INVESTIGATION OF BACTERIAL COMMUNITY COMPOSITION IN OXIC AND ANOXIC SEDIMENT CORES ALONG REDOX GRADIENTS OF THE BLACK SEA

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INVESTIGATION OF BACTERIAL COMMUNITY COMPOSITION IN OXIC AND ANOXIC SEDIMENT CORES ALONG REDOX GRADIENTS OF THE BLACK SEA

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

INVESTIGATION OF BACTERIAL COMMUNITY COMPOSITION IN OXIC AND ANOXIC SEDIMENT CORES ALONG REDOX GRADIENTS OF THE BLACK SEA

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Biogeochemical cycles are essential for the maintenance of life on Earth. However, little is known about the underlying processes, which hinders the estimation of future states of cycles in a changing environment. Microorganisms are essential in biogeochemical cycles by controlling reactions, rates, and products. This study compared bacterial community compositions by amplicon sequencing of V3-V4 regions of the 16S rRNA genes in two sediment cores (oxic vs. anoxic) from the southwestern Black Sea. To understand the interactions between bacterial diversity and environmental parameters, nutrient, organic carbon, and major seawater ions analyses were performed and used in Non-Metric Multidimensional Scaling. Dissolved oxygen was found as the main driver for community composition. However, organic carbon became one of the main drivers of differentiating bacterial diversity after oxygen consumption. While the community composition in deeper sediments were correlated with the concentrations of H_2S, NH_4^+, PO_4^{3+}, and major ions; the microbial community in the middle and upper sediments were related to more energetic molecules such as NO_3^-, NO_2^-, and dFe, SO_4^{2-}, and TOC. Results

show that decreases in oxygen concentration in the water column would change the bacterial community composition through anaerobic metabolisms, producing greenhouse gases.

This study suggests that climate change and anthropogenic effects related to the oxygen and carbon cycle will directly affect bacterial communities. The results suggest that sediment bacterial communities should be considered in the climate change models. The Black Sea is a suitable habitat for further analysis to study anaerobic microbial metabolisms. This is one of the first studies investigating the sediment bacteria in the Black Sea to predict the changes in bacterial communities under the redox shift from oxic to the anoxic water column. Also, whole bacterial diversity was analyzed by amplicon sequencing in the Black Sea sediments for the first time without targeting specific groups/metabolisms. This study is also a pioneer in its contribution to the overall biodiversity record in the Turkish seas.

Keywords: Black Sea Bacteria, 16S rRNA, Community Composition, Microbial Ecology, Sediment Biogeochemistry

ÖZ

OKSİK VE ANOKSİK KARADENİZ SEDİMANLARINDAKİ BAKTERİ KOMÜNİTE KOMPOZİSYONLARININ REDOKS GRADYANI BOYUNCA İNCELENMESİ

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Biyojeokimyasal döngüler, Dünya'daki yaşamın sürdürülebilmesi için gereklidir; ancak bu döngülerin gerçekleştiği reaksiyonlar ve bu reaksiyonların değişen çevre koşullarına nasıl tepki vereceğine dair yeterince bilgimiz bulunmamaktadır. Mikroorganizmalar biyojeokimyasal döngülerde reaksiyonları, hızlarını ve ürünlerini kontrol ederek önemli rol oynarlar. Bu çalışmada, Güneybatı Karadenizdeki iki farklı sediman örneğinin (oksik ve anoksik) bakteri kompozisyonları 16S rRNA genlerinin V3-V4 bölgelerinin amplikon dizilemesi ile araştırıldı. Bakteri kompozisyonu ve çevresel parametreler arasındaki etkileşimleri anlamak için besin tuzu, CHN ve iyon kromatografisi analizleri yapıldı ve bunlar Metrik Olmayan Çok Boyutlu Ölçeklemede (NMDS) kullanıldı. Oksijen varlığının farklı bakteri komünitelerine yol açmasının yanında organic karbonun da komünite farklılaşmalarında önemli rol oynadığı ortaya konuldu. Derin sediman örnekleri H_2S, NH_4^+, PO_4^{3+} ve majör iyonlarla ilişkilendirilirken; orta ve üst sediman katmanları NO_3^-, NO_2^-, dFe, SO_4^{2-} ve TOC gibi daha enerjik moleküllerle ilişkilendirilmiştir. Analiz sonuçları su kolonunda bulunan oksijen miktarındaki azalmanın bakteri

komünite yapısını anaerobik metabolizmalar yönünce değiştirebileceğini ortaya koymuştur.

Bu çalışma, iklim değişikliğinin denizlerdeki oksijen ve karbon döngülerine olan etkilerinin deniz tabanında bulunan bakteri komünite yapısını doğrudan değiştirebileceğini ve iklim değişim modellerine sediman mikrobiyolojisinin eklenmesi gerektiğini önermektedir. Ayrıca Karadeniz'in şu anki ve gelecekte oksijensizleşme potansiyeli olan denizlerin mikroorganizmalarını çalışmak için uygun bir habitat olduğu görülmüştür. Bu çalışma, su kolonunda değişen redoks koşullarının sediman bakteri komünitesinde oluşturacağı değişimleri araştıran Karadeniz sedimanlarında yapılan ilk çalışmadır. Ayrıca Karadeniz sedimanında bakterilerde spesifik bir grubu veya metabolizmayı hedeflemeden amplikon dizileme ile yapılan öncü çalışmalardan birisidir. Bu çalışmanın Türkiye deniz mikrobiyolojisi çeşitliliğinin kayıt altına alınmasına önemli katkılar sağlamaktadır.

Anahtar Kelimeler: Karadeniz Bakteri Kompozisyonu, 16S rRNA, Sediman Mikrobiyolojisi, Mikrobiyal Ekoloji, Sediman Biyojeokimyası

This thesis is dedicated to all those who have supported me and believed in me since I was a child.

“There is a difference between knowing the path and walking the path.”

Morpheus, “The Matrix”

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LIST OF ABBREVIATIONS

Anammox: Anaerobic Ammonium Oxidation

AOM: Anaerobic Oxidation of Methane

CIW: Cold Intermediate Water

Cmbsf : Centimeters Below Surface

dFE: Dissolved Iron

MA: Methanogenic Archaea

NMDS : Non-metric Multidimensional Scaling

OM : Organic Matter

PCR: Polymerase Chain Reaction

Ppm : Parts Per Million

PSU: Practical Salinity Unit

rRNA: Ribosomal Ribonucleic Acid

SMTZ: Sulfate Methane Transition Zone

SRB: Sulfate Reducing Bacteria

SSU: Small Subunit

TN : Total Nitrogen

TOC : Total Organic Matter

TON : Total Organic Nitrogen

CHAPTER 1

INTRODUCTION

The Black Sea is the largest euxinic (both anoxic and sulfidic) sea on Earth and is an essential natural laboratory for studying anoxic events. The Black Sea is an isolated sea, which makes the Black Sea an excellent model to research sea dynamics in semi-enclosed basins. A large amount of anoxic water mass allows for observing redox layers on a large scale.

In the Black Sea, there is a mixed layer with a uniform distribution of hydrological properties such as temperature, density. Heat fluxes, sea ice formation and winds cause the formation of a mixed layer that at a mean depth of 50 meters, all those this changes seasonally and regionally. The upper ~100m of the Black Sea is well ventilated but below it a large anoxic and relatively stagnant water layer is present from ca. 100-150 meters to 2000 meters (150- 200 meters in coastal areas (Stanev et al., 2013)) with an average turnover rate of 387 years and maximum turnover rate of ~2000 years for the deepest waters where water input does not interfere. (Murray et al., 1991). Between oxygenated and anoxic layers (at the ~15.75 kg m−3 potential density anomaly interface), there is a suboxic zone where both O_2 and H_2S decreases below 3 μM (as defined by the limits of the titration methods for both species (Murray et al., 2001). These processes overall prevent the mixing of oxic, suboxic and anoxic zones of the Black Sea. In the suboxic layer, limited rates of vertical oxygen supply are rapidly consumed by organic matter respiration (Jessen et al., 2017).

The general processes in the Black Sea are determined by various biological, physical, and chemical activities such as circulations and the mixing of nutrients (Murray et al., 1991). The water budget of the Black Sea is mainly driven by the

riverine inputs, which affect the chemical composition of the sea (salinity, nutrients, alkalinity, etc.), general circulation and eddies are responsible for the transportation of the materials and nutrients in the sea, hence primary production processes (Özsoy & Ünlüata, 1997). In relation to primary production, marine bacteria have a significant role in organic matter production and degradation dynamics (Lochte & Turley, 1988).

As a semi-enclosed basin, the Black Sea's upper layers rapidly respond to global and regional environmental changes. For example, the pycnocline structure of the Black Sea changed due to extreme cooling events in the year 1992. This single-year climatic event affected the Cold Intermediate Water (CIW) formation with anomalous structures in 1993 and 1995 (Ivanov et al., 1997). The water structure changes affect the biological features of the environment, such as increasing production because of the changing water fluxes. Changes in the flux of the nutrients to the Black Sea change the primary production level and then organic matter flux to the deeper sediments, affecting the distribution and the rates of the biogeochemical reactions (Konovalov et al., 2005).

In such a globally relevant natural laboratory, it is crucial to expand the existing oceanographic and biogeochemical knowledge on the distribution of microbial communities and their interactions with the environmental parameters (Konovalov et al., 2007; Stanev et al., 2018). The aims of this study are (i) to contribute to the knowledge about microbial diversity of the Turkish Seas, (ii) to reveal the interactions between bacteria and environmental parameters in the Black Sea sediments, (iii) to research two spatially close sediment samples' microbial diversity and their roles in the biogeochemical cycles.

1.1. Biogeochemical Insights into the Black Sea

Biogeochemistry is a multidisciplinary scientific discipline that combines biology, geology, and chemistry. The approaches in biogeochemistry try to reveal the complex processes that occur in marine sediments, oceans, agricultural areas, other habitats, and the whole biosphere. Vladimir Vernadsky invented this discipline, and the term "biogeochemistry" was first mentioned in 1926 (Vernadsky, 1998).

Through the novel approaches, biogeochemical studies use genomics, biochemistry, and physiological techniques to understand the fundamental knowledge of microbiologically controlled processes. In oxidation and reduction reactions, these processes occur on various scales, from cubic centimeters to the whole planet. Known life forms use/creates chemical disequilibrium to obtain energy and create molecules, and to generate this equilibrium, organisms use the energy produced by the sun or chemicals. Organisms use proton motive force by free energy obtained by chemically favorable reactions to create energy (Falkowski et al., 2008).

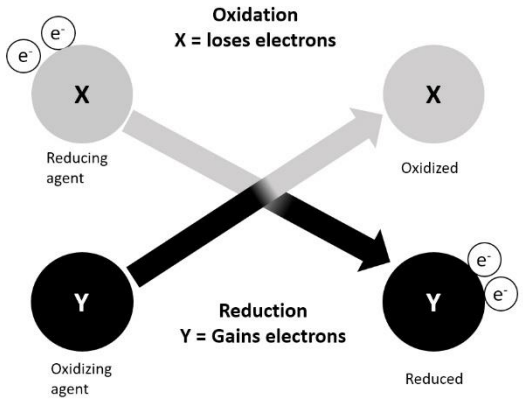

Figure 1. Schematic view of oxidation and reduction process

Organisms produce energetic molecules to continue their biological processes to be alive, and their energy production and consumption of the chemicals are processed by reduction and oxidation reactions. Biogeochemical cycles are essential biological processes to produce and consume energy. Many elements are linked to each other in terms of transformations in nature. Some important biogeochemical reactions are nitrate reduction, sulfate or sulfide reduction, methanogenesis, carbon respiration, and anaerobic ammonium oxidation. Detailed information about important biogeochemical cycles can be found in Table 1.

Table 1. General microbial metabolisms related to biogeochemical cycles (Madsen, 2011).

Cycle	Reaction	Definition
Carbon	Photosynthesis	CO2 fixation into biomass by light energy
	Carbon respiration	Organic C oxidation to CO2
	Methanogenesis	Methane production
	Aerobic methane oxidation	Conversion of methane to CO2
	Anaerobic methane oxidation	
Nitrogen	Nitrogen fixation	N2(g) transforms to ammonia
	Ammonium oxidation	Ammonia transforms nitrate, nitrite
	Anaerobic ammonium oxidation (anammox)	Ammonia and nitrate transforms N2(g)
	Denitrification	Nitrate transforms N2(g)
Sulfur	Sulfur oxidation	Sulfide and sulfur transform sulfate
	Sulfate reduction	Sulfate transforms sulfur and sulfide
Iron	Iron reduction	Fe(III) transforms Fe(II)

Microorganisms are responsible for the occurrence and sustaining of life (including multicellular life) on the Earth by oxygen production, participating in geochemical processes. They consume greenhouse gases such as methane (CH_4) and nitrous oxide (N_2O), whose concentrations and fluxes are not entirely determined yet. Their activities worsen or remediate water quality in two directions (Wakeham, 2020). For example, it is known that microorganisms can adapt to changing conditions, such as changing abundances or gaining resistance genes by horizontal gene transfer (Lupo et al., 2012). Although changing communities were observed in distracting environments (Sharuddin et al., 2018), however, quantitative measurements are not ready to predict future conditions (Liao et al., 2018). Additionally, present but inactive genes are found in the microbial genome. However, these genes can be active in changing conditions, so the ecological roles of these organisms might change (Wakeham, 2020). Consequently, microbial activities are affected by anthropogenic activities that should be researched in future studies.

Microbial metabolisms are affected by their environmental conditions. These conditions influence the evolution of their community composition (Sharuddin et al.,

2018) and the distribution of the chemicals that determine the environment's physical and chemical structure (Smith et al., 2002). Molecular methods targeting genes are crucial to understanding biogeochemical cycles. When the association between microorganisms and their reactions to the environmental properties and changes, their gene expressions can be understood, then the effects of microorganisms on the biogeochemical cycles can be predicted (Zhu et al., 2017).

Relationships between the environment and the microorganisms suggest that any significant changes in marine microorganisms due to climate change have the potential to alter the ecosystem. For example, in the aphotic zone of marine environments, chemolithoautotrophic microorganisms fix CO_2 (Pachiadaki et al., 2017). Methanogenic and methanotrophic organisms are essential methane consumers and producers. However, their contribution to the atmospheric flux of greenhouse gases is unresolved yet (Boetius & Wenzhöfer, 2013). Besides marine bacteria and archaea, marine viruses are vital for the carbon cycle in the marine food web by affecting carbon sequestration and deposition to the deep oceanic habitats (Guidi et al., 2016). The quantity and distribution of seafloor microorganisms change as a response to the downward flux of particulate carbon. These responses affect functional processes (e.g., ammonium oxidation) and related biogeochemical cycles (Danovaro et al., 2016).

Figure 2. The geographic location of the Black Sea (Taken from Wikipedia)

The Black Sea has 4.2 x 10^5 km^2 surface area and 5.3 x 10^5 km^3 total volume; its maximum depth is 2243 meters (Özsoy & Ünlüata, 1997), and the average depth was calculated as 1240 m (Ross et al., 1974). While 25% of the Black Sea basin seafloor is a continental shelf, more than 60% of the Sea is an abyssal plain (Özsoy & Ünlüata, 1997). Danube river contributes to more than half of the total river runoffs.

There is permanent stratification in the Black Sea water column (Figure 3). The surface water is colder but is less saline, hence having low density because of the high amounts of riverine inputs in the Black Sea. The lower layer is warmer and has highly saline Mediterranean water below the surface water. Freshwater input from important rivers such as the Danube, Dniepr, and Dniestry decreases the surface water salinity to 16-17 PSU. On the other hand, the Mediterranean water input increases the Black Sea deep water density with salinity levels of 35.5 PSU. This permanent salinity difference creates a permanent halocline and pycnocline between the depths of 50 – 200 meters (Murray et al, 1991). Between permanent halocline and seasonal thermocline, Cold Intermediate Water (CIW) occurs. The shallow (~50 m) mixed surface layer is affected by the seasonal temperature changes, so CIW depth changes in different seasons. In summer, thermal isolation causes upward

movements of the CIW (~30 m). However, in winter, cooling causes downward movement of the CIW to 70-80 meters or deeper (Özsoy & Ünlüata, 1997). Although traditionally CIW is accepted as the layer below 8 °, recent studies has shown that due to the warming of the Black Sea CIW is defined as the waters with the temperature below 8.35 °C (Capet et al., 2014; Stanev et al., 2013). In addition, the chemical properties of the Black Sea along the isopycnals are equal, allowing the tracing of the water layers and biogeochemical processes across the basin. Oxycline (sharp changes in oxygen concentration), chemocline (the layer in which chemical properties changes), halocline (sharp changes in salinity concentration), and pycnocline (sharp changes in density concentration) match in similar depth intervals because similar mechanisms in the Black Sea affect these features' vertical settlements. The redox reactions occur in similar density values, so density gradients are taken into account instead of depth (Emil V. Stanev et al., 2018). For example, the suboxic zone in the Black Sea water column occurs between 15.2 – 16.2 (Lewis & Landing, 1991; Tugrul et al., 1992)

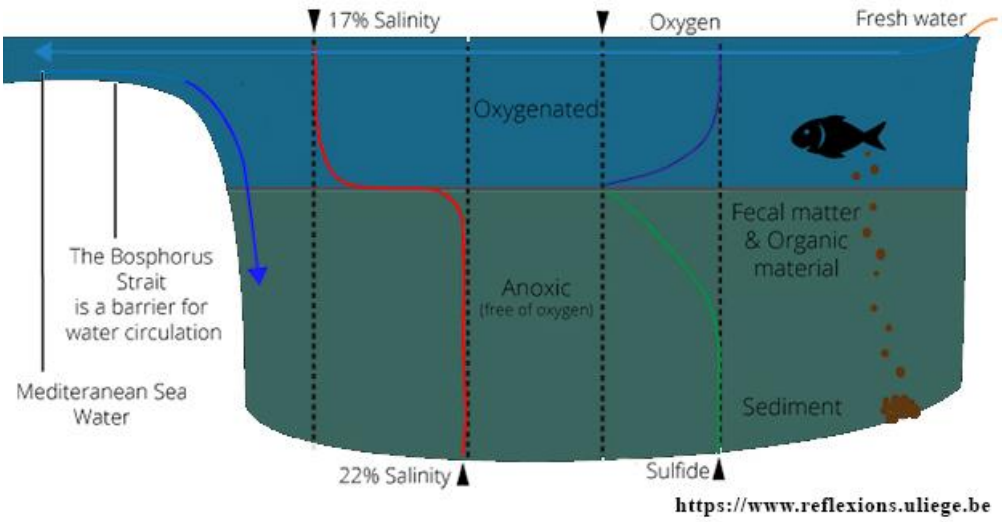

Figure 3. Overview of the Black Sea processes. Blue arrows indicate water flows. Red, purple, and green lines show stratification in the Black Sea water column. (Credit: University of Liège website)

The Bosphorus Strait connects the Black Sea to the Aegean Sea. There is a two-layer counter-flow through the Bosphorus Strait; while the Mediterranean Sea inflows to the Black Sea, the Black Sea water outflows to the Marmara Sea above the denser Mediterranean Sea. The outflow/inflow ratio through the strait was calculated at roughly 2 (Özsoy & Ünlüata, 1997). When the salinity (Figure 4) at the exit of the Bosphorus Strait is around 38 PSU, this value decreases sharply to 22.8 PSU along with the Black Sea shelf break (Özsoy et al., 1993).

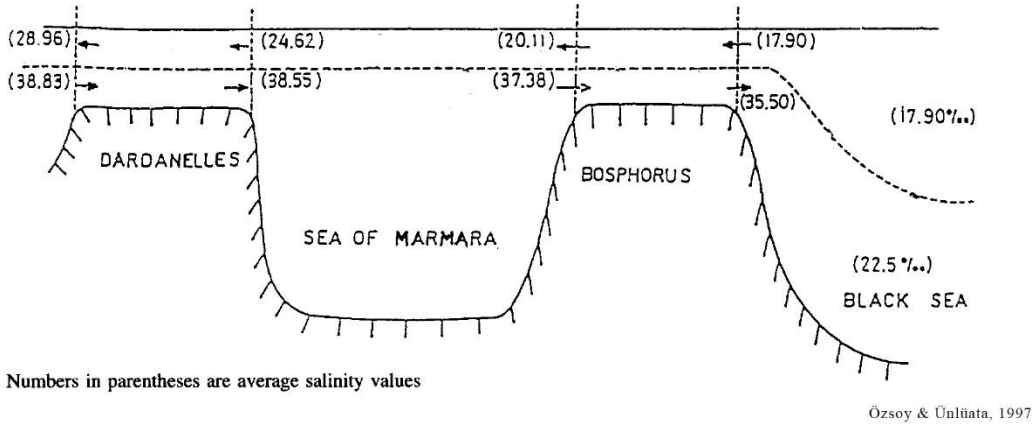

Figure 4. Water fluxes through Bosphorus Strait by Özsoy & Ünlüata, 1997.

Wind patterns determine the general circulation of the Black Sea, and Rim Current is a characteristic oceanographic feature of the Black Sea circulation (Oguz et al., 1993). It is a cyclonic and boundary current whose coastal lines determine its dynamics (indicated with the red line in Figure 5). Performed modeling studies have addressed essential explanations about the driving forces of cyclonic currents. In general, cyclonic currents are mainly driven by cyclonic wind patterns (Trukhchev &Demin, 1992; Klimok & Makeshov, 1993; Sadighrad et al., 2021). Besides wind patterns, seasonal surface fluxes may impact seasonal thermohaline circulations (Emil V. Stanev, 1990). The Rim Current has a vital role in the nutrient distribution, oxygen, fish eggs, and larvae (Fach, 2014). The movement of the current determines the distribution of primary production by lateral transportation of the nutrients, which are necessary for the primary production. Additionally, cyclonic eddies cause upwelling and vertical movement of the nutrients. In general, interior water masses

are controlled by surface currents and boundary mixing processes (Özsoy & Ünlüata, 1997).

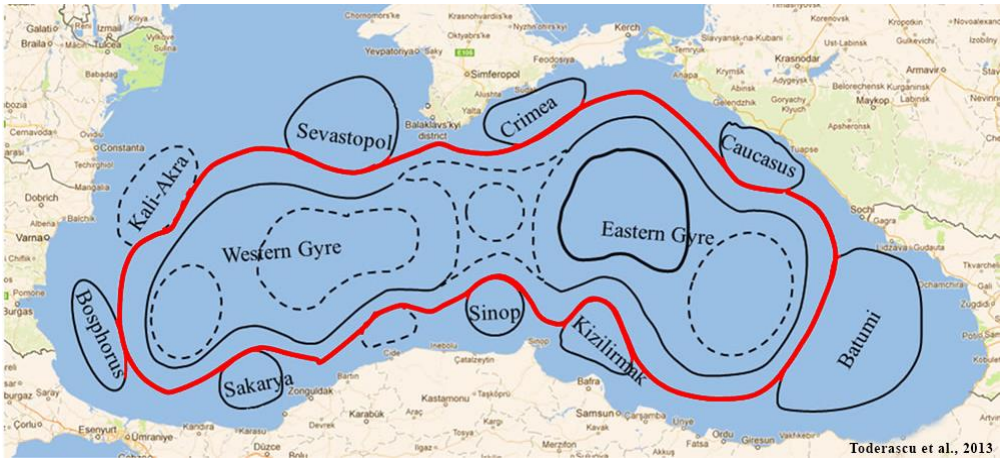

Figure 5. Overview of the Black Sea currents (Toderascu et al., 2013)

Important Biogeochemical Cycles

In oxygenic habitats, organisms use molecular oxygen as an electron acceptor and produce energy by using sinking organic matter. However, in anoxic regions (<1 µM dissolved oxygen), microorganisms use nitrate and sulfate mostly to gain energy. Some important molecules such as H_2S, CH_4, and N_2O are released during these anoxic processes. By expanding hypoxic regions (< 60µM dissolved oxygen), anoxic processes will be more critical for greenhouse gases emissions (Bianchi et al., 2018).

Organisms prefer O_2 as the primary electron acceptor in organic matter respiration. After oxygen depletion, thermodynamically less stable e- acceptors are used gradually (Jørgensen, 2000). The most important e^- acceptors are nitrate, manganese, iron, sulfate, and carbon dioxide (Figure 6). Microbial diversity enables bacteria and archaea to use various e- acceptors in suboxic and anoxic conditions under gradual geochemical gradients (Bertagnolli & Stewart, 2018; Hawley et al., 2014), which is called redox-driven niche partitioning (Wright et al., 2012). Recent developments in molecular biology and bioinformatics enable new insights into the microbial

processes, such as which microorganisms and under which conditions affect their functions.

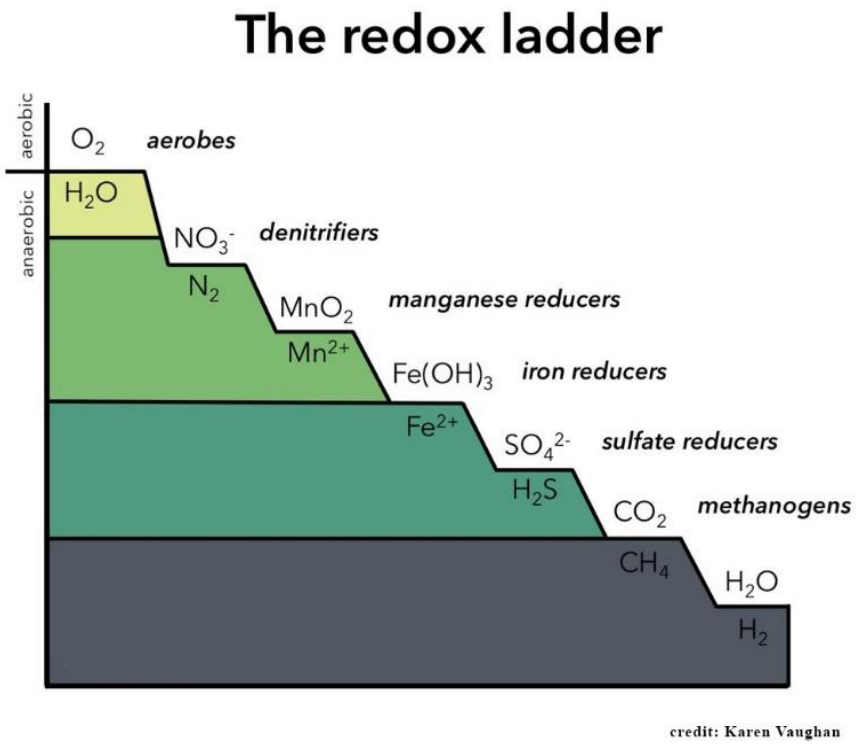

Figure 6. Description of common reduction reactions conducted by microorganisms in marine sediments. The most energetically favorable molecules and reactions are at the top, and the least favorable is at the bottom. Reproduced with permission (Karen Vaughan, personal communication, 2022).

The nitrogen cycle is dependent on oxygen concentrations (Lam & Kuypers, 2010). For example, nitrogen removal of fixed N forms occurs in suboxic zones, and this removal rate is estimated between 25 to 50 % of the total fixed N (Gruber, 2008). Shortly, in the upper limits of the suboxic zone, nitrate is the primary electron acceptor, and the remineralization of organic matter generates ammonium. NH_4^+ is converted to nitrogen dioxide (NO_2) and then nitrate by ammonium oxidizing bacteria. Microorganisms conduct organic matter respiration by nitrate reduction (denitrification) to nitrous oxide (N_2O) and/or $N_{2(g)}$ in suboxic conditions.

Additionally, dissimilatory nitrate reduction to ammonium (DNRA) is conducted by the reduction of nitrate (or nitrite) to ammonium. This produced ammonium can be oxidized by anaerobic ammonium oxidizer (anammox) bacteria. Although oxygen deficient zones (ODZs) are characterized by oxygen concentrations (<20 μM), it was suggested that denitrification and anammox can be an alternate criteria for ODZs (Paulmier & Ruiz-Pino, 2009). Both denitrification and anammox processes cause the removal of fixed nitrogen. In the Black Sea suboxic zone, ammonium oxidation by archaea can be accepted as a source of NO_2^- for the anammox process (Coolen et al., 2007). The first evidence of anaerobically ammonium oxidation with nitrite to N_2 (anammox) was shown in the Black Sea suboxic zone. According to Kalvelage et al. (2011), bacteria conducting anammox reactions have differences according to habitat. Anammox-conducting bacteria that live in deeper, open ocean suboxic zones may be more oxygen-sensitive than those in coastal waters. This is an important suggestion for microbial insights into biogeochemical cycles because the same processes can be affected differently in various habitats.

Different processes control variations in the suboxic layer of the Black Sea. On the one hand, oxygen is consumed by the oxidation of particulate organic matter (POM) in the upper limit of the suboxic zone; on the other hand, the upward flux of sulfide compounds from the anoxic zone and lateral flux of oxygen-containing Bosporus water determines processes in the lower limit of the suboxic zone. Therefore, the reactions to changes in environmental conditions, such as changes in the upward fluxes by seafloor destruction because of anthropogenic activities, would affect the suboxic zone differently to upper and lower boundaries (Konovalov & Murray, 2001).

Insufficient downward flux of O_2 and NO_3^- within the suboxic zone and upward flux of sulfide, ammonium and other compounds to the suboxic zone cause anoxia in the Black Sea (Konovalov et al., 2005). Ammonium concentrations increase from the deep sea to the lower boundary of the suboxic zone. However, rapid ammonium consumption in the suboxic zone decreases its concentrations in the suboxic zone. In the upper parts of the anoxic layer, sulfide is highly produced; however, deeper than

16.9 (sigma-t), sulfide consumption is much higher than its production. It was suggested that a high amount of vertical decrease in the sulfide concentrations should be related to the lateral flux of the Bosporus plume, which can oxidize two-thirds of the sulfide (Konovalov et al., 2005).

The suboxic zone in the Black Sea has essential functions for the sea. Murray et al., (1995) defined the suboxic zone as the layer with less than 10 μM of oxygen at the starting level of the sulfidic zone. The suggested conditions to create a suboxic zone are (i) running out of oxygen due to consumption of the organic matter from the upper layers and (ii) sulfide flux should not be more than the oxidation potential of the Bosporus plume. In general, lateral input of the Bosporus plume through pycnocline enables the suboxic zone's existence. The suboxic zone includes several cases within it besides lateral input of the oxic water. Redox cycling of nitrogen compounds and redox Mn and Fe transformations occur. Anammox and denitrification are essential reactions for the Black Sea suboxic zone, and the system loses bioavailable N compounds, which decreases the fuel for eutrophication in the upper layers. Mn transformations in the suboxic zone are also crucial because Mn(II) – Mn (IV) transformation can support sulfide and iron oxidation (Konovalov et al., 2003). On the other hand, phosphate is adsorbed and precipitated during these transformations, so the upward flux of the phosphate is limited to control the primary production levels.

All the biogeochemical and oceanographic properties mentioned above are connected with microbiological features of the Black Sea to understand the whole environment and the ongoing processes there. For example, metagenomics analysis reveals unexpected processes in many processes. Oxygen production by photosynthesis near the upper limits of the suboxic zone may cause a downward flux of oxygen to anoxic waters; as a result, oxygen consumption and production can be coupled (Garcia-Robledo et al., 2017). Although it is traditionally accepted that oxygen-dependent nitrification is conducted in the oxic zone, however, metagenomics studies showed that oxygen-dependent nitrification might be active below oxycline (Stewart et al., 2012). production of sulfate reduction, sulfide, can

be reoxidized in anoxic waters (Canfield et al., 2010; Wright et al., 2012), making sulfide hard to detect. This process is called the cryptic sulfur cycle. In the enclosed basins such as the Black Sea, high rates of SO_4^{2-} reduction cause euxinia to occur. The produced sulfide flows upward (or lateral advection) to other zones where biological sulfide oxidation by aerobic chemolithotrophic bacteria and anaerobic phototrophic sulfur bacteria or abiotic processes occur (Percy et al., 2008). Other studies have supported that microbial groups are highly flexible in various metabolic potentials (Carbonero et al., 2014; Grzymski et al., 2008). Sulfate-reducing bacteria use SO_4^{2-} as an electron acceptor to degrade organic matter. However, they have the capability of nitrate usage in low oxygen habitats or the capability of fermentation of organic acids under sulfate deficient zones (Plugge et al., 2011). As discussed in Yücel et al. (2010), there are various sulfur species in the Black Sea sediments, such as humic S, pyrite-sulfur, elemental sulfur, and organic polysulfides. Sulfur oxidizing bacteria detected in the Black Sea can use different substrates such as H_2S, elemental sulfur, and SO_3^{2-}. Some can oxidize H_2S by coupling oxidized manganese and iron (Jorgensen et al., 1991).

Furthermore, filamentous bacteria have an important role ecologically. They have an oxidation capacity of H_2S by O_2 or NO_3^-. In the Black Sea, the impact of cable bacteria (Hermans et al., 2020) and in the Baltic Sea impact of *Beggiatoa* (Yücel et al., 2017) on N, Fe, Mn, and S cycles was mentioned. These filamentous bacteria can dissolve siderite and pyrite molecules to form H_2S, which is required by bacteria for maintaining their activity. They oxidize environmental H_2S, which is toxic for complex organisms, and their capture process of H_2S was named a *benthic filter* by Yücel et al. (2017).

There are important sediment geochemistry studies in the Black Sea due to its permanent anoxic conditions showing its richness in redox properties. Although there were variations in porewater features, oxic sediments showed spatially similar porewater chemistry except for manganese concentrations. The porewater of the suboxic zone included highly enriched manganese and iron molecules, which are important for microbial iron and manganese cycles, and their richness was suggested

as the reason of absence reduced sulfur due to major control of organic carbon mineralization by manganese and iron. Anoxic sediments had high sulfide concentrations (Konovalov et al., 2007). Besides biotic processes, abiotic processes also affect sediment's biogeochemical properties. For example, the transportation of iron oxides by turbidity currents can cause sulfide oxidation (Yücel et al., 2010). These changing conditions can have biotic and abiotic effects such as pyrite formation.

Pyrite (FeS_2) is formed by the interaction of sulfide and iron minerals, and sulfate-reducing bacteria mainly produce these sulfide minerals in the anoxic sediments (Schoonen, 2004). It was shown that the Black Sea hosts sulfate-reducing bacteria responsible for sulfide formation (Vetriani et al., 2003). Due to the anoxic features of the prior earth during the formation of the life on it, iron pyrite molecules can be used as biosignatures and as a paleoenvironmental proxy (Shen & Buick, 2004). FeS_2 is stable thermodynamically without oxygen in low-temperature habitats (Schoonen, 2004). In this context, the Black Sea has a vital role in understanding the evolution of early life and its interactions with pyrite formation (Duverger et al., 2020). If the sediment layer is poor in terms of iron minerals, organic sulfur compounds are formed.

The methane zone occurs below the sulfate zone in the sediments. There are many methane seeps and gas hydrate habitats in the Black Sea, and these areas are essential for the methane diffusion to the water column (Ivanov et al., 1998; Luth et al., 1999). Gas seeps have ecological and environmental impacts that affect the sediment and water column's chemical composition and microbial community composition (Luth et al., 1999). The relationship of methane with sulfate is that anaerobic oxidation of methane (AOM) is mediated by methane oxidizer archaea and sulfate-reducing bacteria. This reaction is $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$. Sulfate-dependent anaerobic methane oxidation results in the formation of carbonates (Reitner et al., 2005). Diffusion of the CH_4 up to the transition zone enhances sulfate reduction. This reaction supplies energy around 7-18% to the transition zone (Jørgensen et al.,

2001), so sulfide production increases because of the coupling of methane and sulfate (Yücel et al., 2017).

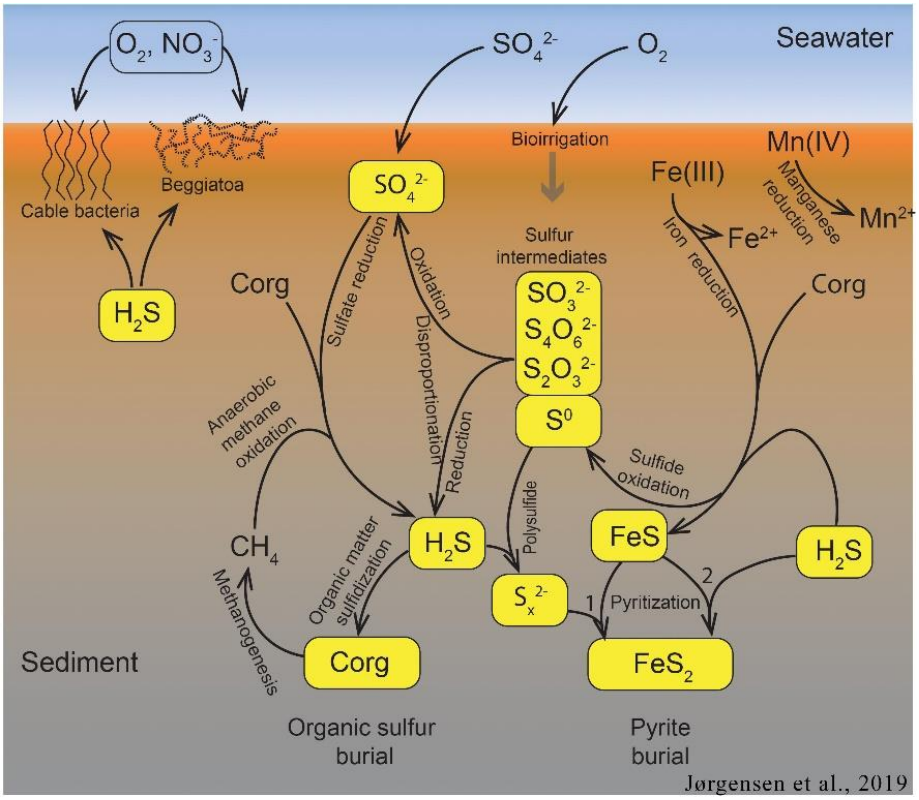

Figure 7. Schematic view of the sulfur cycle in marine sediments. Arrows indicate the fluxes and paths of the reactions. (Jørgensen et al., 2019)

Oceans have a crucial role in the methane cycle (Kirschke et al., 2013; Valentine, 2010). While surface waters include little methane (~80 nM in the Arabian Sea), anoxic basins have much higher methane concentrations (~10 µM in the Black Sea). Therefore, the Black Sea, the largest anoxic basin, plays a crucial role in the methane cycle (Naqvi et al., 2010). Methane is produced by methanogenesis and oxidized by methanotrophy. Although Archaea are responsible for almost half of all methane production on Earth, they cooperate with sulfate-reducing bacteria, as mentioned above. The methane is produced in the last step of OM fermentation and under sulfate-methane transition zones (Evans et al., 2019). However, in the oxygenated and sulfate-rich habitats, methane is not extensive. Surprisingly, cyanobacteria and *Pelagibacter* can release methane by demethylation of methylphosphonates

(Beversdorf et al., 2010; Gomez-Garcia et al., 2010; Metcalf et al., 2012). The increase of cyanobacterial activity due to eutrophication of the seas can cause increased methane emissions from surface waters to the atmosphere (Bižić et al., 2020). On the other hand, the cooperation of sulfate reduces bacteria and methanotrophic archaea and reduces the release of methane from sediments. When methane reaches the oxic or suboxic layers, it can be oxidized by bacterial aerobic methane oxidation (Valentine, 2010). As happens in the S cycle, methane oxidation can be coupled with various reactions such as denitrification, DNRA, and sulfide oxidation (Milucka et al., 2015; Padilla et al., 2017).

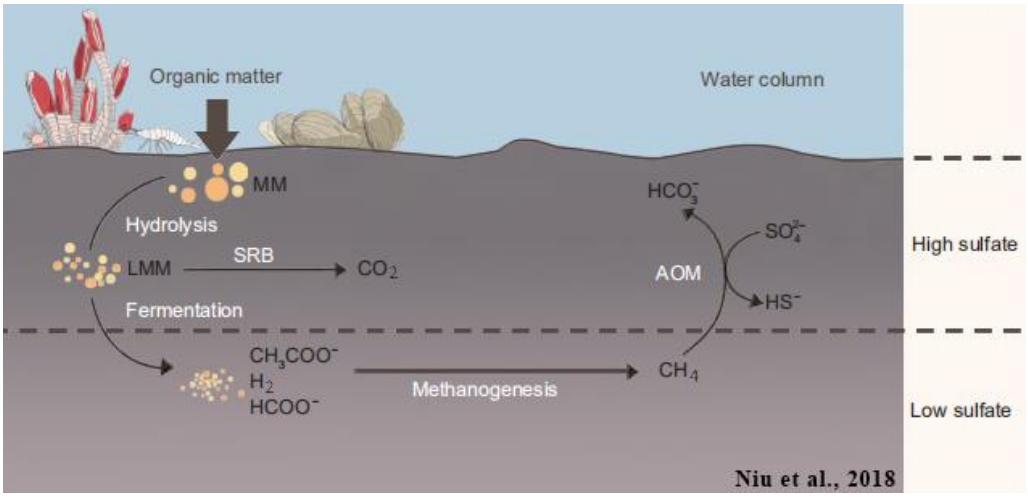

Figure 8. Microbial processes of organic carbon in sulfidic marine sediments. MM: macromolecules; LMM: low molecular weight molecules; SRB: sulfate-reducing bacteria; AOM: anaerobic methane oxidation. Taken from (Niu et al., 2018).

Several studies detected that suboxic zones have been expanding due to climate warming and anthropogenic inputs of nutrients to the seas (Tugrul et al., 1992a; Stramma et al., 2008; Schmidtko et al., 2017; Breitburg et al., 2018). In one of the studies conducted in the Arabian Sea, increasing effects of climate change will increase monsoon wind stress that expands oxygen minimum zones and increase denitrification by 72% (Lachkar et al., 2018).

These reasons make oxygen-depleted zones crucial for research, such as the Black Sea, the largest anoxic basin on Earth. It was shown that the upward trend of the

hypoxic water layer and the tendency of the occurrence eutrophication phenomenon (Mee, 1992) cause change in the macro biological species composition (Zaitsev, 1994). In recent years, increasing nutrient input to the Black Sea increases eutrophication and changes the nutrient regime and transport to the interior water columns (Saydam et al., 1993; Tugrul et al., 1992a). Due to the permanent halocline, oxic and anoxic water bodies are separated (Özsoy & Ünlüata, 1997a), and the oxygen input to the deeper parts of the Black Sea is limited. Increasing anthropogenic inputs increases primary production and organic matter degradation, which causes rapid oxygen consumption, enlarging the suboxic layer, and changing nutrient profiles (Tugrul et al., 1992b).

1.2. Microbial Oceanography

Microbial oceanography is a sub-discipline of some of the integrated disciplines: ecology, oceanography, and biogeochemistry to understand the roles of microorganisms and their dynamics in the biogeochemical cycles. Through this approach, observation and understanding of their physiological and ecological roles and their effects on the climatic variability and the potential of anthropogenic impacts on microbial processes in the oceans (Karl, 2007).

The Earth's surface includes more than 70% of the oceans, which host various habitats for microorganisms. The physical and chemical properties of the oceans differ spatially and temporally. These features (temperature, nutrient concentrations, oxygen, pressure, etc.) impact microbial diversity. The microbial communities drive physical and chemical processes in the ocean and help maintain life on the planet (Dickey, 1991). Their combination affects carbon and nutrient cycling and causes gradients in the environment.

There is a significant challenge to understanding organisms' metabolisms with their biotic and abiotic interactions, shaping their community composition and the biogeochemical cycles. Some of the standard omics technologies are described

below in Table 2. The combination of evolution, microbial ecology, and bioinformatics help to reveal system-level processes.

Table 2. Overview of the important techniques of omics and their major molecules being used for different purposes.

Molecules	Technology	Definition
DNA	Genomics	Structural & functional analysis of genome
RNA	Transcriptomics	Expression profiling
Protein	Proteomics	Identification and quantification of proteins,
Metabolite	Metabolomics	Metabolite profiles, hormones, signaling molecules

Modern approaches in biotechnology and microbiology have increased understanding of microbial oceanography. “Omics” technologies (metagenomics, proteomics, etc.) have opened a new era in this discipline (JiaSong & Li, 2011). High throughput DNA sequencing allows a high resolution to understand microbial diversity and their potential roles in their ecosystem. Metagenomics allows studying the whole genetic material (including different organisms) in a sample. The most significant advantage of this approach is to study all microorganisms regardless of their cultivability. Metagenomics results give information about the present species and their potential functions. The term, “metagenomics”, was coined in Handelsman et al., (1998). Until now, extensive data sets have been created using metagenomics (Sunagawa et al., 2015). However, well-established questions representing large-scale systems and evidence from experiments that can apply to the other habitats are often missing. While metagenomics answers the questions “Who is there” and “what potentials do they have?” mechanisms and the in situ roles of the microorganisms are not sufficient (Grossart et al., 2020).

Previous studies have dramatically changed our understanding of life in deep-sea habitats in terms of diversity and the importance of deep-sea organisms. Although these microorganisms grow slowly, their metabolisms affect the biogeochemical cycles and enable life in the upper oceanic layers. Further studies will reveal new habitats with extensive microbial diversity for natural product research and deep subsurface resources (Orcutt et al., 2013).

In this study, amplicon sequencing technology has been used. This technology amplifies the small subunit (SSU) of ribosomal RNA genes. Carl Woese (Woese, 1987) created a robust method of phylogenetic classification of the organisms by a molecular tree of life. Until then, SSU rRNA gene sequencing has become the gold standard for biological classification (Figure 9). By accumulating the data from amplicon sequencing, it was revealed that microbial diversity is much more than culture collections (Rappé & Giovannoni, 2003). Due to cost-effectiveness, amplicon sequencing is one of the most used techniques to understand a sample's whole diversity. Such studies give clues about crucial biogeochemical cycles mediated by aquatic microorganisms (Duret et al., 2020).

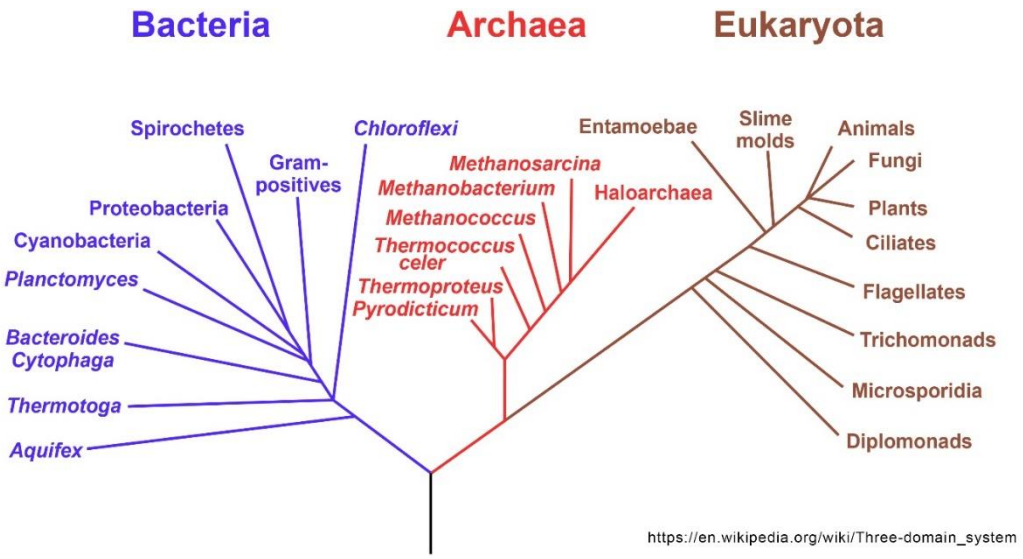

Figure 9. Overview of the phylogenetic tree based on rRNA sequence analysis. Organisms are classified into three domains.

Prokaryotic ribosomes have large and small subunits with nine hypervariable regions (V1-V9), including evolutionarily conserved regions. The ubiquity of 16S rRNA genes conserved sequences allows microbiologists to identify and categorize organisms. With the novel developments in technology, this technique has become rapid, cost-effective, and highly precise, and all these features help establish well-documented databases. Shortly, suitable universal primers of hypervariable regions of the 16S rRNA gene are amplified by next-generation sequencing technology (Case et al., 2007; Yang et al., 2016).

Deep marine environments host various microorganisms in both numbers and diversity. The evidence of life in deep-sea habitats comes from numerous approaches, such as geochemical profiles (D'Hondt et al., 2002; Røy et al., 2012), cell counting (Cragg et al., 1992), RNA and intact polar lipid extractions (Lipp et al., 2008; Mills et al., 2012; Orsi et al., 2013), stable isotope labeling (Morono et al., 2011). Although quite a few studies, current data is insufficient to understand deep-sea microbial habitats because of the different results, genetic material extraction biases, and sampling difficulties (Biddle et al., 2006; Lipp et al., 2008; Mills et al., 2012). Increasing accuracy and collected data can help to better understand Earth's history, past climatic variations, future predictions, and changes (Orcutt et al., 2013).

1.3. Microbial Studies in the Black Sea Sediments

The Black Sea is an essential habitat for researching microorganisms. Especially organisms related to sulfur and methane cycles have become the focus of many research studies. There are several studies all around the Black Sea sediments in this context. In Ince et al. (2007), sulfate-reducing bacteria (SRB) and methanogenic archaea (MA) were researched in the Black Sea sediments using FISH technique. They showed that these organisms were highly diverse in all sampling locations from east to west, north to south (total 6 stations), and the deeper sediment represented the more abundant SRB and MA populations. The most common three SRB genera were *Desulfobotulus, Desulfosarcina,* and *Desulfococcus,* and their relative abundances

increased with sediment depth. Leloup et al., (2007) focused on the diversity and abundance of sulfate-reducing microorganisms and chemical measurements such as pore water analysis sulfate reduction rate measurement in the sulfate and methane zones of a Black Sea sediment to understand their distribution along chemical zonation. They measured abundance by quantitative real-time PCR (Q-PCR) of 16S rRNA genes related to sulfate reduction gene, *dsrA*, and the diversity was detected by 16S rRNA-based microarray analysis. According to their results, *Desulfobacteraceae* are mostly affiliated with *dsrAB* clone libraries in the sulfate methane transition zone (SMTZ), in the upper sulfur-rich layer, and deeper sulfur poor sediment layers were dominated by novel sequences which might have sulfate or sulfite reduction capacity. Finally, they suggested that sulfate reducer microorganisms were responsible for terminal carbon mineralization in surface sediments of the Black Sea. Another study by Schippers et al., (2012) was performed in the Black Sea sediments to analyze quantitative microbial community composition by total cell count, catalyzed reported deposition – fluorescence *in situ* hybridization (CARD-FISH), and qPCR. Their results showed that cell counts were reduced by depth, JS-1 was the dominant group, and *Anaerolineae* and *Caldilineae* were other dominant families. They suggested that the higher organic carbon content of the sediment includes higher cell counts. Another study showed that subsurface Fe oxide layers might indicate cable bacteria in the Black Sea sediments (Hermans et al., 2020). Holmkvist et al. (2011) measured sulfate reduction rates below SMTZ in the Black Sea sediment, and they found that SRB was active below the SMTZ even several meters.

1.4. Aims of the Study

Global climate change and anthropogenic activities have various impacts on the marine ecosystem. The increasing deoxygenation trend in the water column and changes in the organic carbon production inevitably affect microbial community composition. This study aimed to analyze bacterial community compositions in two sediment cores with different redox properties. This study aimed to compare the bacterial community composition under similar surface water properties to predict future changes in the oxic sediments by losing their oxygen. Finally, the possible reasons and results of taxonomic differences were targeted to explain in the light of biogeochemical cycles.

This study focuses on various bacterial groups instead of specific groups such as sulfate reducers. The results will give a snapshot of sediment bacteria by contributing to their little-known distribution and possible ecological roles in the Black Sea sediments and give hints about possible changes in the oxygen-losing sediments.
For this purpose, this study was designed to:

- Compare sediment bacterial community composition under different redox conditions
- Indicate transition from oxic communities to anoxic communities
- Understand the interactions between sediment layers with different redox properties and microbial ecology in the Black Sea
- Contribute to the microbial biodiversity record in the Turkish Seas
- Create a baseline for assessing future changes in the bacterial community composition in the Black Sea sediments.

CHAPTER 2

2. MATERIAL AND METHODS

2.1 Sample Collection

Three sediment cores were collected during a research cruise in November 2020 from the western coast of Turkey in the Southern Black Sea. Samplings were done in three locations representing oxic, suboxic, and anoxic deep-water conditions. The geographic locations (Table 3) and schematic view of the locations on the map (Figure 10) can be seen below. Physical measurements of the water column were done *in situ*; temperature, salinity, density, and dissolved oxygen were measured by a CTD (Seabird, 911). Stations 9, 10, and 11 have water depths of 85, 142, and 330 m, respectively.

Table 3. Geographic locations of the samples from the Black Sea Sediments

Sampling Locations in the Black Sea Sediments			
Station	Latitude (N)	Longitude (E)	Water Column Depth (m)
Station 9	41°36'46.8"N	28°53'02.4"E	85
Station 10	41°40'18.0"N	28°49'48.0"E	142
Station 11	41°38'38.4"N	28°55'19.2"E	330

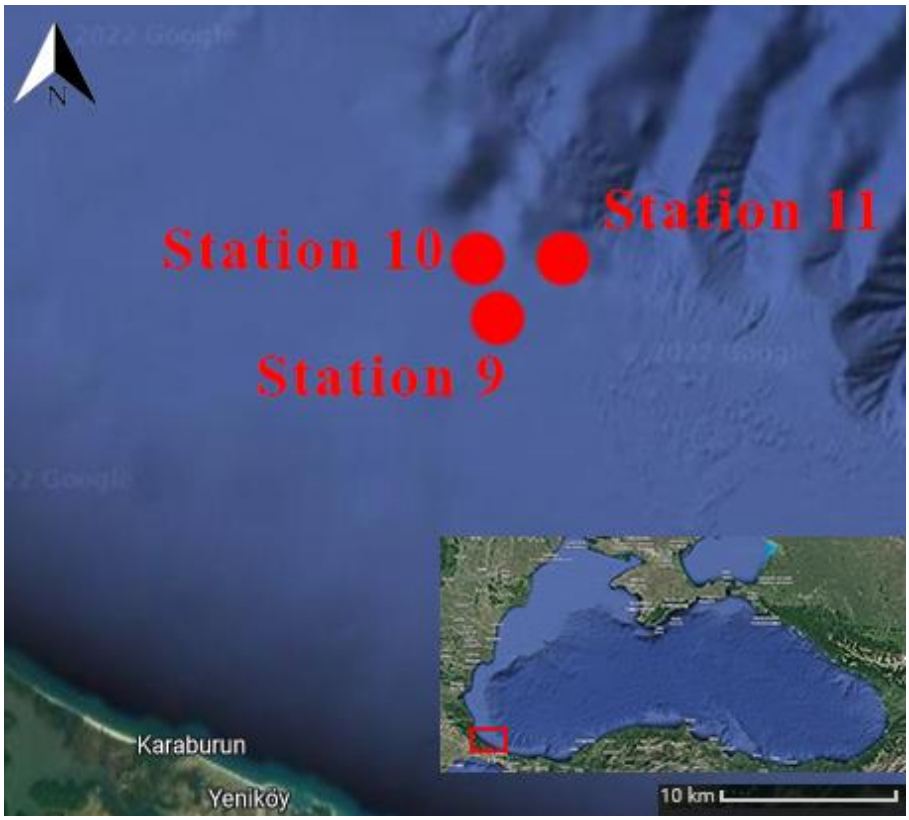

Figure 10. Overview of the sampling sites in the Black Sea sediments

Sediments were collected using a multi-corer sampler (Oktopus, Kiel) to collect <1 m long sediments without disrupting surface sediments, including the overlaying interface water. Sediment cores were collected by polycarbonate tubes, which were cleaned with household grade detergent first and then sterilized by UV-C exposure prior to the expedition to ensure the collection of sediments with full measures for avoiding contamination. The multi-corer was lowered from the research vessel to the seafloor. The messenger weight was attached to the upper part of the device to penetrate the sampling tubes through the seafloor when the device had contact with the seafloor. The spring-loaded lid was closed by sealing onto the tube at the bottom and top openings to preserve the sediment-water interface while collecting the sample.

Seven sediment slice samples were taken from Station 9, six from Station 10, and eight from Station 11 (Table 4). Collected sediment cores were sliced immediately

onboard. Layers were determined to be in increments of 0-1 cm, 1-2 cm, 3-4 cm, 8-10 cm, 10-15 cm, 15-20 cm, 25-30 cm, 30-35 cm starting from the surface sediment and proceeding downwards (Table 4). Technical duplicate sets of 100 µL sediment subsamples from each slice of the core have been collected for microbiological analysis into sterile 2 ml microcentrifuge tubes and were stored at -20 °C until further analysis in the laboratory.

Table 4. Sampling details taken from the Black Sea sediments.

Sampling Details				
Sample Name St.9	***St.9 (cmbsf)***	***St.10 (cmbsf)***	***St.11 (cmbsf)***	***Sample Name St. 11***
A2	0-1	0-1	0-1	D2
A3	1-2	1-2	1-2	D3
A5	3-4	3-4	3-4	D5
A8	8-10	8-10	8-10	D8
A9	10-15	10-15	10-15	D9
A10	15-20	15-23	15-20	D10
A12	25-30		25-30	D12
			30-35	D13
	7 samples	**6 samples**	**8 samples**	

2.2. Chemical Analysis

2.2.1. Nutrient Analysis

Porewater extraction was performed according to methods described in Miyajima et al. (1998). The remaining sediments after microbiological samplings were put into 50 mL falcon tubes, including inert N or Ar gas, to decrease the chance of interactions with oxygen, and centrifuged at 3000 RPM for 30 minutes. The samples' porewaters were extracted by a syringe coupled with GF/F filters (pore size: 0.45 µm) and placed into 15 ml falcon tubes - and stored at -20 °C for nutrient analysis.

Solid phases of the samples were collected for total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) analysis and were stored at -20 °C.

2.2.1.1 Dissolved Inorganic Nutrient Analysis

Dissolved inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and rSi) were measured by a four-channel Autoanalyzer (Bran+Luebbe model) by standardized colorimetric methods (Grasshoff et al., 2007) in the Oceanography Laboratories at METU-IMS, which has successfully passed the International QUASIMEME proficiency tests.

2.2.1.1.1 Nitrite Analysis

Nitrite reacted with sulfanilamide and diazo compounds formed by this reaction. Diazo compounds reacted with N-(1-Naphthyl) ethylenediamine, magenta-like purple, measured at 550 nm wavelength. The detection limit was 0.005 µM for NO_2^- analysis (SEAL Analytical Booklet, No: G-173-96).

2.2.1.1.2 Nitrate and Nitrite Analysis

Nitrate was reduced to nitrite by using a copper cadmium reduction column (Grasshoff et al., 2007; Strickland and Parsons, 1970; SEAL Analytical Booklet, Method No: G-172-96), and under acidic conditions, total nitrite reacted with sulfanilamide to form diazo compounds which reacted with N-(1-Naphthyl) ethylenediamine forming purple azo dyes. NOx concentrations were determined at 550 nm wavelength. The detection limit was 0.04 µM for NOx analysis (SEAL Analytical Booklet, No: G-172-96).

2.2.1.1.3 Ammonium Analysis

Ammonium was detected using Berthelot's reagent to avoid $Mg(OH)_2$ and $Ca(OH)_2$ precipitation. The ammonium in the sample reacted with the reagent to form a blue color product measured colorimetrically at 660 nm (SEAL Analytical Booklet, Method No: G-171-96).

2.2.1.1.4 Dissolved Inorganic Phosphorus

Dissolved inorganic phosphorus in the porewater reacted with the reagent includes molybdic acid, ascorbic acid, sulfuric acid, and antimony. The product complex of the reaction resulted in a blue solution, which was measured at 880 nm (Strickland and Parsons, 1970). The final pH was lower than 1, and the sulfuric acid/molybdate ratio in the solution was between 230 and 330 to quickly colorize and avoid the unwanted effects of silicate (Grasshoff et al., 2007). The detection limit was around 0.015 µg/L (SEAL Analytical Booklet, Method No: G-175-96).

2.2.1.1.5. Reactive Silicate Analysis

Reactive silicate (orthosilicic acid - $Si(OH)_4$-Si) was measured at 820 nm. The silicomolybdate was reduced to molybdenum blue by ascorbic acid. Before adding ascorbic acid, oxalic acid was added to the sample to avoid the effects of phosphates (Grasshoff et al., 2007). The detection limit was 0.05 µM (SEAL Analytical Booklet, Method: G-177-96).

2.2.2. Carbon and Nitrogen Analysis

Carbon and nitrogen analysis of the sediment samples were performed according to the methods described in Grasshoff et al. (2007) using the dry oxidation method at

600 °C to 900 °C and the presence of oxygen by a CHN analyzer (Vario El Cube Elementar Model).

Sediment samples were freeze-dried at first and then crushed into a powder which was then -sieved with 63-µm pore size. For TC and TN analysis, around 30 mg of homogenized sediment was weighed and put into a tin capsule to be measured by the CHN analyzer. To determine TOC concentrations, around 30 mg of homogenized sediment was put into the silver capsule pre-combusted at 400 °C for 6 hours. 10 µL-distilled water was added to wet the samples, and 10 µL HCl (%20 v/v) was added to remove the inorganic carbon as CO2 from the sample until the outgassing ceased. After removing the inorganic carbon, samples were dried at 65 °C overnight for 24 hours. Finally, silver capsules were placed in the CHN analyzer (Nieuwenhuize et al., 1994). The standards were prepared by acetanilide (N-phenylacetamide), including 71.09% carbon and 10.36% nitrogen.

2.2.3. Ion Chromatography

In order to determine ionic concentrations in the porewater of the samples, Ion Chromatography Instrument (Thermo Dionex – ICS-5000) was used. Measured ions are presented in Table 5. Obtained porewater samples were defrosted and diluted by a 1/100 ratio. The cationic ions were detected by CS12-A separation column with methanesulfonic acid eluent (20mM with 1 mL/min flow rate) and DRS600 suppressor. Anions were detected with Dionex AS11-HC separation column, NaOH eluent (12 mM with 1 mL/min flow rate), and DRS600. The particle filtration AG-11 for anion and CG-12 for cation were used as guard columns.

Table 5. Measured ionic compounds from sample sediments.

Cations	Anions
Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}	SO_4^{2-}, Cl^{-}

2.2.4. Dissolved Iron and Hydrogen Sulfide Data

Dissolved iron (dFe) and H_2S data were collected from the same expedition and measured by Nimet Alımlı in 2021 (unpublished). In this thesis, dFe and H_2S data have been used directly from Alımlı's measurements to research these chemicals' effects on the differentiation of microbial community composition by NMDS analysis in this thesis study.

2.3. Microbiological Analysis

2.3.1. DNA Extraction

DNA extractions were done according to the methods described in Karahan et al., (2022) with modifications. Besides collected samples, distilled water was used as a negative control for the DNA extraction process. After defrosting 100 µL subsamples at room temperature, samples were short spun to prevent contamination from the upper parts of microcentrifuge tubes. Firstly, 300 µl lysis buffer (1 M Tris-borate, pH:8.2; 0.5M EDTA; 10% SDS, 5 M NaCl) and 60 µl sodium perchlorate (5M, 0,7 g/ml) were added to the tubes, and samples were mixed at 1500 RPM for 15 minutes at room temperature (~24 °C). After a short spin of the tubes, 360 µL P:C: IAA (25:24:1) were added and mixed well at 1500 RPM for 5 minutes at room temperature. Then the samples were left at room temperature for 4 days, and mixed by hand twice a day. Samples were mixed at 1500 RPM for 10 minutes at room temperature; then they were centrifuged at 14000 RPM for 10 minutes. Upper phases were transferred into new 2 ml microcentrifuge tubes, and then the equal volume (~600 µl) chloroform: isoamyl alcohol (24:1) was added into each tube and mixed well. Samples were centrifuged at maximum RPM (14800) for 10 minutes at +4 °C. Upper phases were transferred into new 2 ml tubes. Two volumes of cold ethanol (%99.9) were added to each tube. After gently mixing, the samples were left overnight at -20°C. Afterward, samples were centrifuged at 14,000 RPM for 30

minutes at +4 °C. Then samples were washed twice with 70% cold ethanol; liquid phases were discarded without disturbing the pellet. 1 ml of cold ethanol was added into tubes, and they were centrifuged at 14,000 RPM for 20 minutes; then, supernatants were discarded. After discarding the liquid phase, sample tubes were placed in a sterile fume hood overnight to dry DNA pellets. Then the DNAs were dissolved in 50 µl of molecular grade water and lived at room temperature overnight. The DNAs were quantified via a Nanodrop spectrophotometer.

2.3.2. DNA Sequencing

DNA sequencing was performed by Macrogen Inc. (Macrogen-Europe). For the amplicon sequencing, the Illumina MiSeq platform was used (2x300 paired-end) to amplify the genes of the samples.

In the beginning, the quality of samples was checked, and the sequencing library was prepared by random fragmentation of the DNA sample. To defeat PCR inhibition of the samples, two PCR amplifications were applied. Two PCR reactions were conducted, and in the end, 15 samples (inhibition still occurred in the samples from St.10) out of 21 were successfully amplified. In the first PCR, sequences of whole 16S rRNA genes were amplified using 27F and 1492R primer pairs (Miller et al., 2013). However, V3-V4 regions were amplified in the second round via 341F and 805R primer pairs (Herlemann et al., 2011). Complete information about raw data statistics can be found in Appendix A. Additionally, due to the absence of DNA in the blank samples, these samples were not sequenced.

2.3.3. Failed Attempts for DNA Extraction & DNA Sequencing

DNA extraction from sediment samples is challenging (Hurt et al., 2001; Klindworth et al., 2013; Lakay et al., 2007; Lloyd et al., 2010). For this study, we applied several methods to overcome this problem. Although the successful DNA extraction and

sequencing methods were mentioned in section 2.3.2, information on the failed efforts is given here for documentation purposes.

Our first attempt was to apply the methods mentioned in Karahan et al. (2022) with modifications, and the resulting DNA concentrations were between 90-2000 ng/µL. However, the sequencing step failed. According to the manufacturer's instructions, the second trial was to clean one of the samples with NucleoSpin Gel and PCR Clean-Up kits. Nevertheless, with this extra process, the DNA concentration of the sample decreased to 25 from 90 ng/µL. The third attempt was purifying the DNA from agarose gel (0.8 %) by electrophoresis, but it was not managed because of the fragmented DNA. The fourth attempt was to precipitate DNA with PEG 6000 (30%) and NaCl (5M). After this protocol, the DNA concentration of the D3 sample was calculated as 200 ng/µL. Then we tried to amplify this sample 16S region via Q-PCR using universal primer; a strong signal was detected. However, the sequencing step had failed with this trial too. DNeasy PowerSoil Kit (QIAGEN) was used in the fifth attempt to extract DNA from two sediment samples; however, the obtained DNA concentrations were too low (24 ng/µL) when compared to the initial amount (1920 ng/µL). Nevertheless, this sample also was sent to sequencing but failed in the library preparation step. According to Karahan et al. (2022), DNA extraction was applied in the final attempt. After two steps of library preparation (full 16S and V3-V4 regions of the 16S rRNA genes) finally sequenced via MiSeq. On the other hand, this method succeeded for 15 samples out of 21, which belong to St.9 and St.11; however, none of the samples from St.10 station could be sequenced.

2.3.4. Bioinformatics and Statistical Analysis

Bioinformatics and statistical analyses were conducted by Qiime 2-2021.4 (Bolyen et al., 2019) and RStudio version 1.2.5033 (Team R, 2020) with phyloseq (McMurdie & Holmes, 2013), vegan (Dixon, 2003), qiime2R (Bisanz, 2018), tidyverse (Wickham, 2017), dplyr (Wickham et al., 2021), readxl, and ampvis2

(Kirkegaard, 2018) packages. For taxonomical analysis Silva 138 (Quast et al., 2013) database was used.

2.3.4.1.Process of replicating samples

All the technical replicates were processed in the Qiime 2 platform. Due to duplicate samples, primarily, duplicate samples were compared with each other for differences. Alpha and beta diversity indices were compared ($p < 0.05$). Then, the samples' reads per sample were controlled, and the highest reads samples were selected to continue analysis.

2.3.4.2.Microbiome Analysis

Paired reads were truncated by the DADA2 pipeline (Callahan et al., 2016) to 274 and 217 for the forward and reverse direction, respectively, and the first 50 and 55 bases were removed. After quality check and trim, amplicon sequence variants were produced (removing low-quality sequences, error correction, merging forward and reverse reads, and removing chimeric sequences). By the results of DADA2 analyses, unique sequences were detected by their relative abundances, and then sequences were aligned, and phylogenetic relationships were produced by align-to-tree-mafft-fasttree pipeline in QIIME 2 to produce rarefaction plots by maximum sequencing depth of 150000. Taxonomical analyses were explored by classify-sklearn classifier in QIIME 2. During classification Silva 138 database (Quast et al., 2013) was used.

2.3.4.3.Diversity Indices

To compare the biodiversity of the samples, alpha and beta diversity analyses were applied. Duplicates and selected samples were analyzed as a two different analysis. Sequencing depth was rarefied to 200000 for replicates and 150000 for selected

samples. For alpha diversity analysis, Faith phylogenetic diversity and Shannon diversity indexes were calculated using the Kruskal-Wallis test (Kruskal & Wallis, 1952) in Qiime 2. Faith phylogenetic diversity indices depend on the number of amplicon sequence variants in the samples, their phylogenetic distances, and relative abundances. Shannon diversity indices depend on the relative abundance and the evenness of the species in the samples. Beta diversity analyses were performed in the Qiime 2 platform to reveal the differences between samples, in other words, calculating the species that are not the same in two different samples. For this purpose, Bray Curtis dissimilarity index and unweighted UniFrac distance (Lozupone et al., 2010; Lozupone & Knight, 2005) were calculated on Qiime 2. Bray Curtis's (BC) dissimilarity index quantifies the compositional dissimilarities between two samples based on the number of amplicon sequence variants. BC is bounded between 1 and 0, where 1 means that the two samples are different in terms of community composition, and 0 means that two samples share all the species (ASVs). On the other hand, unweighted UniFrac analysis includes phylogenetic distances between species with the branch lengths unique to each sample. While UniFrac distance measurement was used for PERMANOVA tests in Qiime 2, the Bray Curtis dissimilarity index was used for non-metric multidimensional scaling (NMDS) analysis.

2.3.4.4.Ordination Analysis

To visualize information about microbial diversity and environmental parameters, the non-metric multidimensional scaling (NMDS) technique was used. To perform this analysis, metaMDS function under the “vegan” package in R. NMDS is an important way to show complex datasets and their interactions with each other. NMDS analysis uses both non-parametric relationship between distance matrix and the Euclidean distances between beta diversity metrics. In this analysis, rank based approach is used so biological distance data is converted to ranks. According to the Bray Curtis distance metric, the calculations are rank-based, and these relationships

are plotted in 2-dimensional space. Due to non-conforming data in this dataset, NMDS is a robust method for ordination analysis. In this study, the NMDS plot helps to understand underlying environmental parameters that are covarying with bacterial community composition, thus possibly driving changes in the community composition. The environmental variables were Total Nitrogen (TN), Total Organic Carbon (TOC), NO_3^-, NO_2^-, Total Carbon (TC), H_2S, PO_4^{3-}, NH_4^+, Si, Cl^-, Na+, Mg^{2+}, K^+, Ca^{2+}, SO_4^{2-}, and dissolved iron (dFe). The abundance of data belonged to the family level. Before NMDS analysis, Hellinger transformed data to obtain meaningful ordination to avoid ordinations that primarily reflect sample size. Then, NMDS analysis was performed by using Bray-Curtis distance metrics because this metric includes taxa's presence, absence, and relative abundance information. The accuracy of the NMDS plot is controlled by stress values which should be below 0.2 (<0.2 significant P*, <0.05 highly significant P**). The NMDS analysis was plotted by using the RStudio by ggplot2 library. In the NMDS plot, closer objects are more similar than others. The arrows indicating the environmental parameters with longer segments have a stronger correlation with the nearby data than those with shorter arrows.

2.3.4.5.Heatmap

To visualize taxonomic classification and compare them within different layers and cores heatmap technique was used. The samples' relative abundances were shown as colors from white (absence) to red (presence) according to the taxa's intensity. To draw the heatmap, the phyloseq object was created by the files of sequence table, rooted tree, taxonomy produced by QIIME 2, and metadata file in RStudio. For the heatmap, the ampvis2 library was used. Although family level taxonomy was used in the heatmap, order-level labels of the samples were added due to many uncultured labels in the taxonomic classification. The 30 most abundant families can be seen on the graph, and they constituted all the samples between 75-50% of the total taxa. However, all the common (>1%) families and lower taxa were scanned.

CHAPTER 3

3. RESULTS

3.1. Chemistry Results

3.1.1. Carbon and Nitrogen Analysis

At the beginning, carbon concentrations along each sediment core was investigated vertically. The total carbon concentration of the surface sediment at St. 9 was 3.47 mmol/g dry weight (dw) which was also the highest measured TC content along St. 9 core, and the TC results had fluctuations up to 10 cmbsf, then decreased by depth. The lowest TC content in St. 9 was 2.12 mmol/g dw in a depth of 25 cm. St.11 had 4.2 mmol/g dw on the surface sediment and increased slightly until 4 cm below seafloor (cmbsf) and peaked with 4.38 mmol/g dw and then decreased to 2.29 until 15 cmbsf with fluctuations, then increased with depth up to 3.2 mmol/g at the 40 cmbsf (Figure 11).

When two stations' total organic carbon (TOC) contents are compared, St.11 had higher TOC values overall. Surface sediment of St.11 had 2.7 mmol/g dw TOC content and decreased down to 15 cmbsf and showed a similar decrease to TC content. While TC content increased after 15 cmbsf, TOC values increased up to 25 cm, decreased rapidly, and stayed around 1.3 mmol/g dw below 25 cm. St. 9 also had similar trends in the upper parts of the sediment. The highest TOC was 2.3 mmol/g dw on the surface sediment and fluctuated down to 2.05 mmol/g dw in the first 8 cm, then decreased until 25. cm, which was the lowest measured TOC amount by 0.78 mmol/g dw and increased a bit more up to 0.91 mmol/g dw in 30 cmbsf.

A comparison of the TOC/TC contents of the sediments indicates that surface layers in both stations had higher TOC contents than total inorganic carbon (TIC).

However, the ratio of TOC/TC decreased with depth below 8 cm in St. 9 and decreased below 10 cm in St. 11. After the decline down to 20 cm of St. 11, the ratio of the TOC/TC increased dramatically in the 25. cm then continued to decrease below 25 cm; the sediment layer's carbon content belonged to 69% organic carbon.

St. 9 had the highest TN values on the surface by 0.26 mmol/g dw when compared other slices of St. 9 and decreased slowly down to 4 cm, then decreased rapidly by depth. Surface sediment of the St. 11 had, 0.28 mmol/g dw TN. TN concentrations stayed around 0,2 mmol/g dw down to 10 cm, then decreased rapidly by depth.

In St. 9, the molar ratio of TOC/TN was 9.1 in the surface layer and generally increased down to 10 cm depth and reached 12.6 molars. However, there was a decrease by around 2-fold in the 15 cmbsf, and the ratio increased up to 15.8 in the 25. cm, but decreased rapidly to 11.4 in 30 cmbsf. Unlike St.9, St. 11 had almost similar TOC/TN molar ratios in the upper sediments, with a slight increase from 9.8 to 13.2. 15 cmbsf faced a similar decrease to the St. 9; however, there was a dramatic increase in the 25 cmbsf, and the molar ratio reached 24. Below this depth, there were similar molar ratios at around 11.5 at 40 cmbsf.

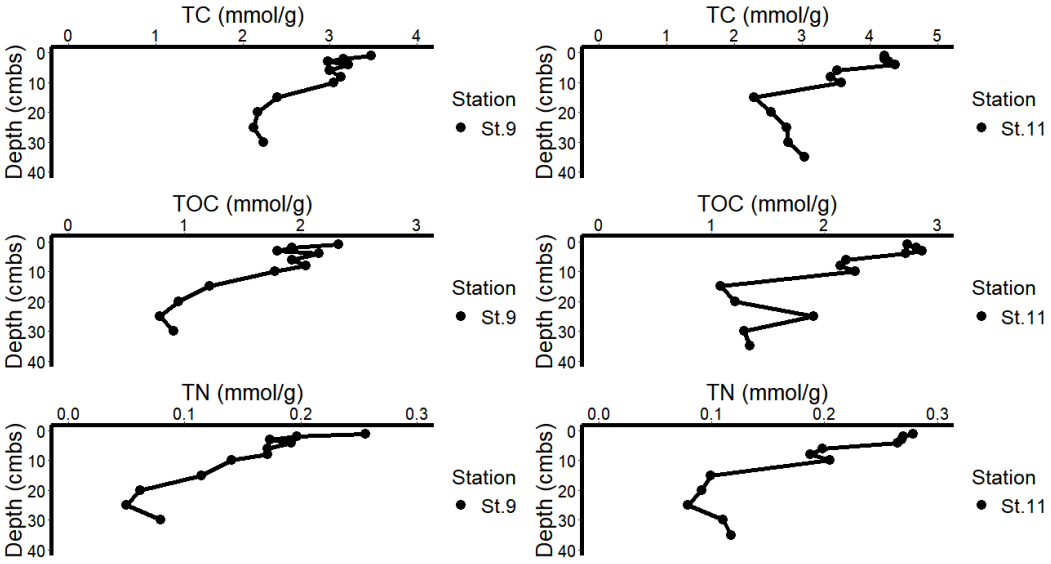

Figure 11. Carbon and Nitrogen measurements from Station 9 and Station 11 according to depth

3.1.2. Nutrient Analysis

In the St. 9, NO_3^- and NO_2^- concentrations were measured as 6.2 µM and 0.5 µM, respectively, just below 1 cm below seafloor (cmbsf); the NO_3^- and NO_2^- decreased to 2 µM and then depleted at around 8 cmbsf. While there was only 1.9 µM NH_4^+ in the surface sediment porewater, NH_4^+ increased to 14.9 µM in 2 cmbsf. By the depletion of NO_3^- and NO_2^-, NH_4^+ had become only dissolved inorganic nitrogen compounds below 8 cmbsf and increased by depth to 207.1 µM in the 30 cmbsf (Figure 12).

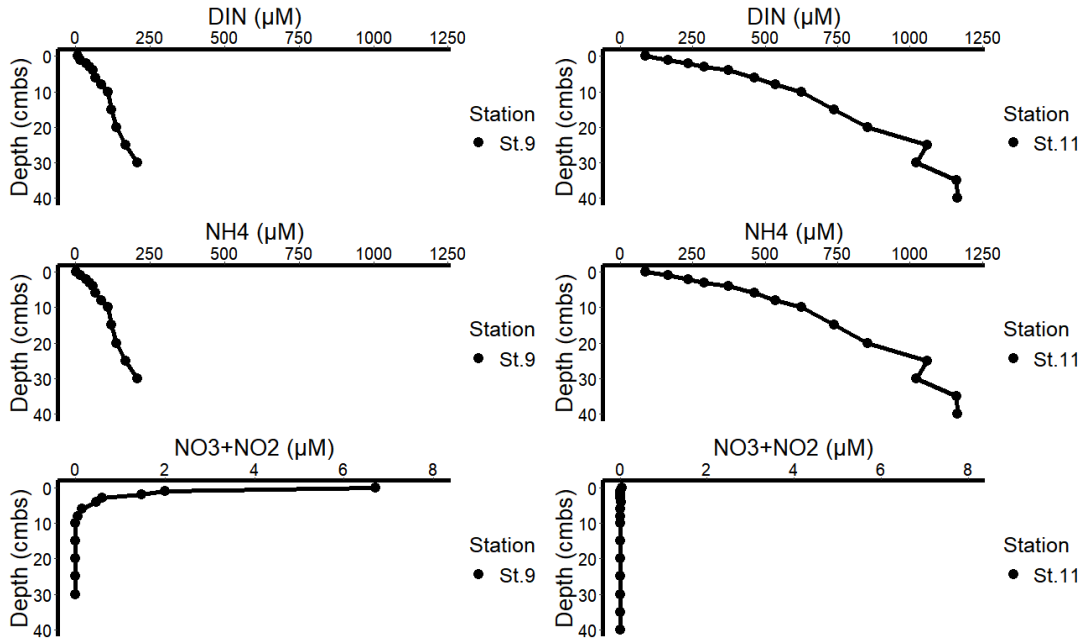

Figure 12. N compounds measured in Station 9 and Station 11 according to depth

In St. 11, NO_3^- and NO_2^- were not detected, but ammonium was detected with an increasing trend by depth. The NH_4^+ concentrations were calculated as 86.4 µM at 1 cmbs and 1164 µM at 40 cmbsf. The only decrease occurred between 25th and 30th cmbsf.

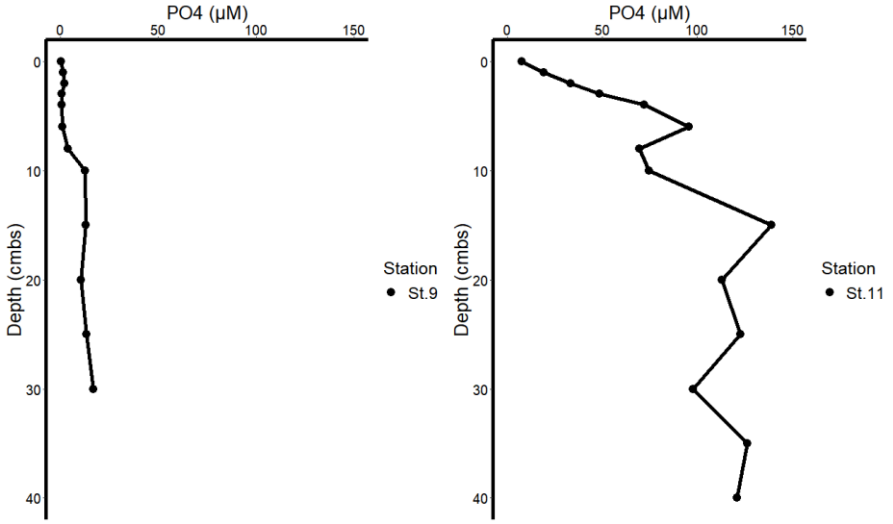

Figure 13. $PO4^{3-}$ measurements in Station 9 and Station 11 according to depth

While PO_4^{3-} was almost depleted in the surface sediment in St. 9, PO_4^{3-} accumulated by depth, after 8 cmbsf, the amount of PO_4^{3-} increased significantly, almost by 4-fold. Unlike St. 9, St. 11 had 7.5 µM PO_4^{3-} in the surface sediment, which increased substantially to 19.4 µM and then increased to 6 cmbsf. After this depth, PO_4^{3-} values decreased to 69.4 µM and then increased to 139 µM in the 15 cmbsf. Below this depth, the PO_4^{3-} values fluctuated between 97 and 126.26 µM until 40 cmbsf (Figure 13).

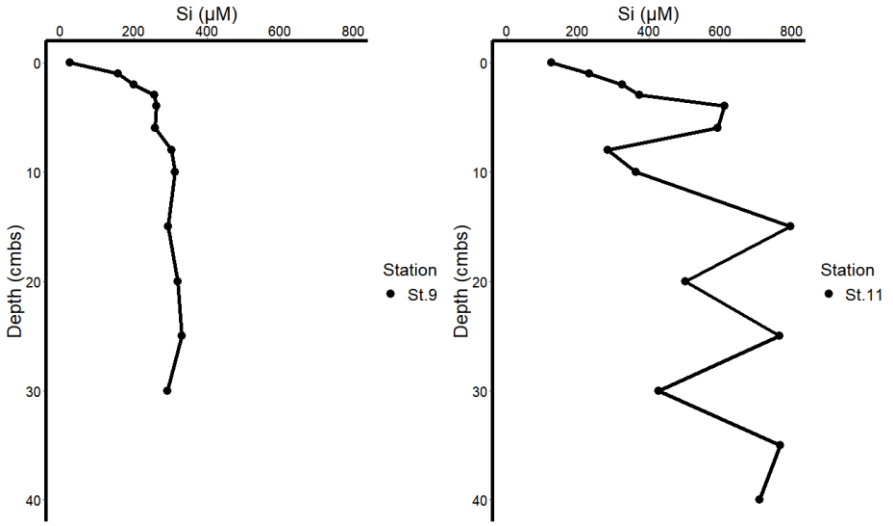

Figure 14. Silicate measurements in Station 9 and Station 11 according to depth

The Si content of the St. 9 started with 25.4 μM on the surface sediment porewater and increased sharply to 262.9 μM in the 4 cmbsf and continued to increase up to 331.8 with variations, and decreased to 291.3 in the 40 cmbsf. In St. 11, Si content oscillated along with sediment depth. The surface sediment had the lowest Si amount by 125.4 μM. In the first four cmbsf, Si concentration raised to 612.4 μM, then decreased sharply to 8 cmbsf. The highest measured value was 797.8 μM in the 15 cmbsf. Below this depth, the values fluctuated from 426.1 to 766.8 μM Si.

3.1.3. Ion Chromatography

Generally, cations increased with depth from the surface to deep layers with fluctuations. While sodium-ion had around 300 mM in the surface layer of Station 9, there was around 370 mM Na^+ in the surface layer of Station 11. In Station 9, Na^+ concentrations increased with fluctuations by depth until 497 mM at 20 cmbsf, Station 11 had the highest level of Na^+ concentrations at 4 cmbsf with 471 mM. Potassium ions and magnesium ions behaved similarly to Na^+. K^+ had 6.3 mM in the surface sediment of Station 9 and 7.2 mM in Station 11. They reached their highest values by 11 mM at 20 cmbsf and 10 mM at 30 cmbsf. Mg^{2+} concentration was 34 mM in the surface sediment of Station 9 and 42.3 mM in Station 11. In 20 cmbsf, Mg^{2+} concentration peaked by 59.8 mM in Station 9 and 57.7 mM in 4 cmbsf in Station 11.

Ca^{2+} had a similar trend in both stations but differed from other major ions mentioned below. In the surface layer, there was ~7 mM Ca^{2+} in Station 9 and ~9 mM in Station 11. The Ca^{2+} concentrations fluctuated around these values. However, there were essential peaks in both stations; station 9 had a Ca^{2+} peak at ~26.2 mM in 6 cmbsf, and station 11 had a Ca^{2+} peak at 23.4 mM in 10 cmbsf.

Cl^- was 18 M in surface sediment of Station 9 and 23 M in Station 11 (Figure 15). Although there were fluctuations in values in both stations, they generally stayed

around 25 M in both stations. In Station 9, Cl^- peaked at 32.4 M in 20 cmbsf and 30.1 M in Station 11.

SO_4^{2-} values behaved differently in both stations (Figure 17). There was around 24 mM SO_4^{2-} in the surface sediment and decreased gradually to 18 mM by depth, then increased significantly to 37.2 mM in 6 cmbsf in Station 9; below this depth, the value decreased to 19.9 mM 8 cmbsf, then increased to 40 mM in 10 cmbsf. Below this depth, the value decreased gradually to around 30 mM in 30 cmbsf. In Station 11, 30.2 mM SO_4^{2-} in the surface sediment peaked at around 33.7 mM in 2 cmbsf. Below 2 cmbsf, SO_4^{2-} concentration decreased by depth with fluctuations to reach ~3 mM value.

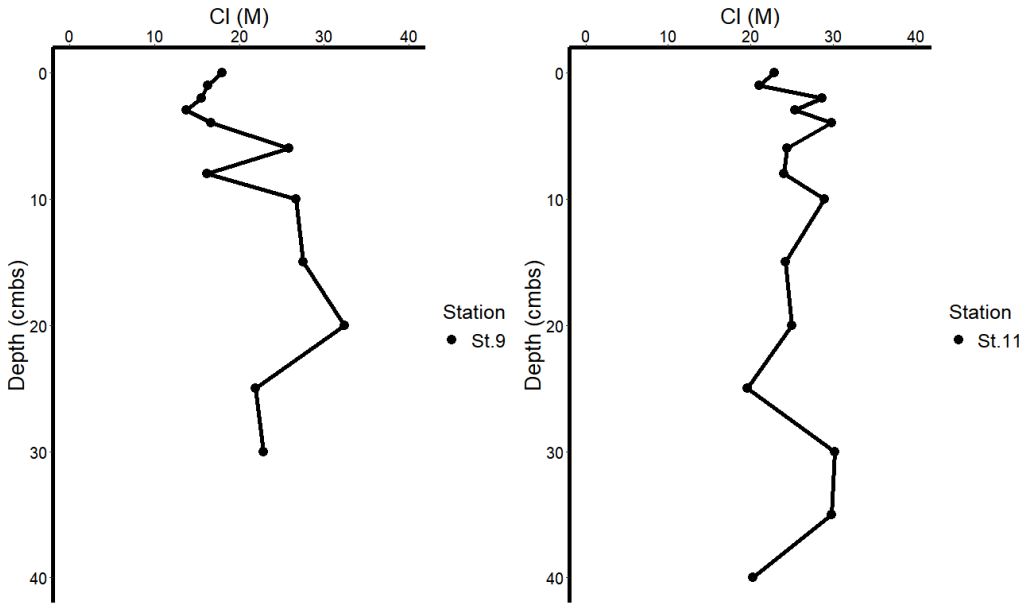

Figure 15. Cl^- concentrations measured in Station 9 and Station 11 according to depth

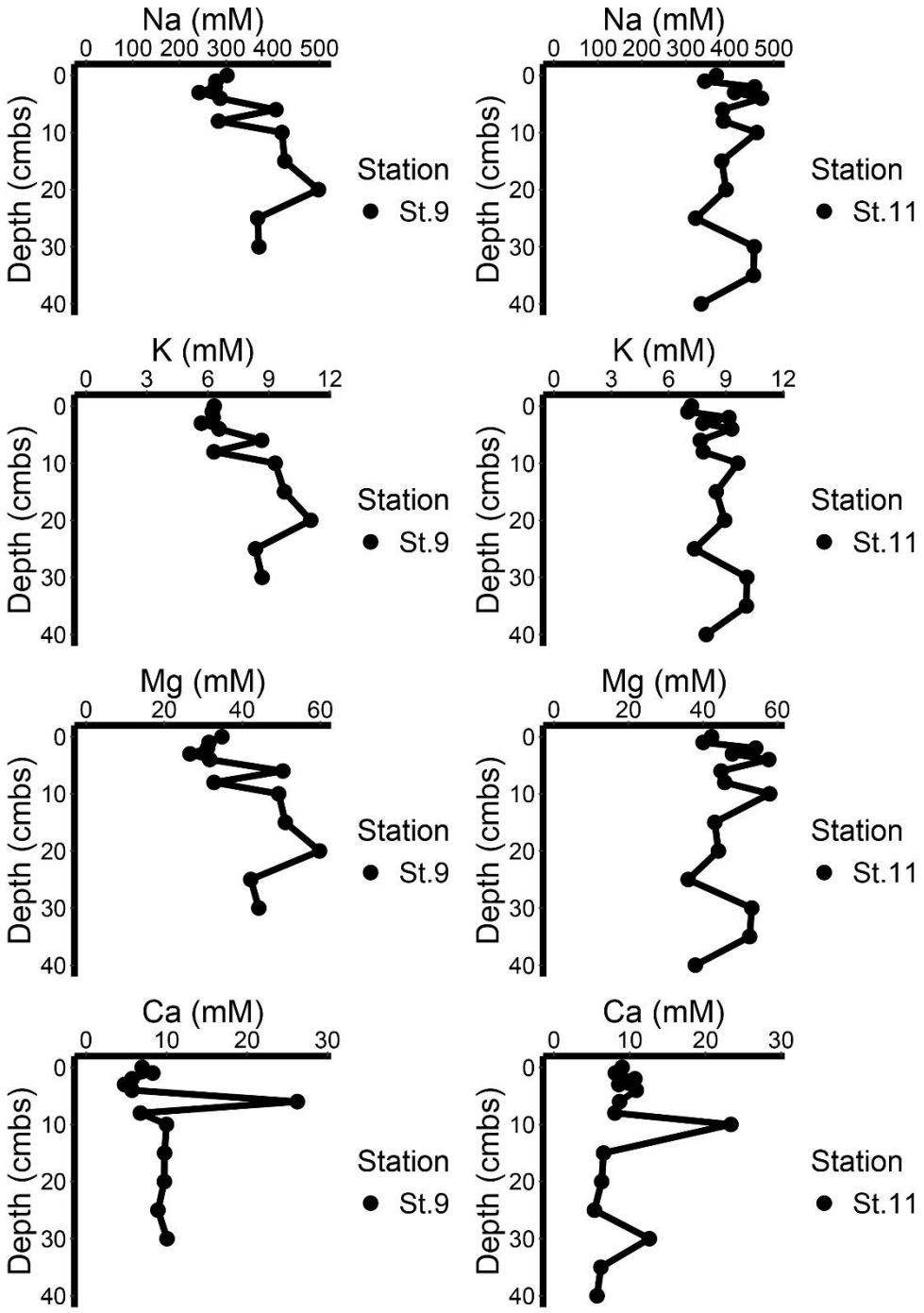

Figure 16. Cations measured in Station 9 and Station 11 according to depth

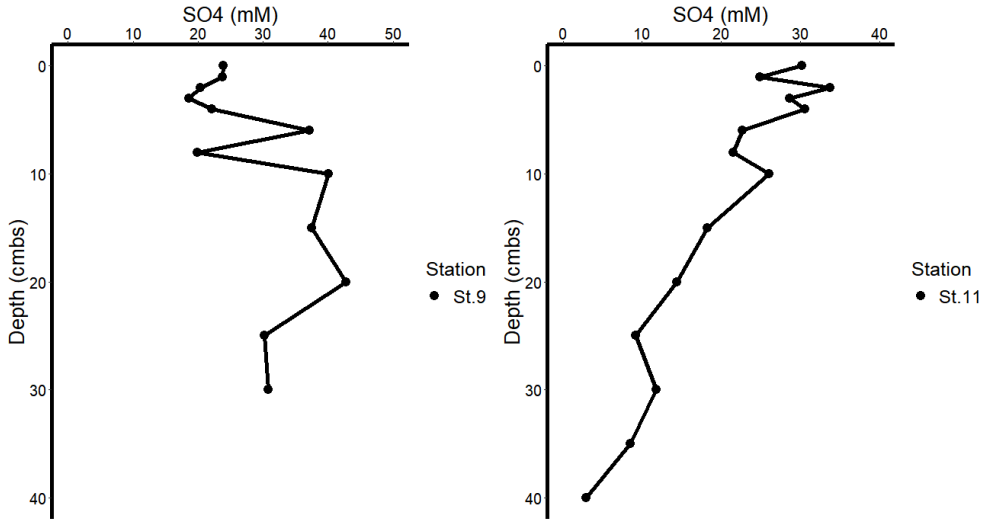

Figure 17. Sulfate concentrations measured in Station 9 and Station 11 according to depth

3.1.4. Dissolved Iron and Hydrogen Sulfide

Fe (II) and Fe (III) were measured as dissolved (dFe) forms in both stations (Figure 18). In the surface sediment of Station 9, 3.6 µM dFe was measured; however, at 2 cmbsf, there was 137.9 µM dFe then decreased by the depth, which dropped to 3 µM at 30 cmbsf. However, dFe concentrations were too low, between 0.334 µM (in the surface sediment) and 0.049 µM (8 cmbsf).

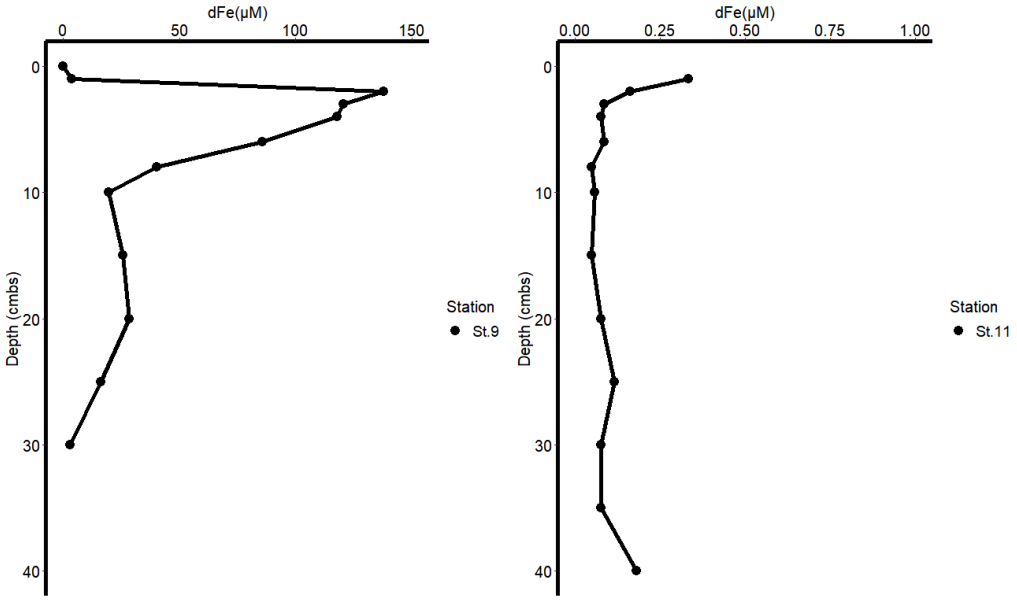

Figure 18. dFe concentrations measured in Station 9 and Station 11 according to depth

Hydrogen sulfide was not detected in all samples of Station 9 (Figure 19). On the other hand, Station 11 was rich in H_2S. In the surface sediment of Station 9, there was 215.4 μM H_2S, and firstly increased suddenly up to 1862.3 μM in 20 cmbsf, next decreased to 1558.3 μM in 25 cmbsf, then increased sharply to 3534.6 μM in 35 cmbsf and finally decreased to 2673.1 μM in 40 cm.

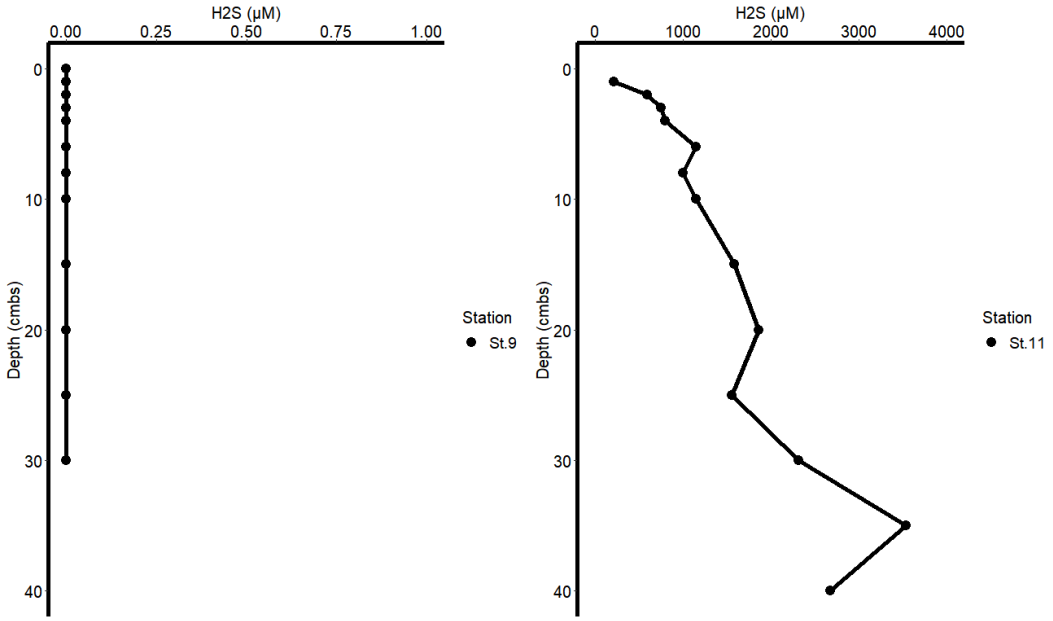

Figure 19. Hydrogen sulfide concentrations measured in Station 9 and Station 11 according to depth

3.2. Microbiology Results

The number of successfully sequenced samples from the two stations was 27. There were two sets of 12 samples; however, three single samples of the three replicates could not be sequenced, so these sequence results were not included prior to replicating the comparison analysis (Table 6).

Table 6. Replication status and details of the sequenced samples

Sample Name	**Station**	**Replication Status**	**Interface Water**
A2	St. 9	Duplicate	Oxic
A3	St. 9	Duplicate	Oxic
A5	St. 9	Duplicate	Oxic
A8	St. 9	Duplicate	Oxic
A9	St. 9	Duplicate	Oxic

A10	St. 9	Single sample	Oxic
A12	St. 9	Duplicate	Oxic
D2	St.11	Duplicate	Anoxic
D3	St.11	Duplicate	Anoxic
D5	St.11	Single	Anoxic
D8	St.11	Duplicate	Anoxic
D9	St.11	Duplicate	Anoxic
D10	St.11	Duplicate	Anoxic
D12	St.11	Duplicate	Anoxic
D13	St.11	Single	Anoxic

Obtained raw data statistics from the Illumina MiSeq platform can be seen in Table 7. 17,749,074 reads were produced, and read bases were 5,342 Mbp in all samples.

Table 7. Raw data statistics of the whole sequences

Sample	**Total Bases**	**Read Count**	**GC (%)**	**AT (%)**	**Q20 (%)**	**Q30 (%)**
1-A2	210,634,984	699,784	55.33	44.67	86.02	75.69
1-A3	220,106,250	731,250	55.70	44.30	83.76	72.72
1-A5	206,393,292	685,692	55.06	44.94	86.91	76.80
1-A8	198,810,500	660,500	55.26	44.74	84.95	73.73
1-A9	172,181,632	572,032	55.92	44.08	86.16	75.83
1-A10	205,651,026	683,226	56.22	43.78	85.61	74.87
1-A12	225,140,776	747,976	56.16	43.84	85.77	75.03
1-D2	264,376,126	878,326	54.62	45.38	86.97	76.88
1-D3	175,236,180	582,180	54.60	45.40	85.90	75.01
1-D5	185,549,644	616,444	54.87	45.13	86.03	75.24
1-D8	206,281,922	685,322	54.90	45.10	86.51	75.94
1-D9	170,105,334	565,134	54.43	45.57	86.50	75.86
1-D10	155,216,068	515,668	54.73	45.27	84.86	73.40
1-D12	232,876,476	773,676	54.56	45.44	86.23	75.51
2-A2	218,603,056	726,256	54.96	45.04	86.63	76.32

2-A3	202,742,162	673,562	55.15	44.85	86.79	76.76
2-A5	195,896,820	650,820	54.86	45.14	85.46	74.67
2-A8	205,061,668	681,268	55.16	44.84	86.23	75.71
2-A9	197,381,954	655,754	55.21	44.79	86.31	75.80
2-A12	212,034,634	704,434	55.89	44.11	85.27	74.37
2-D2	256,686,780	852,780	54.41	45.59	86.73	76.51
2-D3	197,442,756	655,956	54.45	45.55	86.62	76.44
2-D8	164,674,692	547,092	55.04	44.96	86.28	75.59
2-D9	95,267,704	316,504	54.87	45.13	84.29	72.82
2-D10	154,707,980	513,980	54.85	45.15	85.51	74.46
2-D12	186,146,226	618,426	54.94	45.06	85.45	74.40
2-D13	227,264,632	755,032	54.38	45.62	87.01	76.64

After production of the rooted phylogenetic tree with 5000 sequences as sampling depth out of 24 samples, alpha diversities were compared within replicates. To check whether sampling depth was enough or not a rarefaction curve was produced, and all the samples were found to be covered during the sequencing process (Suominen et al. (2021) collected sediment samples from anoxic Baltic Sea sediments and divided the sample core into 4 sections (1-2 cm; 10-11 cm; 20-21 cm; 25-26 cm). According to their 16S rRNA V4 amplicon sequencing results, in the surface sediment Deltaproteobacteria (30.4%) was the most abundant bacterial group, then followed by Bacteroidetes (10.8%), Planctomycetes (9.5%) and Chloroflexi (6.1%); in their deepest sample, Chloroflexi (28.8%) was the most abundant bacterial group, and followed by Planctomycetes (24.3%) and Atribacteria (8.2%). There were similarities and dissimilarities between the Baltic Sea and the Black Sea anoxic sediments. The significant difference was Deltaproteobacteria which was absent in both Stations; however, in the surface sediment of St. 11, Gammaproteobacteria was the dominant class. Bacteroidetes group, similarly to Baltic Sea sediment, had 11.1%, and Chloroflexi also had a similar abundance (6%). Planctomycetota was much less (2.7%) than in the Baltic Sea. When we come to the deep layer of St. 11, Chloroflexi was the most dominant taxa. Its abundance peaked at 29.9% in 20 cm. Planctomycetota had much less abundance (5.9%), and there were no Atribacteria

detected in St. 11's deep sediments, contrary to the Baltic Sea. In the Baltic Sea, almost three-fold more TOC content in the dry weight of surface sediment; however, in the Black Sea sediments, NH_4^+ was almost 2.5-fold higher.

Desulfobacterales is known as sulfate reducer (Pfennig et al., 1981), and this group was abundant in all sediment layers of Bothnian Sea samples (Rasigraf et al., 2020) peaked at SMTZ. In this study, they were present in all layers. However, their abundance decreased with depth, so detected members of Desulfobacterales may not be involved in anaerobic oxidation of methane (ANME) reactions as happened in the Bothnian Sea study (Figure 28).

While the first pair of 1 cm in Station 11 (St.11-1) had the highest sequences, the second pair of the 15 cm in Station 11 (St.11-2) had the lowest sequence numbers.

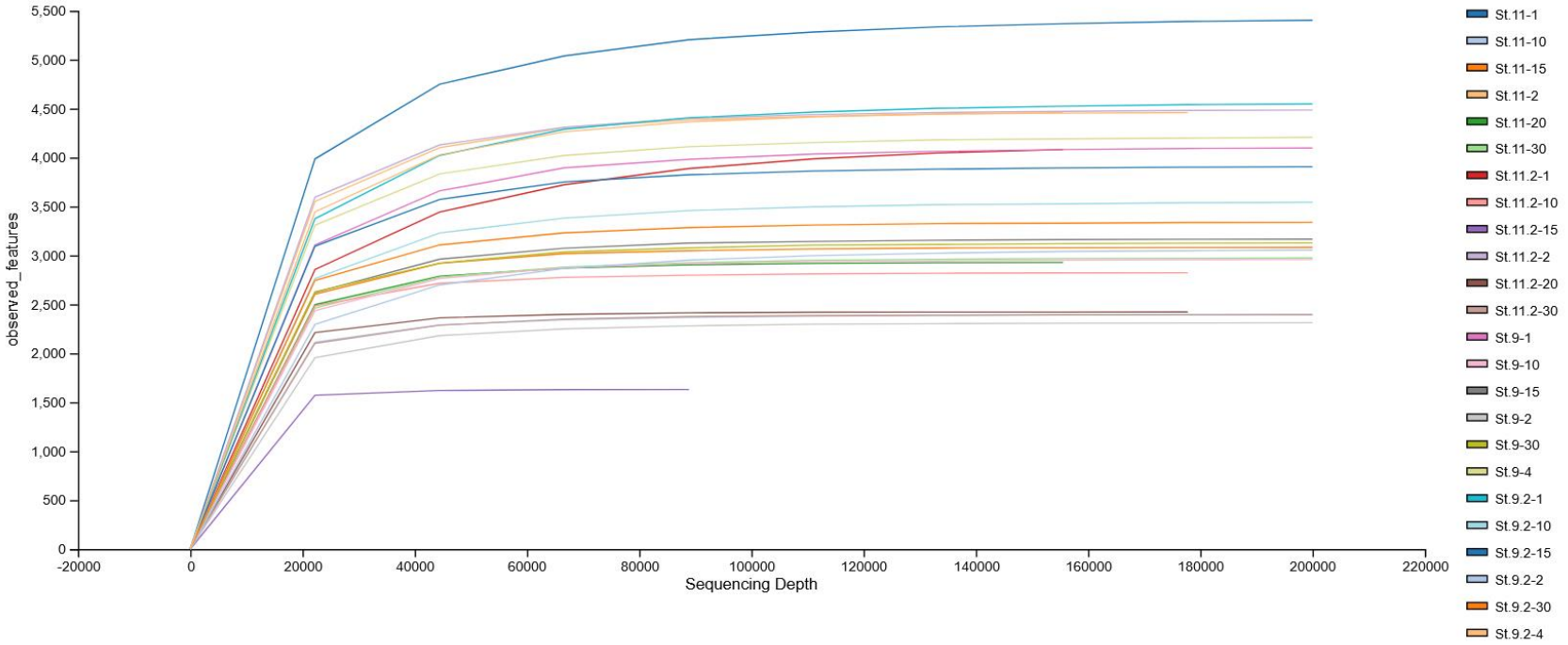

Figure 20. Rarefaction analysis of duplicate samples

Alpha diversity calculations (Observed Features, Faith Phylogenetic Distance, and Shannon entropy) can be seen in Figure 21. Generally, replicates had similar results in every depth except 1 cm of the St. 11. The first replicate of the St.11 had higher results in each diversity indices. According to the Kruskal-Wallis (pairwise) test result to determine similarities between replicates of the stations, there were no statistical differences between St.11-1 and St.11-2 (p-value: 0.34); and St.9-1 and St.9-2 (p-value: 0.42). Furthermore, when beta diversity analysis was done, there were no statistical differences between replicates both in Bray-Curtis dissimilarity results (St.11-1 - St.11-2 (p-value:0.759) and St.9-1 – St.9-2 (p-value: 0.93). Additionally, there were enough reads per sample (higher than 4000 in every sample) allowed to select one of the samples in each replicate with the highest number of reads and proceed with it.

Selected 12 samples from replicates and three samples without replicates were analyzed as mentioned above, and 30,181 ASVs were detected after removing the sequences that belonged to chloroplast and mitochondria. The detailed results can be seen in Table 8. Overall, St.9 had lower ASVs than St. 11. The lowest frequency of ASVs occurred in the 20 cm of St.11. The highest one occurred in 1 cm of St.11.

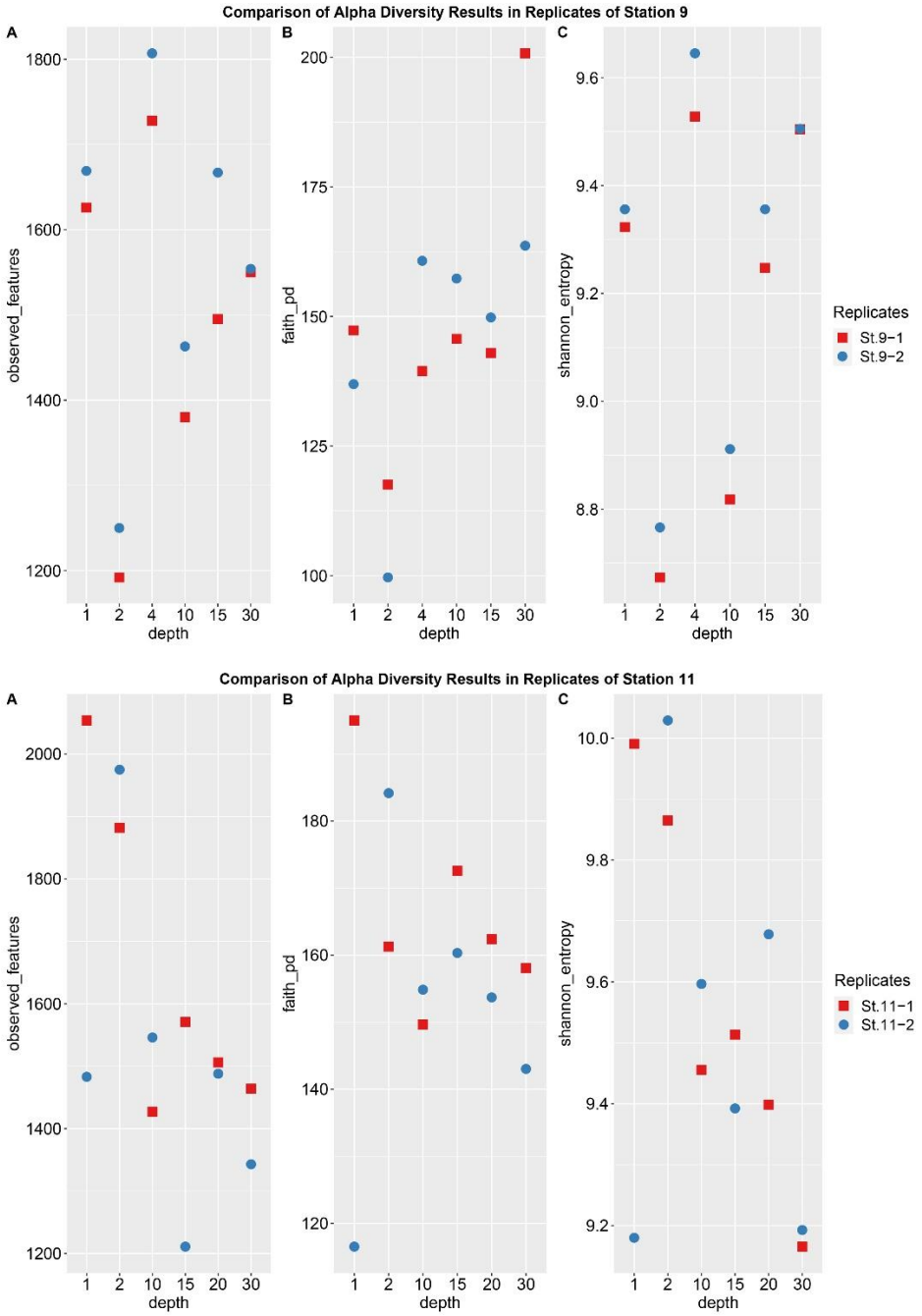

Figure 21. Alpha diversity results of duplicate samples

Table 8. Sequence statistics of the whole samples

Table Summary	
Metric	**Samples**
Number of samples	15
Number of features	30,181
Total frequency	3,494,204
Frequency per sample	
Metric	**Frequency**
Minimum frequency	176,803.0
1st quartile	208,381.0
Median frequency	232,715.0
3rd quartile	249,571.0
Maximum frequency	299,858.0
Mean frequency	232,946.9

To check the proficiency of the sampling depth, a rarefaction curve was produced by 15 samples and the sequencing depth was enough to represent the samples (Figure 22).

Alpha diversity analyses can be seen below (Figure 23). Briefly, the St. 11 was more diverse than the St. 9. The samples belonging to the first 4 cm were much higher than others in St. 11; however, St. 9 had higher diversity in the samples of 1, 4, and 20 cm according to unique observed ASVs. When considering the phylogenetic distances of the samples, upper sediment samples (1 and 2 cmbsf) of St. 11 had higher diversity. However, in other samples, samples belonging to St. 9 had slightly higher results than St. 11. For the Shannon diversity, St. 11 had higher values in the upper 4 cm samples, similar to observed features results, and overall, St. 9 had lower Shannon diversity indices according to the graph. When the Kruskal-Wallis (pairwise) statistical test was done, using each alpha diversity indices, there were no

statistical differences in alpha diversity measurements between the stations. The alpha diversity indices' Kruskal-Wallis (pairwise) test results can be found in Table 9.

Table 9. Kruskal-Wallis test results of alpha diversity indices. P <0.05 is significant.

Kruskal-Wallis (pairwise)				
Observed Features				
Group 1	Group 2	H	p-value	q-value
St.11 (n=8)	St.9 (n=7)	0.33542	0.562485	0.562485
Faith's Phylogenetic Distance				
Group 1	Group 2	H	p-value	q-value
St.11 (n=8)	St.9 (n=7)	0.013393	0.907869	0.907869
Shannon's diversity index				
Group 1	Group 2	H	p-value	q-value
St.11 (n=8)	St.9 (n=7)	3.013393	0.082579	0.082579

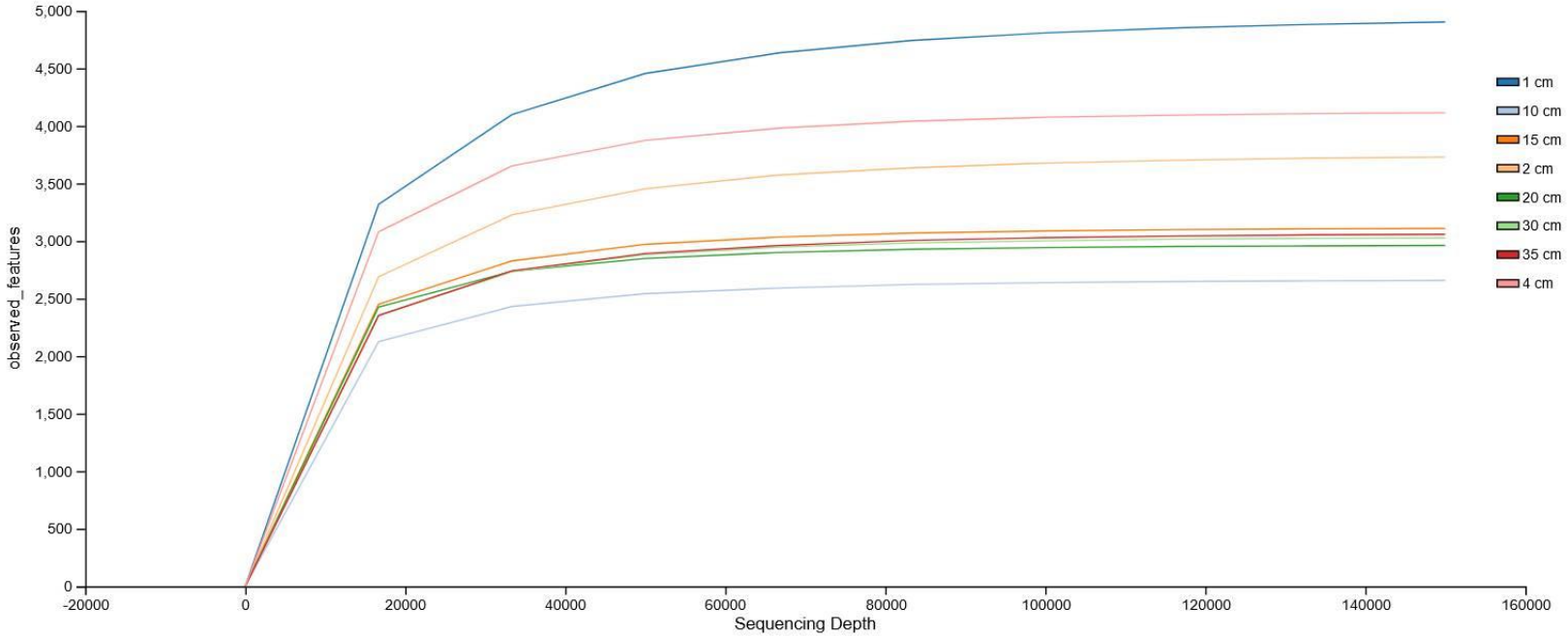

Figure 22. Rarefaction analysis of selected samples according to sampling depths

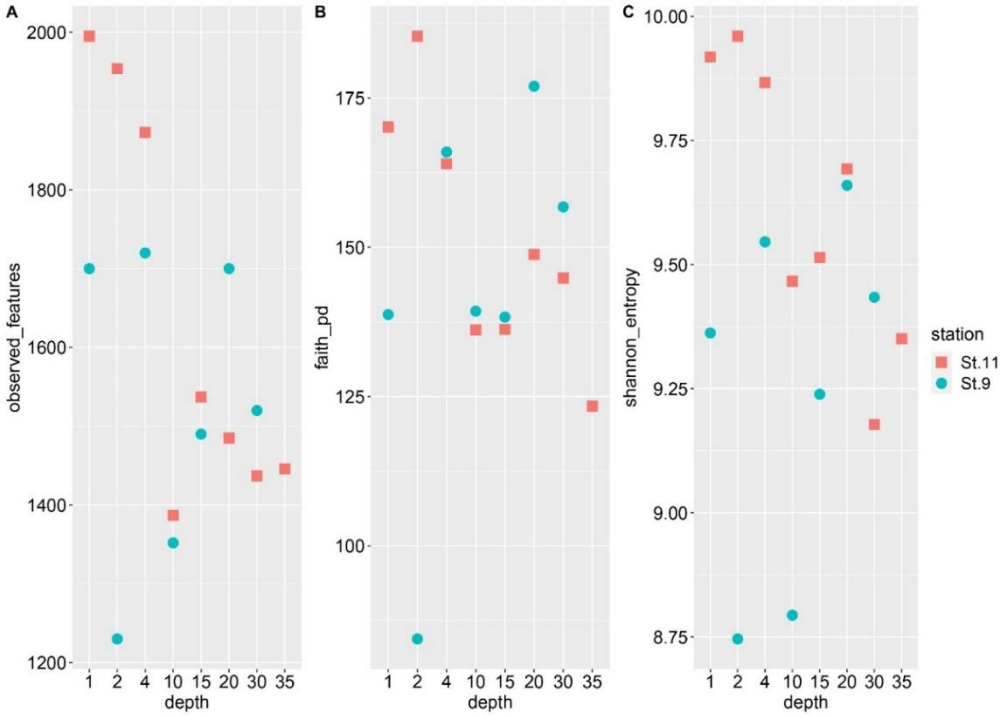

Figure 23. Alpha diversity analysis of selected samples

For the beta diversity analysis, Bray-Curtis (Appendix B) and unweighted UniFrac distances were calculated, then for each analysis, results were tested by pairwise permanova analysis. There were statistical differences between stations for each diversity measure (Table 10).

Table 10. Pairwise permanova results of beta diversity indices between St. 9 and St. 11. P* < 0.05 is significant.

Pairwise permanova results						
Bray-Curtis Dissimilarity						
Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
St.9	St.11	15	999	2.290559	0.045	0.045
Unweighted UniFrac distance						
Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
St.9	St.11	15	999	1.541184	0.029	0.029

3.2.1. Taxonomic Analysis

Phylum level taxonomic analysis revealed that Desulfobacterota was the most abundant phylum by 26.6%, 31.8%, 26.2%, 31.4%, and 23.8%, respectively, from surface sediment to 15 cm sediment layer. Chloroflexi was the most predominant taxa in the 20 (34.4%) and 30 cm (33.5%) samples of Station 9. Otherwise, Proteobacteria had a similar relative percentage of 26.1% to Desulfobacterota in the surface sediment and decreased with depth in Station 9. Other taxa can be seen in Figure 24.

For St. 11, Desulfobacterota and Proteobacteria were the most predominant phyla in the first 4 cm. Below this depth, Chloroflexi became the most abundant phylum. In this station, one of the significant differences is the predominance of Caldatribacteriota. This phylum is almost absent in all the sampling depths from St. 9, but after 10 cm of St. 11, this phylum starts with 7% relative abundance and increases with depth, except in 20 cm, which faced a dramatic decrease to 4.4%. The heatmap belonging to the phylum level shows the most abundant 15 phyla, which account for around 85% of the total phyla.

In the family level taxonomic classification (Figure 25) for St. 9, The most abundant family in the 1 cm was Desulfobulbaceae by 11.2%. Its relative abundance decreases in the second and fourth-centimeter samples to 8.8 and 7.8, respectively. However, it reached 11.2% in the 10 cm of St. 9, and then decreased with depth by reaching zero in the deepest sample (30 cm) of St. 9. Desulfosarcinaceae had 6.1% in the 1 cm sample of St. 9 and increased to 10 cm by reaching 11.8%, then decreased with depth.

In St. 11, upper sediment layers had Desulfosarcinaceae as the most abundant family down to 4 cm, and its abundance decreases with depth. Desulfobulbaceae and Woeseiaceae had around 5% relative abundance separately. However, they were absent below 4 cm sediment layers. While, Anaerolineaceae, Aminicenantales, and Desulfatiglandaceae families had lower percentages in the upper sediment layers

and, generally, increased with depth. On the other hand, Woeseiaceae, Flavobacteriaceae, the uncultured family of Syntrophobacterales, and Sva1033, had higher relative abundances in the upper sediment layers. The heatmap of the families represents 60 to 70 % of the total families.

3.2.2. Ordination Analysis

NMDS analysis revealed possible interactions between microbial diversity and environmental parameters (Figure 26). Stress level is an important indicator for the goodness of fit for the NMDS plot, and the stress level was calculated as 0.024. Sixteen chemical parameters (total nitrogen (TN), total carbon (TC), total organic carbon (TOC), NO_3^-, NO_2^-, H_2S, $PO4^{3-}$, NH_4^+, Si, Cl^-, Na^+, K^+, Mg^{2+}, Ca^{2+}, SO_4^{2-}, dissolved iron (dFe) and Bray-Curtis dissimilarity index were used to perform NMDS analysis. In the NMDS plot, Station 9 was clustered into three groups (1-2 cm & 4-10-15 cm & 20-30 cm), and the samples until 15 cm were positively correlated with NO_3^-, NO_2^-, TOC, TC, dFe, and SO_4^{2-}; however, 20 and 30 cm samples were negatively correlated with these parameters but positively correlated with H_2S, $PO4^{3-}$, NH_4^+, Si and all ions. Station 11 had two clusters (1-2-4 & 10-15-20-30-35). The upper sediment cluster of Station 11 is located on the negative side of the first dimension and showed similar behavior to the first two clusters of Station 9. The second cluster of Station 11 was located on the positive side of the axis and positively correlated H_2S, $PO4^{3-}$, NH_4^+, Si, and ions, and the third cluster of Station 9. Chemical parameters are differentiated on the second axis, but no apparent differentiation in the microbial data.

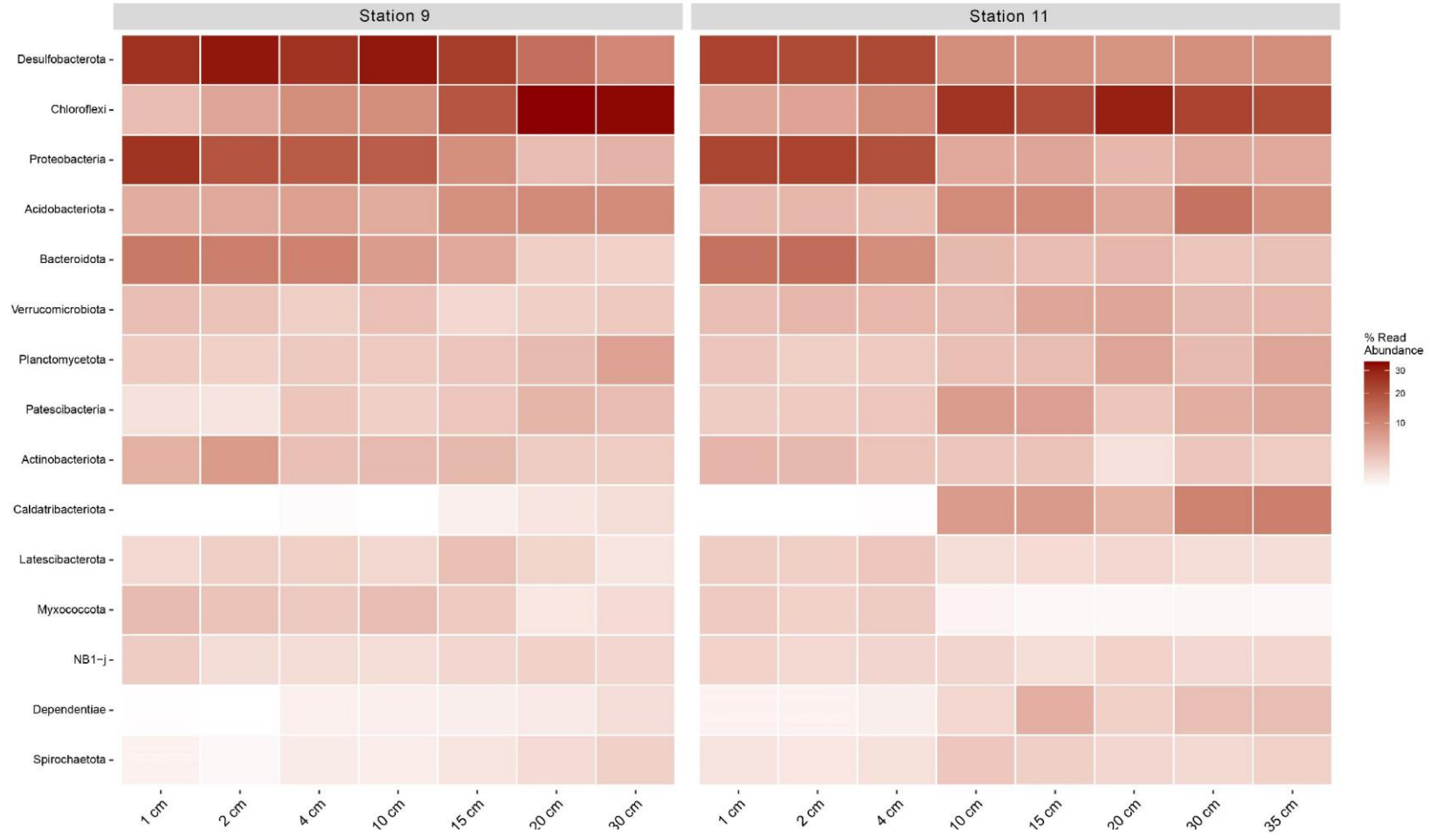

Figure 24. Overview of the taxonomical classification by heatmap according to phylum level

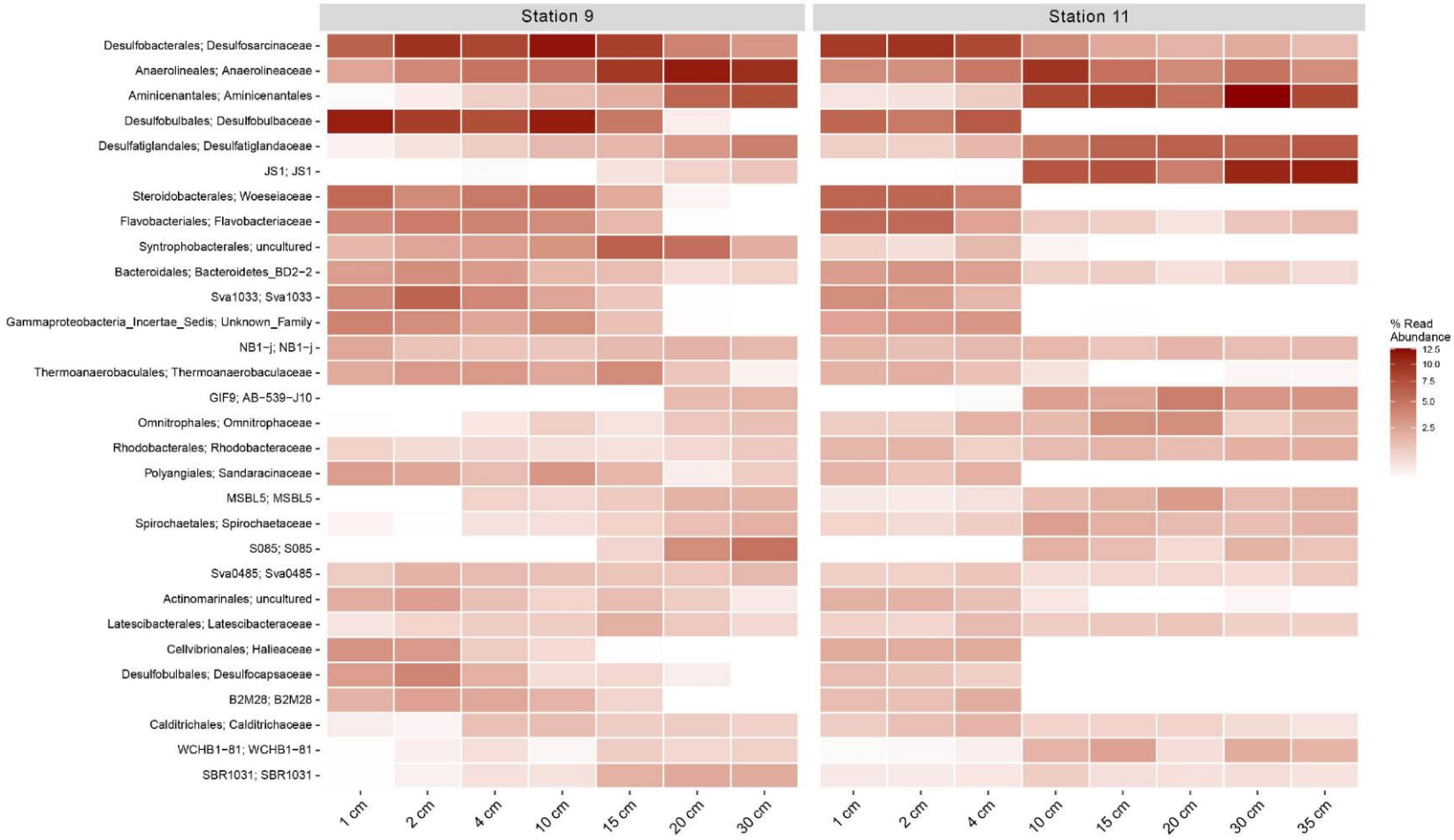

Figure 25. Overview of the taxonomical classification by heatmap according to family level

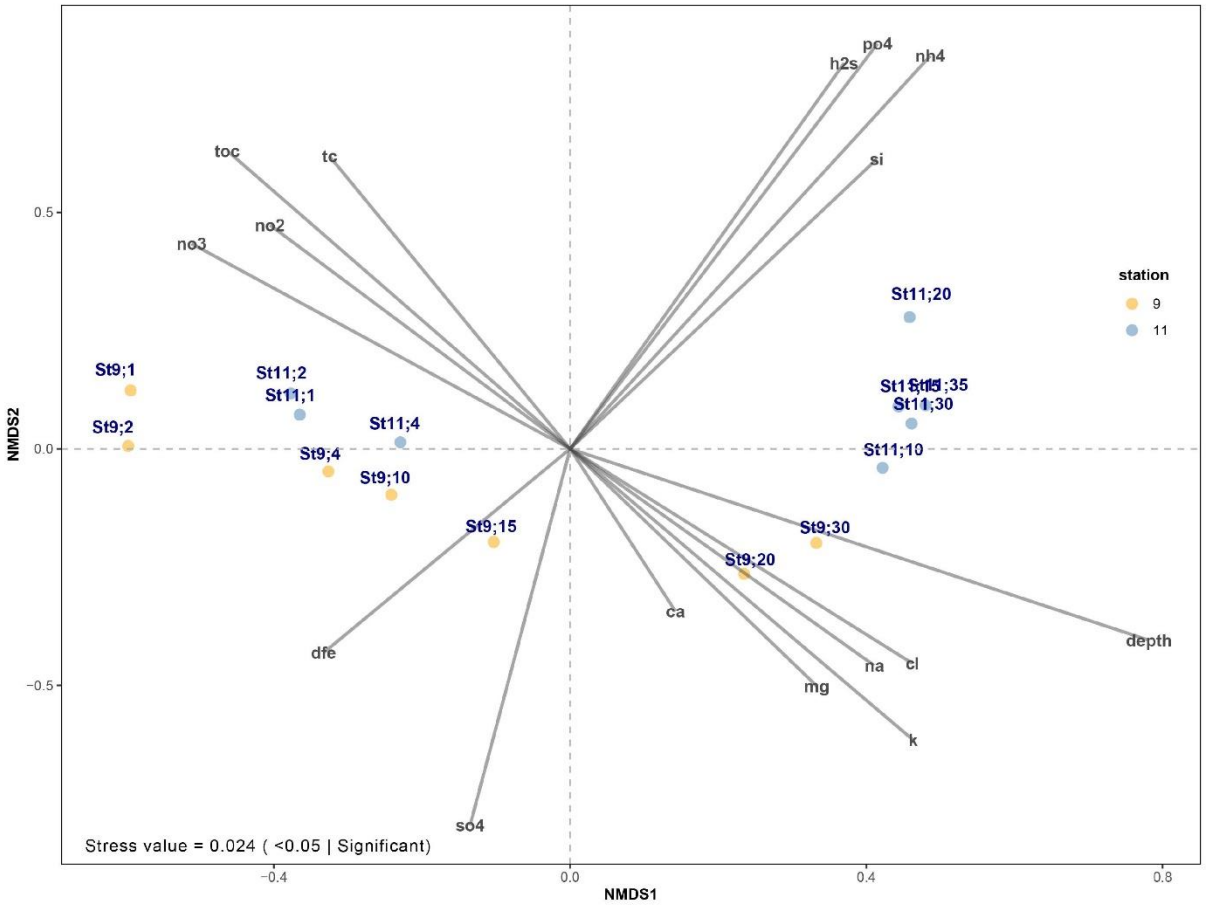

Figure 26. NMDS ordination analysis. Blue and yellow dots indicate Bray-Curtis dissimilarity scores of the samples and the effects of the environmental parameters are shown by gray lines. Stress value is 0.024.

CHAPTER 4

4. DISCUSSION

This study comprehensively researches bacterial diversity in the oxic and anoxic sediment cores in the Black Sea and allows the detailed study of the changes in bacterial community composition over short distances.

4.1. Microbial Diversity

Statistical analysis did not significantly differ between whole sediment samples of St. 9 and St. 11 in terms of alpha diversity. However, beta diversities were different in the stations. These results indicate unique bacterial taxa with similar numbers in each station core. However, they hosted different organisms along with the sediment core. The redox conditions (oxic vs. anoxic) were one of the main driving forces shaping bacterial community composition shown in Figure 26 (NMDS analysis). When all the results were considered, oxygen was the main driver for this differentiation because first 2 cmbsf of the St. 9 was oxic and below oxic layers in St. 9 community compositions were similar to St. 11 according to their redox conditions. Also, there were similar groups even in upper sediment layers. If oxygen were consumed in the water column of St. 9, the sediments of St. 9 and St. 11 may have a similar bacterial community composition.

When vertical diversities were checked, there were statistically significant differences between layers. Diversities and organic carbon contents were much higher in the first 4 cm sediment layer of St. 11 (anoxic). This decrease can be caused by a process put forward by Kallmeyer et al., (2012), which is the proposition that microbial abundance decreases with increasing depth and sediment age, and organic carbon content affects microbial abundance. Anoxic sediment of continental margins with higher organic carbon content hosts more abundant cells than oxic sediment of

open ocean layers with lower organic carbon (D'hondt et al., 2015), and this statement is supported by this study's results when TOC results are compared with alpha diversity metrics. There is more diversity in the sediment with higher TOC content and upper sediment layers except for the 2 cm sediment layer of St. 9, which had the lowest observed ASVs than Station 10.

Bacterial richness was the highest in the surface sediment layer of St. 11, and it can be due to higher organic material (Kallmeyer et al., 2012). Then, in St. 11, 2 and 4 cm samples had similar diversity with surface and 4 cm samples in St. 9, which were the second-highest diversity. Below 4 cm, total richness was similar in all samples. This result confirmed that generally, microbial diversity decreases with increasing sediment depth because of many reasons, such as easily degradable organic matter is consumed rapidly in the upper sediment layers; however, in the deeper sediments, less reactive molecules accumulate, which needs longer time scales and may need particular adaptations to consume less reactive molecules (Biddle et al., 2006).

4.2. Ecological Roles and Ongoing Biogeochemical Processes

In Hoshino et al., (2020) global diversity of sediment microorganisms was investigated, and their results indicated that Proteobacteria were the dominant group in the oxic sediments. Similar to their result, Proteobacteria were predominant in the upper layers (1 to 10 cm) of St. 9 (with oxic interface water). However, Desulfobacterota were higher than Proteobacteria (Figure 25). Nevertheless, while below 4 cm of St. 9 was anoxic, Proteobacteria was the second dominant group below this depth, according to chemical results. Furthermore, even though St. 11 was anoxic, Proteobacteria was one of the most abundant groups in the first 4 cm. This study suggests that the dominance of Proteobacteria was correlated with the total organic carbon content of the sediment layers because the sharp decrease in the TOC content of the sediment layers matches with 10 cm and 4 cm for the Stations 9 and 11, respectively. Their diversity results showed that Chloroflexi, Planctomycetes, and Atribacteria were abundant in the anoxic layer. However, only Chloroflexi was

dominant in both stations below 10 cm and 4 cm for the Stations 9 and 11, respectively, which is a controversial trend of Proteobacteria.

To reveal which environmental parameters drive bacterial community composition along sediment depths, an NMDS analysis was conducted (Figure 26). The communities were divided into two broad groups. Group 1 includes the sediment layers until 15 cmbsf in St. 9 and until 4 cmbsf in St. 11. Group 2 comprises deeper sediment layers: below 15 for St. 9 and below 4 cm for St. 11. The respective groups were associated with 1) organic carbon content, nitrate, dissolved iron and sulfate, 2) hydrogen sulfide, phosphate, ammonium, silicate, and ionic compounds, showing that these environmental parameters drive significantly microbial community composition of marine sediments and a consequence contribute to the biogeochemical reactions in the sediments. These associations between environmental parameters and microbial community composition might result from more reducing conditions occurring in the deeper sediment layers and from oxic to anoxic sediment layers that can decrease nutrient availability and energetically rich substrates with sediment depth (Oni et al., 2015).

In the deep sediments of St. 9 (from 15 cm to 30 cm) and 10 cm of St. 11, Anaerolineaceae was the most abundant family, which was mentioned as it was one of the most frequent groups in petroleum hydrocarbon environments (Liang et al., 2016). This family is essential for organic matter degradation in anoxic sediments, and they have syntrophic relations with methane metabolism organisms. They were also found in oil and heavy metal polluted environments, so they were offered as indicators of ecosystem conditions (Sinkko et al., 2013; Jiang Zhang et al., 2019). Due to the presence of petroleum hydrocarbons in the Black Sea sediments (Balkıs et al., 2012), the dominance of the Anaerolineaceae family may have a relationship with this situation. However, in this study, they occurred in the oxic layers of St. 9 and, also, upper layers of St. 11, and they were mentioned as they have the capacity for aerobic growth (Nakahara et al., 2019).

In the surface layers, mostly known degraders of high molecular weight compounds such as Flavobacteriaceae (Ye et al., 2016), Bacteroidetes_BD2-2 (Li et al., 2020), Sandaracinaceae (Vipindas et al., 2022) families were found, and their abundances decreased with sediment depth. However, low molecular weight compound degraders occurred below the upper layers, such as Sandaracinaceae, Zixibacteria (Campbell et al., 2021), and especially Chloroflexi (Hug et al., 2013) phylum, which was mentioned as essential carbon degraders in the deeper marine sediments, became dominant. This may support the relationship between these taxa and their carbon consumption preferences by stratifying organic molecules according to their molecular weights. Specific utilization of less reactive organic matter may be the lower bacterial diversity in the deep Black Sea sediments (Oni et al., 2015). Additionally, according to Inagaki et al., (2006), the Chloroflexi phylum can potentially have syntropy (relationship of one species relying on the metabolic products of another species) with hydrogenotrophic methanogens or hydrogenotrophic Chloroflexi species (Liang et al., 2015). When the distribution of Chloroflexi is checked on the heatmap (Figure 25), they are found in the more reducing environments such as in SMTZ, and this information supports their activity in the methane present environments. In the upper sediment layers (up to 10 cm), *Bacteroidetes_BD2-2* genus was apparent, and this family's ecological role was suggested as the degradation of high molecular weight compounds (Li et al., 2020). However, below 10 cm, this family was detected in a low abundance, and their suggested interactions with methanotrophic archaea and/or sulfate reducer bacteria may enable them to survive. Sandaracinaceae was reported as a typical aerobic family with some facultative anaerobes (Bradshaw et al., 2020). This family was detected in all sediment cores belonging to St. 9 but only 4 cm in St. 11. They may consume low molecular weight organic matter such as ethanol or acetate and play a role in organic decomposition, leading to C, N, and P transformations in the sediments. Zixibacteria were more abundant in the deeper sediment layers, and the members of this group were detected in anoxic marine sediments (Zinke et al., 2019), hydrothermal vent sites (Dombrowski et al., 2017), and hydrocarbon seep habitats

(Zhao et al., 2020) and by the molecular analysis sulfate reduction pathway was identified in their genome (Vigneron et al., 2021). According to distribution in stations 9 and 11, their sulfate reduction potential can be supported by this study due to other sulfate compounds and sulfate reducer organisms.

Although a high amount of sulfate was measured in St. 9, no H_2S could be measured throughout the sediment core. One alternative explanation may give information about the absence of H_2S: on the one hand, iron (III) was reduced by microorganisms in the sediment; on the other hand, bacteria reduced sulfate. The products of these two reactions were iron (II) and HS^-, which precipitate as FeS, so H_2S does not occur. Another possible alternative is HS^- oxidation by iron oxides so H_2S cannot accumulate (Jørgensen et al., 2019). As mentioned above, iron reducer, sulfate reducer, and sulfate oxidizer bacteria were detected in St. 9, so microbiome results support this situation instead of oxidation of samples mistakenly during samples.

Below 15 cm, GIF9 order (family AB-539-J10) increased in St. 11 and increased below 20 cm in St. 9; however, their abundances decreased below these depths, and GIF9 was offered as a candidate to have a role in the fermentation of plant polymer (Hug et al., 2013) and syntrophic relationships with methanogens to oxidize hydrocarbons (Cohen, 2021). GIF9 was mentioned as it tends to be found in the rich organic sediment layers and was offered as a candidate for chemoautotrophic growth (Coskun et al., 2018). In the euxinic depths of the Cariaco Basin, GIF3 order was identified. Although GIF3 members had a higher abundance in the bottom layers of the core, their values were lower than GIF9 in the bottom layers. However, in the middle depths, GIF3 had a higher abundance than GIF9. *Napoli-4B-65* genus was present below 10 cm of St. 11 and 20 cm of St. 9.

In the deeper sediment layers of St. 11 (30 and 35 cm) JS1 group was prevalent, and this group is accepted as a representative group in methane hydrate environments (Lee et al., 2018). In St. 11, when SO_4^{2-} was almost absent, H_2S peaked at 35 cm, and this zone is accepted as the sulfate methane transition zone, which consists of overlap of both sulfate and methane profiles in low concentrations (Leloup et al.,

2007). According to Inagaki et al., (2006), the JS1 group was more abundant in methane hydrate sites than in hydrate-free sites. Therefore, 30 cm of St. 11 can be accepted as the start of SMTZ. Besides methane hydrate zones, JS1 is found in organic-rich habitats related to hydrates and sulfate depleted zones. This situation suggests that JS1 may have metabolic flexibility with another metabolism besides sulfate reduction. In this study, TOC concentration increased significantly below 20 cm. In addition, the relative abundance of JS1 increases parallel to TOC concentration. This result offers that bacterial communities stratified in marine sediments and geochemical features (e.g., pore water compounds, sulfate concentration, the molecular weight of organic matter) impact community composition in subseafloor sediments.

Although microbial separation according to their roles occurs throughout the redox ladder, in the organic matter-rich sediments, sulfate reduction and methanogenesis can coexist (Mitterer et al., 2001). Even if there is no detected methane, there may be methane consumption because of the relationship between methane oxidizers and sulfate reducers (Boetius et al., 2000). Below this depth, $H_2 - CO_2$ methanogenesis intensifies, and acetate becomes the apparent substrate for methanogenesis, which means organic matter activation in that depth plays a warning role in carbon release (Wellsbury et al., 1997). The acetate can be the product of anaerobic methane oxidation or recycling of the cells (Wellsbury et al., 2000).

By the 4 cm depth from the sediment surface, families related to methanogenesis or syntrophic with methanogens became apparent. Anaerolineaceae had around 5% abundance in the 4 cm layer in both stations. Their relative abundance increases with depth in St. 9; however, it peaked at 10 cm and decreased below 10 cm in St. 11. Members of Anaerolineaceae were reported to have syntrophic interactions with methanogenic Archaea, which uses acetate to transform methane and CO_2 (Liang et al., 2016). Genus *Desulfatiglans* were also abundant in the mid-deep layers of St. 11, and their abundances were reported to increase by increasing SO_4^{2-} and decreasing organic carbon substances (Vipindas et al., 2022). However, they also perform acetogenesis in energy and SO_4^{2-} limited sediment layers. Spirochaetaceae was

detected in the 10 cm of St. 11, and they have sulfate-reducing potential in the methanogenic conditions (Trembath-Reichert et al., 2016). Relative abundance of Aminicenentales increases with depth below 10 cm in St. 11 and below 20 cm in St. 9. This increase shows that they tend to be in reduced conditions such as sulfate and methane zones. Their relationship with hydrogenotrophic methanogens was suggested, and they were abundant in high SO_4^{2-} conditions, which may provide substrates to sulfate reducers (Ara et al., 2020). Other bacterial groups related to sulfur metabolisms decreased, and methane metabolism-related groups increased apparently below 15 cm. This information shows that in St. 11, methane occurs below 15 cm and sulfate reducers and methane oxidizers coexist. However, St. 9 had these groups below 20 cm with lower abundances than St. 11, so SMTZ may not be reached in St. 9 up to 30 cm.

In the upper sediment layers of the stations, similar taxa were apparent. In St. 9, the Desulfobulbaceae family was dominant in the surface sediment. The family's ecological roles are related to acetate assimilation in the oxic, suboxic transition zone, or sulfate reduction to sulfite in the anoxic zones (Dyksma et al., 2018). Cable bacteria, which have filaments to connect electron acceptor and donor molecules, were detected in this layer. Two known genera (*Candidatus_Electrothrix* and *Candidatus_Electronema*) have filaments, but the novel studies expect novel discoveries. In this study, *Candidatus_Eletrothrix* was detected to belong to the Desulfobulbaceae family. Although their relative abundance was lower than 1%, metabolic rates and their contribution to the biogeochemical cycles are unknown. They reduce oxygen or NO_3^- in the upper zone to sulfide oxidation in the deeper and anoxic sediment habitats. According to chemical results, there is oxygen and nitrate in the first 4 cm of St. 9. However, no hydrogen sulfide was detected throughout the whole sediment core of St. 9. It can be hypothesized that cable bacteria transform hydrogen sulfide to sulfate, so this can be one of the reasons for the failure to detect H_2S produced in a small amount. SO_4^{2-} concentrations can support this hypothesis. When there was a sharp increase in SO_4^{2-} concentrations, cable bacteria were detected until 10 cm depth from the surface layer. Oxygen depletion is an ongoing

problem in all the seas and oceans, and depletion of oxygen sometimes causes the occurrence of H_2S, which is toxic to complex organisms (Jørgensen & Jorgensen, 1980). While metal oxides can oxidize H_2S by several reactions conducted by microorganisms (Kristensen et al., 2003), the dangerous situation can be buffering (Zhang et al., 2010).

Additionally, cable bacteria in sediments can be beneficial for the life in the water column by trapping H_2S by oxidation with oxygen or nitrate. With the increasing hypoxia threat in the seas, oxygen depletion has become an essential issue for many biological and economic purposes. Studies revealed that cable bacteria would be more critical soon for the balance in oxygen-depleting regions in the seas (Nielsen, 2016).

On the other hand, the Desulfobulbaceae family was detected in the first 4 cm layers of St. 11. They may reduce sulfate to sulfite. Genus *Sulfurovum* became apparent in the mid-depths of St. 9 (4-15 cmbsf) and 4 cm in St. 11. *Sulfurovum* was offered to sulfur compounds reduction with denitrification, and this can be the case in St. 9; however, due to the absence of nitrate in St. 11, their capacity of sulfide dependent chemolithoautotrophy can be another strategy for their survival. They also have sulfide oxidation capacity, playing another ecological role in sediments.

Other dominant taxa, for St. 9, had essential roles in sediment geochemistry. For example, Desulfosarcinaceae was abundant in surface sediments with fresh organic matter, and they can oxidize sulfur compounds in oxygen minimum zones (Vipindas et al., 2022). However, the same family was much higher in St. 11 than in St. 9. Due to the anoxic conditions of St. 11, they may reduce sulfate to H_2S. *SEEP-SRB1* genus from the Desulfosarcinaceae family was detected in all depths. This genus was reported as sulfate reducers, and they may have syntrophic relationships with anaerobic methane oxidizer (ANME) archaea. This genus was obtained from cold-seep environments in other studies. However, they were thought to be responsible for non-methane hydrocarbon degradation (Petro et al., 2019). The relative

abundance of *SEEP-SRB1* increases with depth up to 5.5% in St. 9 until 15 cm but stays at around 2% until 10 cm, then decreases and disappears in St. 11.

Due to nitrate (only in St. 9) and dFe in the upper sediments, microbial clades were detected related to iron and nitrogen metabolisms. *Woeseia* (genus) was relatively abundant in the upper sediment layers, especially in St. 9 (up to 15 cm in St.9 and 4 cm in St. 11). This family was related to denitrification in the oxic-anoxic transition zone, and N_2O emissions were detected because of *Woeseia* activity (Bacosa et al., 2018; Mußmann et al., 2017). Due to $NO3^-$ in the 4 cm in St. 9, they may be responsible for NO_3^- depletion, and their sulfur oxidation capacity may help their survival in anoxic St. 11. Gammaproteobacteria_Incertae_Sedis (order) and *NB1-j* (genus) had a similar trend with *Woeseia,* and their nitrate reduction and nitrifying capacity were suggested in different sources. *NB1-j* was detected in the suboxic zone of the Black Sea water column (Fuchsman et al., 2011). SVA1033 and Desulfocapsaceae families were detected in a decreasing trend by the depth and disappeared in reduced conditions. Their abundance trend matches dFe concentrations and sediment cores, and they have iron reduction capacity.

Furthermore, uncultured members of the Actinomarinales order were reported in Mn nodules and Fe-rich sediments (Krause et al., 2020). They have the capacity to conduct the feammox process, which involves ammonium oxidation with iron oxides to transform NH_4^+ to NO_2^- and/or N_2(Rios-Del Toro et al., 2018). When SO_4^{2-} concentration graph (Figure 17) and changes in Actinomarinales relative abundance along with sediment cores, this order may impact increasing SO_4^{2-} levels along with sediment due to sulfide oxidation by iron oxide reduction.

Below 10 cm in St. 11 and 20 cm in St. 9 hosted AB-539-j10 family belongs to the GIF9 order, and this family can be responsible for autotrophy connecting CO_2 fixation with acetogenesis in the sediments. However, its ecological roles are unknown (Coskun et al., 2018). Even though Spirochaetaceae was detected in almost all depths except in 1 and 2 cm of St. 9, they were apparent in the similar depths of *AB-539-j10*.

Syntrophobacterales were present in all sediment layers of St. 9 and the first 4 cm of St. 11. This family was reported as strict anaerobes in previous studies with the capacity for sulfate reduction and complete oxidation of organic matter to CO_2. Even its absence in the possibly methane present layers can fuel methanogenic archaea (In 't Zandt et al., 2019). The presence of oxygen in the interface water in St. 9, at least surface sediment was oxic; however, Syntrophobacterales was detected so that they may have facultative aerobic or aerobic species in the group.

SBR1031 order was absent in the first 2 cm of St. 9; however, they were present in two stations in other sediment layers. This order was suggested with their sulfate reduction and biofilm production features (Wang et al., 2022). SBR1031 was relatively abundant in the sediment layers between 15-30 cm of St. 9 but had a very low abundance below 15 cm in St. 11. This can be explained by their sulfate reduction potential because there was abundant SO_4^{2-} in St. 9 at that depth. However, low SO_4^{2-}in St. 11 and other sulfate reducers may win the competition with this family.

S085 (family) was detected in oxic clay containing sediment layers of the South-Eastern Mediterranean Sea, and their ecological roles are unknown (Rubin-Blum et al., 2022). In contrast to that study, this group was detected in anoxic sediment layers in the two stations, and they were absent in the upper sediments (first 10 cm in St. 9 and first 4 cm in St. 11). This situation indicates that S085 may have anoxic metabolism under reduced conditions.

Some sulfur reducers but predominantly methane metabolism-related groups became dominant below 20 cm of St. 11. In St. 9, N and Fe related groups disappeared, and sulfur-related metabolisms became dominant below 15 cm. Especially deep layers of St. 11, primary producers with chemoautotrophic features. The rate and amount of primary production in marine sediments should be measured for environmental effects and to understand the food web in the marine sediments.

Apart from the generally known ecological roles of the dominant taxa, there were many taxa with little to no information. Reporting unknown roles of the groups'

presence would be essential to help reveal their roles in further studies. Rhodobacteraceae was present in each sediment layer in each station. This group was highly abundant in the pelagic zone of the marine environments, and they were mentioned as they can be found in anoxic habitats. Although their ecological roles are unknown, different metabolism potentials were suggested, such as sulfur oxidation and CO_2 oxidation (Pohlner et al., 2019). *Ilumatobacter* was present in St. 9 from surface sediment to 30 cm, and its relative abundance reached ~2% in 2 and 10 cm samples. The members of the *Ilumatobacter* genus were reported as aerobic (Matsumoto et al., 2009); however, in this study, its members were found in the anoxic zone of the Black Sea sediments. Uncultured members of the *B2M28* genus were present primarily in both stations' upper sediment layers. Their relative abundance reached ~2.5 % in the 2^{nd} cm of St. 9 and ~2 % in the 4^{th} cm of St. 11. Calditrichaceae members were present in each sediment layer. *SM23-31* and *Calorithrix* genera and *Cloacimonetes_bacterium* species were detected. In the deeper sediment layers, Omnitrophaceae members were detected. Below the 15 cm of St. 11, its relative abundance reached ~3.5%, and 30 cm of St. 9 peaked at around 1.5 %. They were not detected in the upper zone of St. 9. Their magnetotactic capacity was reported and detected in groundwater samples (Bruno et al., 2021). *WCHB1-81* became apparent by the mid-depth in both stations, and its relative abundance increased by depth. Their ecological roles are unknown, but they were reported in contaminated CH_4 and hydrocarbon habitats (Karaevskaya et al., 2021). Thermoanaerobaculaceae was abundant in the upper sediment layers in both stations. They were reported in hypoxic marine sediment layers, and their possibility was suggested to have a critical role in the N cycle. Although members of this family were detected in oxic layers of St. 9, their metabolisms were mentioned as anaerobic and may have syntrophy with Archaea in the previous studies (Vipindas et al., 2022). Members of phylum Latescibacterota were detected in all depths. There were three different groups detected to belong to this phylum. Members of Latescibacteria class and Latescibacterota behaved controversially, such as while the one had a low abundance other one had a higher abundance. In general, the class of Latescibacteria

was more abundant than other classes, and Latescibacterota was more abundant in the upper sediment layers than Latescibacteria. While *MSBL5* family members had very low abundance in the upper zone, their relative abundances increased by depth. In the previous studies (Vuillemin et al., 2020), they were mentioned as anaerobic, which parallels this study's results. In 20 cm of St. 11, genus *Thermoflexus* was detected with ~1.5% relative abundance. In the previous studies, this group was accepted as thermophilic bacteria and dependent on complex organic matter (Thomas et al., 2021). *Sg8-4 (genus), Vermiphilaceae* (genus), and *Dojkabacteria* (genus) were apparent in the bottom layers of each core. RuBisCo enzyme was detected in *Dojkabacteria,* which had H_2 and metal transformation potential (Hernsdorf et al., 2017).

Oceans have crucial roles in buffering capacity against climate change (Sundquist et al., 1979). By the photosynthesis taking place in the euphotic layer, oceans control most of the atmospheric CO_2 and O_2 fluxes and, consequently, organic matter burial in the sediments. Either oceanic, terrestrial, or atmospheric inputs can be the sources of organic matter in the water column. Terrestrial material is transported to the oceans via river discharges, mainly. River discharge is exceptionally high in the Black Sea basin. Hence, Station 9 and Station 11 are expected to represent the anthropogenic pressures that oceans are facing.

Most of the organic matter that arrives at the basin is consumed, and the remaining particles sink and get buried. Although organic matters produced in the euphotic layer of the water column sink to the deeper ocean, higher than 99.5% are remineralized throughout the water column, and around 0.5% to 1% are buried in the marine sediments (Burdige, 2007). In high aerobic respiration, lower organic matter preservation takes place (Archer et al., 2002). As the carbon emissions increase due to the anthropogenic impacts, the buffering characteristic of the oceans becomes more crucial. On the flip side, oceans are depleted in oxygen, and the dead zones increase. With the presence of more anoxic conditions, as represented by Station 11 sampled in this thesis, the sediment bacterial compositions change towards releasing greenhouse gasses. This comparison highlights the importance of bacteria and the

implications on carbon burial hence climate change rates studying the community compositions.

The surface layer of the marine sediments plays an important role in the fluxes from/to the water column in terms of oxygen and nutrient budget. For example, organic matter can be consumed aerobically, and CO_2 production decreases porewater pH, which can dissolve the calcium carbonate and increase the effects of organic matter oxidation. Methanogenic microorganisms consume buried organic matter to produce CH_4. Oceanic habitats behave as a buffering system that controls the atmospheric O_2 and CO_2 levels. Changes in the organic matter production and/or atmospheric gases will affect the sediment-water interface exchanges and surface water atmosphere. This study shows that there are different amounts of organic carbon in St. 9 and St. 11. Due to the sampling from close areas (about 1.5 miles) under similar surface water properties, the presence and absence of oxygen determine the organic carbon concentrations in the stations. As aerobic respiration causes low preservation of organic matter, Station 9 had lower content. Combining microbial diversity and organic carbon results in sediment suggests that different bacterial groups can produce different products by utilization of organic matter:

1) $CH_3COO^- + SO_4^- > 2HCO_3^- + HS^-$

2) $CH_3COOH > CH_4 + CO_2$

According to bacterial distribution along with sediment cores, there was similar diversity in both St.9 (below oxic slices) and St. 11, so a decrease in the oxygen concentration in the water column of St. 9 can cause changes in electron acceptors according to the redox ladder. As shown in Figure 6, various microbial redox reactions can produce greenhouse gases or toxic products for higher trophic levels. Combining microbial abundance and diversity data with their metabolic rates would be an essential input for the ecosystem assessment models to determine the current situation and predict future changes spatially and temporally.

Increasing temperatures and nutrient concentrations promote more primary production to produce organic matter in the euphotic zone. Aerobic degradation of organic matter decreases oxygen concentrations in the water column and causes expansion of hypoxic regions because of the enhanced oxygen demand. Also, higher temperatures in the oceans decrease O_2 and CO_2 solubility, so atmospheric fluxes from water would increase. As microbial abundance is correlated with sedimentation rate and organic matter concentrations, the microbial dynamics that are altered with the changing environment have significant effects on the alteration of elemental cycles. Therefore, the metabolic diversity of sediment bacteria is critical to be researched further to predict their effect in the face of a climate crisis. Seeing as the microbes have such an essential role in the carbon budget of the oceans, future projections will have a better foundation if their buffering/ greenhouse gas-producing shifts are well calculated and integrated into models. To achieve this, further knowledge should be accumulated in the oxic and anoxic sediment conditions, which is one of the main aims of this study and unveiling the metabolic plasticity of these bacteria, which should be the focus of the research effort that follows.

According to NMDS analysis (Figure 26), organic carbon is one of the main drivers of community composition. Warming in the atmosphere will cause more stratification in the water column and prevent the upward and downward exchange of dissolved organic carbon, nutrients, and gases. Changes in the organic carbon budget in the sediments can cause differentiation in bacterial community composition. The reported metabolisms belong to abundant families of the sediment slices with lower organic carbon content. They are mostly related to sulfate reduction and methane oxidation which produces important greenhouse gas, CO_2.

4.3. Comparison with Other Seas

Ince et al., (2006) collected two shallow sediment samples, and their organic carbon content in the surface sediment was around 2%, respectively. St. 9 had 2.79% (dw) in this study, and St. 11 had 3.3% (dw) organic carbon. They found that members of Desulfosarcinaceae and Desulfobulbaceae were dominant sulfate reducer bacterial groups which were also dominant in St. 9 and St. 11. On the other hand, they collected four sediment cores with more than 1700 m water columns above the sediment. Their results were that *Desulfococcus* and *Desulfosarcina* were two abundant genera in all stations. They found that sediment core depth did not affect the sulfate reducer bacterial diversity, but organic carbon changed the abundance of sulfate reducer bacteria. In this study, relative abundances and dominant families changed along with the sediment core. Their method directly targeted sulfate reducer bacteria; this study is not that precise in terms of sulfate reducer organisms due to targeting whole bacterial communities instead of sulfate reducers.

Figure 27. Sampling locations of the previous studies in the Black Sea sediments.

Suominen et al. (2021) collected sediment samples from anoxic Baltic Sea sediments and divided the sample core into 4 sections (1-2 cm; 10-11 cm; 20-21 cm; 25-26 cm). According to their 16S rRNA V4 amplicon sequencing results, in the surface sediment Deltaproteobacteria (30.4%) was the most abundant bacterial group, then

followed by Bacteroidetes (10.8%), Planctomycetes (9.5%) and Chloroflexi (6.1%); in their deepest sample, Chloroflexi (28.8%) was the most abundant bacterial group, and followed by Planctomycetes (24.3%) and Atribacteria (8.2%). There were similarities and dissimilarities between the Baltic Sea and the Black Sea anoxic sediments. The significant difference was Deltaproteobacteria which was absent in both Stations; however, in the surface sediment of St. 11, Gammaproteobacteria was the dominant class. Bacteroidetes group, similarly to Baltic Sea sediment, had 11.1%, and Chloroflexi also had a similar abundance (6%). Planctomycetota was much less (2.7%) than in the Baltic Sea. When we come to the deep layer of St. 11, Chloroflexi was the most dominant taxa. Its abundance peaked at 29.9% in 20 cm. Planctomycetota had much less abundance (5.9%), and there were no Atribacteria detected in St. 11's deep sediments, contrary to the Baltic Sea. In the Baltic Sea, almost three-fold more TOC content in the dry weight of surface sediment; however, in the Black Sea sediments, NH_4^+ was almost 2.5-fold higher.

Desulfobacterales is known as sulfate reducer (Pfennig et al., 1981), and this group was abundant in all sediment layers of Bothnian Sea samples (Rasigraf et al., 2020) peaked at SMTZ. In this study, they were present in all layers. However, their abundance decreased with depth, so detected members of Desulfobacterales may not be involved in anaerobic oxidation of methane (ANME) reactions as happened in the Bothnian Sea study.

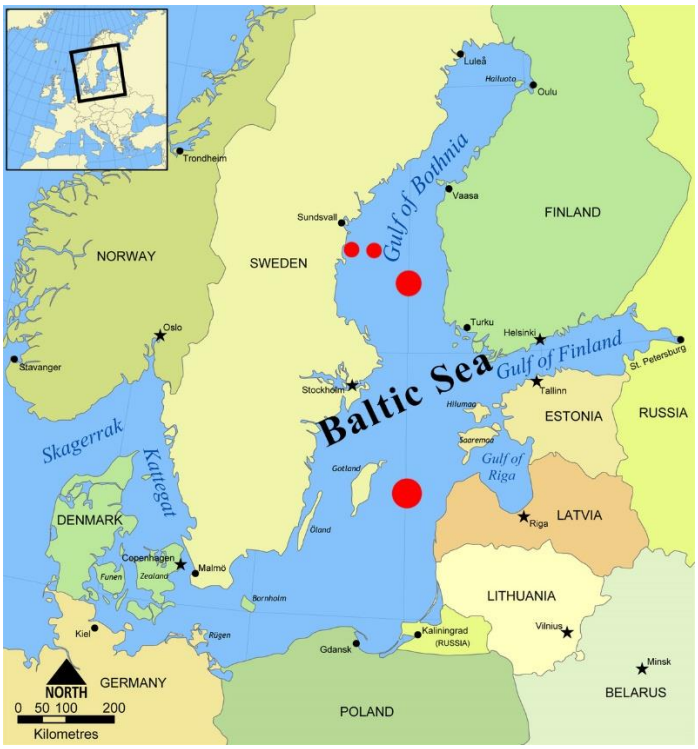

Figure 28. Sampling locations of the previous studies in the Baltic and Bothnian Sea.

All given information above clearly indicates that St. 9 had similar microbial diversity to St. 11 except for upper sediment layers. When microbial diversity is checked from surface to bottom layers of St. 9, the change from less reduced to a more reduced environment can be seen, and microbial diversity results support this change. The upper layers of St. 9 had unique diversity compared to St. 11; however, the mid-depths of St. 9 started to be similar to the upper layers of St. 11 and continued this trend down to the deepest sediment layer of St. 9.

5. CONCLUSION

The Black Sea has an important role, especially for microorganisms living in suboxic and anoxic environments. It is the most extensive euxinic sea on Earth and allows the investigation of anoxic metabolisms in large water bodies and sediment layers. Due to interactions between organisms and their environments, environmental parameters should be included in studies to get more realistic results. Three sediment cores with oxic, suboxic, and anoxic interface water were collected from the southwestern part of the Black Sea; however, DNA isolation and sequencing failed in the suboxic sediment core, and 15 sediment slices were analyzed as duplicate samples from oxic and anoxic cores. Nutrients, organic carbon and nitrogen, and major ion analyses were performed to understand the biogeochemical context of the microbial communities. Microbial communities were analyzed by amplicon sequencing of V3-V4 regions of bacterial 16S rRNA. Finally, taxonomical and statistical analyses were conducted by QIIME 2 and R. Due to the presence of oxygen in the upper layers of the oxic core, community composition differed; however, general diversity was not statistically different between the two cores. Ordination analyses were matched with detected taxa and their ecological roles. The microbial community in the upper and middle sediment layers were related to more energetic molecules. After consumption of the oxygen in the oxic sediment, bacterial community composition shifted through anaerobic bacterial groups similar to anaerobic station's bacterial composition. This study contributes to the microbial diversity record in the Turkish Seas, reveals the effects of environmental parameters on bacterial community structure in the sediments, and can be a baseline study to understand the community shift from oxic to anoxic habitats.

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7. APPENDICES

A. Raw Data Statistics

Software: Illumina package bcl2fastq

* Fastq Quality Encoding: Sanger Quality (ASCII Character Code = Phred Quality Value + 33)

Appendix A

Sample	Total Bases	Read Count	GC (%)	AT (%)	Q20 (%)	Q30 (%)
1-A2	210,634,984	699,784	55.33	44.67	86.02	75.69
1-A3	220,106,250	731,250	55.70	44.30	83.76	72.72
1-A5	206,393,292	685,692	55.06	44.94	86.91	76.80
1-A8	198,810,500	660,500	55.26	44.74	84.95	73.73
1-A9	172,181,632	572,032	55.92	44.08	86.16	75.83
1-A10	205,651,026	683,226	56.22	43.78	85.61	74.87
1-A12	225,140,776	747,976	56.16	43.84	85.77	75.03
1-D2	264,376,126	878,326	54.62	45.38	86.97	76.88
1-D3	175,236,180	582,180	54.60	45.40	85.90	75.01
1-D5	185,549,644	616,444	54.87	45.13	86.03	75.24
1-D8	206,281,922	685,322	54.90	45.10	86.51	75.94
1-D9	170,105,334	565,134	54.43	45.57	86.50	75.86
1-D10	155,216,068	515,668	54.73	45.27	84.86	73.40
1-D12	232,876,476	773,676	54.56	45.44	86.23	75.51
2-A2	218,603,056	726,256	54.96	45.04	86.63	76.32
2-A3	202,742,162	673,562	55.15	44.85	86.79	76.76
2-A5	195,896,820	650,820	54.86	45.14	85.46	74.67
2-A8	205,061,668	681,268	55.16	44.84	86.23	75.71

2-A9	197,381,954	655,754	55.21	44.79	86.31	75.80
2-A12	212,034,634	704,434	55.89	44.11	85.27	74.37
2-D2	256,686,780	852,780	54.41	45.59	86.73	76.51
2-D3	197,442,756	655,956	54.45	45.55	86.62	76.44
2-D8	164,674,692	547,092	55.04	44.96	86.28	75.59
2-D9	95,267,704	316,504	54.87	45.13	84.29	72.82
2-D10	154,707,980	513,980	54.85	45.15	85.51	74.46
2-D12	186,146,226	618,426	54.94	45.06	85.45	74.40
2-D13	227,264,632	755,032	54.38	45.62	87.01	76.64

B. Bray-Curtis Dissimilarity Matrix

Appendix B. Bray Curtis Dissimilarity Matrix Between Samples.

Bray-Curtis Dissimilarity Matrix Between Samples

	St.11-1	St.11-10	St.11-15	St.11-2	St.11-20	St.11-30	St.11-35	St.11-4	St.9-1	St.9-10	St.9-15	St.9-2	St.9-20	St.9-30	St.9-4
St.11-1									0.5652	0.6494	0.753	0.5752	0.9168	0.9378	0.5988
St.11-10	0.9256							0.9004	0.9332	0.9108	0.855	0.926	0.7712	0.7702	0.8934
St.11-15	0.9374	0.596						0.9172	0.9466	0.932	0.8966	0.9426	0.8206	0.798	0.9168
St.11-2	0.5024	0.923	0.935			0.9396		0.6082	0.5942	0.665	0.7396	0.5992	0.9046	0.9386	0.6048
St.11-20	0.9564	0.7112	0.6654	0.9552		0.657		0.9452	0.9628	0.9562	0.9268	0.9616	0.8572	0.8464	0.9452
St.11-30	0.9386	0.6228	0.5474					0.922	0.949	0.934	0.907	0.9426	0.8402	0.812	0.9282
St.11-35	0.947	0.659	0.5872	0.943	0.6504	0.5384		0.9314	0.9502	0.9404	0.9236	0.948	0.8606	0.8358	0.9306
St.11-4	0.6008								0.6266	0.5942	0.6762	0.6166	0.863	0.9038	0.5616
St.9-1	0.5652	0.9332	0.9466	0.5942	0.9628	0.949	0.9502	0.6266		0.6254	0.741		0.9152	0.9432	0.6004
St.9-10	0.6494	0.9108	0.932	0.665	0.9562	0.934	0.9404	0.5942							0.555
St.9-15	0.753	0.855	0.8966	0.7396	0.9268	0.907	0.9236	0.6762		0.6222					0.6148
St.9-2	0.5752	0.926	0.9426	0.5992	0.9616	0.9426	0.948	0.6166	0.464	0.5748	0.668		0.8846	0.9336	0.5566
St.9-20	0.9168	0.7712	0.8206	0.9046	0.8572	0.8402	0.8606	0.863		0.837	0.689				0.8348
St.9-30	0.9378	0.7702	0.798	0.9386	0.8464	0.812	0.8358	0.9038		0.8952	0.815		0.6394		0.8992
St.9-4	0.5988	0.8934	0.9168	0.6048	0.9452	0.9282	0.9306	0.5616							