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Interactions between microorganisms and oxic-anoxic transitions

Diao, M.

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General Introduction

Hypoxia and anoxia

Every summer, 'dead zones' without fish, shrimp and crabs occur across vast areas spanning thousands of km² in the Gulf of Mexico, the Baltic Sea, the Arabian Sea, the East China Sea, and many other coastal waters (Rabalais *et al.*, 2002; Diaz and Rosenberg, 2008). Similar dead zones are observed in Lake Erie and many smaller inland waters (Conroy *et al.*, 2011; Jenny *et al.*, 2016). They are caused by oxygen depletion (Diaz and Rosenberg, 2008). Oxygen is a critical component for aquatic ecosystems. Many fish and aquatic invertebrates suffer from a lack of oxygen if dissolved oxygen (DO) concentrations decline below 5 mg l⁻¹, and further depletion below 2 mg l⁻¹ often leads to fish kills and mass mortalities of aquatic invertebrates.

For practical applications, when dissolved oxygen is still present, but decreases below 2 mg l⁻¹, one speaks of **hypoxia** (Rabalais *et al.*, 2002; Vaquer-Sunyer and Duarte, 2008). When oxygen levels have been fully depleted, it is termed **anoxia** (Karlson *et al.*, 2002). Anoxia stimulates the formation of hydrogen sulfide, which is highly toxic for many organisms. The combination of anoxia and high sulfide concentrations is called **euxinia**. Prolonged hypoxia, and especially anoxia and euxinia, are detrimental for most aerobic organisms (Diaz and Rosenberg, 1995; Vaquer-Sunyer and Duarte, 2008).

Hypoxia and anoxia have existed throughout geologic time (Rabalais *et al.*, 2002). However, during the past decades, the frequency, intensity and duration of hypoxia and anoxia have increased in both lakes and coastal waters due to eutrophication and global warming (Diaz and Rosenberg, 2008; Middelburg and Levin, 2009; Jenny *et al.*, 2016).

Eutrophication is caused by high nutrient (nitrogen and phosphorus) inputs from, for instance, agriculture and urban wastewater (Conley *et al.*, 2009; Chislock *et al.*, 2013). Nutrient enrichment stimulates dense phytoplankton blooms of cyanobacteria and algae (Paerl and Huisman, 2008; Michalak *et al.*, 2013). The subsequent microbial degradation of senescent blooms and other sources of organic material may deplete DO in waters, killing fish and other aquatic organisms. Rising temperatures decrease the solubility of oxygen in water (Deutsch *et al.*, 2011). Furthermore, rising temperatures strengthen thermal stratification of lakes and seas, which effectively suppresses oxygen exchange between the atmosphere and deeper water layers (Rabalais *et al.*, 2002; Livingstone, 2003; Thackeray *et al.*, 2008).

Rising temperatures and CO₂ levels also promote phytoplankton blooms in eutrophic waters (Paerl and Huisman, 2008; Verspagen *et al.*, 2014), further increasing the risk of hypoxia and anoxia when these blooms die off. Therefore, eutrophication and climate change are main drivers of the expanding zones of hypoxia and anoxia in lakes and coastal waters.

In many eutrophied lakes and seas in temperate latitudes, oxygen depletion is an annually recurrent phenomenon in deeper water layers (a.k.a. the hypolimnion), when the water column stratifies in summer. In case of mild oxygen depletion in the hypolimnion, usually hypoxia rapidly disappears during fall turnover when the hypolimnion mixes with oxygen-rich surface waters. In more severe situations with anoxia and euxinia, however, low-oxygen conditions may spread out from the hypolimnion throughout the entire water column in the fall and it may take several days to weeks before the oxic conditions are restored (Ciglenc̆ki *et al.*, 2005; Pjevac *et al.*, 2015). Hence, oxic-anoxic transitions are frequently observed in seasonally stratified ecosystems (Rabalais *et al.*, 2007; Garcia *et al.*, 2013; Yu *et al.*, 2014).

Although transitions between oxic and anoxic water are accompanied by major changes in microbial community structure and biogeochemical processes, a comprehensive understanding of the different biological and chemical feedbacks involved in oxic-anoxic transitions is still lacking. Therefore, it is imperative to investigate how microorganisms interact with oxic-anoxic transitions since hypoxia and anoxia are increasingly threatening aquatic ecosystems.

Microbial communities in freshwater lakes

It is well known that microorganisms drive the major biogeochemical cycles on Earth (Azam and Worden, 2004; Falkowski *et al.*, 2008; Fuhrman, 2009). Hence, aquatic microorganisms, including bacteria and archaea, have been extensively studied for decades (Blaauboer, 1982; Christensen and Sorensen, 1986; Eiler and Bertilsson, 2004; Kent *et al.*, 2007; Salcher *et al.*, 2008; Nelson, 2009; Auguet *et al.*, 2011; Auguet *et al.*, 2012; Yang *et al.*, 2017). Common bacterial groups in lakes are *Actinobacteria*, *Bacteroidetes*, *Chlorobi*, *Cyanobacteria*, *Firmicutes*, *Proteobacteria* (*Alpha-*, *Beta-*, *Gamma-*, and *Delta-*) and *Verrucomicrobia* (Newton *et al.*, 2011; Karhunen *et al.*, 2013; Dai *et al.*, 2016). The dominant archaeal groups in lakes are

Crenarchaeota and *Euryarchaeota* (Liros *et al.*, 2008). To date, the ecological roles of many of these microbial groups are still elusive because of the difficulties in culturing and isolating them (Newton *et al.*, 2011).

In recent years, high-throughput DNA and RNA sequencing has provided more detailed and clear insights into the composition, dynamics and activities of microbial communities (Lauro *et al.*, 2011; Fortunato *et al.*, 2013). Consequently, nowadays a much higher taxonomic resolution of the composition and dynamics of lake bacteria and archaea can be achieved than before (Eiler *et al.*, 2012; Pester *et al.*, 2012). Furthermore, functional genes indicate the biogeochemical transformations in which these microorganisms are potentially involved, and metatranscriptomic analysis provides insights into the expression of these functional activities at the level of the lake microbiome (Llorens-Marès *et al.*, 2015; Steffen *et al.*, 2015; Yang *et al.*, 2016). Hence, it has now become feasible to explore community dynamics of microorganisms as well as their roles in ecosystem functioning with the help of advanced molecular techniques and bioinformatics approaches (Fortunato *et al.*, 2013; Lima-Mendez *et al.*, 2015; Harke *et al.*, 2016).

Microbial sulfur cycle

The sulfur cycle is of paramount importance to aquatic microbial communities, because sulfur compounds can be used as electron donors and/or acceptors by a variety of different microorganisms. In addition, sulfur is an important component of proteins. Sulfur oxidation states in aquatic environments span a wide range, from -2 (sulfide and reduced organic sulfur) to +6 (sulfate). Sulfate is the most commonly used sulfur resource for plants and microbes, whereas sulfide is very toxic to most multi-cellular organisms and some microorganisms (Lavik *et al.*, 2009). Microbially-mediated transformations between sulfide and sulfate can occur via a variety of pathways (**Figure 1.1**). Specifically, dissimilatory sulfur and sulfate reduction and chemolithotrophic sulfur oxidation are ancient microbial metabolisms (Canfield and Raiswell, 1999).

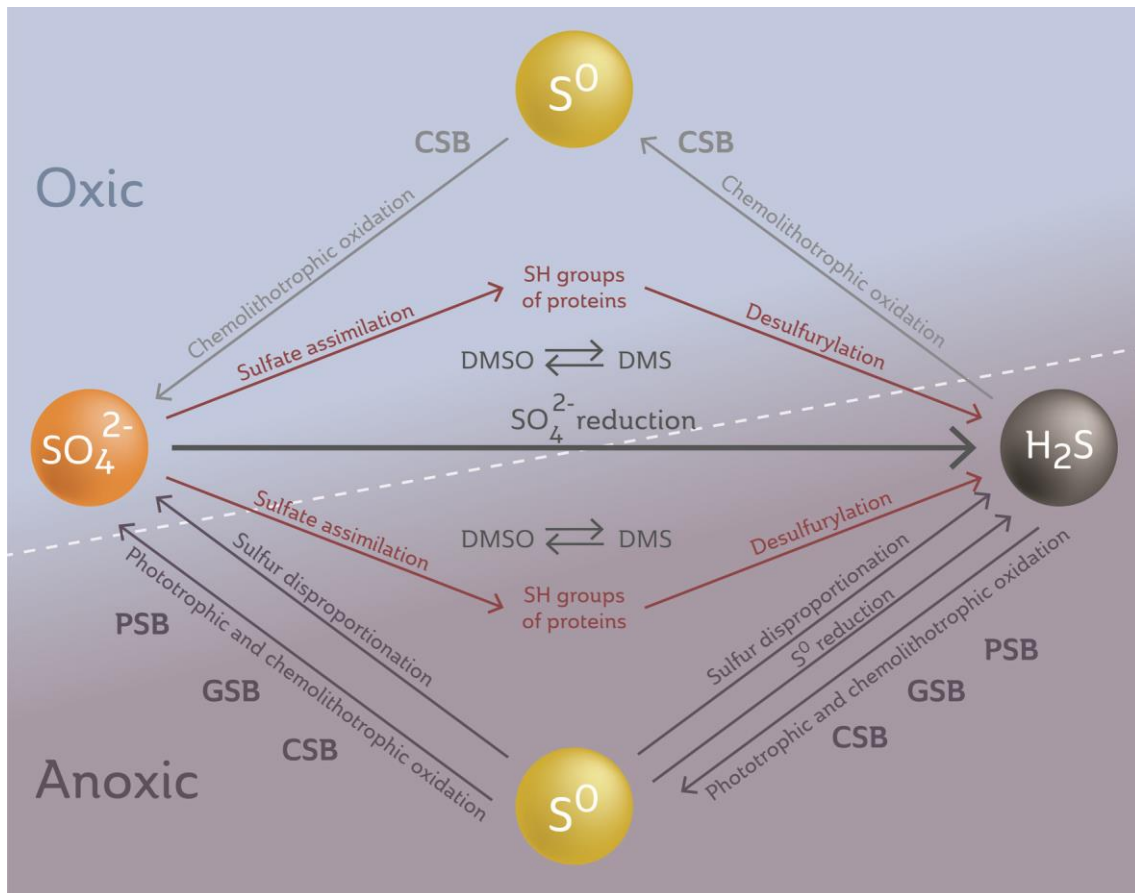


Figure 1.1. Overview of the microbial sulfur cycle. This figure is adapted from Muyzer and Stams (2008).

Sulfate-reducing bacteria

Sulfate-reducing bacteria (SRB) are anaerobic bacteria and archaea that respire organic carbon compounds using sulfate as terminal electron acceptor (Muyzer and Stams, 2008). SRB play important roles in carbon cycling since they can degrade organic matter in anoxic environments (Jørgensen, 1982). Furthermore, SRB can co-exist with methanogens and affect methane production (Sela-Adler *et al.*, 2017). In freshwater sediments, sulfate reduction is also a dominant force in iron cycling (Hansel *et al.*, 2015).

SRB mainly belong to the *Deltaproteobacteria*, *Nitrospirae*, *Clostridia*, *Thermodesulfobiaceae*, *Thermodesulfobacteria*, *Euryarchaeota* and *Crenarchaeota* (Muyzer and Stams, 2008). In the eutrophic meromictic Lake Harutori, SRB were observed in anoxic water layers below the chemocline (Kubo *et al.*, 2014). Seasonal fluctuations in the populations of SRB and their sulfate-reducing activity were reported in the sediment of Lake Kizaki in Japan (Li *et al.*, 1999).

Sulfur-oxidizing bacteria

Sulfur-oxidizing bacteria (SOB) oxidize reduced sulfur compounds, such as hydrogen sulfide (H_2S) to elemental sulfur (S^0) or sulfate (SO_4^{2-}). Based on the energy source, SOB comprise 2 functional groups: phototrophic sulfur bacteria and chemolithotrophic sulfur-oxidizing bacteria. Bacteria of the latter group are also termed colorless sulfur bacteria (CSB) (Muyzer *et al.*, 2013). Phototrophic sulfur bacteria use light as energy source and sulfide, elemental sulfur or thiosulfate as electron donor for anoxygenic photosynthesis (Frigaard and Dahl, 2009), whereas CSB use reduced sulfur compounds as energy source (Muyzer *et al.*, 2013; Ghosh and Dam, 2009).

Phototrophic sulfur bacteria include green sulfur bacteria (GSB) and purple sulfur bacteria (PSB) (Frigaard and Dahl, 2009; Gregersen *et al.*, 2011). GSB belong to the phylum *Chlorobi*, while PSB belong to the class *Gammaproteobacteria* within the phylum *Proteobacteria* (Frigaard and Dahl, 2009). GSB and PSB can make important contributions to primary production in several ecosystems (Takahashi and Ichimura 1968; Storelli *et al.*, 2013). Furthermore, many GSB and PSB can fix atmospheric nitrogen, indicating their important roles in the nitrogen cycle (Madigan, 1995; Ohkouchi *et al.*, 2005).

GSB and PSB contain specific pigments for photosynthesis, which can be used for their detection by microscopic techniques (Tonolla *et al.*, 2003; Decristophoris *et al.*, 2009). GSB (*Chlorobiacea*) dominated in the chemocline of Lake Suigetsu (Mori *et al.*, 2013) and anoxic water layers of Lake Mekkajärvi (Saarenheimo *et al.*, 2016). Similarly, PSB were observed in the chemocline of the meromictic Lake Shunet and Mahoney Lake (Rogozin *et al.*, 2012; Hamilton *et al.*, 2014). Coexistence of GSB and PSB in the metalimnion has been observed in several stratified lakes, for instance in Lake Cadagno (Tonolla *et al.*, 2005; Decristophoris *et al.*, 2009).

CSB are taxonomically diverse, including bacteria from *Alpha*-, *Beta*-, *Gamma*-, and *Epsilonproteobacteria* (Friedrich, 1998; Ghosh and Dam, 2009; Muyzer *et al.*, 2013). Accordingly, the metabolic pathways in CSB are quite diverse and versatile (Ghosh and Dam, 2009). CSB are often the dominant primary producers in aphotic environments (Grote *et al.*, 2008; Noguerola *et al.*, 2015). In seasonally stratified lakes, it has been observed that phototrophic sulfur bacteria were replaced by CSB

when the lake was mixed after stratification (Noguerola *et al.*, 2015; Pjevac *et al.*, 2015).

Microbial nitrogen cycle

The element nitrogen (N) is an essential nutrient for all living organisms, as it is a crucial constituent of proteins and nucleic acids. It is well known that microorganisms play vital roles in transforming nitrogen compounds in both aquatic and terrestrial environments (Falkowski *et al.*, 2008; Nelson *et al.*, 2016). These transformation processes are of paramount importance for ecosystem functioning. Therefore, numerous studies have been performed on the diversity, abundance and distribution of nitrogen microorganisms in lakes (Hastings *et al.*, 1998; Schubert *et al.*, 2006; Halm *et al.*, 2009). Based on their functions in nitrogen transformations, the microorganisms involved in nitrogen cycling are classified into different groups (Figure 1.2).

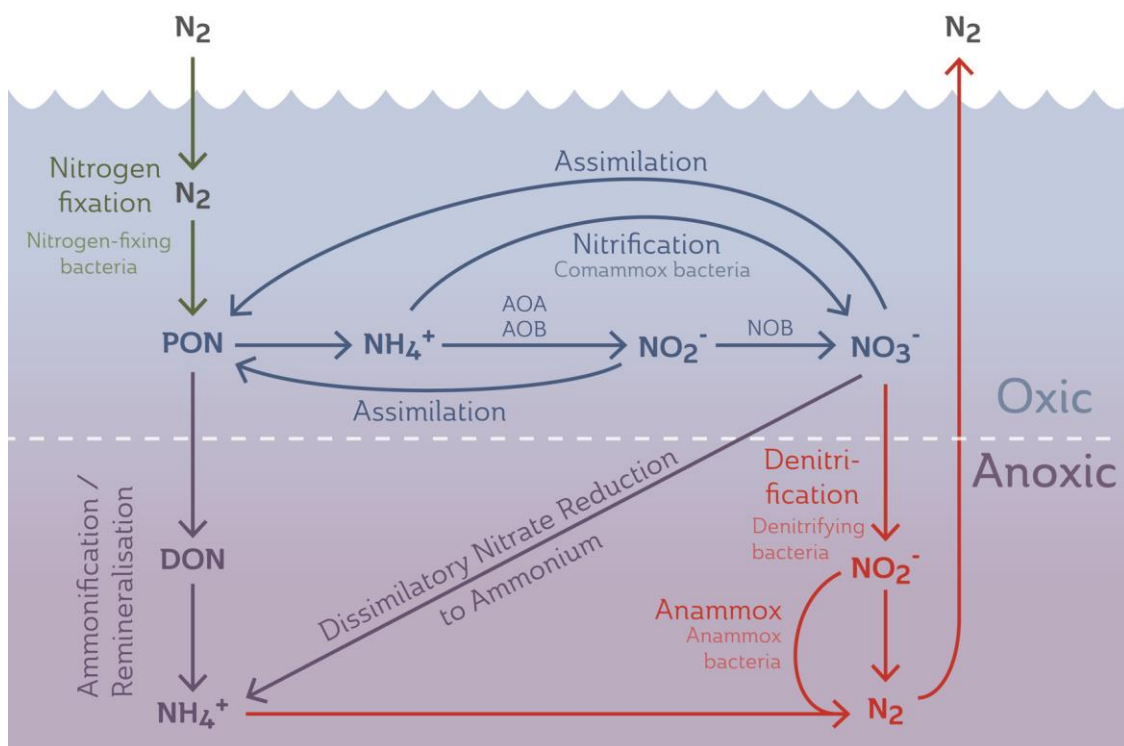


Figure 1.2. Overview of the microbial nitrogen cycle. This figure was adapted from Rush and Sinninghe Damsté (2017). PON: particulate organic nitrogen; DON: dissolved organic nitrogen.

Ammonia-oxidizing bacteria and archaea

Nitrification is the process in which ammonia (NH_3) is oxidized to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-). The first and rate-limiting step of nitrification is ammonia oxidation, which is catalyzed by the enzyme ammonia monooxygenase (Rotthauwe *et al.*, 1997). For a long time, it was believed that ammonia-oxidizing bacteria (AOB) were the only microorganisms that could perform ammonia oxidization, until the discovery of ammonia-oxidizing archaea (AOA) belonging to the new phylum *Thaumarchaeota* (Konneke *et al.*, 2005; Leininger *et al.*, 2006). Recent work shows that AOA and AOB coexist in various environments, including soils, marine and freshwater ecosystems (Li *et al.*, 2015; Lu *et al.*, 2016; Ouyang *et al.*, 2017).

The relative abundance of AOA and AOB in lakes shows seasonal fluctuations (Vissers *et al.*, 2013; Lu *et al.*, 2015; Yang *et al.*, 2016) and is determined by environmental conditions, such as oxygen (French *et al.*, 2012; Lu *et al.*, 2015), trophic states (Hou *et al.*, 2013; Bollmann *et al.*, 2014), and ammonia availability (Herrmann *et al.*, 2009; French *et al.*, 2012).

Nitrite-oxidizing bacteria

Nitrite-oxidizing bacteria (NOB) oxidize nitrite (NO_2^-) to nitrate (NO_3^-), providing nutrient resources for primary production in aquatic environments (Yool *et al.*, 2007). *Nitrobacter*, *Nitrococcus*, *Nitrospira* and *Nitrospina* are the most well-known NOB (Koch *et al.*, 2014; Levipan *et al.*, 2014). *Nitrolanceetus hollandicus*, which belongs to the phylum *Chloroflexi*, can also catalyze the oxidation of nitrite (Sorokin *et al.*, 2012). Previously, nitrite oxidization was believed to be a chemolithotrophic process only. However, it has been recently discovered that the purple sulfur bacterium *Thiocapsa* can perform phototrophic nitrite oxidation (Hemp *et al.*, 2016). NOB often live together with ammonia oxidizers, which facilitates the complete oxidization from ammonium to nitrate (Gieseke *et al.*, 2003; Matsumoto *et al.*, 2010).

Comammox bacteria

For more than 100 years, nitrification was assumed to be carried out by the stepwise activity of two groups of bacteria. That is, first ammonia is oxidized to nitrite by

ammonia oxidizers, and subsequently nitrite is oxidized to nitrate by nitrite oxidizers (Winogradsky, 1890; Costa *et al.*, 2006). Recently, however, *Nitrospira* strains that can conduct complete ammonium oxidation (comammox) have been discovered (Daims *et al.*, 2015; van Kessel *et al.*, 2015). Isolated bacteria as well as metagenomics analysis of an uncultivated bacterium proved that comammox bacteria can oxidize ammonia completely to nitrate (Daims *et al.*, 2015; van Kessel *et al.*, 2015; Pinto *et al.*, 2016).

Comammox bacteria have been detected in drinking water systems and wastewater treatment bioreactors (Gonzalez-Martinez *et al.*, 2016; Pinto *et al.*, 2016). The isolated comammox strain, *Nitrospira inopinata*, exhibits a higher affinity for ammonia, a lower maximum ammonia oxidation rate, and a higher growth yield than most AOA (Kits *et al.*, 2017). These results indicate that AOA are not necessarily the dominant ammonia oxidizers in oligotrophic environments as previously assumed (Stahl and de la Torre, 2012), whereas comammox bacteria might have significant roles in nitrification in oligotrophic and dynamic environments.

Anammox bacteria

The discovery of anaerobic ammonia oxidizing (anammox) bacteria, which can convert ammonium and nitrite into dinitrogen gas, has expanded our understanding of the microbial nitrogen cycle (Strous *et al.*, 1999). Increasing evidence shows that anammox bacteria play significant roles in the nitrogen losses from marine and freshwater habitats (Kuypers *et al.*, 2003; Schubert *et al.*, 2006).

The distribution and abundance of anammox bacteria in the water column and sediment of lakes have been investigated before (Schubert *et al.*, 2006; Yang *et al.*, 2017). In a permanently stratified lake, the abundance and activity of anammox bacteria showed seasonal variations (Hamersley *et al.*, 2009). Furthermore, it was observed that the abundance of anammox bacteria was much higher in summer than in spring in both eutrophic Dianchi Lake and mesotrophic Erhai Lake (Yang *et al.*, 2017).

Denitrifying bacteria

Denitrification is the process which reduces oxidized nitrogen compounds (NO_3^- / NO_2) to dinitrogen gas (Zumft, 1997), which can counterbalance the nitrogen input

from biological nitrogen fixation and artificial nitrogen fertilizers produced for agriculture (Schlesinger, 2009). The reduction from NO_3^- to N_2 includes 4 steps which are catalyzed by 4 functional enzymes, including nitrate reductases, NO-generating nitrite reductases, N_2O -generating nitric oxide reductases, and nitrous oxide reductases (Zumft, 1997; Shapleigh, 2013). The reduction of nitrate to nitrite can be performed by a taxonomically diverse set of bacteria, whereas the reduction of nitrite to nitric oxide is solely carried out by denitrifying bacteria and archaea that contain nitrite reductases (Zumft, 1997; Kandeler *et al.*, 2006). Most denitrifying bacteria and archaea are facultative anaerobes (Zumft, 1997).

Many denitrification studies were performed in marine environments (Mosier and Francis, 2010; Lee and Francis, 2017; Marchant *et al.*, 2017) whereas related research in lakes has been relatively scarce (e.g., Christensen and Sorensen, 1986; Sweerts *et al.*, 1990).

Nitrogen-fixing bacteria

Biological nitrogen fixation converts atmospheric dinitrogen (N_2) into ammonia, which is an exclusively prokaryotic metabolic process only performed by bacteria and archaea containing the enzyme nitrogenase (Turk *et al.*, 2011; Gaby and Buckley, 2012). Nitrogen fixation is energetically expensive and it can only proceed in the absence of oxygen. Furthermore, many microorganisms cease their nitrogen fixation activity when other forms of nitrogen (especially ammonium) are available.

Previous research revealed that nitrogen-fixing bacteria are phylogenetically diverse, including members of the *Cyanobacteria*, *Alpha-* *Gamma-* and *Deltaproteobacteria* (Zani *et al.*, 2000; Zehr *et al.*, 2000). Furthermore, green sulfur bacteria (*Chlorobi*) can also conduct nitrogen fixation (Madigan, 1995; Ohkouchi *et al.*, 2005). Long-term observations of biogeochemical processes in the North Pacific Gyre have revealed that biological nitrogen fixation supplied up to half of the nitrogen demand by planktonic production (Karl *et al.*, 1997). In lakes and estuaries, nitrogen-fixing *Cyanobacteria* may form dense blooms when other nitrogen compounds, such as nitrate and ammonium, are limited (Hendzel *et al.*, 1994; Bentzon-Tilia *et al.*, 2015). Furthermore, it has been reported that nitrogen-fixing bacteria can show high activity at the chemocline of meromictic lakes (Halm *et al.*, 2009).

Lake Vechten

This thesis investigates how oxic-anoxic transitions affect the microbial nitrogen and sulfur cycle in Lake Vechten (**Figure 1.3**), which is a eutrophic lake near the village of Bunnik (52°04'N, 5°05'E) in the center of the Netherlands. As one of many artificial lakes in the Netherlands, Lake Vechten was originally created by sand excavation during 1938-1939 (Best *et al.*, 1978). It has a total surface area of 4.7 ha, consisting of a western basin and eastern basin. The maximum depth of Lake Vechten is 11.9 m and the average depth is 6.0 m.



Figure 1.3. Panorama view of Lake Vechten in summer (taken on July 19, 2013).

There are no surface in- or effluents of Lake Vechten and the water level is determined by rainfall, evaporation and horizontal groundwater flow (Steenbergen and Verdouw, 1982). Therefore, Lake Vechten can be considered a seepage lake. Lake Vechten becomes stratified with an oxic epilimnion, a hypoxic metalimnion and an anoxic, sulfidic hypolimnion during summer and autumn, whereas the lake is well mixed during winter and early spring (Best *et al.*, 1978; Blaauboer, 1982; Steenbergen and Verdouw, 1982). Thus, oxic-anoxic regime shifts happen seasonally in Lake Vechten.

Enclosed environments with strong seasonality are ideal systems to perform a clear and detailed study of dynamic changes in microbial community structure as

many potential sources of variability are limited (Nelson, 2009). Therefore, Lake Vechten has been extensively investigated by scientists of the former Dutch Limnological Institute (now part of the Netherlands Institute of Ecology) in the 1960s and 1970s. The morphometry, hydrology, physicochemical conditions, and organisms of Lake Vechten have been studied (Best *et al.*, 1978; Blaauboer, 1982; deGraaf and Cappenberg, 1996). The role of microorganisms in the biogeochemical cycles of nitrogen (Verdouw and Dekkers, 1982), carbon and sulfur (Cappenberg, 1975) in Lake Vechten were investigated at a low taxonomic resolution because our modern molecular techniques of DNA sequencing and bioinformatics were not yet available at that time. Overall, the vertical gradient in physicochemical conditions during summer stratification, relatively stable hydrological environment, and the previous studies make Lake Vechten an ideal 'model ecosystem' to investigate how oxic-anoxic transitions interact with the dynamics of microbial communities in seasonally stratified lakes.

Outline of this thesis

Climate change and eutrophication affect aquatic ecosystems at a global scale (Walther *et al.*, 2002; Conley *et al.*, 2009; Pecl *et al.*, 2017). In particular, global warming can expand the anoxic zone and prolong the stratification period of seasonally stratified lakes, altering the oxic-anoxic transitions (Arvola *et al.*, 2010; North *et al.*, 2014). Meanwhile, human activities are increasingly disturbing the nitrogen and sulfur cycle worldwide, especially in freshwater environments (Galloway *et al.*, 2004; Erisman *et al.*, 2008; Finlay *et al.*, 2013). However, our understanding of how oxic-anoxic transitions affect the microbial nitrogen cycle and sulfur cycle in seasonally stratified lakes is still limited.

Therefore, this thesis focuses on the interactions between microbial communities and oxic-anoxic transitions in the seasonally stratified Lake Vechten. The main purpose of the thesis is to: (a) detect the microbial diversity and dynamics during oxic-anoxic transitions in the water column and sediment; (b) elucidate the roles of microorganisms in these oxic-anoxic transitions; and (c) investigate the microbial nitrogen and sulfur cycle during oxic-anoxic transitions.

Chapter 2 describes the dynamics of various bacterial groups in different water layers and the sediment over one year. The bacterial community composition of

epilimnion, metalimnion and hypolimnion diverged during summer stratification and converged when the lake was mixed. Conversely, bacterial communities in the sediment remained relatively stable over the year. In general, the results show large spatio-temporal changes in bacterial community composition, especially during transitions from oxic to anoxic and from sulfidic to nonsulfidic conditions.

Regime shifts are defined as abrupt, large and persistent changes in the structure and function of ecosystems triggered by gradual changes in environmental conditions (Chavez *et al.*, 2003; Scheffer and Carpenter 2003; Biggs *et al.*, 2009). In **Chapter 3**, the role of species interactions in microbial communities during oxic-anoxic transitions was investigated by mathematical modelling and field observations. We discovered that gradual environmental changes induced by, for example, eutrophication or global warming can induce major oxic-anoxic regime shifts. In particular, interactions between microbially-mediated biogeochemical processes and dynamic changes in microbial community composition (*Cyanobacteria*, phototrophic sulfur bacteria and SRB) can cause hysteresis during oxic-anoxic transitions.

Although sulfur bacteria play vital roles in biogeochemical cycles and oxic-anoxic regime shifts, the succession of sulfur bacteria in seasonally stratified lakes is still largely unresolved. **Chapter 4** studies the diversity, dynamics and abundance of SRB, GSB, PSB and CSB in Lake Vechten. We found that sulfur bacteria exclusively inhabited the sediment during the mixing period in winter. After the water column stratified, various SRB species expanded into the anoxic hypolimnion, while PSB and GSB bloomed in the metalimnion and hypolimnion. During fall turnover, SRB and GSB vanished from the water column, whereas CSB (mainly *Arcobacter*) and PSB (*Lamprocystis*) became dominant and oxidized the accumulated sulfide under micro-aerobic conditions.

Seasonal succession of bacteria and archaea involved in the nitrogen cycle is studied in **Chapter 5**. AOA, AOB and anammox bacteria were abundantly present in the sediment in winter. Nitrogen-fixing bacteria and denitrifying bacteria increased in the water column in spring, when nitrate was gradually depleted and the hypolimnion became anoxic. After the lake was mixed during fall turnover, AOA, AOB and anammox bacteria increased to high abundances in the sediment again. Overall, nitrogen bacteria and archaea in the water column and sediment displayed a pronounced seasonal succession during the oxic-anoxic transitions induced by seasonal stratification of the lake.

In **Chapter 6**, the results from this thesis are synthesized, and future research directions are discussed.