A guide for closed circular aquaculture systems farming North Sea biota: An overview on the key biogeochemical cycles and the role of the North Sea benthic microbial communities in the nitrogen cycle.

© North Sea Region Climate Change Assessment

Sofia Mayorga
Student number: 1248286

Table of Contents

Summary for laymen

Aquaculture is a rapidly evolving field with a great potential to form part of the solution for the provision of sufficient animal protein for a growing global human population whilst reducing our dependence on overexploited fish stocks. Bluelinked, a research and consultancy company from the Netherlands, is part of the transition to a more sustainable and animal-friendly form of aquaculture. Bluelinked is currently creating and optimizing a closed and circular aquaculture system for turbot fish fry, taking inspiration from how natural aquatic systems work to create this system.

The aim of this review is to provide a comprehensive understanding of the biogeochemical cycles of carbon, nitrogen, phosphorus, and sulfur and the role of these elements in living organisms, whilst going into detail on the role of the North Sea seafloor microorganisms on the nitrogen cycle. Whilst in the natural world every compound is typically kept at balanced concentrations due to the microorganisms present in the sediment and water column, the intensive nature of aquaculture means toxic products can build much faster as the naturally occurring microorganisms are in imbalance or insufficient population size. By providing an in-depth understanding of biogeochemical processes in the natural ecosystem; we may aid aquaculture facilities aiming to improve fish culture management and performance in closed circular aquaculture systems farming North Sea biota such as Bluelinked to move towards the creation of a more natural, balanced, and healthy aquaculture system. The reason why we focused on understanding the role of the North Sea's seafloor microorganisms exclusively in the N cycle is due to this element's crucial importance to the marine ecosystem and its dominant role as a limiting nutrient in the marine world, and our need to limit the scope of the research within the existing time frame. Moreover, the review's views, conclusions, and suggestions for aquaculturists and researchers can be partially translated to the other biogeochemical cycles.

The review makes evident that the dynamic and permeable North Sea surface sediments are characterized by diverse and versatile microorganism communities, whose impact on the nitrogen cycle is influenced by biotic and abiotic factors including varying sand grain characteristics, fluctuating redox conditions, seasonality,

temporality, biota characteristics, substrate availability, and resource competition; and that multiple key factors must be taken into consideration to optimize future research on this topic.

Abstract

The oceans are dominated by microbial communities which play a central role in the biogeochemical cycling of elements on Earth. In order to create natural, circular, animal-friendly, and resilient aquaculture systems, aquaculturists must strive to understand these complex biogeochemical processes to replicate natural conditions and maintain resilience to disequilibrium. The aim of this review is to provide a comprehensive understanding of the biogeochemical cycles of carbon, nitrogen, phosphorus, and sulfur and the role of these elements in living organisms, whilst going into detail on the role of the North Sea seafloor microorganisms on the nitrogen cycle to improve their understanding and management in closed circular aquaculture systems farming North Sea biota. The paper also reviews the physical and ecological characteristics of the North Sea seafloor sediments, which consist of permeable sands, also referred to as ´biocatalytic filters` due to their ability to stimulate microbial activity by trapping dissolved and particulate organic matter from the water column. We subsequently highlight the reasons for the ecological importance of maintaining the biogeochemical cycles, especially the N cycle, in a healthy balance, and present the key concepts aquaculturists farming North Sea biota must take into account to maintain this balance. We conclude the review with a summary of the critical knowledge gaps which must be considered in future research on this topic to aid in the management and creation of more sustainable and self-supporting closed circular aquaculture systems.

Introduction

Marine microbial ecosystems play an important role in marine biogeochemical cycling, which is defined as the complex biological, chemical, and physical processes involved in cycling key chemical elements within the ocean and between the ocean and the seafloor, land, and atmosphere (Achterberg, 2014). Continental shelves, which are shallow submarine terraces that connect continents to the open ocean, have an enormous role in global biogeochemical cycles due to their high microbial activity. Although they only extend around 65 km from shore and comprise a mere 5% of the total ocean area, they are zones of strong land-ocean interactions and intense nutrient

cycling, comprising productive systems that fuel around 15-20% of global primary production (Jahnke, 2010; Walsh, 1991, Simpson and Sharples, 2012).

The North Sea is a continental shelf sea with a surface area of about 575 000 km^2. It borders the North-East Atlantic, the English Channel, and several nations including France, Belgium, Netherlands, Germany, Scandinavia, and Great Britain (see Figure 1) (Otto et al. 1990). It is a heavily exploited sea, with a large number of activities taking place such as shipping, fishing, and energy production. Water mass exchange with the Atlantic occurs through both the English Channel and the North-West opening of the North Sea. The southern part of the North Sea also receives freshwater from the Rhine, Elbe, and Meuse rivers (Lenhart and Pohlmann, 1997). The North Sea's average depth is 90 meters, with the southern part being the shallowest area (around 40 m in depth), and the central and northern parts having a relatively much greater depth (<80 m in depth) (The European MSP Platform, 2022). By far the deepest area is the Norweigen Trench (725 m in depth). The southern part of the North Sea is the most productive due to its shallow depth and anthropogenic nutrient enrichment due to the riverine input. On the other hand, the water masses in the northern North Sea have lower nutrient concentrations due to their greater depth, distance to the riverine discharges, and dilution by water exchanges with the Northern Atlantic waters (Lenhart and Pohlmann, 1997).

Figure 1: Map of the North Sea in Northern Europe. The map shows bordering countries, sea depths, exclusive economic zones (EEZs), and labels the different regions within the North Sea. (Source: Wikipedia, 2010)

Although high microbial activity is present in both the water column and seafloor, this review focuses on the microbial activity in the North Sea's benthic sediments. Permeable sandy sediments cover more than 50% of continental shelves, and the North Sea is no exception. Figure 2 shows how North Sea seafloor sediments consist mainly of permeable sands and to a lower extent muddy sand and mud (Huettel et al. 2014; Boudreau et al. 2001).

Organic matter remineralization rates and oxygen uptake is high in sandy sediments (de Beer et al. 2005; D'Andrea et al. 2002). Part of the reason for a high diversity in redox conditions in sandy benthic environments is due to benthic-pelagic coupling – the tight coupling between the water column and the benthos (Huettel et al. 2004; Hunter et al. 2006). Sandy sediments are often referred to as "biocatalytic filters" due

to two key properties: (1) their ability to trap dissolved and particulate organic matter (e.g. particles, algae, and bacteria) and (2) their rich diversity of microorganisms (microbial cell numbers range between 10^8-10^9 cells cm^{-3}) containing a wide variety of enzymes capable of degrading different substrates (Miksch et al. 2021; Böer et al, 2009; Mayer, 1994; Gobet et al. 2012).
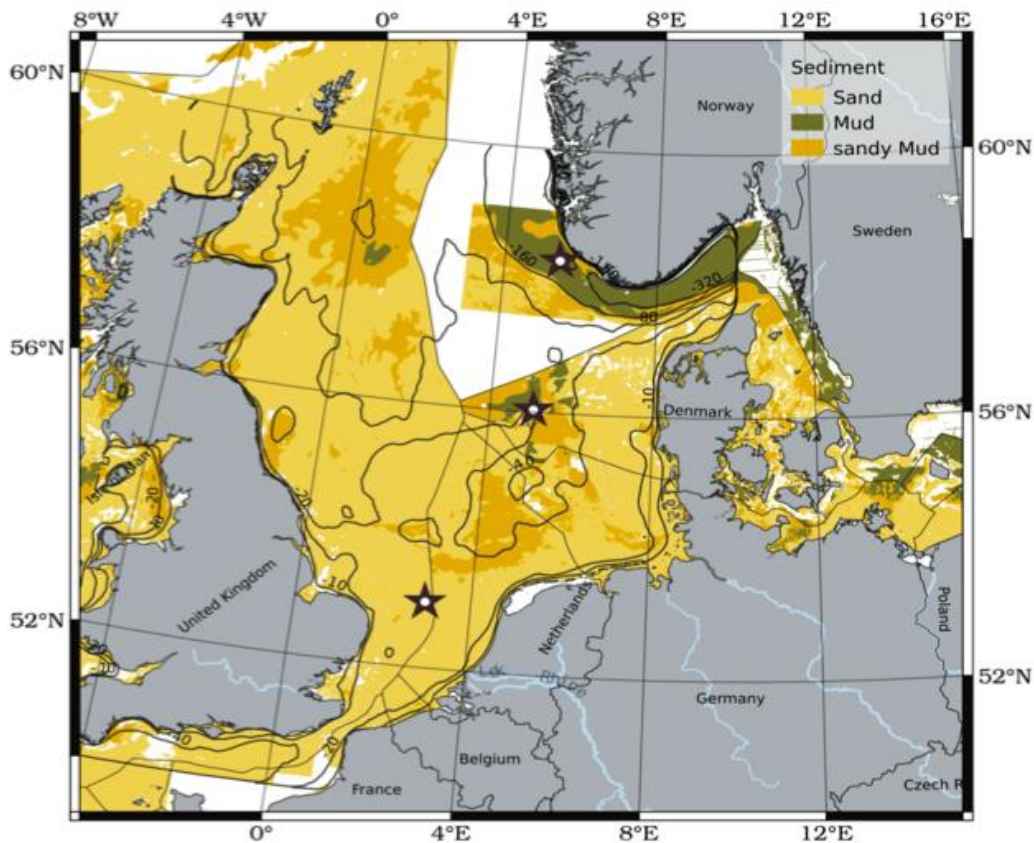
Figure 2: Map showcasing North Sea sediment types, revealing the seafloor is mostly made up of sandy sediment. (Source: Ahmerkamp (2016), data provided by *Geopotential Deutsche Nordsee and EMODnet*)

Carbon, nitrogen, phosphorus, and sulfur are crucial elements for life. They are always in circulation, moving between non-living and living components of the ecosystem in a circular fashion known as biogeochemical cycling. In the case of carbon, nitrogen, and sulfur, they are cycled between the biota, the atmosphere, the ocean, soil, and sediment. Carbon is essential for all living organisms, as it is used to (i) create energy when carbon macromolecules are broken down during the metabolism of food, (ii) in

the production of macromolecules (e.g. proteins, nucleic acids, carbohydrates, and lipids); and (iii) in the synthesis of carbohydrates during photosynthesis by photosynthetic organisms (Waring and Running, 2007; Katz and Falkowski, 2007). Nitrogen, the fourth most abundant element in living biomass, is also an essential element for all forms of life as it is a key structural component of the nucleic acids DNA and RNA and of amino acids from which animal tissues, enzymes, and many hormones are made of (Hanrahan and Chan, 2005; Howarth, 2021; Sterner and Elser, 2002). In the marine world, it is a vital growth-limiting nutrient for photosynthetic organisms (primary producers) because of its key role in the composition of chlorophyll (Bracken et al. 2015). Although nitrogen gas (N_2) is incredibly abundant in the air we breathe, it cannot be assimilated by most organisms in this form. The two major sources of biologically available, or "fixed" forms of nitrogen in the ocean are (i) the fixation of atmospheric N_2 by nitrogen-fixing microbes and (ii) anthropogenic nitrogen riverine input (Mahmud et al. 2020; Swaney et al. 2012). Like nitrogen, phosphorus can also be a key nutrient limiting rates of primary productivity due to its essential role in the creation of RNA machinery necessary for protein synthesis, and in the formation of ATP within cells (Elser et al. 2000; Guignard et al. 2017). The main source of phosphate is phosphate rock, and unlike carbon and nitrogen, phosphorus is not found atmospherically (Guignard et al. 2017; Schröder et al. 2010). Lastly, sulfur is an essential element to life because it is a component of essential organic compounds including proteins (especially in cysteine and methionine amino acids), some coenzymes, metalloproteins, and bridging ligands (Sievert et al. 2007).

The marine world has a crucial role in all the cycles, in large part because it harbors an immense diversity of microorganisms able to transform C- N-, P- and S- containing compounds. Thus, understanding the diversity and functions of these microbial communities has a large impact on the comprehension of the ecosystem function of the North Sea (Glöckner et al. 2012). In this review, we will describe the biogeochemical cycles for carbon, phosphorus, nitrogen, and sulfur in the marine world and the abiotic and biotic influences on the benthic seafloor microenvironment (Part 1 and 2); explain in-depth the role of the North Sea's seafloor microorganisms in the nitrogen cycle (Parts 3); we conclude by highlighting recommendations for aquaculture centers identified in the review and key knowledge gaps. We have focused on understanding what is currently known about the role of the North Sea's

seafloor microorganisms exclusively in the N cycle due to this element's crucial importance to the marine ecosystem and its dominant role as limiting nutrients in the marine world, and our need to limit the scope of the research within the existing time frame. Moreover, the review's views, conclusions, and suggestions for aquaculturists and researchers can be partially translated to the other biogeochemical cycles.

The carbon, nitrogen, and phosphorus cycles are closely interlinked. This interlinkage occurs by a key stoichiometric ratio in ocean biogeochemistry called the Redfield ratio, which is the roughly consistent ratio of 106C:16N:1P (Anderson and Sarmiento, 1994). The three elements are all commonly found both in the environment and within living organisms in these relatively fixed proportions, and whilst all three elements are interlinked, it is nitrogen that is typically the key limiting nutrient in many aquatic ecosystems (Bristow et al. 2017). However, it's important to note that surprising divergences from this ratio sometimes occur in the marine world, such as the large variations of N:P ratios in cyanobacteria depending on ecological conditions (Klausmeier et al. 2004).

The main aim behind this review is to utilize the knowledge obtained on the role of North Sea seabed microbial communities on the nitrogen cycle to aid aquaculture centers, principally those farming North Sea biota in closed circular systems, in the creation of more circular, sustainable, and efficient fish cultures. Aquaculture is a rapidly growing and highly valued field of the seafood industry necessary to provide sufficient animal-sourced protein to a growing population and can reduce our dependence on overexploited fish stocks (SOFIA, 2020). However, many steps are necessary for it to become a more sustainable, ethical, and circular practice. Research on aquaculture, although with a focus on mariculture in fish cages, suggests it has adverse effects on seabed microbiota, which in turn has an adverse effect on the cultured species. Adverse effects include: (1) the accumulation of heavy metals and organic matter due to sedimentation of uneaten feed and fish faeces on the seafloor, which in turn affects fish performance due to O_2 depletion in sediments, (2) the reduction in bacterial biodiversity and the overgrowth of seabed microbes able to flourish in anaerobic carbon-rich conditions in the sediments, (3) shifts in nutrient and carbon fluxes and pH decline; and (4) the increase in toxic products (e.g. sulfide and ammonium) due to the accumulation of bacteria linked to the

production of these products (Moncada et al. 2019; Kalantzi et al. 2021; Diego-McGlone et al. 2008, Holmer et al. 2003, Hornick and Buschmann, 2018, Rubio-Portillo et al. 2019, Shi et al. 2019, Zhang et al. 2020). Studies have shown similar outcomes in shrimp farms, where the toxic nitrogenous substances produced by the excretion of shrimp and uneaten feed increase shrimp disease susceptibility, causing a reduction in body weight and higher mortalities (Barbieri et al. 2014; Schuler, 2008; Thompson et al. 2002).

Understanding the function of different seabed microorganisms in the North Sea's N biogeochemical cycle may improve fish culture performance by providing an in-depth understanding of the process in the natural ecosystem. Future research on the seabed microbial diversity and performance in aquaculture can then be used to compare the processes in the natural system and fish farms. Whilst in the natural ecosystem every compound is usually kept at balanced concentrations due to the microorganisms present in the sediment and water column, the intensive nature of aquaculture means toxic products can build up at a much faster rate as the naturally occurring microorganisms are in insufficient population size. Thus, if there is a significant difference in performance and microbial species diversity between the natural conditions and aquaculture, this opens up the opportunity to modify the seabed microorganisms in the fish farm, most likely through the addition of necessary microbes, to replicate the one in natural conditions and to create optimal farming conditions, moving towards the creation of a more natural, balanced, and healthy aquaculture system.

A great example of an aquaculture center that may benefit from this research is Bluelinked, a research and consultancy company from the Netherlands run by Michaël Latveer that is currently investigating the creation of a sustainable, inland, animal-friendly, closed circular system for turbot fish fry, although they aim to expand to other marine organisms. As can be observed in Figure 3, their system includes a sand filter teamed with microbial life to provide services such as controlling nutrient fluxes and water quality (Bluelinked, n.d.). Researching the sand filter's microbial community and their roles is one of their next steps to improve the resilience and sustainability of their concept.
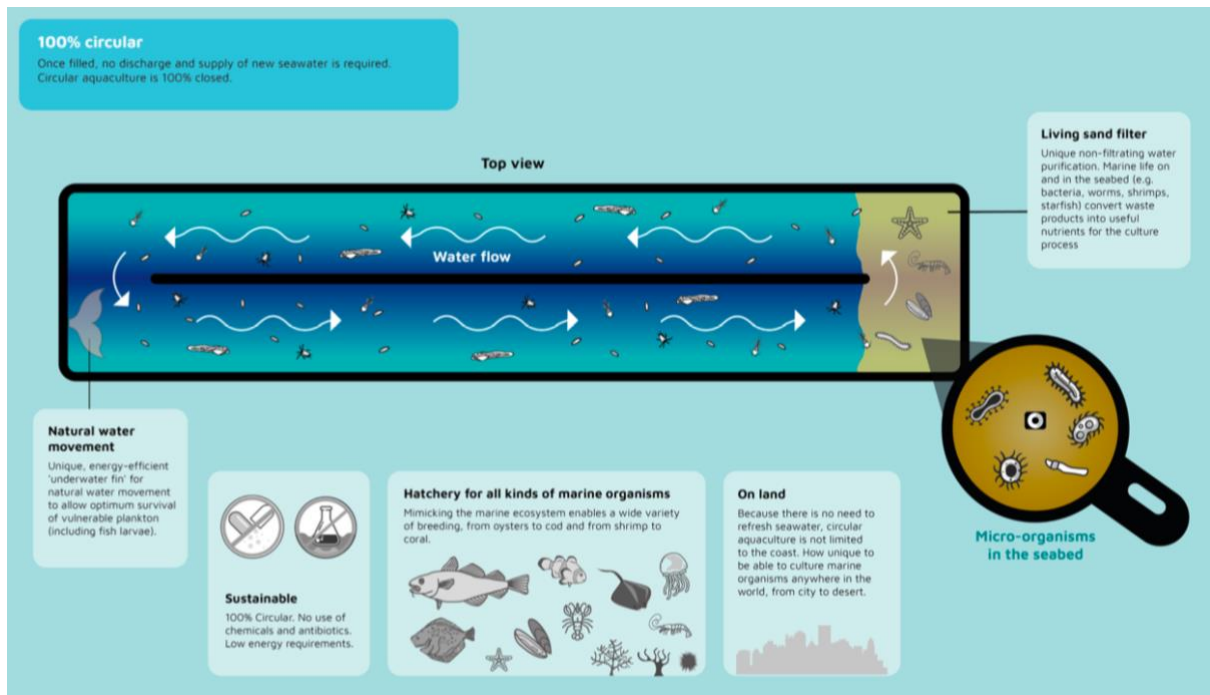
Figure 3: Circular aquaculture system created by Bluelinked, a research and consultancy company in the Netherlands. (Source: Bluelinked, n.d.)

Part 1: An Overview of the four key Biogeochemical cycles

1.1 Carbon cycling in the Ocean

The global carbon cycle can be divided into two components, the biological and the geological pathway. Whilst the geological pathway refers to the process of carbon storage in the atmosphere, ocean, rocks, sediments, and fossil fuels over millions of years; the biological pathway operates at a much smaller timescale of days to thousands of years.

The oceans play an important role in the carbon cycle, acting as a carbon sink by taking up more carbon from the atmosphere than it releases (Hedges, 2002). When CO_2 from the atmosphere dissolves in the ocean surface waters, autotrophs (e.g. bacteria and algae) take up and convert part of the carbon dioxide or bicarbonate ions in the water into organic compounds (Zhang et al. 2018). In the temperate seas and higher latitudes, including the North Sea, organic matter deposition (i.e. capturing inorganic carbon and free nutrients into organic matter) is dependent on the time of year, with phytoplankton blooms occurring in Spring (April-May) due to the increase in irradiance and temperatures. During this time, inorganic carbon and free nutrients are captured by algal blooms and converted into organic matter. The organic carbon is then transported up the food chain (initiated by the consumption of autotrophs by heterotrophs) and converted back into carbon dioxide gas and released into the atmosphere by cellular respiration (Zhang et al. 2018; Irigoien et al. 2014).

1.1.1 The Microbial Loop and the Microbial Carbon Pump

The microbial loop and the microbial carbon pump are opposing concepts with significant influence on the carbon cycle dynamics. The microbial loop is the process by which DOC from the water is converted into particulate organic matter (POC) due to the assimilation by bacteria (Wang, 2018). This transformation returns the carbon to the food chain, allowing it to reach higher trophic levels.

On the other hand, the microbial carbon pump (MCP) entails the transformation of labile dissolved organic carbon (LDOC) into the long-lived recalcitrant dissolved

organic carbon (RDOC) (Jiao et al. 2010). The formation of RDOC acts as a biological carbon sequestration mechanism as this form of carbon is resistant to biological degradation by decomposition or assimilation, and thus it remains in the water for decades or millennia in this form (Ogawa et al. 2001; Jiao et al. 2014; Wang, 2018). The ocean dissolved organic carbon (DOC) pool is one of the greatest organic matter reservoirs on the Earth's surface (Hedges, 2002).

It's important to note that many factors are still unknown about MCP and the ML, including the influence of abiotic and biotic factors on their dynamics and their quantitative contribution to climate modulation (Boyd, 2015; Robinson et al. 2018).

1.1.2 The Biological Pump

Some of the fixed carbon accumulates in marine aggregates also referred to as "marine snow" and sinks to the ocean seafloor (Boyd et al. 2019). This process is crucial to the carbon biological pump, which is defined as the transfer of CO_2 from the surface of the ocean surface to the deep (Honjo et al. 2014; Boyd et al. 2019; Sarmiento and Gruber, 2006). Once it reaches the seafloor sediment, organic carbon burial is low and thus most of it is not kept there for long-term storage. Instead, most of it is degraded relatively quickly by the benthic bacterial community (Middelburg, 2019). The strength of the biological pump is influenced by the ratio between sedimentation (carbon export to the ocean floor) and remineralization (conversion of biomass to gaseous form, water, salts, and minerals, and residual biomass, leading to the release of carbon back into the atmosphere) (Boyd et al. 2019; DeVries et al. 2012; Kumari & Chaudhary 2020). Benthic remineralization is an important mechanism involved in recycling nutrients in the ocean, and in the case of the North Sea, remineralization rates are so high, recycled nutrients are available to the water column within weeks or months after deposition, and nutrient build-up is low (Wild et al. 2004, Sansone et al. 2008; De Borges et al. 2021). It's important to note that remineralization rates decrease from south to north of the North Sea, whereas the importance of denitrification increased with increasing latitude. (De Borges et al. 2021). In the southern shallower North Sea, remineralization rates are so high it is considered a source of carbon dioxide, whilst the northern deeper North Sea is a strong CO_2 sink (Thomas et al. 2004). The carbon cycle is also influenced by the formation of calcium

carbonate ($CaCO_3$), by marine organisms (e.g. coccolithophores, corals, pteropods) mostly in the forms of calcite and aragonite, which also sink as DIC (Tanhua et al. 2013).
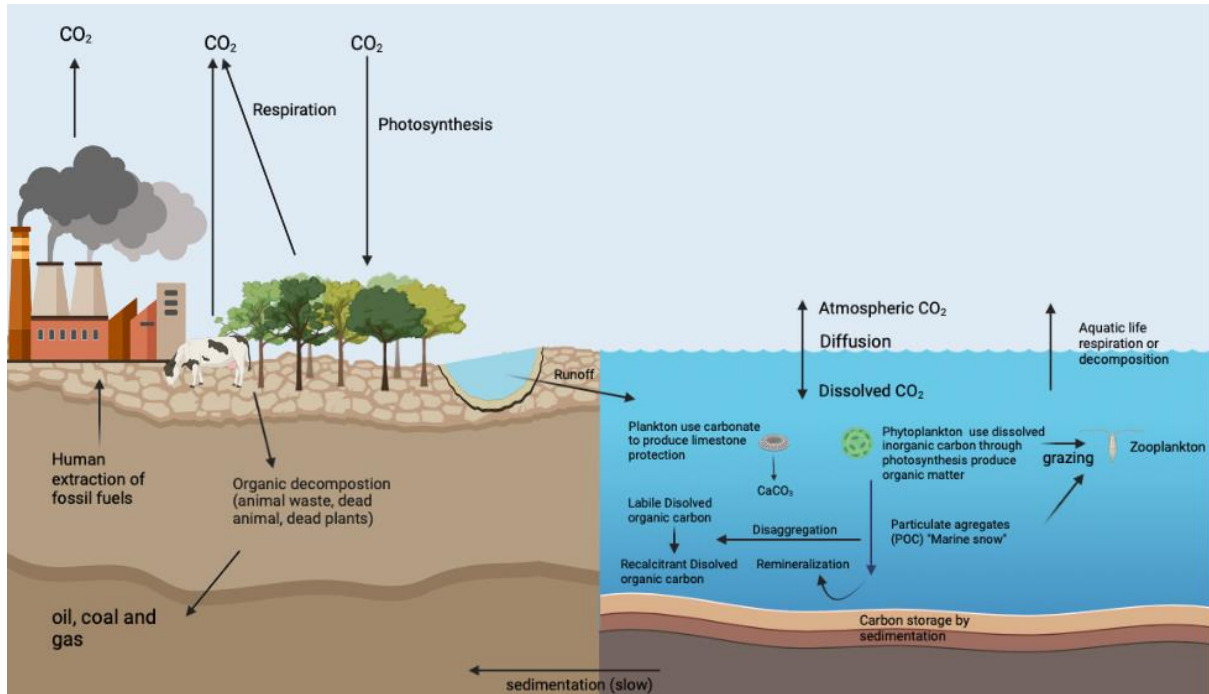

Figure 4: Simplified diagram of the carbon cycle (Based on Steinberg and Landry, 2017; Tanhua et al. 2013)

1.2 Nitrogen cycling in the Ocean

The marine nitrogen cycle is driven by an array of complex biogeochemical processes. It is an integral feature regulating the ocean's productivity and is closely linked to the carbon cycle because of its involvement in both the fixation of atmospheric CO_2 and carbon export from the ocean's surface (Falkowski et al. 1998; Zehr and Kudela, 2011). We will focus on the processes involved in the marine nitrogen cycle.

1.2.1 Nitrogen fixation

Although nitrogen (N_2) is abundant in the Earth's atmosphere, this atmospheric nitrogen reservoir is not available to most organisms in this form (Zehr and Kudela, 2011). A diverse set of specialized microorganisms called nitrogen fixers (diazotrophs), found both in the terrestrial and marine biosphere have the ability to convert this N_2 gas into the more bioavailable form of ammonia (NH_3). All nitrogen fixers can catalyze the nitrogen fixation reaction due to the possession of the enzyme nitrogenase (Dekas et al. 2009; Hutchins and Capone, 2022; Bernhard, 2010). As nitrogenase is easily inactivated by O_2, nitrogen fixers living in aerobic environments require cellular protective strategies against O_2 (reference; Bernhard, 2010). It's important to note that although nitrogen fixation has an important role as a source of nitrogen to support the biological production of nitrogen in the ocean, this process can be limited by factors such as iron or phosphate limitation (Hutchins and Boyd, 2016).

1.2.2 Nitrification

Another important step in the marine nitrogen cycle is the aerobic process of nitrification, used by some microorganisms as an energy source. Nitrification is composed of two distinct processes: (1) the oxidation of ammonia (NH_3) to nitrite (NO_2^-), carried out by ammonia oxidizers; and (2) the oxidation of nitrite (NO_2^-) to nitrate (NO_3^-), carried out by another set of microorganisms named nitrite-oxidizers (Bernhard, 2010).

1.2.3 Bioavailable N-gain and retention in marine organisms

Most phytoplankton can take up nitrogen in the inorganic forms of nitrite, nitrate, and ammonia, which are referred to as dissolved inorganic nitrogen (DIN). However, dissolved organic nitrogen (DON) in the form of small organic molecules such as urea and free amino acids are also recognized as an important N source for phytoplankton (Owen and Watts, 2020; Moschonas et al. 2017). In this way, nitrogen is taken up and incorporated into the cells of microorganisms and can be moved up the food chain by the consumption of phytoplankton by heterotrophs. The nitrogen in the organism's tissues is then released back into the environment in the form of organic nitrogen (e.g amino acids, DNA) when the organism excretes waste or dies. Some fungi and prokaryotes, known as ammonifiers, decompose these organic molecules into ammonia or ammonium, which is then even more bioavailable for re-uptake (Bernhard, 2010; Graham et al. 2011).

1.2.4 Denitrification

During the respiratory process of denitrification, nitrate is converted into nitrogen gas which is then released into the atmosphere. Denitrification is an anaerobic process, occurring in the near or total absence of oxygen, mostly in sediments and oxygen minimum zones (OMZ) (Voss et al. 2013).

1.2.5 Anammox

Anammox refers to the anaerobic production of N_2 gas from ammonia using nitrite as the electron acceptor (Bernhard, 2010). This anaerobic oxidation of ammonia occurs in wastewater treatment plants, and marine areas such as bottom sediments, and oxygen minimum zones (OMZs) (Jetten et al. 2009; Rich et al. 2008; Hamersley et al. 2009).

1.2.6 Dissimilatory Nitrate Reduction to Ammonium (DNRA)

DNRA is an energy-releasing process in which NO_3^- is reduced into NH_4^+. The NH4+ produced can then contribute to nitrification and anammox (Pajares and Ramons 2019). NO_3^- can be reduced to NO_3^- directly, although other times it is first reduced to

NO_2^-. As DNRA is an anaerobic process carried out by both autotrophs and heterotrophs, it is restricted to anaerobic environments such as anoxic sediments and low oxygen zones. Although DNRA and denitrification compete for NO_3^-, DNRA has a lower energy yield than denitrification. Thus, DNRA will only be favored in conditions of high S^{-2} concentration, high $C:NO_3^-$ ratio, elevated temperatures, high salinity, and low oxygen (Pajares and Ramos, 2019).
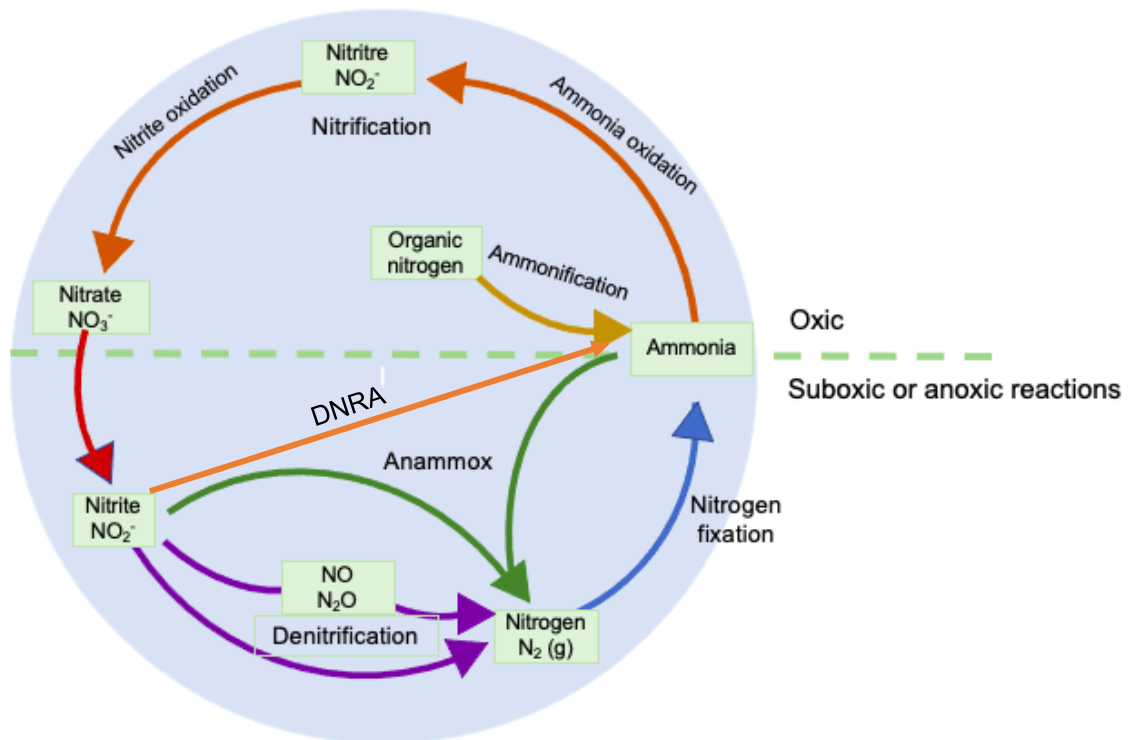
Figure 5: Simplified diagram of the nitrogen cycle. Whilst the major biologically available sources of nitrogen are atmospheric deposition and nitrogen fixation; major nitrogen sinks are denitrification and anammox (Adapted from Bernhard, 2010; Pajares and Ramos, 2019).

1.3 Phosphorus cycling in the Ocean

Although the phosphorus cycle is one of the slowest biogeochemical cycles, this nutrient has a vital role in the ocean by regulating primary productivity and is considered the primary limiting nutrient in the ocean's productivity over geological timescales (Föllmi, 1996; Toggweiler, 1999; Tyrrell, 1999). Although most of the phosphorus is not available to organisms and is locked up in sediments and rocks, it

is converted into bioavailable forms through a combination of geo- and biochemical reactions (Ruttenberg, 2001). Here, we describe the phosphorus cycle, whilst focusing on the cycle's oceanic component.

1.3.1 Phosphorus delivery into the oceans

The main phosphorus supplier to the ocean is river runoff. River runoff provides phosphorus released during rock weathering (i.e. the physical, chemical, and biological erosion of phosphorus-containing rocks), and phosphorus released as a result of human activities (e.g. fertilizer use, animal feeds, agricultural crops) (Elena et al. 2001). Rivers transport phosphorus in both the dissolved and particulate forms (Benitez-Nelson, 2000; Delaney, 1998). Unlike nitrogen, the atmosphere is not a phosphorus storage zone, and thus does not have an important role in the supply of phosphorus in the ocean, with phosphorus entering from atmospheric deposition only minimally through aerosols, volcanic ash, and mineral dust. However, in some oligotrophic regions and areas that are P-limited, atmospheric phosphorus depositions are a major external supply of phosphorus (Paytan and McLaughlin, 2007; Wu et al. 2000; Krom et al. 1991).

1.3.2 Marine cycling of phosphorus

Most of the bioavailable phosphate reaching the ocean is immediately used by the ocean surface phytoplankton community during photosynthesis, where it is incorporated into organic matter for the formation of new biomass and as an energy carrier (Arning, 2008). Most of the phosphorus is assimilated in its dissolved inorganic form, mainly in the most bioavailable form, orthophosphate (PO_4^{3-}). Phosphorus in orthophosphate is present at an oxidation state of +5. (Karl, 2014; Cotner and Wetzel, 1992). Dissolved organic phosphorus (DOP) may also be the dominant form of phosphorus, especially in the surface water of the ocean's oligotrophic gyres (Björkman et al., 2000). DOPs are mostly found in the form of phosphonates, and have been found to be synthesized, processed, and/or assimilated by several cyanobacteria and heterotrophic bacteria and archaea (Mooy et al. 2015; Villarreal-Chiu et al. 2012; Metcalf et al. 2012; Dyhrman et al. 2008; Martinez et al. 2010; Young and Ingall, 2010).

Once the phytoplankton has assimilated the dissolved inorganic phosphorus, transforming it into organic phosphorus compounds by incorporating it into organic matter, it then goes up the food chain when the phytoplankton are ingested by detrivores or zooplankton. This organic phosphorus is released back into the environment as dissolved organic and inorganic phosphorus when the zooplankton excrete it back into the water column and when phytoplankton die (Cotner and Biddanda, 2002). Whilst the inorganic phosphorus can be rapidly assimilated by the phytoplankton, bacterial and phytoplanktonic enzymes must hydrolyze the organic phosphorus compounds to allow them to be assimilated (Cotner and Biddanda 2002; Azam et al. 1983). This permits the continuous active recycling of phosphorus in the water.

1.3.3 Phosphorus sinks in the ocean

Although around 90% of the particulate phosphorus reaching the seafloor is remineralized by microbial heterotrophs inhabiting the sediment and reutilized in the water column, a percentage of it sinks to the ocean floor and is buried in the bottom marine sediments (Soudry, 2000). The phosphorus can be buried as particulate phosphorus associated or attached to organic matter or as phosphate oxide/carbonate minerals, which predominately occur as authigenic carbon fluorapatite (CFA) (Föllmi et al. 1996). Phosphogenesis refers to the burial of phosphorus and formation of phosphate sediments called phosphorites in marine sediments, and has been observed in suboxic and anoxic sediments of some ocean upwelling regions (Arning, 2008; Föllmi, 1996). Sink switching between labile phosphorus phases (e.g. organic phosphorus) to more stable authigenic phosphate minerals occurs in many oceanic environments. (Gunnars et al. 2004; Benitez-Nelson, 2000; Ruttenberg and Berner, 1993; Delaney 1998; Wallmann, 2010).
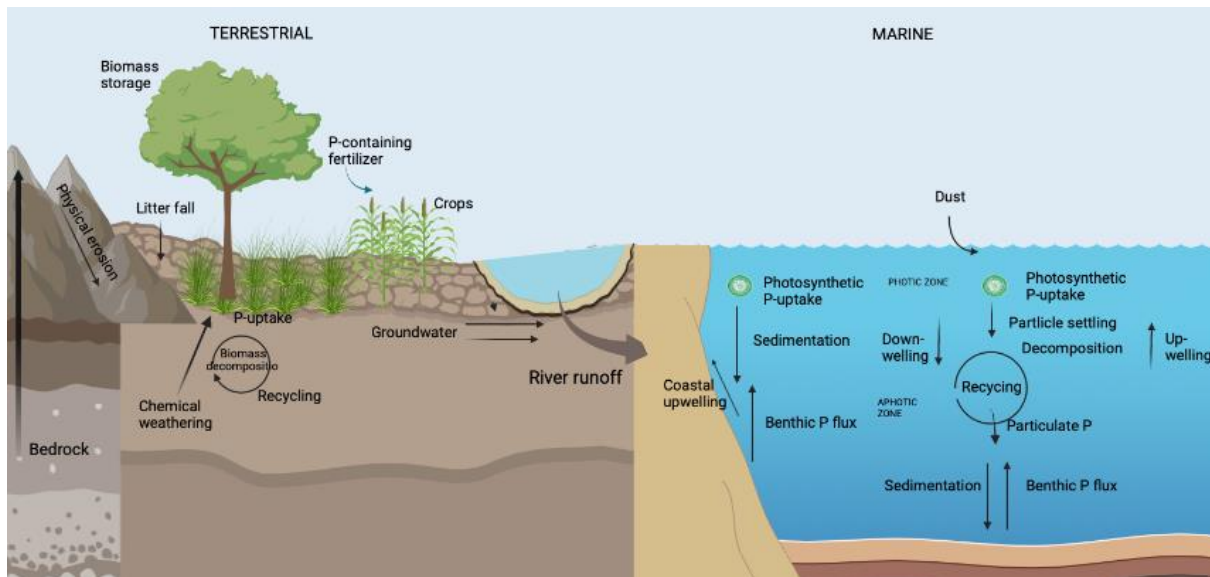
Figure 6: Simplified diagram of the phosphorus cycle. (Adapted from: Ruttenberg, 2019)

1.4 Sulfur Cycling in the Ocean

The ocean plays a major role in the sulfur cycle and is an important reservoir of this element in both its dissolved and sedimentary form. Although rarely a limiting nutrient, sulfur has a very important role in the function of global ecosystems. Sulfur occurs in oxidation states ranging from +6 (in sulfates, SO_4^{2-}) to -2 (in sulfides, H_2S) and in states in between. A large variety of microorganisms, mostly from the bacterial domain, are involved in the transitions of S between redox states (Sievert et al. 2015). The archaea involved in the sulfur cycle are commonly found in high-temperature environments (e.g. hydrothermal vents) (Sievert et al. 2015).

1.4.1 Sulfur delivery into the ocean

One way sulfur is delivered to the ocean is when the weathering of sulfur-containing rocks (which originate to a large extent from ocean sediments moved to land due to the geological uplifting) release sulfur into the environment, which eventually ends in the marine environment via leaching and run-off (Bharathi, 2008). Sulfur may also be released to aquatic ecosystems due to long-term sulfur additions to crops in fertilizers, pesticides, and soil conditioners (Hinckley et al. 2020). Sulfur can also enter the ocean via atmospheric fallout and precipitation. Sulfur is delivered in the atmosphere during the release of hydrogen sulfide gas (H_2S) by volcanoes and some hot springs, during

the death and decomposition of animals, and by petroleum refinery activity (Gnana Thanga Vincent et al. 2021). Petroleum refineries are also responsible for the release of sulfur dioxide gas (SO_2), which can be delivered to the ocean when dissolved in rain. When SO_2 dissolves in rain, it forms a weakly corrosive rain due to the formation of sulfuric acid (H_2SO_4). Finally, H_2S ends up in the oceans when emitted by underwater geothermal vents (Gnana Thanga Vincent et al. 2021).

1.4.2 Assimilatory uptake of sulfur by phytoplankton

Sulfate (SO_4^{2-}) is highly abundant in coastal and marine systems. Phytoplankton in the ocean's photic zone assimilate inorganic sulfur (mainly in the form of sulfate) to form organic compounds for the synthesis of structural cell material. This energy-dependent process is needed to form the amino acids methionine and cysteine. Some phytoplankton transform methionine into the highly stable and soluble dimethylsulfoniopropionate (DMSP), which is then decomposed into dimethylsulfide (DMS) gas and released into the atmosphere when the phytoplankton die.

1.4.3 Dissimilatory sulfur metabolism

Dissimilatory sulfur metabolism (DSM) refers to the energy-yielding reduction of sulfate to sulfide by anaerobic sulfate-reducing microorganisms which occurs in marine sediments (Glöckner et al. 2012; Jorgensen and Kasten, 2006; Hofer, 2018). This sulfur reduction is undertaken within 20 phyla of bacteria and archaea, include those from the genera *Desulfomaculum*, *Desulfovibrio, Desulfobulbus, Desulfobacterium, Desulfosarcina,* and *Thermodesulfobacterium,* as well as archaea from the genus *Archaeoglobus* (Simon and Kroneck, 2013; Rabus et al. 2013; Anantharaman et al, 2018).

1.4.4 The "mini sulfur cycle"

The sulfide produced can then be oxidized by anoxygenic phototrophic bacteria, which use the reduced sulfur compound as an electron donor for CO2 fixation in anoxygenic photosynthesis to produce elemental sulfur (Glöckner et al. 2012). The sulfide can also be used by colorless sulfur bacteria for chemosynthesis (see Section X below for more information on colorless sulfur bacteria). The sulfur produced can

then be reduced back to sulfide by anaerobically respiring bacteria (Glöckner et al. 2012). The quick anaerobic reactions transitions between sulfide to elemental sulfur and back to sulfide are referred to as the "mini sulfur cycle" (Glöckner et al. 2012). Although most of the sulfide is eventually converted back to sulfate via oxidation by phototrophic or colorless sulfur bacteria, a small proportion of it is deposited and buried deep in the sediments when it precipitates as organic matter, or with iron (pyrite formation) and other metals. (Schippers and Jorgensen, 2002; Jorgensen and Nelson, 2004; Holmkvist et al. 20011a; Jorgensen et al. 2019).

1.4.5 Colorless sulfur bacteria

Colorless sulfur bacteria comprise a large and diverse group of microorganisms that grow chemolithoautotrophically, using reduced sulfur compounds to generate energy by oxidation. These bacteria, who are described as colorless due to their lack of photopigments, play an essential role in the oxidative parts of the sulfur cycle (see Figure below) (Muyzer et al. 2013; Robertson and Kuenen, 2006). They include bacteria from the genera Acidithiobacillus, *Sulfolobus, Sulfurimonas, Sulfuricurvum, Thioalkalimicrobium, Thioalkalivibrio, Thiobacillus, Thiomicrospira,* amongst many others (Muyzer et al. 2013; Robertson and Kuenen, 2006).

1.4.6 Disproportionation or sulfur fermentation

Disproportionation refers to the chemolithotrophic process in which the intermediate compounds formed during the partial oxidation of sulfide, such as elemental sulfur (S^o), sulfite (SO_3^{2-}), and thiosulfate ($S_2O_3^{2-}$), function as both electron donors and acceptors, creating sulfide and sulfate (Finster, 2008; Glöckner et al. 2012).

The ability to disproportionate intermediate sulfur species is widespread amongst marine surface sediments and is relatively common among sulfate reducers and includes those from the genera *Desulfobacterium, , Desulfomaculum, Desulfovibrio,, Desulfobulbus,* amongst others *(Finster, 2008;* Bak and Pfennig, 1987; Thamdrup et al., 1993; Finster et al., 1998).
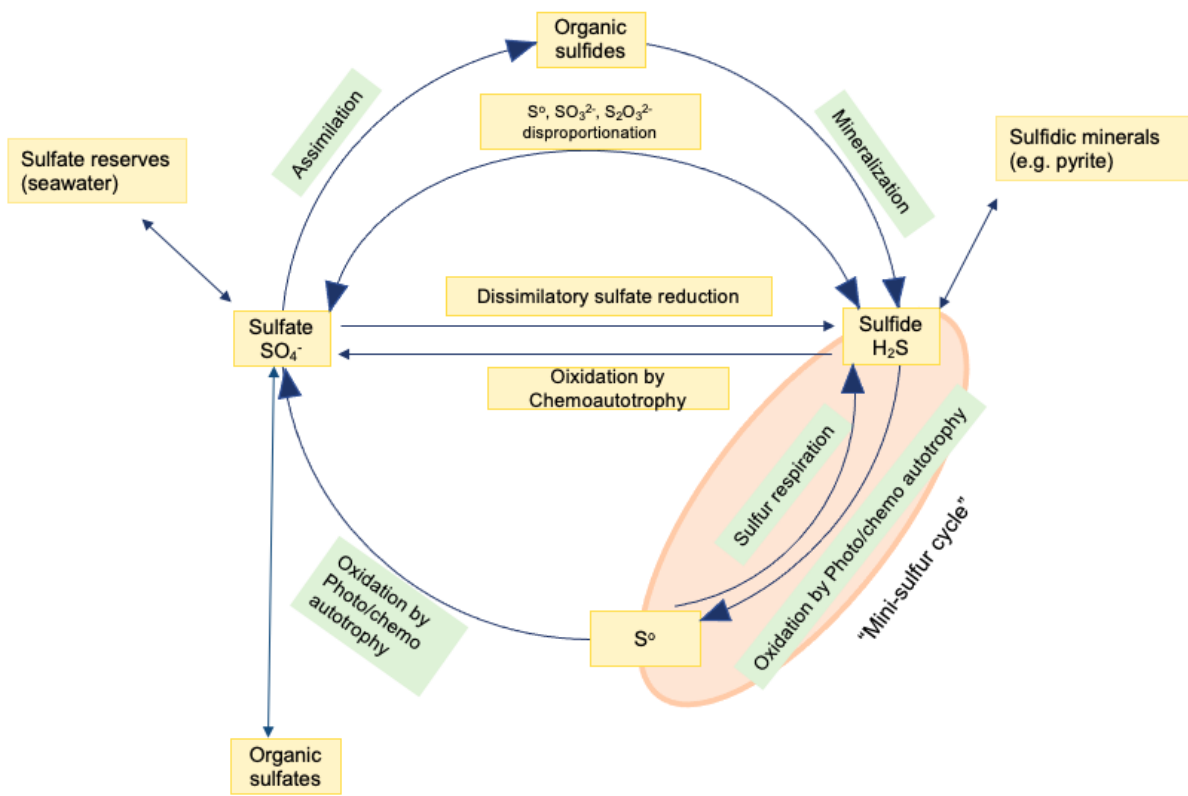
Figure 7: Schematic overview of the sulfur cycle. (Based on Glöckner et al. 2012)

Part 2. Abiotic and biotic influences on the North Sea seabed & its microbial community

The permeable sandy sediments making up the North Sea seabed play a role in the biogeochemical cycles discussed above. Although sandy sediments have low organic carbon concentrations, in the last two decades they have started to be recognized as sites where biogeochemical reaction rates are high. Thus, the low organic carbon content is likely a reflection of high biogeochemical reaction rates instead of low seabed microbe content and activity (Boudreau et al. 2001; Sansone et al. 2008). In this next section, we will explain the abiotic and biotic influences on the North Sea benthic community and the transport processes affecting biogeochemical cycling on the seabed.

2.1 Abiotic and biotic influences on the sandy sediment microbial community

The sandy microbial community is quite heterogeneous, mainly living in high densities attached to sand cracks and local depressions. This uneven distribution, which likely results due to naturally occurring mechanical abrasion, promotes intra- and interspecies communication and interactions and protects the bacteria from abrasion, grazing pressure by protists, and viral lysis (Hamann et al. 2017; Berninger and Epstein, 1995; Vandieken et al. 2017; Dang and Lovell, 2016; Flemming and Wuertz, 2019; Ahmerkamp et al. 2020) (see Figure 3).
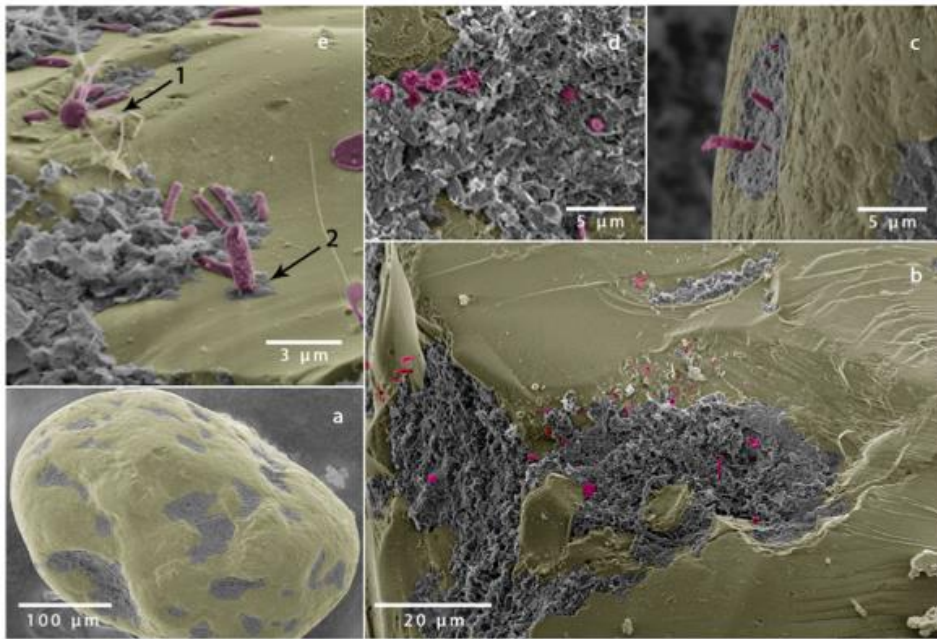
Figure 8: Scanning electron micrographs of the microbial colonization of a single sand grain shows reveals dense microbial colonization (colored magenta) in cracks and depressions (Source: Ahmerkamp et al. 2020).

The marine benthic community is influenced by various biotic and abiotic factors. For instance, the abundance and diversity of these microbes decrease with (i) increasing sediment depth; (ii) decreasing organic matter availability; and (iii) increasing grazing frequency, although the latter is not a major process controlling the fate of benthic microbes (Guilini et al. 2010, Parkes et al. 1994; Hoshino et al. 2020). Another influential factor on benthic microbial colonization is sediment size and shape. Golf-like particles with high sphericity facilitate microbial colonization, which is likely because of their preference for life in sand grain cracks and depressions (Ahmerkamp et al. 2020). Whether the sediment is coarse- or fine-grained will also influence seabed biogeochemical cycling and the microbial community composition by influencing sediment permeability (Probandt et al., 2017; Ahmerkamp et al., 2020). The less permeable fine-grained sediment allows less oxygen penetration, and thus promotes anoxic mineralization and the trapping of organic matter (Jørgensen, 1982; Canfield et al., 1993). On the other hand, highly permeable coarse-grained sediment has a high oxygen penetration rate and depth, and thus more organic matter is remineralized (Brenner et al. 2016) Although the North Sea seafloor mainly consists of medium and

fine sandy permeable sediments, coarse permeable sand can also be found at certain locations spread throughout the North Sea (Brenner et al. 2016).

The significance of the effect of seasonal temperature changes on the shelf benthic communities is currently disputed. This is surprising when compared to the unquestionable influence of seasonal fluctuations on water-column microbes in temperate waters. In the water column, the seasonal increase in sea temperatures at the beginning of spring cause nutrient enhancement, spurring strong algal blooms lasting from spring until early summer which stimulates a marked increase in water column microbe activity and abundance (Teeling et al. 2016; Teeling et al. 2012; Fuhrman et al. 2006). In the case of the shelf sand microbial community, Musat et al. (2006) revealed that Wadden Sea intertidal shelf sediments showed a clear seasonal variation of bacteria, with higher values in July and October versus March. Tait et al. (2015) also found indicators for seasonality in bacterium genus Flavobacteria in surface sediments of the English Channel. However, both studies have a small sample size. Musat et al. (2006) was based solely on 4 sampling dates, and Tait et al. (2015) on results from a single year. In contrast, a recent study by Miksch et al. (2021) on the changes in the North Sea sandy community over two annual cycles did not find seasonal fluctuations in the sandy communities. This may be because: (i) the bacteria may have responded to spring algal blooms simply by increasing their exoenzymatic activity instead of changing bacterial structure and abundance, and/or because (ii) the limited space in sand grains keep the bacterial population in a steady state (Miksch et al., 2021; Braeckman et al. 2018). In conclusion, although Miksch et al. (2021)'s study suggests that pelagic and benthic bacterial communities may respond to seasonal temperature and phytoplankton productivity changes very differently, further research is needed on this topic. The relevance of seasonality for the North Sea seabed microorganisms will be discussed in more detail in Section 3.2.

2.2 Transport Processes Affecting Benthic Biogeochemical Cycling

Biogeochemical processes in the seafloor sediment are closely coupled to those in the water column, referred to as benthic-pelagic coupling. Transport goes both ways – oxygen, electron acceptors and donors, nutrients, dissolved and suspended particles,

phytoplankton, and bacteria are transported from the water into the sediment; whilst organisms, degradation products, and reduced matter are released back into the water column (Boudreau et all. 2001; Huettel et al. 1996, 2014; Rusch et al. 2001; Ahmerkamp et al. 2017; Santos et al. 2012a). These transport processes are responsible for the formation of oscillating oxic/anoxic conditions and stimulating reaction rates in both systems (Ahmerkamp et al. 2017; Huettel et al. 2014). Below, we describe the transport processes driving the efficient exchange of dissolved and particulate compounds between the two systems.

2.2.1 Diffusion

Diffusion, the small-scale movement of chemical species from areas of high to low concentration is responsible for the transport of chemical species into the sand grain microenvironment and eventually into the microbial cells (Santos et al. 2012a; Addiscott and Leeds-Harrison, 2005). As it is the primary factor determining the microbe microenvironment solute concentration, it may lead to divergence between the solute concentration of the microbe microenvironment and that of the bulk porewater (Jahnke, 1985, Jørgensen, 1977).

2.2.2 Porewater Advective transport

On a larger scale, the transport of solutes and particulate compounds is dominated by advective transport, which refers to the bulk motion of bottom water pumped through the permeable sediment pore spaces (Huettel et al. 2014). Porewater advection can be driven by various physical processes. One of the principal physical processes is flow- and topography-induced pressure gradients, which is when the interaction of bedforms (i.e. morphological features at the fluid-seabed interface as a result of the interaction of fluid flow with the seabed materials) with the overlying currents causes a local acceleration of these currents generating a pressure gradient that drives the bottom water into the sediment's pore spaces (see Figure 4) (Charru et al. 2013; Santos et al, 2012a). Whilst this process is dominant in slower-moving beds, bedform migration is thought to be the major mechanism driving porewater exchange in fast-moving rippled beds (Santos et al. 2012a; Elliott and Brooks, 1997a; Elliott and Brooks, 1997b). Bedform migration occurs when wave or current stress increases beyond the

seabed erosion threshold, causing seabed morphology changes and advection during sediment resuspension/deposition (Santos et al. 2012a).

(A)   (B)
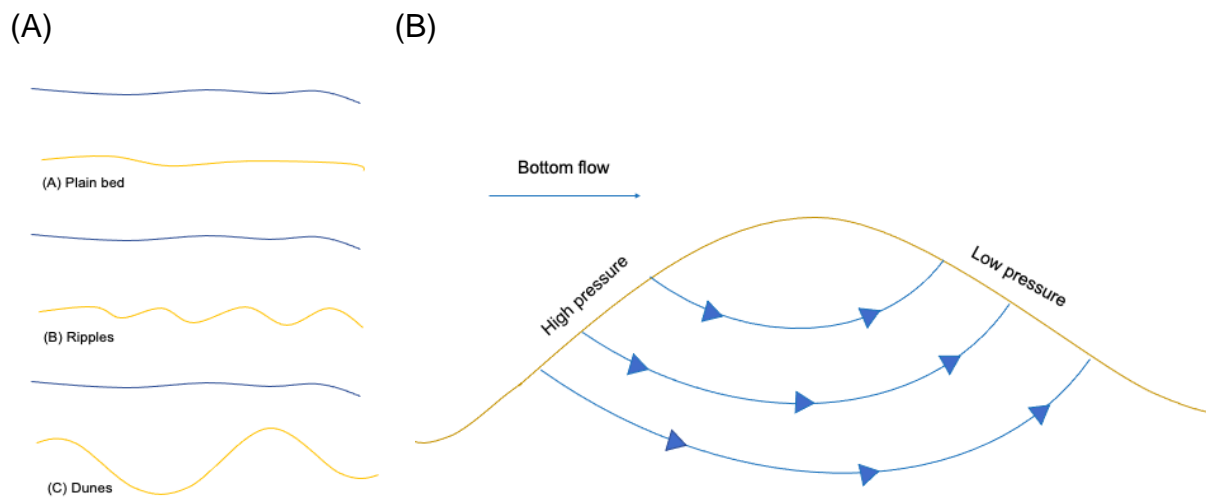
Figure 9: (A) Diagram comparing a plain bed with two types of bedforms, ripples and dunes; (B) Advective pore-water fluxes in permeable sediments on bedform. Arrows represent direction. Adapted from Wörman et al. (2002).

Other physical processes driving advection include (i) tidal pumping (when changing tides drives water recirculation into beach sandy sediments) (Santos et al. 2012a); (ii) wave pumping (when waves generate undulating differences in hydrostatic pressure), (iii) convection (when the density of the porewater differs from that of the water column due to differences in temperature and/or salinity) (Santos et al. 2012a, Webster et al. 1996), (iv) bioirrigation (when bottom-dwelling organisms flush their burrows with overlying water) (Aller, 1980; Santos et al. 2012a), and (v) bioturbation (burrowing activity from benthic organisms) (Rhoads, 1963; Santos et al. 2012 a).

Part 3: The role of the North Sea seabed microbial community on the nitrogen cycle

In Part 3, we compiled all relevant up-to-date studies on the role of seabed microorganisms in the nitrogen cycle in the North Sea, discussing key findings and limitations and explaining how these conclusions can improve the sustainability and resilience of aquaculture.

Table 1: Relevant up-to-date (2010-2022) published studies on the role of the seabed on the nitrogen cycle in the North Sea.

Study	Sediment location within North Sea	Site sediment type	Study methodology	Nitrogen cycle process studied	Were nitrogen cycle-related microorganisms studied?	Was advective transport considered in the study?	Period of study	Main aim of study
Toussaint et al. 2021	Belgian Part of the North Sea (Southern North Sea)	Subtidal benthic sediments representative of a wide permeability gradient (muddy to fine sand)	Combination of incubation of sediment cores and modelling	Nitrification N-mineralization (i.e. ammonification)	X	Yes	September 2016 and 2017 (no seasonality)	To understand the sedimentological and faunal control of benthic organic matter mineralization and nitrification in the Southern North Sea

Lipsewers et al. 2014	The Oyster Grounds (Southern North Sea)	Subtidal benthic sediments consisting of organic rich and muddy sediments	Incubation of sediment cores and quantification of specific intact polar lipids and the abundance and gene expression of their 16 rRNA gene, the *amoA* gene of AOA and AOB, and the *hzsA* gene of anammox bacteria	Nitrification Anammox	Ammonia oxidisers (both AOA and AOB) and annamox bacteria	No (Not relevant)	February, May and August 2011 (seasonality)	To determine the seasonal and depth distribution of the abundance and potential activity of archaeal and bacterial ammonia oxidizers and anammox bacteria in coastal marine sediments of the southern North Sea.
Yazdani Foshtomi et al. 2015	Belgian part of North Sea (Southern North Sea)	Subtidal benthic habitats representative of a wide permeability gradient (muddy to fine sand)	Combination of incubation of sediment cores, modelling, and microbial community analyses by amplification of bacterial 16S rRNA genes and denaturing gradient gel electrophoresis (DGGE)	Nitrification Denitrification, N-mineralization (i.e. ammonification)	Ammonia oxidisers (both AOA and AOB)	No	February to October 2014, monthly (seasonality)	To investigate spatio-temporal patterns in microbial community composition and diversity, macrofaunal abundance and their sediment reworking activity, and N-cycling in the Southern North Sea
Rosales Villa et al. 2019	The Wash, a large area of intertidal sand and mud banks found	Intertidal and subtidal sites representative of a wide permeability gradient	Incubation of sediment cores and ^{13}N isotope analysis	Denitrification Anammox DNRA	x	No	The Wash: May, June, September and October 2013 (seasonal)	To determine the rates of sedimentary denitrification and its component processes (canonical denitrification, anammox, and DNRA) in intertidal and subtidal sites of the North Sea

	(South-west North Sea) Other sites spread all around the North Sea						Other sites of North Sea: August 2013	
Neubacher et al. 2013	North of Dogger Banks (Southern North Sea)	Subtidal site with muddy sand (low permeability)	Mesoscom experiment. Sediment core samples collected and then cores were exposed to different oxygen concentrations	Denitrification Anammox	x	No	Mesoscom 1: Sample from January 2008, experiment lasted 70 days Mesoscom 2: Sample from April 2008, experiment lasted 29 days (no seasonality)	To understand the potential effect of sustained hypoxia on the production of N_2 gas through denitrification and anammox, as well as the sediment-water exchange of nitrite, nitrate and ammonia, oxygen consumption and penetration in sediments of southern North Sea
Neubacher et al. 2011	Southern North Sea	Subtidal sediments representative of a wide permeability gradient (muddy to fine sand)	incubation of sediment cores and subjected to different oxygen regimes. ^{13}N isotope analysis done to determine oxygen uptake, denitrification and anammox rates	Denitrification Anammox	x	No	February, April, May, September, October (2007) April 2008	To determine denitrification, anammox, oxygen uptake, nutrient exchange, and pore-water profiles of oxygen in intact sediments at three sites in the southern North Sea experimentally exposed to different oxygen saturations acutely
Deek et al 2012	German Wadden Sea	Intertidal sediments with	incubation of sediment cores	Denitrification	X	No	February 2009 June 2009	To improve the seasonal and spatial resolution of sedimentary N_2

	(South-East of the North Sea	two different sand types each site– coarse and fine sands	in a flow-through incubation set-up Measuring N_2 fluxes by the N_2/Ar technique				November 2009 April 2010 (seasonality)	production measurements as an indication of nitrogen removal in intertidal sediments of the Wadden Sea.
Gao et al. 2012	Wadden Sea (south east North Sea)	Intertidal sand flat sites	incubation of sediment cores and Combination of stable N isotope incubation experiments and a flux model based on annual monitoring data of NOx concentrations and bottom current velocities	Denitrification	x	Yes	October Autumn 2006 and spring (March) and summer (August) 2007 (seasonality)	To determine the nitrogen loss rates in permeable sediments of the Wadden Sea
Ahmerkamp et al. 2020	German Bight, (southeast North Sea)	Subtidal sediments representative of a wide permeability gradient	Incubation of sediment cores in flow-through reactors	Denitrification	x	Yes	Autumn 2014 (no seasonality)	To investigate how sand properties influence microbial distribution and microbial respiration rates under advective conditions
Probandt et al. 2018	Southern North Sea	Subtidal sediments representative of a wide permeability gradient	Analysis of the individual bacterial diversity on sand grains by 16S ribosomal RNA gene sequencing and	Nitrification	Ammonia- and nitrite-oxidizing Bacteria and Archaea	No (not relevant)	June 2016 (no seasonality)	To analyze single grains to address the microbial community directly in its micro-habitat

			visualization using in situ hybridization					
Marchant et al. 2017	Wadden Sea (south east North Sea)	Intertidal sediments from sand flat	Incubation of sediment cores which are exposed to different O_2 regimes for short and long exposures.	Denitrification	Denitrifying bacteria	Yes	April 2012, December 2014, February 2015 (No seasonality)	To investigate aerobic denitrification in permeable sediments of the Wadden Sea
Fan et al. 2015	North Sea coast of the Dutch Barrier island Schiermonnikoog, (south east North Sea)	Tidal and Intertidal coastal sediments	Incubation of sediment cores for 15N isotope and nucleic acid analysis	Nitrification	Ammonia-oxidizing archaea (AOA) and ammonium-oxidizing bacteria (AOB)	No	July, September, November (2010) January and April 2011 (seasonality)	To identify ammonia oxidizing communities in different types of microbial mats and elucidate factors that determine the abundance and activity of ammonia oxidizers
Bale et al. 2014	Southern North Sea	Subtidal sediments representative of a wide permeability gradient	Incubation of sediment cores for a combination of intact polar lipid and nucleic acid analysis, and 15N isotope labeling.	Anammox	Anammox bacteria	Yes (and determined no sediment sampled was influenced by advection)	November 2010 February 2011 May 2011 August 2011 (seasonality)	To investigate the seasonality of occurrence and activity of anammox bacteria in sandy and muddy sand sediments of the southern North Sea
Fan et al. 2015a	Dutch Coast, Oyster Grounds and	Subtidal sediments representative	incubation of sediment cores for 15N isotope	Nitrification Denitrification	Diazotrophs Denitrifiers	No	February 2011 May 2011 August 2011	To measure N_2 fixation and denitrification in the bottom sediment and to measure the

	Dogger Banks (southern North Sea)	of a wide permeability gradient	and DNA sequence analysis Quantitative Polymerase Chain reaction (qPCR) and quantitative reverse transcription polymerase chain reaction (qRT-PCR)				(seasonality)	diversity, abundance and activity of nitrogen-fixing and denitrifying organisms at three different stations in the southern North Sea during three seasons
Neumann et al. 2017	Elbe estuary, Wadden Sea, and adjacent German Bight (South East North Sea)	Subtidal sediments representative of a wide permeability gradient	Incubation of sediment cores for the collection of pore water samples and analysis of sediment characteristics. Measurement of nitrate pore water profiles and use of models	Denitrification	x	Yes	March 2009 May 2009 September 2009 February 2010 (seasonality)	To analyze the nitrate removal in surface sediments of the Elbe estuary, Wadden Sea and adjacent German Bight during spring and summer along a eutrophication gradient ranging from a high riverine nitrate concentrations at the Elbe estuary to offshore areas with low nitrate concentration.

3.1 Spatial changes in nitrogen cycle processes in the North Sea seabed

Spatial differences in sedimentary nitrogen cycling rates across the North Sea are influenced by various factors including tidal influence, sediment type and composition, seabed depth, nutrient distribution, and resource and space competition. In terms of sediment tidal location, Rosales Villa et al. (2019) demonstrated that despite seasonal differences in denitrification rate between intertidal and subtidal sediments, the overall average denitrification rate in the intertidal Wash sediment was not significantly different from those in subtidal North Sea sites. This is surprising as the source of nitrate for denitrification in each site differs greatly: whilst denitrification in the intertidal site is sustained by nitrate in the water column, the source of nitrate for denitrification in the subtidal sites is nitrification by nitrifying organisms (Rosales Villa et al. 2019). Moreover, Rosales Villa et al. (2019) demonstrated that in both locations, canonical denitrification was the dominant process (>90%) at all times compared to anammox or DNRA. Contrarily, when Fan et al. (2015) studied the effect of tidal influence on nitrification, they found that this process's rate is higher in the tidal and intertidal sites compared to the supratidal mat (irrespective of the season); and that similarly, the AOB and AOA communities in tidal and intertidal stations were alike but significantly different from those in supratidal microbial mats. Interestingly, this trend of similarity between the tidal and intertidal mat community compared to that of the supratidal mats prevails in both the community of denitrifiers (Fan et al., 2015a) and nitrogen fixers (Severin et al. 2012), suggesting all three communities are determined by the same physical and geochemical factors.

Sedimentary nitrogen cycling in the North Sea is also influenced by the sediment type and composition, especially by the distribution and abundance of organic carbon in the bottom sediment. Fan et al. (2015a) demonstrated that denitrification rates were higher in the muddier, organic carbon-rich sands of the muddy depression Oyster Grounds than at the sandy low-carbon Dogger Banks; and this finding was supported by Deek et al. (2012) who showed that N_2 production was higher in the finer North Sea sand stations (which has a higher substrate quality due to higher organic carbon and amino acid content) than at the coarse sand station. Sedimentary characteristics also play an important role for anammox, which shows a higher abundance and rates of

activity at the muddy stations Oyster Grounds and the Frisian Front than at the sand stations Dogger Banks and the Dutch Coast (Bale et al., 2014).

Other factors impacting nitrogen cycling in the North Sea include: (a) phosphate nutrient concentration, demonstrated by the positive linear correlation between benthic N_2 fixation rate and phosphate concentration in the North Sea due to its crucial role (Fan et al. 2015a); (b) sediment depth, with denitrification rates decreasing with depth and the ratio of anammox to denitrification increasing with depth (referenced therein, Rosales Villa et al. 2019) most likely strongly influenced by the decreasing oxygen supply; and (c) heterotrophic processes competing for organic matter (i.e. a reduction in rate when rates of other heterotrophic processes such as the reduction of sulfate or aerobic respiration are higher) (Fan et al. 2015).

Another factor hugely impactful on the rate of nitrogen cycle processes, especially the rate of denitrification, is the distance of the seabed from the river. Rivers are responsible for discharging anthropogenically-produced nitrate, which triggers higher rates of denitrification due to the use of nitrate for this process to occur. Neumann et al. (2017)'s study highlights this trend clearly, demonstrating how the closer the site is to the river Elbe, the higher the nitrate concentration and thus the greater the rate of sediment denitrification. The role of North Sea sediment denitrifiers in the utilization of anthropogenically-produced and released nitrate is a valuable ecosystem service. Although nitrogen is essential for all living things, a period of industrialization from the 1960s to the mid-80s led to a large and excessive input of bioavailable nitrogen into the North Sea (Pätsch and Radach, 1997). Bordered by multiple densely populated industrialized countries, the North Sea suffers from excessive anthropogenic nutrient input via rivers, sewage, groundwater, and atmospheric deposition, fueling unregulated nutrient levels and eutrophication (Burson et al. 2016; Lenhart et al. 2010). This excess nutrient input led to multiple adverse consequences including eutrophication (the mass production of algae), changes in species composition, and severe oxygen depletion in the German Bight (Cadée and Hegerman, 2002; Philippart et al. 2000; Westernhagen and Dethlefsen, 1983; Lancelot et al. 1987). In response, nutrient loads were actively reduced, although whilst phosphorus inputs were reduced by more than 50%, nitrogen loads were only reduced by 20-30% (OSPAR, 1988; Lenhart et al. 2010; Passy et al. 2013; Grizzetti et al. 2012). By removing fixed nitrogen

released from the river and converting it into the unavailable gaseous form, the North Sea is an important sink for fixed nitrogen, mitigating the harmful effects of eutrophication and acting as a barrier for river nutrient inputs to the open ocean (Fennel et al. 2010),

3.2 Seasonality of nitrogen cycle processes in the North Sea

Many of the studies on the nitrogen cycle processes in the North Sea observed and analyzed seasonal nitrogen process rates changes. Their results are summarized in Table 2 below. In the case of denitrification, results varied widely. Whilst some studies found that denitrification rates peaked in the warmer seasons due to higher temperatures and organic matter availability (Rosales-Villa et al. 2019; Neubacher et al. 2011; Fan et al. 2015a), other studies observed the opposite trend, detecting the highest rates of nitrification in the colder seasons due to an increase in riverine discharge stimulating an increase in nitrate concentration in the water (Neumann et al. 2017; Deek et al. 2012; van Beusekom et al. 2009). On the other hand, Gao et al. (2012) observed a consistent rate of nitrification all throughout the year, stating this is due to the continuously saturated capacity of the permeable sediments to host the microbial community due to microorganism's ability to exclusively colonize the sand grain surface.

With respect to nitrification, Fan et al. (2015) and Yazdani Foshtomi et al. (2015) showed nitrification rates peaked in autumn, likely as a consequence of increased photosynthesis rates due to increased light intensity and temperatures resulting in: (a) a higher benthic oxygen concentration stimulating the aerobic process of nitrification (b) the acceleration of organic matter degradation producing ammonium as a source of energy for nitrifying organisms. Despite high rates of photosynthesis in summer, Fan et al. (2015) showed a surprisingly low nitrification rate during this season, which is likely because of the competition of ammonia oxidizers and cyanobacteria for ammonium during this season. In contrast, Lipsewers et al. (2014) found a constant nitrification rate during all seasons, seeing this as a consequence of the stable presence and activity of aerobic ammonia oxidizers due to the continuous oxygen supply to deeper anoxic layers due to bioturbation.

Studies on the seasonal changes in sediment anammox rate have all shown a peak in anammox rate in summer, which is most likely due to the higher temperatures, superior organic carbon content, and lower oxygen availability in the sediment as a consequence of stratification during this season (Bale et al. 2014; Neubacher et al. 2011; Lipsewers et al. 2014).

Lastly, the singular study undertaken on seasonal changes in nitrogen fixation in North Sea sediment showed an increased nitrogen fixation during late summer most likely due to higher temperatures and increased organic matter availability; and a decrease in nitrogen fixation rate just after post-bloom deposition in late spring presumably due to the alteration in the deposited organic matter because of this post-bloom deposition (Fan et al. 2015a). However, more research is needed exploring the seasonal changes in nitrogen fixation to increase the reliability of these results.

There are multiple reasons which could be responsible for the observed variations in seasonal patterns across studies for the nitrogen cycle processes, including (a) differences in the type of sediment analyzed between studies (e.g. coarse or fine sand; highly bioturbated or slightly bioturbated sediment); (b) differences in the distance of the sediment from the river, which will be influenced differently by riverine nutrient discharges (Deek et al. 2012; Rosales Villa et al. 2019); (c) differences in the methodology used to determine the nitrogen process rate (e.g. modeling, ^{13}N isotope analysis, the analysis changes in gene expression abundance), and the limitations brought by different methodologies. Moreover, although various studies have shown the influence of various factors on seabed microbial communities which has been used to explain various trends, there is a need of more studies directly exploring the influence of these factors on the seasonality of their nitrogen cycle process rate changes.

Table 2: Studies on the influence of seasonal changes on individual nitrogen cycle process rates in the North Sea. Table row colors are representative of the nitrogen cycle process studied: Denitrification is in green, anammox is in yellow, nitrogen fixation is in red, and nitrification is in blue.

Study	Process	Season with Highest process rate	Season with Lowest process rate
Neumann et al. 2017	Denitrification	Late winter	Late summer
Gao et al. 2012	Denitrification	Consistent	Consistent
Deek et al. 2012	Denitrification	Fall, Winter, and early spring	Summer
Rosales-Villa et al. 2019	Denitrification	Early Summer (June)	Autumn (September)
Neubacher et al. 2011	Denitrification	Late summer and early autumn	Late winter/early spring
Fan et al. 2015a	Denitrification	Late Spring (May)	Winter (February)
Fan et al. 2015	Nitrification	Autumn	Summer (July)
Yazdani Foshtomi et al. 2015	Nitrification	September	Undefined
Lipsewers et al. 2014	Nitrification	Relatively Stable	Relatively Stable
Bale et al. 2014	Anammox	Summer	Winter
Neubacher et al. 2011	Anammox	Late summer and early autumn	Late winter/early spring
Lipsewers et al. 2014	Anammox	Late summer (August)	February and May (Winter to Spring)
Fan et al. 2015a	Nitrogen fixation	Late summer (August)	Late spring (May)

3.3 Seabed microorganism diversity in the North Sea

A small number of studies detected and identified North Sea seabed microorganisms involved in the nitrogen cycle. Due to the differences in the nature of the experiments (i.e. DNA hybridization, PCR bases gene amplification, whole-genome sequencing, ect.) and the small number of studies undertaken, direct comparison between these studies is not possible. We will discuss the results of these studies in the context of current global knowledge on seabed microorganisms involved in the marine nitrogen cycle.
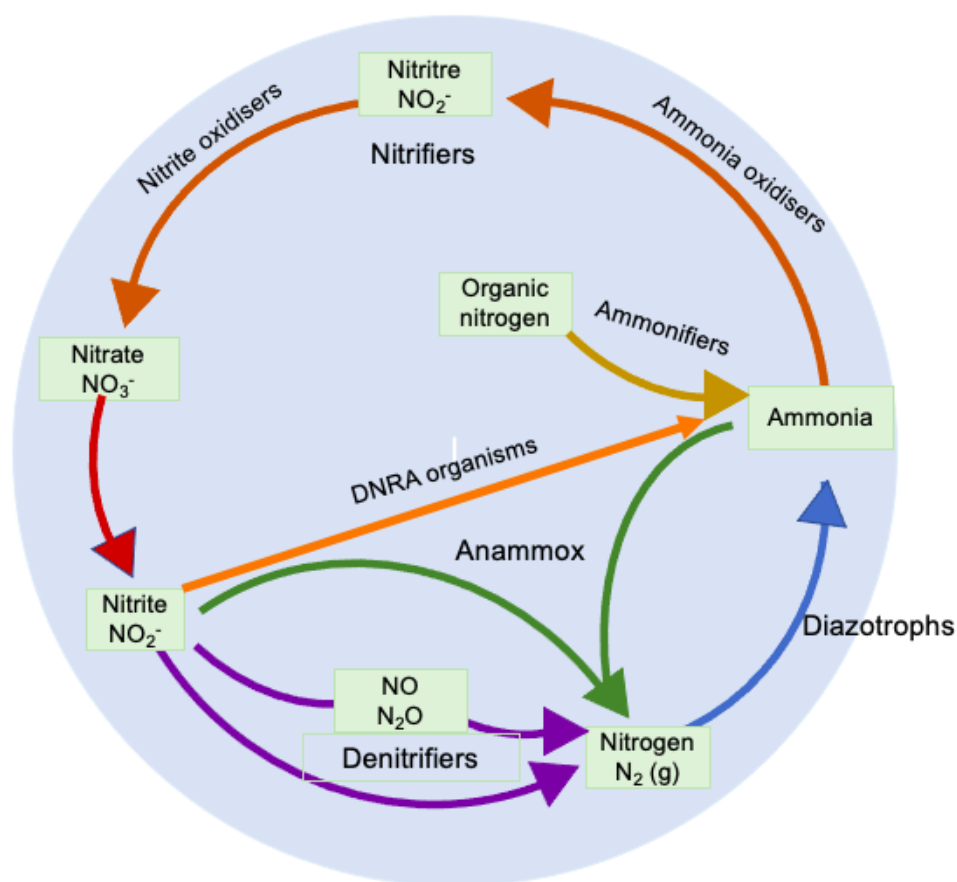

Figure 10: Simplified diagram showing the microorganisms involved in different nitrogen cycle processes.

3.3.1. Diazotroph microorganism diversity

Nitrogen fixers occur both in the water column and the seafloor of a wide variety of marine environments (e.g. open ocean, deep sea, coastal waters, estuaries, tropical

lagoons, and cold Arctic waters), and include a wide physiological and phylogenetic diversity of microorganisms (Mehta et al. 2003; Man-Aharonovich et al. 2007; Jenkins et al. 2004; Hewson et al. 2007; Jabir et al. 2021). Nitrogen fixers include both symbiotic and free-living cyanobacteria groups, and even some heterotrophic microorganisms (Hutchins and Capone, 2022). Although heterotrophic N_2 fixation is ubiquitous throughout the oceans (Farnelid et al. 2011), as nitrogen fixation requires a high energy input to break the N_2 triple bond, autotrophic microorganisms are likely to have an advantage over other diazotrophs as they are not restricted by available organic carbon concentrations (Herbert, 1975; Nedwell, 1980; Chakraborty et al. 2021).

Fan et al. (2015a) investigated the diversity and abundance of denitrifying organisms at three sites in the southern North Sea: the Dutch Coast, Oyster Grounds and Dogger Banks using the marker gene *nifH*. Interestingly, their study demonstrated that the dominant diazotrophic microorganisms were *Desulfovibrio salexigens* and *Desulfovibrio vulgaris,* two sulfate-reducing bacteria, which have been shown to have the genetic potential for undertaking nitrogen fixation (Fan et al. 2015a; Zehr et al., 1995). A large proportion of *nifH* homologs also belonged to *P. carbinolicus*, a strictly anaerobic bacterium belonging to the deltaproteobacterial family *Peleobacteraceae* (Fan et al., 2015a). Although no study has yet observed N_2 fixation by *P. carbinolicus*, the phylogenetically related *Geobacter metallireducens* fixes N_2 (Bazylinski et al., 2000).

3.3.2. Nitrifier diversity

3.3.2.1 Ammonia oxidizer diversity

The first step of nitrification is carried about by ammonia oxidizers transforming ammonium to nitrate (Fan et al. 2015). Two types of ammonia oxidizers exist; ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). AOBs fall within three genera within the classes Betaproteobacteria (i.e. *Nitrosomonas* and *Nitrosopira*) and Gammaproteobacteria (i.e. *Nitrosococcus*) classes (Purkhold et al. 2000; Pajares and Ramos, 2019). Ammonia-oxidizing archaea (AOA) include *Nitrosopumilus maritimus* and *Cenarchaeum symbiosum* (Könneke et al., 2005;

Hallam et al., 2006; Pajares and Ramos, 2019). Both organisms are chemolithoautotrophic, meaning that they obtain energy from the oxidation of inorganic substances (Pajares and Ramos, 2019).

Fan et al. (2015) studied ammonia oxidizer diversity in the North Sea, demonstrating that AOB *amoA* genes were significantly more abundant than AOA *amoA* genes by two to four orders of magnitude in the Southern North Sea microbial mats. This indicates that AOB are mainly responsible for ammonium oxidation in this region (Fan et al., 2015). The most abundant AOB in the region were Betaproteobacteria belonging to the *Nitrospira* clusters B and C, and included species such as *Nitrospira multiformis, Nitrospira briensis* and *Nitrospira sp. REGAU*. Many sequences also belonged to *Nitrosommas.* Whilst there was a broad distribution of archael *amoA* genes, the most dominant orders were *Nitropumulis* and *Nitrosphaera* (Fan et al., 2015). A positive correlation was also found between AOA *amoA* gene abundance and organic matter, suggesting that AOA may not be obligate ammonia-oxidisers (Fan et al., 2015).

3.3.2.2 Nitrite oxidizers diversity

Nitrite-oxidizing bacteria (NOB) are a diverse group of bacteria responsible for nitrite oxidation, which all have a key enzyme called nitrite oxidoreductase (NXR). The known NOBs mainly belong to six bacterial genera: *Nitrobacter*, *Nitrotoga*, *Nitrospira*, *Nitrococcus*, *Nitrospina*, and *Nitrolancea* (Daims et al. 2016; Watson and Waterbury, 1971; Daims et al., 2001; Alawi et al., 2007; Sorokin et al., 2012). Interestingly, *Nitrospira* have a reciprocal feeding interaction with ammonia oxidizers. *Nitrospira* convert urea into ammonia and CO_2. The ammonia produced is used by ammonia oxidisers, that in return provide nitrite to the NOBs (Koch et al. 2015).

Haaijer et al. (2013) explored Dutch coastal North Sea nitrite oxidizer diversity in bioreactors and highlighted the presence of both (a) *Nitrospira*-like NOB (80% of the bacterial population), mostly from a novel species called *Nitrospira salsa,* and (b) *Nitrospina*-like NOB (10% of the bacterial pop.). No *Nitrospina* were detected. However, *in situ* data must be collected to identify their respective relevance as nitrite oxidisers in the North Sea.

3.3.3 Denitrifier diversity

Denitrification is carried out by a diverse group of mostly heterotrophic prokaryotes, although some autotrophic denitrifiers have been found in the marine environment (Voss et al. 2013; Lam and Kuypers, 2011). The genes encoding for two nitrite reductases, that is NirK and NirS (*nirK* and *nirS*) are used as marker genes for denitrifiers (Wei et al. 2015; Sakurai and Kataoka, 2007; Cutruzzola et al. 2001). Bacteria that have been pointed out as denitrifiers include species from the genera *Bacillus*, *Paracoccus*, and *Pseudomonas*, amongst others (Bernhard, 2010). Recent evidence also demonstrated that some eukaryotes such as foraminifera are also capable of denitrification (Risgaard-Petersen et al. 2006, Bernhard, 2010). Some Archaea from the phyla Euryachaeota and Crenarchaeots have also been observed to possess *nirK* or *nirS*.

Fan et al. (2015) phylogenetically analyzed sequences for *nirS* gene fragments in the North Sea. Although the *nirS* marker gene could not be assigned to a specific group of microorganisms, the sequences related to those from various environments and was most similar to those belonging to *Thiothrix lacustris* and *Azoarcus tolulyticus* (Fan et al. 2015).

3.3.4 Anammox bacteria diversity

Anammox is carried out by a number of anammox bacteria from the phylum *Planctomycetes* (Chen et al. 2019), including the *Candidatus* genera *Brocadia* (Strous et al. 1999), *Kuenenia* (Schmid et al. 2000), *Scalindua* (Kuypers et al. 2003), *Anammoxoglobus* (Kartal et al. 2007), *Jettenia* (Zhe-Xue et al. 2010) and *Anammoximicrobium* (Khramenkov et al. 2013; Connan et al. 2017, Chen et al. 2019). Moreover, anammox bacteria have been found to be able to co-exist with ammonia-oxidizing bacteria (AOB) in a single reactor in which AOB simultaneously creates an anoxic environment for anammox and provides nitrite to the anammox bacteria (Chu et al., 2015; Chen et al. 2019).

Lipsewers et al (2014) analyzed anammox bacteria species diversity in the North Sea. Anammox was performed by bacteria from the Planctomycetes phylum (Lipsewers et

al, 2014). The sequences were closely related to the Plactomycetes members *Candidatus Scalindula marina* and *Candidatus Scalindula brodae* (Lipsewers et al, 2014). Surprisingly, anammox activity was also detected in the upper oxic layers of the sediment, suggesting that anammox bacteria can tolerate the presence of oxygen or have the ability to create anoxic niches (Lipsewers et al, 2014). It's important to note that the discordance between anammox bacteria 16S rRNA gene transcriptional activity and *hzsA* gene abundance indicates *hzsA* transcript abundance may not be an adequate biomarker for anammox bacteria activity (Bale et al. 2014; Lipsewers et al. 2014).

3.3.5 DNRA microorganism diversity

A large diversity of microorganisms, both prokaryotic and eukaryotic, are capable of DNRA. The main DNRA groups include anaerobic and aerobic prokaryotic Proteobacteria, Firmicutes, Verrucomicrobia, Planctomycetes, Acidobacteria, Chloroflexi, and Chlorobia (Tiedje, 1988; Welsh et al. 2014; Pajares and Ramos, 2019).

So far, DNRA microorganism species diversity has not been identified for the North Sea. However, DNRA rates have been observed to be variable, ranging from 0% to 17% of the denitrification rate (Rosales Villa, 2019), and have been observed to increase in importance relative to denitrification during both periods of high nitrate concentration but low organic matter concentration (as denitrification is exclusively heterotrophic); and in well-irrigated sediments (as DNRA is better at dealing with oxic conditions than denitrification) (Toussaint et al. 2021).

Conclusion and future recommendations

In this review, we addressed the key biogeochemical cycles and the role of the North Sea benthic microbial communities in the nitrogen cycle for use in closed circular aquaculture systems farming North Sea biota. Aquaculture is a fast-growing system that is playing an increasingly important role in the supply of marine organisms for consumption, currently making up around 52% of fish for human consumption (FAO, 2020). Thus, research on how to improve its sustainability and efficiency is of great use. Presently, most aquaculture centers do not consider microorganism seabed diversity of great importance to the performance of the system, which is partly why few studies can be found on this subject area (i.e. Quero et al. 2020; Hornick et al. 2018; Stoeck et al. 2018; Chen et al. 2019). The Dutch company Bluelinked is currently undertaking research in this field to aid in the creation of an optimal benthic microorganism seabed community to create an efficient and sustainable indoor aquaculture system where the in-depth comprehension and the possible modification of seabed microorganisms will be used to increase the system's performance and sustainability largely through (1) the prevention of the need for the constant replacing of water and through (2) the recycling the nutrients to prevent the dangerous build-up of nutrients in the water (Michaël Latveer, personal communication). Bluelinked is not working with North Sea sediment and is currently working with a system whose bottom sediment consists of microorganism-inoculated calcium carbonate. However, both the water column temperature and species used will be in line with the conditions found in the North Sea (Michaël Latveer, personal communication).

We hope this review assists Bluelinked and other aquaculture centers to become equipped with relevant information and guidance on the role of seabed microorganisms in the nitrogen cycle, and their potential importance in the performance of aquaculture centers. This review makes evident that the dynamic and permeable North Sea surface sediments are characterized by diverse and versatile microorganism communities influenced by varying sand grain characteristics (fine or coarse sand), fluctuating redox conditions, seasonality, temporality, biota characteristics, substrate availability, and resource competition. The following factors represent the key recommendations for aquaculture centers identified in the review

and factors that must be taken into consideration to optimize future research on this topic.

- o There is an unequal distribution of studies focused on different nitrogen cycle processes, with denitrification and nitrification by far the most studied processes (see Figure 11). It would be of high interest to perform more studies on all the various components of the nitrogen cycle, as they are closely interlinked and mutually influenceable. To give an example, the most understudied process in the nitrogen cycle, DNRA, competes for nitrite with denitrifiers and thus may have a strong influence on denitrification rates (Pajares and Ramos, 2019).

■ Denitrification ■ Nitrification ■ Anammox
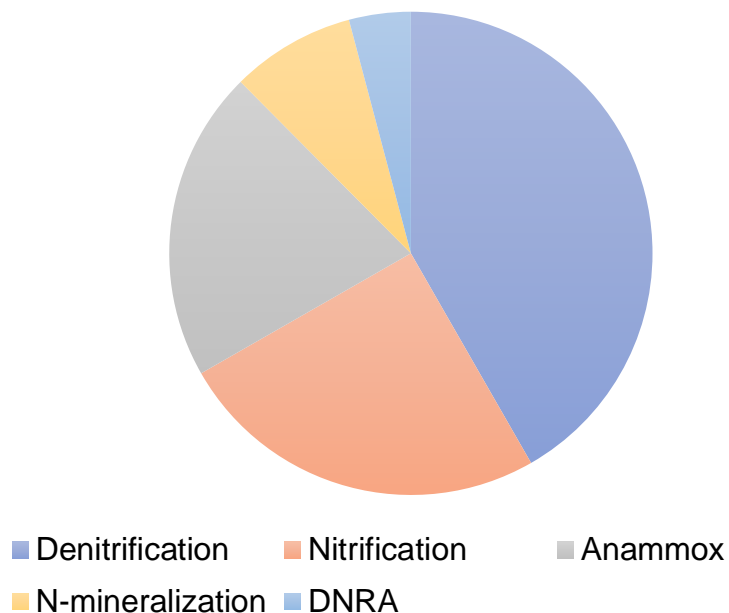■ N-mineralization ■ DNRA

Figure 11: Pie chart showing the proportion of studies on the North Sea seabed working on different nitrogen cycle processes. Whilst denitrification was the most studied process (66.7% of total studies), the competing process DNRA only made up 6.6% of all studies.

- o The experimental procedure and monitoring methods should be kept the same across studies to permit quantitative and reliable comparisons between them. For example, in the case of accurately estimating nitrogen cycle rates, ^{15}N isotope analysis has been identified as a recommended experimental procedure for the accurate estimation of rates with fewer uncertainties; and

therefore we recommend it should be utilized by all studies (Rosales Villa et al. 2019).

o A large proportion of the studies on the North Sea seabed microorganisms did not take advective pore water transport into consideration in their experimental set-up, which has been shown to have a significant influence on seabed microorganism diversity and performance. Future studies on the North Sea seabed microorganisms should take porewater advective transport into account to reduce potentially significant errors in their approach and data set.

o Future studies should undertake field and laboratory experiments together with experiments in indoor aquaculture systems (Spicer, 2014). For example, it would be necessary to compare the seabed microorganism community and process rates between the calcium carbonate sediment in the aquaculture tanks in Bluelinked with the North Sea sandy sediment. If there is a significant difference in performance and microbial species diversity between the natural conditions and aquaculture, this opens up the opportunity to modify the seabed in the fish farm, such as through the addition of necessary microbes or bioturbating fauna, to replicate natural conditions and to create optimal farming conditions. However, it's important to note that environmental conditions in the field and laboratory are not exactly the same as those experienced within the aquaculture environment. For example, abiotic and biotic influences such as advective pore water transport, which plays a major role in the influence of the seabed microorganism community and process rates in the North Sea, are not present in aquaculture.

o Future studies should assess aquaculture microorganism diversity and performance change over time, as it would be of interest to find out to what extent microorganism evolution and adaptation in the calcium carbonate seabed enables them to replicate the role played by North Sea microorganisms over time and whether they are capable of self-equilibration after disruptions (i.e. a nutrient imbalance or an increase in competition for substrate) through changes in microorganism activity or abundance to maintain a healthy system.

- Although multiple studies assessed the presence and activity of microorganisms in response to a range of environmental variables, more studies are needed to shed light on the influence of interconnections between various microorganisms (i.e. competition or synergism) and the ecosystem functions played by microorganisms both individually and collectively.

- Future studies should assess the possible adverse consequences brought by aquaculture seabed microorganisms and their involvement in the nitrogen cycle on fish health and the environment, and how to mitigate these effects. To give an example, nitrous oxide (N_2O) formation occurs during various processes involved in the nitrogen cycle, which is concerning due to N_2O's contribution to greenhouse gas emissions, ozone depletion, and air pollution (Bernhard, 2010). The main producers of N_2O are both bacterial and archaeal nitrification, where ammonia oxidizers are an important contributor (Löscher et al. 2012, Santoro et al., 2011); and as an intermediate gas produced during denitrification (Ji et al., 2015; Babbin et al., 2015; Elkins et al., 1978).

Bibliography

Achterberg, E.P. (2014). Grand challenges in marine biogeochemistry. Front. Mar. Sci., Front. Mar. Sci. 1:7.

Ahmerkamp S, Winter C, Krämer K, de Beer D, Janssen F, Friedrich J.,. Kuypers M.M.M., Holtappels M. (2017). Regulation of benthic oxygen fluxes in permeable sediments of the coastal ocean. Volume: 62, Pages: 1935-1954

Ahmerkamp, S., Marchant, H.K., Peng, C. Probandt D., Littmann S., Kuypers M.M.M., Holtappels M. (2020). The effect of sediment grain properties and porewater flow on microbial abundance and respiration in permeable sediments. Sci Rep 10, 3573

Alawi, M., Lipski, A., Sanders, T., Pfeiffer, E. M., and Spieck, E. (2007). Cultivation of a novel cold-adapted nitrite oxidizing betaproteobacterium from the Siberian Arctic. ISME J. 1, 256–264.

Anantharaman, K., Hausmann, B., Jungbluth, S.P. Kantor R.S., Lavy A., Warren L.A., Rappé M.S., Pester M., Loy A., Thomas B.C., Banfield J.F. Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. (2018). ISME J 12, 1715–1728.

Anderson, L. A. & Sarmiento, J. L. (1994) Redfield ratios of remineralization determined by nutrient data-analysis. Glob. Biogeochem. Cycles. 8, 65–80

Arning E. (2008) Phosphogenesis in coastal upwelling systems - bacterially-induced phosphorite formation. . (Ph.D./ Doctoral thesis), Universität Bremen, Bremen, Germany, 137 pp.

Babbin, A. R., Bianchi, D., Jayakumar, A., & Ward, B. B. (2015). Rapid nitrous oxide cycling in the suboxic ocean. Science, 348(6239), 1127–1129.

Bak, F., and Pfennig, N. (1987). Chemolithotrophic growth of Desulfovibrio sulfodismutans sp. nov. by disproportionation of inorganic sulfur compounds. Arch. Microbiol. 147, 184–189.

Bale, N. J., Villanueva, L., Fan, H., Stal, L. J., Hopmans, E. C., Schouten, S., et al. (2014). Occurrence and activity of anammox bacteria in surface sediments of the southern North Sea. FEMS Microbiol. Ecol. 89, 99–110.

Barbieri E, Bondiol ACV, de Melo CB, Henriques MB. (2014) Nitrite toxicity to Litopenaeus schmitti(Burkenroad, 1936, Crustacea) at different salinity levels. Journal of Aquaculture Research. 47(4):1-9.

Bazylinski, D. A., Dean, A. J., Schüler, D., Phillips, E. J. P., and Lovley, D. R. (2000). N2-dependent growth and nitrogenase activity in the metal-metabolizing bacteria, Geobacter and Magnetospirillum species. Environ. Microbiol. 2, 266–273.

Benitez-Nelson, C. R. (2000) Biogeochemical cycling of phosphorus in marine systems. Earth Sci. ReV. 51, 109-135

Bernhard A., (2010). The Nitrogen Cycle: Processes, Players, and Human Impact. Nature Education Knowledge 3(10):25

Berninger, U. G. & Epstein, S. S. (1995) Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea sediment. Aquatic Microbial Ecology 9(3), 229–236

Bharathi L.P.A. (2008) Sulfur Cycle, from Encyclopedia of Ecology. Pages 3424-3431, Academic Press.

Björkman K., Thomson-Bulldis A. L., Karl D. M., (2000) Phosphorus dynamics in the North Pacific subtropical gyre. Aquat. Microb. Ecol. 22, 185–198

Bluelinked nd. Available at: https://www.bluelinked.eu/projects/fish-fry-hatchery.html [Accessed the 18th of March 2022]

Böer, S., Arnosti, C., Van Beusekom, J. & Boetius, A. (2009) Temporal variations in microbial activities and carbon turnover in subtidal sandy sediments. Biogeosciences 6, 1149–1165

Boudreau BP, Huettel M, Forster S, Jahnke RA, McLachlan A, Middelburg JJ, et al. (2001) Permeable marine sediments: overturning an old paradigm. Eos Trans AGU. 82:133–6

Boudreau BP, Huettel M, Forster S, Jahnke RA, McLachlan A, Middelburg JJ, Nielsen P., Sansone F.J., Taghon G.L., Van Raaphorst W. (2001) Permeable marine sediments: overturning an old paradigm. Eos Trans AGU. 82:133–6.

Boyd P.W., Claustre H., Levy M., Siegel D.A., Weber T. (2019) Multi-faceted particle pumps drive carbon sequestration in the ocean. Nature.. 568:327–335.

Boyd, P. W. (2015). Toward quantifying the response of the oceans' biological pump to climate change. Front. Mar. Sci. 2:77.

Bracken MES, Hillebrand H, Borer ET, Seabloom EW, Cebrian J, Cleland EE, Elser JJ, Gruner DS, Harpole WS, Ngai JT, Smith JE. (2015). Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. Oikos. 124:113–121.

Braeckman U, Janssen F, Lavik G, Elvert M, Marchant H, Buckner C, et al. (2018) Carbon and nitrogen turnover in the Arctic deep sea: in situ benthic community response to diatom and coccolithophorid phytodetritus. Biogeosciences. 15:6537–57.

Brenner, H. Braeckman, U. Le Guitton, M., Meysman F.J.R. (2016) The impact of sedimentary alkalinity release on the water column CO2 system in the North Sea. Biogeosciences, 13, pp. 841-863

Bristow L.A., Mohr W., Ahmerkamp S., Kuypers M.M.M. (2017). Nutrients that limit growth in the ocean, Current Biology, Volume 27, Issue 11, Pages R474-R478, ISSN 0960-9822

Burson,A., Stomp, M., Akil, L., Brussaard, C.P.D. Huisman J. (2016) Unbalanced reduction of nutrient loads has created an offshore gradient from phosphorus to nitrogen limitation in the North Sea. Limnol. Oceanogr., 61, pp. 869-888

Cadée, G. C., and J. Hegeman. 2002. Phytoplankton in the Marsdiep at the end of the 20th century; 30 years monitoring biomass, primary production, and Phaeocystis blooms. J. Sea Res. 48: 97–110.

Canfield, D. Jørgensen, B., Fossing, H., Glud, R., Gundersen, J. Ramsing, N., Thamdrup, B., Hansen J.,Nielsen, L., Hall P.O. (1993) Pathways of organic carbon oxidation in three continental margin sediments

Chakraborty, S., Andersen, K.H., Visser, A.W. Inomura K., Follows M.J., Riemann L. (2021) Quantifying nitrogen fixation by heterotrophic bacteria in sinking marine particles. Nat Commun 12, 4085

Charry F., Andreotti B., Claudin P., (2013). Sand Ripples and Dunes. Annual Review of Fluid Mechanics 45(1):469-493

Chen C. Chang Z., Zhang L., Jiang Y., Ge H., Song X., Chen S., Zhan F., Li J., (2019). Effects of water recirculation rate on the microbial community and water quality in relation to the growth and survival of white shrimp (Litopenaeus vannamei) BMC Microbiology, 19:192

Chen R., Yao J., Ailijiang N., Liu R., Fang L., Chen Y., (2019) Abundance and diversity of nitrogen-removing microorganisms in the UASB-anammox reactor. Page num. PLOS ONE.

Chu, Z.-R. , Wang, K. , Li, X.-K. , Zhu, M.-T. , Yang, L. , and Zhang, J.
(2015) Microbial characterization of aggregates within a one-stage nitritation–anammox system using high-throughput amplicon sequencing. Chem Eng J 262: 41–48

Connan R., Dabert P., Chapleur O., Bridoux G. Magrí A., Beline F.,Vanotti M.B., Le Roux S., (2017) Characterization of a combined batch-continuous procedure for the culture of anammox biomass. Ecological Engineering. 106: 231–241.
Cotner, J. B.; Wetzel, R. G. (1992) Uptake of dissolved inorganic and organic bphosphorus compounds by phytoplankton and bacterioplankton. Limnol. Oceanogr. 37, 232.

Cotner, J., Biddanda, B. Small Players, Large Role: Microbial Influence on Biogeochemical Processes in Pelagic Aquatic Ecosystems.
(2002) Ecosystems 5, 105–121

Cutruzzola F, Brown K, Wilson EK, Bellelli A, Arese M, Tegoni M. Cambillau C., Brunori M. (2001). The nitrite reductase from Pseudomonas aeruginosa: Essential role of two active-site histidines in the catalytic and structural properties. PNAS 98: 2232–2237.

D'Andrea, F.A., Aller, R.C., Lopez G.R., (2002). Organic matter flux and reactivity on a South Carolina sandflat: the impacts of porewater advection and macrobiological structures. Limnol. Oceanogr., 47, pp. 1056-1070

Daims, H., Lücker, S., and Wagner, M. (2016). A new perspective on microbes formerly known as nitrite-oxidizing bacteria. Trends Microbiol. 24, 699–712.

Daims, H., Nielsen, J. L., Nielsen, P. H., Schleifer, K. H., and Wagner, M. (2001). In situ characterization of Nitrospira-like nitrite oxidizing bacteria active in wastewater treatment plants. Appl. Environ. Microbiol. 67, 5273–5284.

Dang, H. & Lovell, C. R. (2016) Microbial surface colonization and biofilm development in marine environments. Microbiology and Molecular Biology Reviews 80, 91–138.

de Beer, D., Wenzhöfer, F., Ferdelman, T., Boehme, S.E., Huettel, M., van Beusekom, J.E.E., Böttcher, M.E., Musat, N., Dubilier N., (2005). Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Romo Basin, Wadden Sea. Limnol. Oceanogr., 50, pp. 113-127

De Borges E., Braeckman U., Soetaert K. (2021). Rapid organic matter cycling in North Sea sediments. Continental Shelf Research, Volume 214, 104327, ISSN 0278-4343

Deek, Astrid, Kay Emeis, and Justus van Beusekom. (2012) "Nitrogen removal in coastal sediments of the German Wadden Sea." Biogeochemistry 108.1. 467-483.

Dekas AE, Poretsky RS& Orphan VJ. (2009) Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. Science 326, 422–426

Delaney, M. L. (1998) Phosphorus accumulation in marine sediments and the oceanic phosphorus cycle. Global Biogeochem. Cycles, 12, 563.

DeVries, T., Primeau, F. & Deutsch, C. (2012) The sequestration efficiency of the biological pump. Geophys. Res. Lett. 39.

Diego-McGlone M.L.S., Azanza R.V., Villanoy C.L., (2008) Jacinto G.S. Eutrophic waters, algal bloom and fish kill in fish farming areas in Bolinao, Pangasinan, Philippines. Mar. Pollut. Bull; 57:295–301.

Dyhrman S. T., Chappell P. D., Haley S. T., Moffett J. W., Orchard E. D., Waterbury J. B., Webb E. A., (2006) Phosphonate utilization by the globally important marine diazotroph Trichodesmium. Nature 439, 68–71

Elena M. Bennett, Stephen R. Carpenter, Nina F. Caraco, Human Impact on Erodable Phosphorus and Eutrophication: A Global Perspective: Increasing accumulation of phosphorus in soil threatens rivers, lakes, and coastal oceans with eutrophication, (2001) BioScience, Volume 51, Issue 3, Pages 227–234.

Elkins JW, Wofsy SC, McElroy MB, Kolb CE, Kaplan WA (1978) Aquatic sources and sinks for nitrous oxide. Nature 275: 602–606

Elliott, A.H., Brooks N.H., (1997b) Transfer of nonsorbing solutes to a streambed with bed forms: laboratory experiments. Water Resources Research, 33, pp. 137-151

Elser, J. J., Sterner R.W., Gorokhova W.F., Fagan W.F., Markow T.A., Cotner J.B., Harrisson J: F.,Hobbie S.E., Odell G.M., Weider L.W. (2000) Biological stoichiometry from genes to ecosystems. Ecol. Lett. 3: 540–550.

Falkowski, P. G., Barber, R.T. and Smetacek. V. (1998) Biogeochemical controls and feedbacks on ocean primary production. Science 281:200-206.

Fan, H., Bolhuis, H. and Stal, L.J., (2015). Nitrification and nitrifying bacteria in a coastal microbial mat. Frontiers in Microbiology, 6, p.1367.

Fan, H., Bolhuis, H., and Stal, L. J. (2015a). Drivers of the dynamics of diazotrophs and denitrifiers in North Sea bottom waters and sediments. Front. Microbiol. 6:738.

FAO. (2020) The State Of World Fisheries And Aquaculture (SOFIA). Available at: https://reliefweb.int/sites/reliefweb.int/files/resources/The%20State%20of%20World%20Fisheries%20and%20Aquaculture%202020.%20In%20brief.pdf

Fennel, K. (2010) The role of continental shelves in nitrogen and carbon cycling: Northwestern North Atlantic case study, Ocean Sci., 6, 539–548,

Finster K. (2008) Microbiological disproportionation of inorganic sulfur compounds, Journal of Sulfur Chemistry, 29:3-4, 281-292

Finster, K., Liesack, W., and Thamdrup, B. (1998). Elemental sulfur and thiosulfate disproportionation by Desulfocapsa sulfoexigens sp. nov., a new anaerobic bacterium isolated from marine surface sediment. Appl. Environ. Microbiol. 64, 119–125.

Flemming, H.-C. & Wuertz, S. (2019) Bacteria and archaea on Earth and their abundance in biofilms. Nature Reviews Microbiology. 17, 247–260

Föllmi K.B., (1996) The phosphorus cycle, phosphogenesis and marine phosphate-rich deposits, Volume 40, Issues 1–2, Pages 55-124

Fuhrman JA, Hewson I, Schwalbach MS, Steele JA, Brown MV, Naeem S. (2006), Annually reoccurring bacterial communities are predictable from ocean conditions. Proc Natl Acad Sci USA. 103:13104–9.

Gao, Hang, Matyka M., Liu B., Khalili A., Kostka J.E., Collins G., Jansen S., et al. 2012. "Intensive and extensive nitrogen loss from intertidal permeable sediments of the Wadden Sea." Limnology and Oceanography 57.1: 185-198.
Geochim. Cosmochim. Acta, 75, pp. 3581-3599

Glöckner F.O., Stal L.J., Sandaa R.-A., Gasol J.M., O'Gara F., Hernandez F., Labrenz M., Stoica E., Varela M.M., Bordalo A., Pitta P. (2012). Marine Microbial Diversity and its role in Ecosystem Functioning and Environmental. ISBN: 978-2-918428-71-8. European Scientific Foundation (ESF): Ostend, Belgium.

Gnana Thanga Vincent et al. (2021) Chapter 4 - Biogeocycling of nutrients (C, N, P, S, and Fe) and implications on greenhouse gas emissions, Microbial Communities in Coastal Sediments, Elsevier, Pages 119-145, ISBN 9780128151655.

Gobet, Böer S.I., Huse S.M., van Beusekom J.E.E., Quince C., Sogin M.L., Boetius A., Ramette A. (2012) Diversity and dynamics of rare and of resident bacterial populations in coastal sands. The ISME Journal 6, 542

Graham J.E., Wantland N.B., Campbell M., Klotz M.G. (2011) Chapter Fourteen - Characterizing Bacterial Gene Expression in Nitrogen Cycle Metabolism with RT-qPCR, Academic Press, Volume 496, Pages 345-372

Graham, J.E., Wantland, N.B., Campbell M., Klotz M.G. Chapter Fourteen - Characterizing Bacterial Gene Expression in Nitrogen Cycle in Methods in Enzymology, Volume 496, Pages 345-372, ISSN 0076-6879, ISBN 9780123864895, Academic Press.

Grizzetti B., Bouraoui F., Aloe A. (2011) Changes of nitrogen and phosphorus loads to European seas. Global Change Biology, 18, 769–782

Guignard MS, Leitch AR, Acquisti C, Eizaguirre C, Elser JJ, Hessen DO, Jeyasingh PD, Neiman M, Richardson AE, Soltis PS, Soltis DE, Stevens CJ, Trimmer M, Weider LJ, Woodward G and Leitch IJ (2017) Impacts of Nitrogen and Phosphorus: From Genomes to Natural Ecosystems and Agriculture. Front. Ecol. Evol. 5:70.

Guilini K, Oevelen DV, Soetaert K, Middelburg JJ, Vanreusela A. (2010) Nutritional importance of benthic bacteria for deep-sea nematodes from the Arctic ice margin: results of an isotope tracer experi5ment. Limnol Oceanogr. 55:1977–89

Gunnars, A., Blomqvist, S., Martinsson, C., (2004). Inorganic formation of apatite in brackish seawater from the Baltic Sea: an experimental approach. Mar. Chem. 91, 15-26.

Haaijer S., Ji K., van Niftrik L., Hoischen A., Speth D., Jetten M.S.M., Sinninghe J.S., Damsté S., Op den Camp H.J.M., 2013. A novel marine nitrite-oxidizing Nitrospira species from Dutch coastal North Sea water. Frontiers in Microbiology. Volume 4.
Hallam, S. J., Mincer, T. J., Schleper, C., Preston, C. M., Roberts, K., Richardson, P. M., DeLong E.F. (2006). Pathways of carbon assimilation and ammonia oxidation suggested by environmental genomic analyses of marine Crenarchaeota. PLoS Biol. 4:e95.

Hamann, E. Tegetmeyer H.E., Riedel D., Littmann S., Ahmerkamp S., Chen J., Hach P.F., Strous M. (2017) Syntrophic linkage between predatory Carpediemonas and specific prokaryotic populations. The ISME journal, 11(5), p. 1205

Hamersley MR, Woebken D, Boehrer B, Schultze M, Lavik G, Kuypers MMM. (2009). Water column anammox and denitrification in a temperate permanently stratified lake (Lake Rassnitzer, Germany). Syst. Appl. Microbiol. 32: 571– 82

Hanrahan G. and Chan G. (2005). Nitrogen, in Encyclopedia of Analytical Science. Second Edition. Elsevier.

Hedges, J. I. (2002). "Why Dissolved organics matter," in Biogeochemistry of Marine Dissolved Organic Matter, 1–33. New York, USA: Academic Press

Herbert RA (1975) Heterotrophic nitrogen fixation in shallow estuarine sediments. J exp mar Biol Ecol 18: 215–225

Hewson I, Moisander PH, Morrison AE, Zehr JP . (2007). Diazotrophic bacterioplankton in a coral reef lagoon: Phylogeny, diel expression, and response to phosphate enrichment. ISME J 1: 78–91.

Hinckley, EL.S., Crawford, J.T., Fakhraei, H. Driscoll C.T. (2020) A shift in sulfur-cycle manipulation from atmospheric emissions to agricultural additions. Nat. Geosci. 13, 597–604

Hofer, U. (2018) New diversity in the sulfur cycle. Nat Rev Microbiol 16, 261.

Holmer M., Duarte C.M., Heilskov A., Olesen B., Terrados J. (2003) Biogeochemical conditions in sediments enriched by organic matter from net-pen fish farms in the Bolinao area, Philippines. Mar. Pollut. Bull.;46:1470–1479. doi: 10.1016/S0025-326X(03)00281-9

Holmkvist, L., Ferdelman, T.G.,.Jørgensen. B.B. (2011) A cryptic sulfur cycle driven by iron in the methane zone of marine sediment (Aarhus Bay, Denmark)

Honjo S., Eglinton T.I., Taylor C., Ulmer K.M., Slevert S.M., Bracher A., German C.R., Edgromb V., Francois R., Iglesias-Rodriguez D., Van Mooy B., Repeta D.J. (2014) Understanding the Role of the Biological Pump in the Global Carbon Cycle: An Imperative for Ocean Science. Oceanography 27(3):10-16

Hornick K.M., Buschmann A.H. (2018) Insights into the diversity and metabolic function of bacterial communities in sediments from Chilean salmon aquaculture sites. Ann. Microbiol. 68:63–77.

Hoshino T, Doi H, Uramoto G-I, Wörmer L, Adhikari RR, Xiao N, et al. Global diversity of microbial communities in marine sediment. (2020) Proc Natl Acad Sci USA. 117:27587–97.

Howarth W. (2021). Nitrogen, in Reference Module in Earth Systems and Environmental Sciences, Elsevier.

Huettel, M., Berg, P. & Kostka, J. E. (2014) Benthic exchange and biogeochemical cycling in permeable sediments. Annual Review of Marine Science, 6, 23–51

Huettel, M., W. Ziebis, and S. Forster. (1996). Flow-induced uptake of particul ate matter in permeable sediments. Limnol. Oceanogr. 41: 309–322.

Hunter EM, Mills HJ, Kostka JE . (2006). Microbial community diversity associated with carbon and nitrogen cycling in permeable shelf sediments. Appl Environ Microb 72: 5689–5701.

Hutchins, D. & Boyd, P. (2016) Marine phytoplankton and the changing ocean iron cycle. Nat. Clim. Chang. 6, 1072–11079

Hutchins, D.A., Capone, D.G. (2022) The marine nitrogen cycle: new developments and global change. Nat Rev Microbiol.

Irigoien, X., Klevjer, T., Røstad, A. Martinez U., Boyra G., Acuña J.L., Bode A., Echevarria F., Gonzalez-Gordillo J.I., Hernandez-Leon S., Agusti S., Aksnes D.L., Duarte C.M., Kaartvedt S. (2014). Large mesopelagic fishes biomass and trophic efficiency in the open ocean. Nat Commun 5, 3271

Jabir, T., Vipindas, P.V., Krishnan, K.P. Hatha M.A.A. (2021) Abundance and diversity of diazotrophs in the surface sediments of Kongsfjorden, an Arctic fjord. World J Microbiol Biotechnol 37, 41

Jahnke R. (1985) A model of microenvironments in deep-sea sediments: Formation and effects on porewater profiles. Limnology and Oceanography. 30:966–971.
Jahnke, R. A. (2010), Global synthesis, in 'Carbon and Nutrient Fluxes in Continental Margins', Springer, pp. 597–615.

Jenkins BD, Steward GF, Short SM, Ward BB, Zehr JP . (2004). Fingerprinting diazotroph communities in the Chesapeake Bay by using a DNA macroarray. Appl Environ Microbiol 70: 1767–1776.

Jetten MSM, van Niftrik L, Strous M, Kartal B, Keltjens JT, Op den Camp HJM. (2009). Biochemistry and molecular biology of anammox bacteria. Crit. Rev. Biochem. Mol. Biol. 44:65–84

Ji Q., Babbin A.R., Kayakumar A., Oleynik S., Ward B.B., (2015). Nitrous oxide production by nitrification and denitrification in the Eastern Tropical South Pacific oxygen minimum zone. Geophysical Research Letters. Volume 42, Issue 24, Pages 10,755-10,764

Jiao, N., Herndl, G. J., Hansell, D. A., Benner, R., Kattner, G., Wilhelm, S. W., et al. (2010). Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nat. Rev. Microbiol. 8, 593–599.

Jiao, N., Robinson, C., Azam, F., Thomas, H., Baltar, F., Dang, H., et al. (2014). Mechanisms of microbial carbon sequestration in the ocean – future research directions. Biogeosciences 11, 5285–5306.

Jørgensen B. (1977) Bacterial sulfate reduction within reduced microniches of oxidized marine sediments. Marine Biology. 41:7–17.

Jørgensen B. B., Kasten S. (2006). "Sulfur cycling and methane oxidation," in Marine Geochemistry, eds Schulz H. D., Zabel M. 271–309. Springer: Berlin.

Jørgensen B.B. (1982). Mineralization of organic matter in the sea bed—the role of sulphate reduction, Nature, 296, pp. 643-645

Jørgensen B.B., Nelson D.C. (2004) Sulfide oxidation in marine sediments: Geochemistry meets microbiology. Geol. Soc. Am. Spec. Pap., 379, pp. 63-81
Jørgensen BB, Findlay AJ, Pellerin A. (2019) The Biogeochemical Sulfur Cycle of Marine Sediments. Front Microbiol;10:849.

Kalantzi I., Rico A., Mylona K., Pergantis S.A., (2021) Tsapakis M. Fish farming, metals and antibiotics in the eastern Mediterranean Sea: Is there a threat to sediment wildlife? Sci. Total. Environ,;764:142843.

Karl D. M., (2014) Microbially mediated transformations of phosphorus in the sea: New views of an old cycle. Annu. Rev. Mar. Sci. 6, 279–337

Kartal B., Rattray J., van Niftrik L. A., Van d. V. J., Schmid M. C., Webb R. I., Schouten S., Fuerst J. A., Damste J. S. and Jetten M. S.. (2007) Candidatus "Anammoxoglobus propionicus" a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. Systematic and Applied Microbiology. 30: 39–49. pmid:16644170

Khramenkov S. V., Kozlov M. N., Kevbrina M. V., Dorofeev A. G., Kazakova E. A., Grachev V. A., Kuznetsov B. B., Polyakov D. Y. and Nikolaev Y. A.. (2013) A novel bacterium carrying out anaerobic ammonium oxidation in a reactor for biological treatment of the filtrate of wastewater fermented sludge. Microbiology. 82: 628–636.

Klausmeier, C., Litchman, E., Daufresne, T. et al. (2004) Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature 429, 171–174

Koch H., Lücker S., Albertsen M., Kitzinger K., Herbold C., Spiek E., Nielsen P.H., Wagner M., Daims H., (2015) Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira. PNAS. 112(36):11371-6.

Könneke, M., Bernhard, A. E., de la Torre, J. R., Walker, C. B., Waterbury, J. B., and Stahl, D. A. (2005). Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546.

Krom MD, Brenner S, Kress N, Gordon LI, Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. (1991) Limnol Oceanogr 36, 424–432

Kumari A., Chaudhary D.R., (2020). Engineered microbes and evolving plastic bioremediation technology, Pages 417-443. First Edition. Bioremediation of Pollutants.

Kuypers M. M., Sliekers A. O., Lavik G., Schmid M., Jørgensen B. B., Kuenen J. G., Sinninghe Damsté J. S., Strous M. and Jetten M. S.. (2003) Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature; 422: 608. pmid:12686999

Lam, P., and Kuypers, M. M. M. (2011). Microbial nitrogen cycling processes in oxygen minimum zones. Ann. Rev. Mar. Sci. 3, 317–345.

Lenhart, H.J. and Pohlmann, T. (1997), 'The ICES-boxes approach in relation to results of a North sea circulation model', Tellus A: Dynamic Meteorology and Oceanography, 49:1, 139-160

Lenhart, H.J., Mills, D.K., Baretta-Bekker, H., van Leeuwen S.M., van derMolen J., Baretta, J.W., Blaas, M., Desmit, X., Kühn, W., Lacroix, G., Los, H.J., Ménesguen, A., Neves, R., Proctor R., Ruardij, P., Skogen, M.D., Vanhoutte-Brunier, A., Villars M.T.,, Wakelin S.L. Predicting the consequences of nutrient reduction on the eutrophication status of the North Sea. J. Mar. Syst., 81 (2010), pp. 148-170

Lipsewers YA, Bale NJ, Hopmans EC, Schouten S, Sinninghe Damsté JS and Villanueva L (2014) Seasonality and depth distribution of the abundance and activity of ammonia oxidizing microorganisms in marine coastal sediments (North Sea). Front. Microbiol. 5:472.

Löscher, C. R., Kock, A., Könneke, M., Laroche, J., Bange, H. W., and Schmitz, R. A. (2012). Production of oceanic nitrous oxide by ammonia-oxidizing archaea. Biogeosciences 9, 2419–2429.

Mahmud, K., Makaju, S., Ibrahim, R., & Missaoui, A. (2020). Current Progress in Nitrogen Fixing Plants and Microbiome Research. Plants (Basel, Switzerland), 9(1), 97.

Man-Aharonovich D, Kress N, Bar Zeev E, Berman-Frank I, Beja O . (2007). Molecular ecology of nifH genes and transcripts in the eastern Mediterranean Sea. Environ Microbiol 9: 2354–2363.

Marchant, H., Ahmerkamp, S., Lavik, G. et al. (2017) Denitrifying community in coastal sediments performs aerobic and anaerobic respiration simultaneously. ISME J 11, 1799–1812

Martinez A., Tyson G. W., Delong E. F., (2010) Widespread known and novel phosphonate utilization pathways in marine bacteria revealed by functional screening and metagenomic analyses. Environ. Microbiol. 12, 222–238

Mayer, L. M. (1994) Surface area control of organic carbon accumulation in continental shelf sediments. Geochimica et Cosmochimica Acta 58, 1271–1284

Mehta MP, Butterfield DA, Baross JA . (2003). Phylogenetic diversity of nitrogenase (nifH) genes in deep-sea and hydrothermal vent environments of the Juan de Fuca ridge. Appl Environ Microbiol 69: 960–970.

Metcalf W. W., Griffin B. M., Cicchillo R. M., Gao J., Janga S. C., Cooke H. A., Circello B. T., Evans B. S., Martens-Habbena W., Stahl D. A., van der Donk W. A., (2012) Synthesis of methylphosphonic acid by marine microbes: A source for methane in the aerobic ocean. Science 337, 1104–1107

Middelburg J.J. (2019) Carbon Processing at the Seafloor. In: Marine Carbon Biogeochemistry. SpringerBriefs in Earth System Sciences. Springer, Cham.

Miksch, S., Meiners, M., Meyerdierks, A. Probandt D., Wegener G., Titschack J., Jensen M.A., Ellrott A., Amann R., Knittel K. (2021) Bacterial communities in temperate and polar coastal sands are seasonally stable. ISME COMMUN. 1, 29

Moncada C., Hassenrück C., Gärdes A., Conaco C. (2019) Microbial community composition of sediments influenced by intensive mariculture activity. FEMS Microbiol. Ecol. Volume 95, Issue 2

Moschonas, G., Gowen, R.J., Paterson, R.F. Mitchell E., Stewart B.M., McNeill S., Glibert P.M., Davidson K. (2017) Nitrogen dynamics and phytoplankton community structure: the role of organic nutrients. Biogeochemistry 134, 125–145

Muyzer G., Kuenen J.G., Robertson L.A. (2013) Colorless Sulfur Bacteria. In: Rosenberg E., DeLong E.F., Lory S., Stackebrandt E., Thompson F. The Prokaryotes. Springer: Berlin.

Nedwell D, Aziz S (1980) Heterotrophic nitrogen fixation in an intertidal salt marsh sediment. Estuart cstl mar Sci 10: 699–702

Neubacher E.C., Parker R.E., Trimmer M. (2011). Short-term hypoxia alters the balance of the nitrogen cycle in coastal sediments. Limnol. Oceanogr. 56:651-665

Neubacher, Elke C., Ruth E. Parker, and Mark Trimmer. (2013) "The potential effect of sustained hypoxia on nitrogen cycling in sediment from the southern North Sea: a mesocosm experiment." Biogeochemistry 113.1: 69-84.

Neumann, A., van Beusekom, J.E.E., Holtappels, M., Emeis, K.C., (2017). Nitrate consumption in sediments of the German bight (North Sea). J. Sea Res. 127, 26–35. https://doi.org/ 10.1016/j.seares.2017.06.012.

Ogawa, H., Amagai, Y., Koike, I., Kaiser, K., and Benner, R. (2001). Production of refractory dissolved organic matter by bacteria. Science 292, 917–920.

Otto, L., Zimmerman, J., Furnes, G., Mork, M., Saetre, R. and Becker, G. (1990), 'Review of the physical oceanography of the north sea', Netherlands Journal of Sea Research 26(2), 161–238.

Owens, N. & Watts, L. (2020) in Stable Isotopes: The Integration of Biological, Ecological and Geochemical Processes 257–283, Garland Science: London. Pages 203-211.

Pajares S and Ramos R (2019) Processes and Microorganisms Involved in the Marine Nitrogen Cycle: Knowledge and Gaps. Front. Mar. Sci. 6:739

Parkes R.J. Cragg B.A:, Bale S.J., Getlifff J.M., Goodman K., Rochelle P.A., Fry J.C., Weightman A.J., Harvey S.M. (1994) Deep bacterial biosphere in Pacific Ocean sediments. Nature 371, 410–413

Pätsch, J., and G. Radach. (1997). Long-term simulation of the eutrophication of the North Sea : Temporal development of nutrients, chlorophyll and primary production in comparison to observations. J. Sea. Volume 38, Issues 3–4, , Pages 275-310
Paytan A., McLaughlin K., (2007) The oceanic phosphorus cycle. Chem Rev 107, 563–576

Philippart, C. J. M., G. C. Cadée, W. van Raaphorst, and R. Riegman. 2000. Long-term phytoplankton-nutrient interactions in a shallow coastal sea : Algal community structure, nutrient budgets, and denitrification potential. Limnol. Oceanogr. 45: 131–144.

Probandt, D., Eickhorst, T., Ellrott, A. et al. (2018) Microbial life on a sand grain: from bulk sediment to single grains. ISME J 12, 623–633

Probandt, D., Knittel, K., Tegetmeyer, H.E., Ahmerkamp, S., Holtappels, M. Amann N. (2017) Permeability shapes bacterial communities in sublittoral surface sediments. Environ. Microbiol., 19, pp. 1584-1599,

Quero GM, Ape F, Manini E, Mirto S and Luna GM (2020) Temporal Changes in Microbial Communities Beneath Fish Farm Sediments Are Related to Organic Enrichment and Fish Biomass Over a Production Cycle. Front. Mar. Sci. 7:524.

Rabus R., Hansen T.A., Widdel F. (2013) Dissimilatory Sulfate- and Sulfur-Reducing Prokaryotes. In: Rosenberg E., DeLong E.F., Lory S., Stackebrandt E., Thompson F. The Prokaryotes. Springer: Berlin

Rhoads D. C. (1963). Rates of sediment reworking by Yoldia limatula in Buzzards bay, Massachusetts and long island Sound

Rich JJ, Dale OR, Song B, Ward BB. (2008). Anaerobic ammonium oxidation (Anammox) in Chesapeake Bay sediments. Microb. Ecol. 55: 311– 20

Risgaard-Petersen, N. Langezaal A.M., Ingvardsen S., Schmid M.C., Jetten M.S.M. Op den Camp J.M., Derksen J.W.M, Piña-Ochoa E., Eriksson S.P., Nielsen L.P., Revsbech N.P., Cedhagen T., van der Zwaan G.J. (2006) Evidence for complete denitrification in a benthic foraminifer. Nature. 443(7107):93-6

Robertson L.A., Kuenen J.G. (2006) The Colorless Sulfur Bacteria. In: Dworkin M., Falkow S., Rosenberg E., Schleifer KH., Stackebrandt E. The Prokaryotes. Springer: New York.

Robinson, C., Wallace, D., Hyun, J. H., Polimene, L., Benner, R., Zhang, Y., et al. (2018). An implementation strategy to quantify the marine microbial carbon pump and its sensitivity to global change. Natl. Sci. Rev. 5, 474–480.

Rosales Villa, A.R. Jickells, T.D. Sivyer, D.B. Parker, E.R. Thamdrup, B. (2019) Benthic nitrogen cycling in the North Sea, Continental Shelf Research, Volume 185, 2019, Pages 31-36, ISSN 0278-4343, Academic Press: London.

Rubio-Portillo E., Villamor A., Fernandez-Gonzalez V., Antón J., Sanchez-Jerez P. (2019) Exploring changes in bacterial communities to assess the influence of fish farming on marine sediments. Aquaculture; 506:459–464.

Rusch A, Forster S, Huettel M. (2001) Bacteria, diatoms and detritus in an intertidal sandflat subject to advective transport across the water-sediment interface. Biogeochemistry. 55:1–27.

Ruttenberg K.C. (2001). Phosphorus cycle in Encyclopedia of Ocean Sciences. (1rst edition). Volume 4, pp 2149-2162. Elsevier.

Ruttenberg, K.C. (2019). Marine Biogeochemistry, in Encyclopedia of Ocean Sciences (3rd edition). Volume 1, 2019, Pages 447-460. Academic Press.

Ruttenberg, K.C., Berner, R.A., (1993). Authigenic apatite formation and burial in sediments from non-upwelling, continental margin environments. Geochim. Cosmochim. Acta 57, 991-1007.

Sakurai T, Kataoka K . (2007). Structure and function of type I copper in multicopper oxidases. Cell Mol Life Sci 64: 2642–2656.

Sansone, F.J., Pawlak, G., Stanton, T.P., McManus, M.A., Glazer, B.T., Decarlo, E.H., Bandet, M., Sevadjian, J., Stierhoff K., Colgrove, C., Hebert, A.B., Chen IC. (2008). Kilo Nalu: Physical/biogeochemical dynamics above and within permeable sediments. Oceanography, 21, pp. 173-178

Santoro, A. E., Buchwald, C., McIlvin, M. R., and Casciotti, K. L. (2011). Isotopic signature of N_2O produced by marine ammonia-oxidizing archaea. Science 333, 1282–1285.

Santos, I. R., D. Eyre, and M. Huettel. (2012a). The driving forces of porewater and groundwater flow in permeable coastal sediments: A review. Estuar. Coast. Shelf Sci. 98: 1–15.

Sarmiento, J. L. & Gruber, N.(2006) Ocean Biogeochemical Dynamics Ch. 8. Edition x. Princeton Univ. Press: Princeton.

Schippers, A., Jørgensen B.B. (2002) Biogeochemistry of pyrite and iron sulfide oxidation in marine sediments. Geochim. Cosmochim. Acta, 66, pp. 85-92

Schmid M., Twachtmann U., Klein M., Strous M., Juretschko S., Jetten M., Metzger J. W., Schleifer K. H. and Wagner M.. (2000) Molecular evidence for genus level diversity of bacteria capable of catalyzing anaerobic ammonium oxidation. Systematic and Applied Microbiology. 23: 93–106. pmid:10879983

Schröder J.J., Cordell A. Rosemarin A., (2010). Sustainable use of phosphorus. EU Tender ENV.B.1/ETU/2009/0025. Plant Research International, Wageningen University, the Netherlands.

Schuler DJ. (2008) Acute toxicity of ammonia and nitrite to white shrimp (L. vannamei) at low salinities [thesis]. Blacksburg, Virginia, USA: Virginia Polytechnic Institute and State University; 76 p

Severin, I., Confurius-Guns, V., and Stal, L. J. (2012). Effect of salinity on nitrogenase activity and composition of the active diazotrophic community in intertidal microbial mats. Arch. Microbiol. 194, 483–491.

Shi R., Xu S., Qi Z., Zhu Q., Huang H., Weber F. (2019) Influence of suspended mariculture on vertical distribution profiles of bacteria in sediment from Daya Bay, Southern China. Mar. Pollut. Bull. 146:816–826.

Sievert, S.M., R.P. Kiene, and H.N. Schulz-Vogt. 2007. The sulfur cycle. Oceanography 20(2):117–123

Simon J., Kroneck P.M.H., (2013) Chapter Two - Microbial Sulfite Respiration, in Advances in Microbial Physiology, Volume 62, Pages 45-117, ISSN 0065-2911, Simpson, J. H., and Sharples, J. (2012). Introduction to the Physical and Biological Oceanography of Shelf Seas. Cambridge: Cambridge University Press.

Sorokin, D. Y., Lücker, S., Vejmelkova, D., Kostrikina, N. A., Kleerebezem, R., Rijpstra, W. I. C., Sinninghe J.S., Damsté S., Le Paslier D., Muyzer G., Wagner M., van Loosdrecht M.C.M., Daims H. (2012). Nitrification expanded: discovery, physiology and genomics of a nitrite-oxidizing bacterium from the phylum Chloroflexi. ISME J. 6, 2245–2256.

Soudry D (2000) Microbial phosphate sediment. In Microbial Sediments (eds. Riding RE, Awramik SM) Springer, Berlin, Heidelberg, pp. 127-136.

Steinberg D.K. and Landry M.R. (2017). Zooplankton and the Ocean Carbon Cycle. Annu. Rev. Mar. Sci. 9:413–44

Sterner, R. W. and Elser, J. J. (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. – Princeton Univ. Press: USA.

Stoeck, T., Frühe, L., Forster, D., Cordier, T., Martins, C. I., and Pawlowski, J. (2018). Environmental DNA metabarcoding of benthic bacterial communities

indicates the benthic footprint of salmon aquaculture. Mar. Pollut. Bull. 127, 139–149.

Swaney DP., Hong B., Ti C., Howarth R.W., Humborg C, (2012) Net anthropogenic nitrogen inputs to watersheds and riverine N export to coastal waters: a brief overview. Current Opinion in Environmental Sustainability, Volume 4, Issue 2, T.M. Addiscott, P. and Leeds-Harrison, (2005) DIFFUSION, from Encyclopedia of Soils in the Environment, Pages 389-394, ISBN 9780123485304, Elsevier

Tait K, Airs RL, Widdicombe CE, Tarran GA, Jones MR, Widdicombe S. (2015) Dynamic responses of the benthic bacterial community at the Western English Channel observatory site L4 are driven by deposition of fresh phytodetritus. Prog Oceanogr. 137:546–58.

Tanhua T., Bates N.R., Körtzinger A., (2013). Chapter 30 - The Marine Carbon Cycle and Ocean Carbon Inventories, in: Ocean Circulation and Climate – A 21st Centry Perspective. Volume 103, Pages 787-815, ISSN 0074-6142, ISBN 9780123918512,

Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrecht A, Bennke CM, Kassabgy M, Huang S, Mann AJ, Waldmann J, Weber M, Klindworth A, Otto A, Lange J, Bernhardt J, Reinsch C, Hecker M, Peplies J, Bockelmann FD, Callies U, Gerdts G, Wichels A, Wiltshire KH, Glöckner FO, Schweder T, Amann R. (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science. May 4;336(6081):608-11.

Teeling H, Fuchs BM, Bennke CM, Kruger K, Chafee M, Kappelmann L, Reintjes G., , Waldmann J., Quast C., Glöckner F.O., Lucas J., Wichels A., Gerdts G., Wiltshire K.H., Amann R.I. (2016) Recurring patterns in bacterioplankton dynamics during coastal spring algae blooms. eLife.5:e11888.

Thamdrup, B., Finster, K., Hansen, J. W., and Bak, F. (1993). Bacterial disproportionation of elemental sulfur coupled to chemical reduction of iron or manganese. Appl. Environ. Microbiol. 59, 101–108.

The European MSP Platform, (2022). The North Sea. Available at: https://maritime-spatial-planning.ec.europa.eu/sea-basins/north-sea-0 [Accessed on: 15/03/2022]

Thomas, H., Y. Bozec, K. Elkalay, and H. J. W. De Baar. (2004). Enhanced open ocean storage of CO2 from shelf sea pumping. Science 304: 1005–1008.

Thompson FL, Abreu PC, Wasielesky W. (2002) Importance of biofilm for water quality and nourishment in intensive shrimp culture. Journal of Aquaculture.;203:203-278

Tiedje, J. M. (1988). "Ecology of denitrification and dissimilatory nitrate reduction to ammonium," in Environmental Microbiology of Anaerobes, ed. A. J. B. Zehnder, (New York, NY: John Wiley & Sons), 179–244.

Toggweiler, J. R. (1999) Oceanography: An ultimate limiting nutrient. Nature, 400 (6744). 511-512

Toussaint E., De Borger E., Braeckman U., De Bracker A., Soetaert K., Vanaverheke. (2021). Faunal and environmental drivers of carbon and nitrogen cycling along a permeability gradient in shallow North Sea sediments, Science of The Total Environment, Volume 767, 144994, ISSN 0048-9697,

Tyrrell, T. (1999) The relative influences of nitrogen and phosphorus on oceanic primary production. Nature 400, 525–531

van Beusekom JEE, Loebl M, Martens P (2009) Distant riverine nutrient supply and local temperature drive the long-term phytoplankton development in a temperate coastal basin. J Sea Res 61:26–33

Van Mooy BAS., Krupke A., Dyhrman S.T., Fredricks H.F., Frischkorn K.R., Ossolinski J.E., Repeta D.J., Rouco M., Seewald J.D., Seewald J.D., Sylva S.P. (2015) Science 348, 783

Vandieken, V., Sabelhaus, L. and Engelhardt, T. (2017) Virus dynamics are influenced by season, tides and advective transport in intertidal, permeable sediments. Frontiers in microbiology, 8, p. 2526

Villarreal-Chiu J. F., Quinn J. P., McGrath J. W., (2012) The genes and enzymes of phosphonate metabolism by bacteria, and their distribution in the marine environment. Front. Microbiol. 3, 19

Voss M., Bange H.W., Dippner J.W., Middelburg J.J., Montoya J.P., and Ward B. (2013). The marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of climate change. Phil Trans R Soc B 368: 20130121.

Wallmann, K., 2010. Phosphorus imbalance in the global ocean? Global Biogeochem. Cycles. Volume 24, Issue 4 GB4030

Walsh, J. J. (1991), 'Importance of continental margins in the marine biogeochemical cycling of carbon and nitrogen', Nature 350 (6313), 53–55.

Wang L., (2018) Microbial control of the carbon cycle in the ocean, National Science Review, Volume 5, Issue 2, Pages 287–291

Waring R. and Running, S. (2007). Forest Ecosystems, Analysis at Multiple Scales. Third Edition. Academic Press: USA

Watson, S. W., and Waterbury, J. B. (1971). Characteristics of two marine nitrite oxidizing bacteria, Nitrospina gracilis nov. gen.nov. sp. and Nitrococcus mobilis nov. gen.nov.sp. Arch. Microbiol. 77, 203–230.

Webster, I.T., Norquay S.J., Ross, F.C., Wooding R.A., (1996) Solute exchange by convection within estuarine sediments. Estuarine, Coastal and Shelf Science, 42, pp. 171-183

Wei, W., Isobe, K., Nishizawa, T. Zhu L., Shiratori Y., Ohte N., Koba K., Otsuka S., Senoo K. (2015) Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. ISME J 9, 1954–1965.

Welsh, A., Chee-Sanford, J. C., Connor, L. M., Löffler, F. E., and Sanford, R. A. (2014). Refined NrfA phylogeny improves PCR-based nrfA gene detection. Appl. Environ. Microbiol. 80, 2110–2119.

Westernhagen, H., and V. Dethlefsen. 1983. North Sea oxygen deficiency 1982 and its effects on the bottom fauna. Ambio 12: 264–266.

Wikipedia, 2010. Bestand : North Sea map. Available at: https://nl.m.wikipedia.org/wiki/Bestand:North_Sea_map-en.png [Accessed the 12th March 2022]

Wild C., Huettel M., Tollrian R. (2004). Rapid recycling of coral mass-spawning products in permeable reef sediments. Mar. Ecol. Prog. Ser., 271 (2004), pp. 159-166

Wörman A, Packman AI, Johansson H, Jonsson K (2002) Effect of flow induced exchange in hyporheic zones on longitudinal transport of solutes in streams and rivers. Water Resources 38:2-1-2–15 .

Wu J, Sunda W, Boyle EA, Karl DM, (2000) Phosphate depletion in the western North Atlantic Ocean. Science 289, 759–762

Yazdani Foshtomi M, Braeckman U, Derycke S, Sapp M, Van Gansbeke D, Sabbe K, et al. (2015) The Link between Microbial Diversity and Nitrogen Cycling in Marine Sediments Is Modulated by Macrofaunal Bioturbation. PLoS ONE 10(6): e0130116.

Young C. L., Ingall E. D., (2010) Marine Dissolved Organic Phosphorus Composition: Insights from Samples Recovered Using Combined Electrodialysis/Reverse Osmosis. Aquat. Geochem. 16, 563-574

Zehr J.P and Kudela R.M. (2011). Nitrogen Cycle of the Open Ocean: From Genes to Ecosystems. Annual Review of Marine Science. Vol. 3:197-225

Zehr, J. P., Mellon, M., Braun, S., Litaker, W., Steppe, T., and Paerl, H. W. (1995). Diversity of heterotrophic nitrogen fixation genes in a marine cyanobacterial mat. Appl. Environ. Microbiol. 61, 2527–2532.

Zhang C., Dang H., Azam F:, Benner R., Legendre L., Passow U, Polimene L., Robinson C., Suttle C.A., Jiao N., Evolving paradigms in biological carbon cycling in the ocean, National Science Review, Volume 5, Issue 4, July 2018, Pages 481–499

Zhang K., Zheng X., He Z., Yang T., Shu L., Xiao F., Wu Y., Wang B., Li Z., Chen P., et al. (2020) Fish growth enhances microbial sulfur cycling in aquaculture pond sediments. Microb. Biotechnol. 13:1597–1610.

Zhe-Xue Q., Sung-Keun R., Jian-E Z., Yang Y., Jin-Woo B., Ja Ryeong P., Sung-Taik L. and Yong-Ha P.. (2010) Diversity of ammonium-oxidizing bacteria in a

granular sludge anaerobic ammonium-oxidizing (anammox) reactor. Environmental Microbiology. 10: 3130–3139.