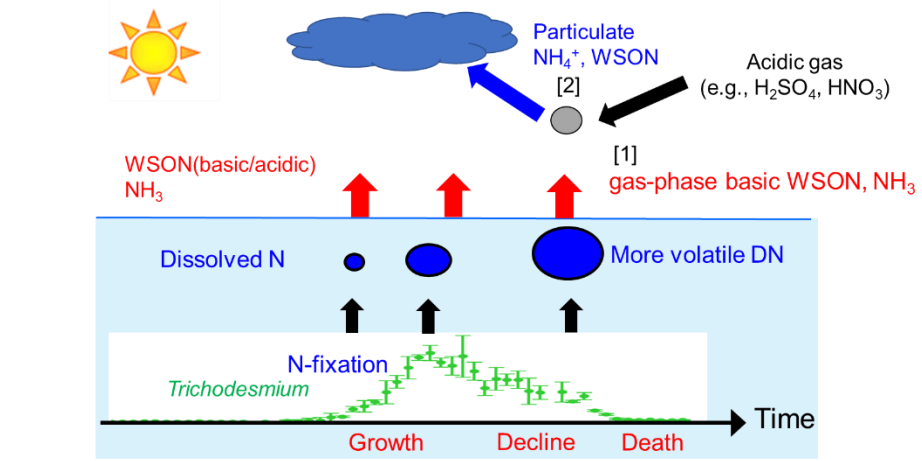
and exuded by nitrogen-fixing microorganisms in SSW likely contributed to the formation of WSON aerosols over the oceanic region.

To prove that reactive nitrogen is emitted into the atmosphere associated with nitrogen fixation, an incubation experiment with simultaneous samplings of atmospheric reactive nitrogen and biological/chemical parameters of artificial seawater was conducted in a laboratory. In the experiment, *Trichodesmium erythraeum* IMS101 was used as representative species of nitrogen-fixing organisms. During the incubation period of two months, the growth and decline phases of the nitrogen-fixing cyanobacterium were observed. During the exponential phase, DN and DOC concentrations in the seawater increased with an increase in Chl-*a* concentrations, suggesting that DN and DOC were released by nitrogen fixation during the growth of *Trichodesmium*.

Throughout the entire period of the experiment, ammonium (62%)/ammonia (86%) were the dominant reactive nitrogen both in particle and gas phases, followed by WSON (particle phase: 33%, gas phase: 13%). A substantial increase in the atmospheric emissions of NH_3 and gas-phase basic WSON was found during the decline and death phases. This result suggests the significant decomposition of DN and dead bacteria cells and/or photochemical reactions in seawater, followed by the production of low-molecular-weight, more volatile, basic DN that were more easily transferred from seawater to the atmosphere. Atmospheric emissions of acidic WSON were larger during the exponential and stationary growth phases of *Trichodesmium*. In contrast, the

emissions of basic reactive nitrogen were more evident during the decline and death phases. The overall results demonstrated sea-to-air emissions of WSON and ammonium/ammonia associated with the growth and decline of *Trichodesmium* for the first time.

The atmospheric implications of this study are summarized in **Figure 4.1**. The acidity of reactive nitrogen emitted into the atmosphere associated with nitrogen fixation may differ depending on the growth phase of nitrogen-fixing microorganisms, which can further affect the acidity of the atmosphere. This effect includes possible reactions of basic WSON and/or ammonia with sulfuric acid and other acidic species. These reactions may affect gas-to-particle conversion in the atmosphere, leading to an increase in particle number concentrations. Changes in the particle number concentration can affect cloud droplet concentrations produced over the oceanic region and are thus crucial for understanding their climate effects.



Sea-to-air emissions of NH_3 and WSON:
[1] Change acidity of marine aerosol, which may affect atmospheric chemistry over the ocean
[2] Increase aerosol particle number concentrations by reacting with acidic gas species, and can affect cloud number concentrations

Figure 4.1 Atmospheric implications of the results obtained in this study.