Impact of saltwater intrusion on paddy soil microbial communities

Dissertation

zur Erlangung des Grades eines

Doktor der Naturwissenschaften

(Dr. rer.nat.)

des Fachbereichs Biologie

der Philipps-Universität Marburg

Vorgelegt von

Mehrdad Rabiei Nematabad

Aus Täbris, Iran

Marburg an der Lahn

2023

The experimental study for this thesis was conducted at the Max Planck Institute for Terrestrial Microbiology, Department of Biogeochemistry, under the guidance of PD Dr. Werner Liesack from November 2017 to April 2021. Afterward, the project was supervised by Mr. Prof. Dr. Robert Junker from November 1st, 2022, to March 31, 2023.

Vom Fachbereich Biologie der Philipps-Universität Marburg (Hochschulkennziffer 1180) als Dissertation angenommen am _____

Erstgutachter(in):   Prof. Dr. Robert Junker

Zweitgutachter(in):   Prof. Dr. Lars Voll

Tag der Disputation: _____

Dedicated to my family.

Table of contents

Summary

The rice farming in wetlands worldwide is facing a significant threat from soil salinization caused by the infiltration of saltwater due to the rising sea levels in coastal areas. The high concentrations of salt in the soil pose a serious risk to the viability of arable land as most crops are not tolerant to such conditions. Despite numerous studies on the growth and yield of rice in saline conditions, little is known about how soil salinity affects the microbial communities, their compositions, and functions in rice paddy soil. Rice cultivation is one of the major sources of methane emission globally due to the use of rice straw as an organic fertilizer. This source accounts for about 10% of the world's methane budget. The microbial communities in the paddy soil can effectively break down rice straw in the absence of oxygen, with the rate-limiting stage being the breakdown of bio-polymers. Seawater intrusion leads to reduced carbon availability and increased recalcitrance of organic matter, resulting in decreased soil CO_2 and CH_4 production and lower enzyme activity involved in the hydrolysis of cellulose and the oxidation of lignin. The aim of my thesis research is to (i) investigate how intermediate salinity affects the structure and function of methanogens, sulfate reducers, and bacterial communities in paddy soil under anoxic conditions, (ii) assess the long-term impact of NaCl and seasalt treatments on microbial communities.

At the beginning, microcosm slurry was set up with 40 gr of the Philippine paddy soil 35 ml distilled water, which amended with 0.5 gr rice straw. The bottles containing microcosm incubated 30°C for seven days as a preincubation. On day seven (week = 0) some microcosms treated with NaCl and seaalt at 150 mM then incubated up to week six. During the experiement, we measured gases and fatty acids concentrations, and also evaluated the copy numbers of three marker genes (16S rRNA, *dsrB* and *mcrA*) and their transctipts. Later, taxonomy assignment at different levels was perfomed.

The NaCl and seasalt treatments at 150 mM had a significant impact on the production of CH_4 and CO_2. In particular, the concentration of these gases was considerably reduced in the group that received the seasalt treatment, as compared to both the control group and the group treated with NaCl. As for the concentration of H_2S gas among the three groups, it was heightened in the seasalt treated group but lower in both the control and NaCl treated groups. The processing of fatty acids digestion (namely, acetate, butyrate, and propionate) was observed to be slower in both the NaCl and seasalt treated groups when compared to the control group. The experimental findings revealed that the NaCl treatment hindered the digestion of propionate and butyrate to a greater extent than the seasalt treatment. Conversely, the digestion of acetate was found to be more impeded in the seasalt treated group compared to the NaCl treated group.

The application of the two salt treatments had a discernible impact on the genes and transcript copies of bacteria, methanogens, and sulfate-reducing bacteria, as revealed by the RT-qPCR and qPCR assays carried out using the three primer sets (namely, 16S rRNA, mcrA, and dsrB). With the exception of the *dsrB* gene and transcript assays, the groups treated with seasalt displayed fewer copies compared to the other two groups. Upon analyzing the 16S rRNA gene at the phylum level, it was found that the treated groups exhibited a higher relative abundance of *Firmicutes* in comparison to the control group. Additionally, the implementation of both the NaCl and seasalt treatments resulted in an escalation of the relative abundance of *Actinobacteria* and *Chloroflexi* in

the 16S rRNA transcript's phylum level. Conversely, the utilization of the two salt treatments caused a decline in the relative abundance of *Proteobacteria* during the entire course of the experiment. In the *dsrB* gene at the phylum level, the two treatments exhibited a marked increase in the relative abundance of *Firmicutes* during the first and second weeks. Similarly, the application of both NaCl and seasalt treatments resulted in an increase in the abundance of *Methanosarcinales* at the order level of the *mcrA* gene. Using order-level mcrA transcripts, the analysis showed an increase in *Methanomassiliicoccales* and *Methanosarcinales* in both the NaCl and seasalt treated groups, while *Methanocellales* demonstrated a decrease throughout the experiment.

The present study investigated the salt treatment effects on the functional and growth characteristics of the Philippine paddy soil microbial communities, including bacteria and methanogens. Our results demonstrate that the treatments disrupted the metabolic functions of the microbial communities, as evidenced by the delayed processing of fatty acid digestion biologically, such as acetate, propionate, and butyrate, and also they mitigated the methane and carbon dioxide concentrations. Furthermore, we observed that intermediate levels of salinity (150 mM; NaCl and seasalt) had an impact on the microbial community structure in anoxic condition. Specifically, the treatments led to a higher diversity of microbial taxa at the 16S rRNA gene, transcript, and *mcrA* gene levels when compared to the untreated control group. Conversely, the dsrB gene and transcript levels were reduced following treatment with intermediate salinity. Of note, we observed an increase in H_2S gas concentration in the seasalt-treated group, which was reflected in the *dsrB* gene expression level resulted in sulfate reduction. These findings underscore the importance of salinity as a key environmental factor in shaping microbial community structure and function. The observed disruption of metabolic functions highlights the potential impact of saltwater intrusion due to sea-level rise or tidal changes on the soil microbial community, which in turn can have significant implications for ecosystem functioning and productivity by changing biogeochemical cycles.

1-Introduction

Soil salinization is a widespread problem in coastal, arid, and semi-arid regions, and it has been shown to have a significant impact on soil microbial communities. Soil microbes are essential for many critical ecosystem processes, including nutrient cycling, decomposition, and soil structure formation. Therefore, changes in the microbial community structure and function can have significant consequences for soil health and productivity. This chapter of my PhD thesis provides an overview of the threats posed by salt stress on soil microorganisms in coastal regions from seawater intrusion, going through the basic knowledge. Additionally, I will generally discuss how salinity influences H_2S gas concentration as well as greenhouse gases such as methane and carbon dioxide production by modifying the structure and function of certain paddy soil microbial guilds, including methanogens and sulfate-reducing bacteria (SRB). Moreover, I will offer additional information on my thesis with relevant topics. The scientific topics pertaining to my thesis are categorised and organised under the following headings:

1.1 The looming climate crisis: understanding the relationship between methane, global warming, and greenhouse gases in the context of rising sea levels

Global warming is referred to as the increase in the Earth's average surface temperature due to increases in the concentration of greenhouse gases (GHGs) such as water vapour, methane, ozone, carbon dioxide, chlorofluorocarbons (CFCs) and nitrous oxide (Venkataramanan, 2011). The greenhouse effect is the main reason why the earth is a suitable place to live; without greenhouse gases, the temperature of the earth's surface would be too low and there would be no life on earth. However, the increase in the amount of greenhouse gases in the atmosphere led to this catastrophic phenomenon, global warming (Anderson et al., 2016). The Earth's atmosphere consists primarily of nitrogen, oxygen, and argon, with small amounts of other gases, including greenhouse gases. The percentage of permanent gases (nitrogen, oxygen, and argon) does not change, while the percentage of trace gases (carbon dioxide, methane, nitrogen oxides, and ozone) changes daily, seasonally, and annually (Khandekar et al., 2005). Greenhouse gases have the ability to absorb and re-emit infrared radiation due to the internal vibrational modes of their atoms, unlike the other main components of the atmosphere (Oktyabrskiy, 2016).

The concentration (i.e., mole fraction) of methane (CH_4) in the atmosphere continues to increase. The average global increase of 14.7 ppb observed in 2020 was the largest in four decades. Since 1750, its relative concentration has increased twice as fast as carbon dioxide (CO_2) and is now more than 2.5 times pre-industrial levels (Lan et al., 2022). Methane is the second most important anthropogenic greenhouse gas after CO_2; the radiative forcing attributable to its direct (0.64 Wm2) and direct plus indirect effects (0.97 Wm2) is 38% and 58%, respectively, of the 1.68 Wm2 for CO_2 (Change, 2014). Global methane emissions approached a record of 600 Tg CH_4 per year-1 in 2017, with anthropogenic sources accounting for 61% of the total (about 365 Tg CH_4 per year; (Jackson et al., 2020a, 2020b; Saunois et al., 2020).

Analysis of in situ sea temperature data collected over the past 50 years from ships and more recently from argo profiling floats (Roemmich et al., 2009) shows that the ocean has been warming and the upper ocean heat content (OHC) is increasing. Therefore, sea level has risen significantly since 1950 due to the thermal expansion of seawater (Domingues et al., 2008; Ishii and Kimoto, 2009; Levitus et al., 2009; Church et al., 2011). The coastal zone, serving as a transitional zone between the ocean and land, plays a crucial ecological

role in connecting marine and terrestrial hydrological ecosystems. As sea levels rise and seawater intrusion (SI) becomes more prevalent, the process of salinization, which involves the excessive accumulation of soluble salts in soils, can adversely affect soil microbial communities, leading to detrimental impacts on environmental health and crop yield (Oryza sativa L.).

1.2 Effect of salt stress on coastal life

Plant response to salt stress depends on the plant growth stage and the duration and intensity of the stress (Zeng and Shannon, 2000; Ali et al., 2004; Hussain et al., 2012, 2013; Hariadi et al., 2015). Exposure of rice to harmful salt stress affects plant growth through (i) osmotic stress, (ii) ionic toxicity, (iii) nutrient disparity, and (iv) cumulative and interactive effects of all these factors (Ashraf and Harris, 2004; Flowers and Blumen, 2005; Siringam et al., 2011). Effects of salt stress on rice growth, nutrient uptake and transport, carbon fixation, and grain formation. When the concentration of toxic levels is reached, it leads to senescence, clipping or death of the leaves (Tester and Davenport, 2003; Horie et al., 2012; Hairmansis et al., 2014). The accumulation of these toxic ions like Sodium (Na^+) and chloride (Cl^-), in high concentrations, injures membranes and other organs, resulting in erratic growth and plant death (Davenport et al., 2005; Quintero et al., 2007; Saqib et al al., 2012). Despite the moderate sensitivity of many crop species, including rice, to salt in the field, it is crucial to investigate the long-term effects of soil salinization and resulting seawater intrusion on microbial communities in paddy soil under anaerobic condition. Previous research has not adequately addressed this critical knowledge gap.

1.3 Seasalt compositions and ions toxicity

Soil salinity refers to the concentration of salts in the soil solution, consisting of four main cations (i.e. Na^+, K^+, Mg^{+2} and Ca^{+2}) and five main anions (i.e. HCO_3^-, Cl^-, NO_3^- , SO_4^{2-} and CO_3^{2-}). Soil salinity is characterized by the concentration and composition of soluble salts and is most commonly measured in the laboratory as the electrical conductivity of the saturation extract in dS m^{-1} (Corwin and Yemoto, 2017). Soil salinity accumulation can lead to reduced plant growth, reduced yields and in severe cases, crop failure. Salinity limits water uptake by plants by reducing osmotic potential, making it more difficult for the plant to extract water. Salinity can also cause specific ion toxicity effects (e.g. Na^+ ion toxicity) depending on soil pH and disrupt the nutritional balance of plants. The salt composition of the soil water influences the composition of the cations on the exchange complex of the soil particles, which affects the soil permeability and soil topsoil. Sodium chloride (NaCl) is the main component of seasalt and can have toxic effects on soil microbes at high concentrations. It can cause dehydration and interfere with the osmotic balance of the cells, leading to cell death. However, at intermediate concentrations, the effects on microbial commuinites of paddy soil require more investigation.

1.4 Determination of bacterial metabolic types by pH and E_H

Redox potential (E_h) clearly affects the development of microorganisms. As early as 1934, Heintze proposed using variations in soil E_h to characterize groups of microorganisms (Heintze, 1934). Bacterial growth correlates directly with changes in E_h (Kimbrough et al., 2006). Microbial and enzymatic activity are negatively correlated with E_h in anaerobic soils (Snakin and AG, 1980; Kralova et al., 1992; Brzeziska, 2004). In addition, the redox state of nodules is considered a referee of legume-rhizobium symbiosis (Marino et al., 2009). Each microorganism type is adapted to specific E_h conditions and is characterized by

its ability to evolve in a broader or narrower E_h range. For example, anaerobic bacteria can only develop in a narrow range of very low E_h values. Aerobic microorganisms such as *Actinomyces* sp. or *Azotobacter* sp. require a higher E_h but can develop over a much larger range (Rabotnova, 1963). Fungi develop more than bacteria under moderately reducing conditions ($E_h > +250$ mV), while bacteria are more abundant than fungi under strongly reducing conditions ($E_h < 0$ mV) (Seo and DeLaune, 2010).

Oxidation-reduction conditions are classically evaluated by measuring the redox potential (E_h), expressed in volts. The zero point for the E_h scale is set by the standard hydrogen electrode (SHE) with the redox couple H^+/H_2. E_h is commonly used in a variety of disciplines dealing with living organisms such as (Szent-Gyorgi, 1957; Mathis, 1995; Gurin, 2004), hydrobiology and the study of marine ecosystems (Meadows et al., 1994), soil science (Chadwick and Chorover, 2001) and physiology and ecophysiology (Dietz, 2003).; Foyer and Noctor, 2005; Lambers et al., 2008; Dessaux et al., 2009; De Gara et al., 2010). Depending on the discipline, E_h and pH are measured on different scales and for different substrates: organelles, cells, plants, rhizosphere, soil, sediments, soil solution or water.
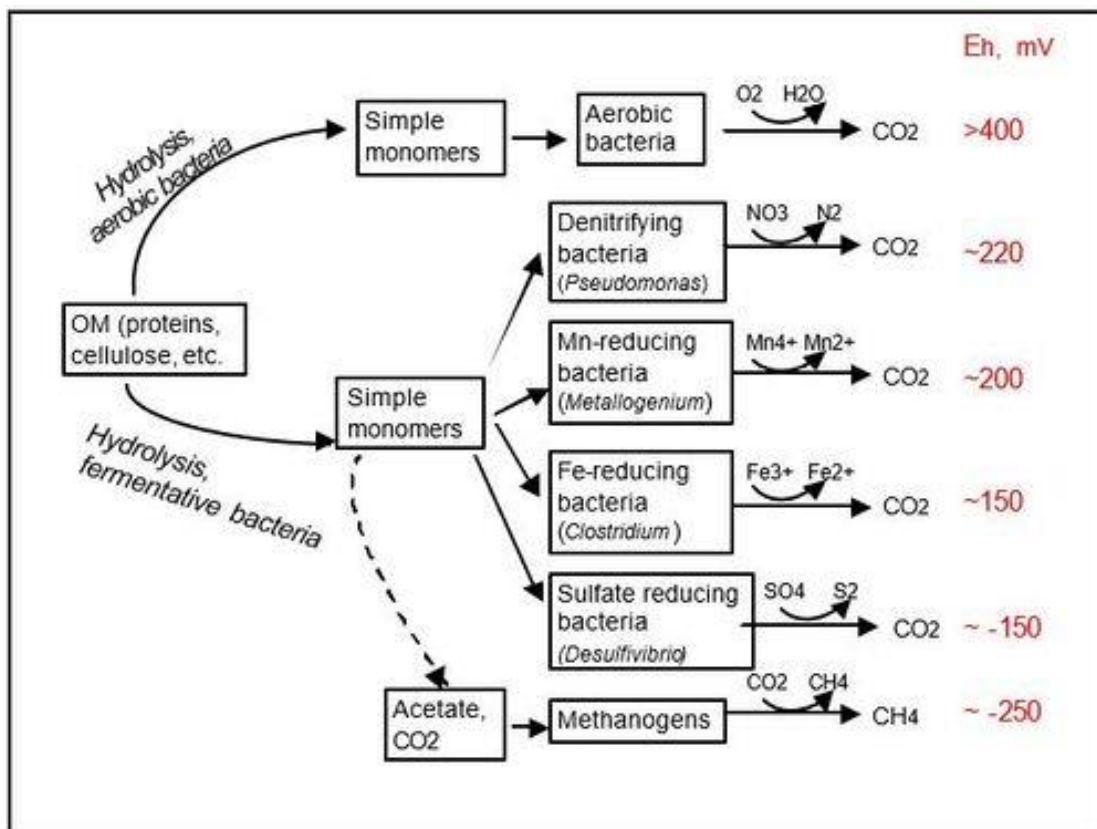
Figure. 1.1. Redox potential is the tendency for a reaction, specifically the movement and transfer of electrons, to occur spontaneously and is reported as E_h in mV (Barich, n.d.).

Redox potentials (E_h) drop rapidly upon inundation in tidal wetland sediments, influencing the buffering capacity of chemical and biochemical processes that support organic carbon mineralization (Mishra et al., 2003). Odum, (1988) also indicated that Eh was lower in saline marshes than in freshwater marshes, and attributed the lower E_h to the presence of HS^-.

Saltwater intrusion can occur through lateral encroachment from coastal waters, vertical movement of saltwater near discharging wells, and withdrawals of freshwater from the groundwater system. Soil oxidation reduction (redox) potential readings can be taken to assess the effects of saltwater intrusion on soil potential redox (Chambers et al., 2014).

1.5 Wetland soils and biogeochemical cycles

1.5.1 Carbon cycle

Biogeochemical processes occurring in wetland soils can be important to the local, regional, and global cycles of elements including carbon, nitrogen, phosphorus, and sulfur. These processes can be influenced by changes in environmental conditions such as temperature, soil moisture, oxygen (O_2) availability, nutrient supply and salinity (Updegraff et al., 1998; Sundareshwar et al., 2003; Baldwin et al., 2006; Bridgham et al., 2008). Environmental changes can have direct impacts on biogeochemical transformations (e.g. the presence of O_2 inhibits methanogenesis; (Segers, 1998)) or the impacts can be indirect and driven by interactions between ecosystem components (e.g. addition of nutrient increases plant productivity and subsequent O_2 transport to the subsoil, thereby enhancing methane (CH_4) oxidation (Keller et al., 2006b)). Rates of C accumulation generally account for a small fraction of the total C inputs to a wetland (Lindroth et al., 2007; Megonigal and Neubauer, 2019), indicating that the vast majority of C inputs are mineralized or is otherwise removed (Mitra et al., 2005). As previously discussed, the carbon cycle can be influenced by environmental changes. Abiotic stressors, such as salt stress in paddy fields, can affect biogeochemical cycles, including the carbon and sulfur cycles. Specifically, the impact of intermediate salinity levels on paddy soil can be critical for these cycles and for the reduction of sulfate, which could be studied through fatty acids and gene expression levels.
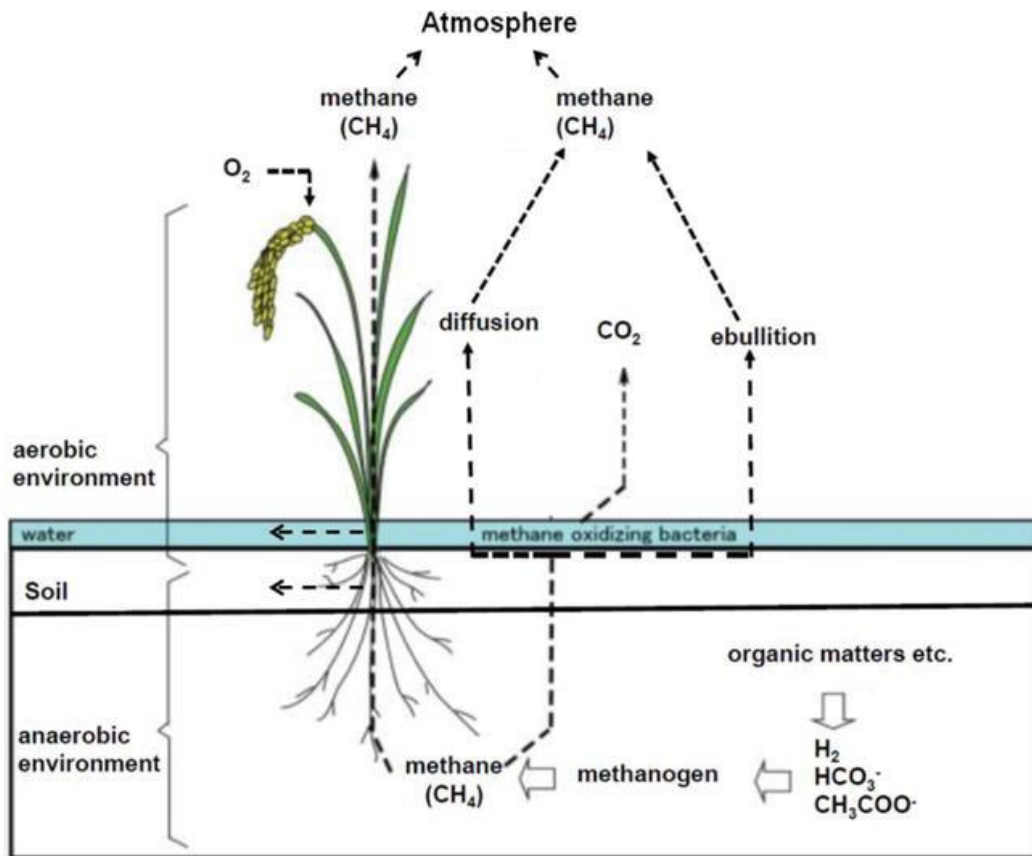
Figure. 1.2. Biogeochemical mechanisms of CH$_4$ production of paddy field and it's cycling (Rahman and Yamamoto, 2020).

1.5.2 Sulfur cycle

Gaining a comprehensive understanding of the interplay between the biotic and abiotic responses in wetlands has been a major challenge in engineered wetland (CW) engineering and operation. The success of the traditional black-box approach to CW operation is a testament to the aforementioned innate versatility of these systems, but a broader understanding of microscale wetland processes would clearly support their design. Research over the past decade has shed light on the complexities of wetland microbiology and geochemistry, but many questions remain. Several recent researchers have identified sulphur-related processes in wetlands as both poorly understood and of paramount importance in promoting a broader understanding of wetland function (Whitmire and Hamilton, 2005; Wiessner et al., 2005). Sulfur can occur in four valences, 2(H_2S), 0(S0), +2($S_2O_3^{2-}$) and +6(SO_4^{2-}); Hence, it is reactive under both oxidized and reduced conditions and in both biotic and abiotic environments. It can function as an electron donor or electron acceptor in energy-producing microbial reactions and reacts with virtually all metals (except gold and platinum) to form metal sulfides. Sulfur is also a macronutrient for the growth of microorganisms and plants. It is typically abundant in CW influents, including municipal and industrial effluents and particularly acid rock drainage. The high reactivity, redox sensitivity, and microbial activity of sulfur combined with the range of conditions found in many CWs results in complex geomicrobial interactions.
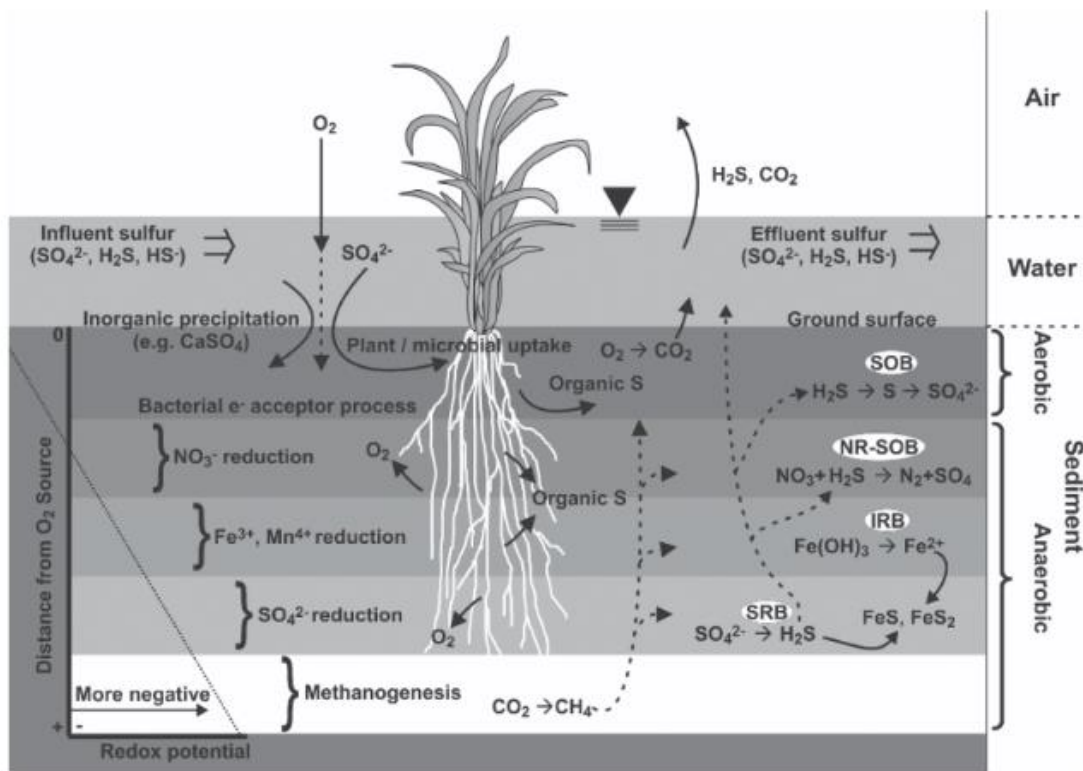
Figure. 1.3. Major biotic and abiotic sulfur transformations in constructed wetlands and their relation to redox potential (Sturman et al., 2008).

Dissimilatory sulfate reduction is considered one of the oldest metabolic processes of life on earth and is found in a large number of gram-positive and gram-negative genera of bacteria. SRB are a phylogenetically diverse group of *Proteobacteria*, comprising over 20 genera and utilizing a range of organic electron donors including H_2, volatile fatty acids (VFAs), and some primary alcohols. SRB are divided into two main groups: (1) incomplete oxidizers (*Desulfovibrio*, *Desulfomicrobium*), which use VFAs such as pyruvate, formate and butyrate and produce acetate; and (2) complete oxidizers (*Desulfobacter*, *Desulfobacterium*) that utilize fatty acids, including acetate, and produce carbon dioxide (Widdel, 1988). It was previously thought that SRBs could only grow using sulfate as an electron acceptor and only in the absence of dissolved oxygen. Recent research has shown that some SRBs can grow using higher-energy electron acceptors, particularly nitrate (Ito et al., 2002; Lopez-Cortes et al., 2006). In addition, some SRBs have been shown to be both low oxygen tolerant and oxygen detoxification mechanisms (Vasconcelos and McKenzie, 2000). Such properties generally ensure the survival of SRB populations in sediments that are periodically (or seasonally) exposed to oxygen or other electron acceptors. The catabolic bacterial sulfate reduction reaction produces one mole of sulfide per mole of sulfate used, as illustrated in the following stoichiometry with acetate as the electron donor:

$$CH_3COO^- + H^+ + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$$

SRB activity also leads to the generation of alkalinity, which can increase the pH of acidic systems. It is important to note that sulfate reduction does not occur in isolation, but rather in concert with other microbial responses, including fermentation and methanogenesis. The use of organic acids as electron donors by SRB implies a close relationship between SRB activity and the activity of fermentative organisms that produce VFAs as a metabolite. These reactions create a strongly reducing environment that can enrich reduced

inorganic species such as Fe^{2+}, Mn^{2+}, NH^{4+} and CH_4 in sediments in addition to sulfide and bicarbonate. Biogenic sulfide can undergo further biotic and abiotic reactions with these compounds. Dissimilatory sulfate reduction can account for half or more of the total organic carbon mineralization in many settings (Jürgensen, 1982).

Salinity can affect sulfate reducing bacteria (SRB) in soil. SRB are a group of anaerobic bacteria that use sulfate (SO_4^{2-}) as an electron acceptor in the process of anaerobic respiration. The presence of high levels of salt can impact the ability of these bacteria to carry out their metabolic processes. Seawater is a source of sulfate that can stimulate the activity of sulfate reducing bacteria (SRB) and lead to competition with methanogenic microorganisms. Interactions between methanogens and SRB at intermediate seawater (150 mM) needs to investigate in paddy soil for a long-term, therefore, further investigation is required to fully understand the mechanisms underlying these interactions.

1.6 Products of Anaerobic Digestion

Anaerobic Digestion (AD) is a biological process that degrades organic matter through the concerted actions of a variety of microbial communities in the absence of oxygen. In a simplified description, AD is divided into four phases: hydrolysis, acidogenesis (acid production), acetogenesis (acetic acid production) and methanogenesis (methane production). (Ngan et al., 2020).
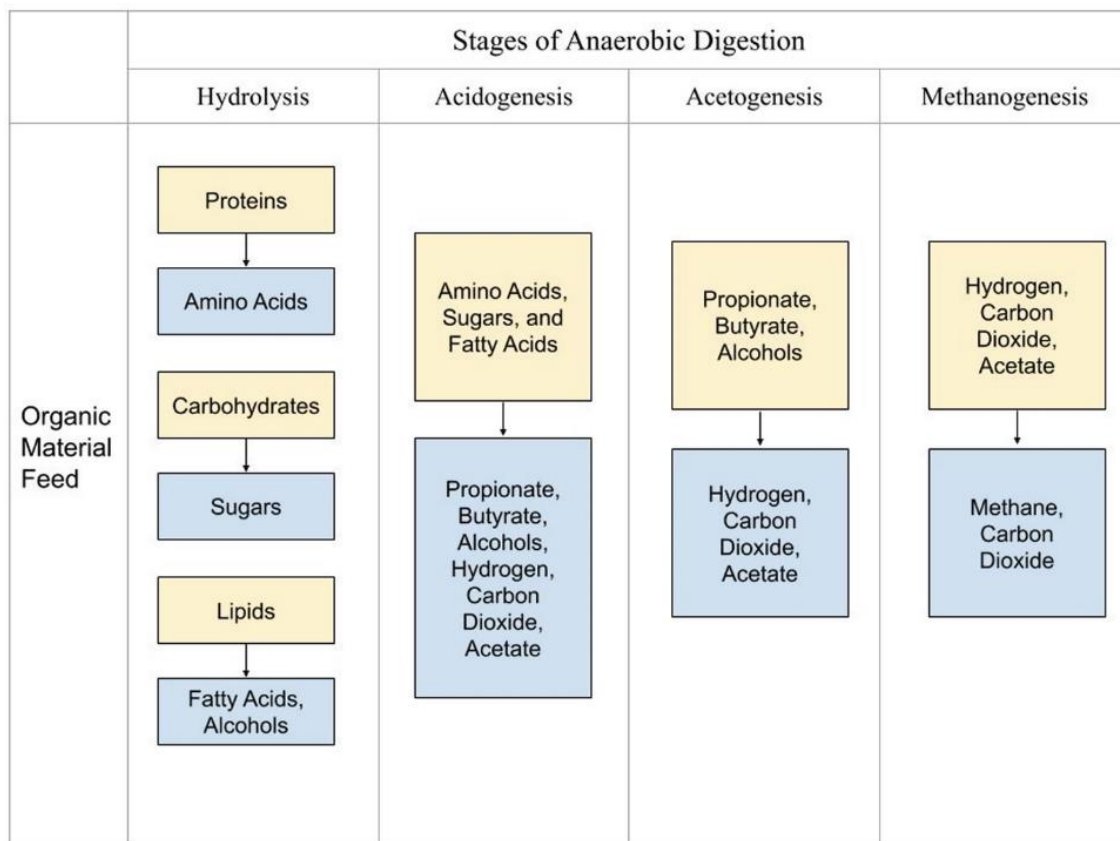
Figure. 1.4. Four stages of anaerobic digestion (AD) process from hydrolysis to methane production (Ngan et al., 2020).

1.6.1 Stage 1: Hydrolysis

Insoluble organic compounds such as cellulose, protein, fat and some insoluble forms of organic compounds are broken down by enzymes (produced by bacteria) and anaerobic bacteria. Small soluble organic molecules produced in this step are the starting material for the bacteria in the next step. Carbohydrate hydrolysis can occur in a matter of hours, while protein and fat hydrolysis can take several days. However, lignocellulose and lignin substances are slowly and incompletely degraded (Deublein and Steinhauser, 2011). Facultative anaerobes consume dissolved oxygen in the water, resulting in a reduction in redox potential that is favorable for the AD process. In this phase, carbohydrates are broken down into simple sugars; Fats are broken down into fatty acids; and proteins are broken down into amino acids (Eastman and Ferguson, 1981; Gerardi, 2003).

1.6.2 Stage 2: Acid-Producing (Acidogenesis)

Simple organic compounds generated during the hydrolysis phase are converted by anaerobes into volatile fatty acids (VFAs), long chain fatty acids, propionate and butyrate (Jrdening and Winter, 2005). The H^+ concentration formed at this stage can affect the fermentation products. A high concentration of H^+ reduces the production of acetate. In general, simple sugars, fatty acids and amino acids are fermented in this phase to form organic acids and alcohol (Gerardi, 2003).

1.6.3 Stage 3: Acetic Acid-Producing (Acetogenesis)

The products from the previous stage are the substrate for bacteria in the acetic acid-producing stage. The products of these intermediate substrates are H_2, CO_2 and acetate. At this stage, acetogenic bacteria grow together with methanogenic bacteria.

1.6.4 Stage 4: Methane-Producing (Methanogenesis)

During this phase, methane is produced under fully anaerobic conditions. This reaction is considered an exothermic reaction. Stage 4 can be divided into two methane production processes: reduction of CH_3COO^- and conversion of H_2 with CO_2. Acetotrophic methanogens are responsible for the reduction of acetate (CH_3COO^-) to methane, while hydrogenotrophic methanogens are responsible for the conversion of H_2 and CO_2 to methane (Ziemiski and Frc 2012). High salt and ammonium concentrations adversely affect biological processes such as anaerobic digestion (Kargi and Dincer, 1996; Chen et al., 2008; Fang et al., 2011; Townsend, 2018). High salt concentrations dehydrate bacterial cells due to osmotic pressure (Alhraishawi and Alani, 2018). Salt toxicity is primarily determined by the type of cation that the salt has.

Wetlands play a crucial role in contributing to atmospheric CH_4, with the production of biogenic CH_4 being regulated by methanogens, as observed by Conrad in 2007 and 2009. Methanogenic microorganisms display variations not only in their CH_4 production pathway and potential but also in their response to intermediate salinity levels, as demonstrated by Jetten, Stams, and Zehnder in 1992. In the UK, Webster et al. (2015) reported a decline in methanogen diversity from low-salinity brackish sediments to high-salinity marine sites. However, the impact of intermediate salinity (150 mM) with two types of salts (NaCl and seasalt) on methanogenic archaea in paddy soil is yet to be fully evaluated (Tong et al., 2017; Yuan et al., 2019).

1.7 Rice straw properties and its compositions

The use of rice straw depends on its properties, which can be divided into three main categories: (1) physical properties, (2) thermal properties, and (3) chemical composition. Physical properties include bulk density, heat capacity, and thermal conductivity. Density is most relevant to handling and storing rice straw. thermal properties and calorific value; these properties are relevant when biomass is converted into energy.

Chemical composition such as lignin, cellulose, hemicellulose/carbohydrate and nutrient levels are relevant to applications such as forage and soil fertility. The characterization of rice straw is useful for life cycle analysis and efficiency calculations. The most common methods for characterizing rice straw can be referenced by the National Renewable Energy Laboratory (NREL) and the American Society for Testing and Materials (ASTM).

1.10.1 Physical Properties

Based on various studies, the bulk density of rice straw can vary depending on the different forms it can take. Loose rice straw collected directly from the field can have a density of 13 to 18 kg m^3 in dry matter (dm) (Migo, 2019). Chopped straw with a length of 2 to 10 mm (Chou et al., 2009) can have a density range of 50 to 120 kg m^3 (Liu et al., 2011) depending on the equipment used. Depending on the baler equipment used, the size of the baled straw and the compression ratio, and therefore the bulk density, will vary. A round bale of rice straw with a length of 70 cm and a diameter of 50 cm has a bulk density of 60 to 90 kg m^3 TS (Van Nguyen et al., 2016). The density of rice straw briquettes with a diameter of 90 mm and a thickness of 7-15 mm is 350450 kg m^3 dm (Munder et al., 2013). The density of rice straw pellets with a diameter of 8 mm and a height of 30-50 mm is 600700 kg m^3 dm (Nguyen et al., 2018).

Compared to rice husk, which has a density between 86 and 114 kg/m^3 (Mansaray and Ghaly, 1997), unprocessed, loose rice straw has a low density. This means a higher volume per kilogram, which means higher shipping and handling costs, as well as more complications in processing, transport, storage and incineration (Liu et al., 2011; Duan et al., 2015). The rice straw volume can be reduced through processing, but this requires additional energy input. Various crushing methods can increase the density of the straw, including the use of pellet mills (Nguyen et al., 2018), roller presses, ram presses, dicers, briquette presses, screw extruders, tableters and agglomerators (Satlewal et al., 2018). When used for bioenergy, the bulk density of rice straw affects the combustion process as it affects the time needed in the reactor (Zhang et al., 2012). Rozainee et al., (2008), as quoted by Zhang, Ghaly, and Li, (2012), reported that low bulk density causes poor mixing and uneven temperature distribution (unfavorable operating conditions), which reduces energy efficiency. The moisture content of rice straw is an important consideration when deciding how it is processed and what it is used for. For example, the moisture content affects the calorific value of the straw, which is important if the by-product is to be used as bioenergy. In addition, if the rice straw volume is to be reduced, the moisture content before compression should be between 12 and 17% (Kargbo et al., 2010). The moisture content can vary greatly due to the type and duration of straw storage (Topno, 2015).

1.10.2 Chemical Compositions

The chemical composition determines the nutritional quality of rice straw, which is important for animal feed, anaerobic digestion and as a soil conditioner. Rice straw has low nutritional value and research has been done to improve it. Jenkins, Bakker and Wei (1996) pointed out that the typical components of plant biomass are moisture cellulose, hemicelluloses, lignin, lipids, proteins, simple sugars, starches, water, hydrocarbons, ash and other compounds. The concentrations of these compounds depend on the plant species, tissue type, growth stage and growth conditions. Rice straw is considered a lignocellulosic biomass containing 38% cellulose, 25% hemicellulose and 12% lignin (Yokohama and Matsumura, 2017). Compared to the biomass of other plants such as softwood, rice straw contains less cellulose and lignin and a higher content of hemicellulose (Barmina et al., 2013).

1.8 Microbial communities of paddy fields

In flooded rice, the need to maintain adequate water depth throughout most of the crop year characterizes the farming system as aquatic. Compared to other aquatic environments such as lakes, ponds and swamps,

the environmental conditions in flooded paddy fields are relatively unstable due to physical, chemical and biological properties that vary depending on current agricultural practices and water supply (Shibagaki-Shimizu et al., 2006). The different physical and chemical properties in this environment could support the growth of microorganisms that possess wide ranges of metabolic plasticity, allowing them to rapidly adapt to changing environmental conditions (Bernhard et al., 2005). Thus, the rice ecosystem can be a prime habitat for microorganisms adapted to fluctuating levels of nutrition and oxygen and light availability.

The phyla *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Bacteroidetes* and *Proteobacteria* have previously been found in soil samples from rice-alfalfa (Lopes et al., 2014) and rice-wheat crops (Lopes et al., 2014). In these agroecosystems, rice exudates and nutrients from straw incorporation have been shown to affect bacterial community composition. Breidenbach and Conrad (Breidenbach and Conrad, 2015) found a uniform bacterial composition in the soil during the rice-growing season, with *Proteobacteria* being the most abundant phylum, while *Christensenellaceae* being the fifth most abundant phylum. *Christensenellaceae* were also present at relatively low frequency when a maize rotation was introduced into an irrigated paddy field (Breidenbach et al., 2016). Although these microorganisms are common inhabitants of agricultural soils, the small numbers of *Christensenellaceae* are intriguing given that this group includes the classes Bacilli and *Clostridia*, which are often very common in agricultural rice soils, where they decompose plant debris with cellulolytic enzymes (Koeck et al., 2014). Furthermore, researchers observed higher bacterial 16S rRNA abundance in the flood phase than in the drainage phase, which was attributed to the rice straw left in the field (Itoh et al., 2013).

The bacterial communities in rice soils have been studied using both crop-independent and crop-dependent molecular techniques (Janssen et al., 1997; Grokopf et al., 1998; Chin et al., 1999; Henckel et al., 1999). Kimura et al., (2001) reported on gram-positive bacteria as the main decomposer of rice straw incorporated into microcosms of rice soil under submerged conditions. In contrast, both gram-negative bacteria and fungi have been implicated in leaf sheath and blade decomposition under oxic conditions in upland soils (Nakamura et al., 2003). Stable RNA isotope studies revealed that the bacteria that actively assimilate C from pulse-tagged rice plants are *Azospirillum* spp. (*AlphaProteobacteria*) and members of the family Burkholderiaceae (Beta*Proteobacteria*). These organisms were abundant in the rice root environment (Lu et al., 2006). Asakawa and Kimura (2008) compared bacterial community structures in different habitats in a Japanese paddy field ecosystem by comparing the DGGE profile data, and they found that dominant bacterial communities varied in diversity and stability and were phylogenetically distinct from each other in their respective habitats. Matsuyama et al., (2007) and Sugano et al., (2005) studied the bacterial community in plant debris in a Japanese paddy field using Denaturing Gradient Gel Electrophoresis (DGGE) and Terminal restriction fragment-length polymorphism (T-RFLP). They found that members of *Christensenellaceae* (Clostridia), α-, γ-, and δ-*Proteobacteria*, *Nitrospira*, *Acidobacteria*, *Bacteroidetes*, *Verrucomicrobia*, and *Spirochaetes* were the predominant microorganisms in the rice residues. In addition, Tanahashi et al. (2005) VERB on the presence of members of these groups during the decomposition of rice straw compost when incorporated into flooded paddy soil. In addition to degradation communities that contribute to the C pool in paddy fields, free-living nitrogen-fixing bacteria contribute significantly to the N pool in natural ecosystems. Biological dinitrogen fixation is considered the second most important biological process on earth after photosynthesis. Microorganisms that can use inert atmospheric N as their own source of nitrogen are referred to as diazotrophs (Zubberer, 2005). This process offers a viable alternative for the development of sustainable agriculture that meets human needs while conserving natural resources (Giller and Cadisch, 1995; Vance, 1997). Previously, very few bacterial species were thought to be nitrogen fixers (Postgate, 1982). Young, (1992) has documented that nitrogen fixation is a trait found in representatives of most bacterial strains and also in methanogenic archaea. Rodrigues et al. (2004) found a strain of *Verrucomicrobium* isolated from termite gut that had nitrogen fixation genes. Two years later,

Martinez-Romero (2006) further documented 6 main lineages or phyla within the domain bacteria with nitrogen-fixing members: *Proteobacteria*, *Cyanobacteria*, *Chlorobi* (green non-sulphur), Spirochetes, Gram-positive bacteria (*Christensenellaceae* and *Actinobacteria*). The application of next-generation sequencing has made it possible to detect a larger number of taxa with a potential N_2-fixing gene. Wartiainen et al., (2008) reported the genetic diversity of free-living N_2-fixing bacteria in paddy fields based on *nifH* gene sequences and assessed their contribution to N input into the paddy field ecosystem.

1.9 Microbial diversity

The most unique thing about Earth is the existence of life, and the most extraordinary aspect of life is its diversity (Cardinale et al., 2012). Biodiversity is the variety of life, including variation between genes, species and functional traits in an ecosystem, and has implications for the functioning of that ecosystem and, in turn, the services that ecosystem provides to humanity. It is often measured as: wealth, which is a measure of the number of unique life forms; evenness, which is a measure of equality between life forms; and heterogeneity, which is the dissimilarity between life forms. It is well known that species richness and abundance of each species can affect ecosystem functions (Niklaus et al., 2006; Cornwell et al., 2008; Reed et al., 2008). Understanding the former relies on accurate species determination that is increasingly dependent on molecular approaches, particularly for microorganisms. Understanding the latter requires knowledge of the functional role each species plays in ecosystem processes (e.g. nutrient cycling) and a way to measure the abundance of each species (Johnson et al., 2009). In rice cultivation, less than half of the total rice biomass is edible, the rest consists of straw, stubble and rice roots. It has been shown in the laboratory that the decomposition rate of the above-ground residues of straw is faster than that of the underground roots (Lu et al., 2003). The different degradation rates are due both to the chemical composition of the residues 21 and to the microbial community involved in the degradation of these residues.

Changes in different residue sources can alter the decomposition process, indicating that understanding the importance of biodiversity for decomposition is essential to assessing the consequences of changing biodiversity on carbon and nutrient cycling (Httenschwiler et al., 2005). Cellulose degradation is one of the most important biological processes due to the large amount of cellulose in the plant dry weight (3050%). This process can take place under aerobic and anaerobic conditions. Both bacteria and fungi are actively involved in this process (Boer et al., 2005). It can be considered irrelevant which group of organisms is responsible for degrading the residues in the soil; however, bacterial or fungal decomposition can lead to different amounts and compositions of decomposition products (Fischer et al., 2006). Aerobic cellulolytic fungi are remarkably effective degraders in cellulolytic systems compared to aerobic bacteria (Boer et al., 2005).

1.10 Effects of salinity on microorganisms

Microorganisms are essential components of the soil ecosystem on the Tibetan Plateau and play a key role in ecosystem health (Li et al., 2015; Che et al., 2019). Microorganisms adapt to high salinity environments primarily through two mechanisms involving the synthesis or absorption of organic osmotic agents and the absorption of K^+ and other inorganic ions to resist osmotic stress (Csonka, 1989; Zhou et al.,2019), thereby maintaining the normal life activities of cells under conditions of high osmotic pressure. Meanwhile, the microbial community also adapts to salinity by adjusting its composition and enhancing interactions (Asghar et al., 2012; Zheng et al., 2017). Soil samples from different regions of high salinity differ greatly

in microbial community structures, and bacteria are more sensitive than fungi (Yu et al., 2019; Zhang et al., 2019). Studies of saline soils around the world have shown that salinity affects not only bacterial community composition, but also metabolic functions. Salinity leads to a significant decrease in soil microbial diversity and biomass, a reduction in soil enzyme activities (Khan et al., 2016; Ma et al., 2017), inhibition of bacterial growth and respiration (Rath et al., 2017), delaying the rate of organic matter degradation and suppressing nitrification (Wichern et al., 2006). The mechanisms of bacteria resisting high salinity environments consume large amounts of energy, and soil organic matter is rapidly depleted (Yan et al., 2015). Bacteria with autotrophic abilities are likely to have survival advantages in saline soil, leading to changes in the metabolic functioning network for the bacterial community. However, no bacteria are specifically adapted to high salinity environments, and finding a bacterial indicator in saline soil is not easy (Li et al., 2016). The soil microbial community on the Tibetan Plateau has responded to extreme environmental stresses via a unique metabolic mechanism (Chu et al., 2014; Qi et al., 2017).

Many studies have shown that salinity reduces microbial activity and microbial biomass and changes microbial community structure (Batra and Manna, 1997; Pathak and Rao, 1998; Rousk et al., 2011; Setia et al., 2011; Andronov et al., 2012). Salinity reduces microbial biomass mainly because osmotic stress leads to drying and lysis of cells (Laura, 1974; Sarig and Steinberger, 1994; Sarig et al., 1996; Batra and Manna, 1997; Pathak and Rao, 1998; Rietz and Haynes, 2003; Yuan et al., 2007). Some studies showed that soil respiration decreased with increasing soil EC (Adviento-Borbe JW and Drijber A, 2006; Yuan et al., 2007; Wong et al., 2009; Setia et al., 2010). Setia et al., (2010) found that soil respiration was reduced by more than 50% at EC1:5 Z5.0 dS m1. However, Rietz and Haynes (2003) reported that soil respiration did not significantly correlate with EC, but as EC increased, the metabolic quotient (respiration per unit biomass) increased. The sensitivity of soil enzyme activities to salinity varies: the activities of urease, alkaline phosphatase, -glucosidase were strongly inhibited by salinity (Frankenberger Jr. and Bingham, 1982; Pan et al., 2013), while dehydrogenase and catalase were less affected (Garcia and Hernández, 1996). As discussed above, microorganisms have the ability to adapt to or tolerate salinity-induced stress by accumulating osmolytes (Quesada et al., 1982; Del Moral et al., 1987; Zahran et al., 1992; Sagot et al al., 2010).

Proline and glycine betaine are the main organic osmolytes, and potassium cations are the most common inorganic solutes used as osmolytes accumulated by salt-tolerant microbes (Csonka, 1989). However, the synthesis of organic osmolytes requires a lot of energy (Killham, 1994; Oren, 2001). The accumulation of inorganic salts as osmolytes can be toxic and is therefore linked to halophytic microbes, which have evolved salt-tolerant enzymes to survive in highly saline environments. Fungi tend to be more sensitive to salt stress than bacteria (Pankhurst et al., 2001; Gros et al., 2003; Sardinha et al., 2003; Wichern et al., 2006), which can increase the bacteria/fungus ratio in saline one's floors. Differences in saline tolerance between microbes lead to changes in community structure compared to non-saline soils (Pankhurst et al., 2001; Gros et al., 2003). Soil salinization is a process of localized accumulation of soluble salts. This phenomenon is now unanimously recognized as a serious threat to agricultural land as it directly undermines the value and quality of the soil (Ammari et al., 2013; Daliakopoulos et al., 2016a). Soil is a complex system in constant evolution and dynamic equilibrium with the other components of the environment, sensitive to the impacts of climate change and human activities (Smith et al., 2012). Nevertheless, it represents an essentially non-renewable resource in the sense that the rate of its degradation is potentially fast (Zewdu et al., 2017), while the soil formation and regeneration processes are extremely slow.

Globally, it is estimated that 33% of the world's irrigated agricultural land and over 20% of the total cultivated land is salinated. If the current salinization trend continues, cropland salinity will be increased approximately 30% by 2050. This means that agricultural productivity is falling due to the decline in arable

land and the number of starving people is increasing. Plants are the first in the food production chain to be affected by salt stress, which hampers their basic physiological and biochemical processes such as water uptake and photosynthesis, resulting in overall reduced growth (Vaishnav et al., 2016). However, plants evolve different morphological, physiological, biochemical and molecular strategies in response to the salinity in their environment (Meng et al., 2018).

Soil salinity can be divided into primary and secondary salinity. The first depends on factors mainly related to the lithology of the substrate (particularly hydrological characteristics), morphological characteristics of the area, intrinsic soil chemistry and climatic factors (Schofield and Kirkby, 2003). If the bedrock of the soil contains carbonate minerals or feldspar, salts are dissolved by water as a result of physical or chemical weathering, which increases their concentration in the groundwater and thus on the wetted topsoil layer. Soil porosity, texture and mineral composition affect the hydrological properties of the soil, which also depend on the accumulation of salts on the soil surface. The amount of saline precipitation is in turn modulated by soil transpiration and the extent and properties of the capillary fringe. This type of accumulation process is reported in different European areas (Schofield and Kirkby, 2003; Kovov and Velskov, 2012; Gkiougkis et al., 2015; Daliakopoulos et al., 2016b).

Various studies have reported that salt stress can alter the structure of microbial communities, particularly bacterial and fungal communities (Yan et al., 2015; Rath et al., 2016). This shift is due to selective pressure from inhibitory effects of osmotic stress (osmotic dehydration of microbial cells) and the effect of specific ions (Yuan et al., 2007; Yan et al., 2015). Increasing salinity leads to low osmotic potentials of the soil solution that limit water availability for soil organisms (Rath et al., 2019), resulting in desiccation and lysis of microbial cells (Yuan et al., 2007). On the other hand, high concentrations of ions (Na^+, Cl^-, HCO_3^-) can induce toxicity of soil microbes (Yan et al., 2015). Several studies showed that fungi are better able to withstand salt stress compared to bacteria in saline soils (Rath et al., 2016, 2019), probably due to the fact that chitinous cell walls of fungi offer protection from low matrix potentials (Manzoni et al., 2012). This is well illustrated in findings involving alteration of fungal cell morphology or accumulation of ergosterol, a key component of fungi (Wichern et al., 2006). In contrast, other studies have reported that fungi may be more sensitive to salt stress than bacteria (Sardinha et al., 2003; Chowdhury et al., 2011b), where significant reductions in ergosterol levels from salt supplementation have been reported (Sardinha et al., 2003). Such contrasting findings could be attributed to differences in ground-level conditions, biomes and salinity ranges, but more research on this topic is still needed to clearly understand the susceptibility of fungal salinity to bacteria (Rath et al., 2019). It should be noted that changes in the soil microbial community in response to salt stress could impact the C cycle. For example, fungi can degrade more complex SOM (Roman et al., 2006; Paterson et al., 2008), while fungal necromass can have a longer turnover time in soil than bacterial residues (Six et al., 2006; Strickland and Rousk, 2010). In addition, Morrissey et al. (2014) found a significant positive effect of salinity on C-degrading enzymes as reported in tidal wetlands and suggested that changes in bacterial abundance and community structure were associated with increased C-degrading enzymes. Effects of salinity on the structure of bacterial communities have recently been reported based on an NGS method. For example, Chen et al., (2017) found non-responsive (*Gemmatimonadetes* and *Acidobacteria*), decreased (*Christensenellaceae*, *Bacteroidetes*) and increased (*Proteobacteria*, *Actinobacteria* and *Chloroexi*) bacterial strains to salt stress in arid agricultural soils. Yang et al., (2020) showed that high salinity increased the relative abundance of *Gemmatimonadetes* and *Bacteroidetes* but decreased the bacterial taxa *Proteobacteria* and *Christensenellaceae* in grassland. In addition, they reported an increased relative abundance of the fungal strain *Ascomycota* in soils with high salinity. High salinity increased the relative abundances of *GammaProteobacteria*, *Bacteroidetes*, and *Christensenellaceae* with increasing salinity, while the relative abundances of *Acidobacteria*, *Chloroexi*, and Cyanobacteria decreased in wet coastal estuaries (Zhang et al., 2020). Rath, Murphy, and Rousk (2019) found that high

community salt tolerance is positively correlated with OTUs belonging to *GammaProteobacteria* and *Bacteroidetes* and suggested that the majority of OTUs belonging to these taxa (*GammaProteobacteria* and *Bacteroidetes*) are associated with a high salt tolerance. Salinity also directly and/or indirectly affects microbial diversity and richness by altering the edaphic properties of the soil. Li et al., (2021) reported decreasing bacterial diversity with increasing salinity and suggested that the disappearance of species susceptible to high salinity was related to decreasing bacterial diversity. The authors also suggested that high salinity significantly reduced soil nutrients, which could contribute to reduced bacterial diversity. Similarly, a decrease in bacterial diversity with increasing salinity has been observed in lake sediments, suggesting that only a limited group of bacterial taxa can withstand the significant stresses imposed by highly saline conditions (Rath et al., 2019). Wan et al., (2021) suggested that the response of rare bacterial taxa and more common bacterial taxa to salt stress is different, further emphasizing the importance of studying rare and more common taxa separately. Salt-tolerant microbes counteract osmotic stress by synthesizing organic (proline, glycine betaine) and inorganic (potassium cations) osmolytes (Csonka, 1989), which allows them to maintain their cell turgor and metabolism (Yan et al., 2015). The addition of organic residues can mitigate the negative effects of salinity by synthesizing osmolytes that counteract osmotic pressure or investing resources in metabolic processes for detoxification and cell repair (Wichern et al., 2006). N supply is particularly important for microbial adaptation to salinity (Hasbullah and Marschner, 2015).

1.11 Soil salinity effects on soil CH_4 emission

The majority of previous studies found that soil salinity reduces soil CH_4 emissions in various ecosystems including semi-arid farmland (Maucieri et al., 2017), paddy field (*Oryza sativa* L.), paddy field (Theint et al., 2016), tidal marshes (Poffenbarger et al., 2011), tidal forest (Marton et al., 2012) and riparian zones of wetlands. For example, in soils collected in Canada's Prairie Pothole region, soil CH_4 emissions decreased (19.2, 5.0, and 1.4 CH_4, respectively) with increasing soil salinity (0.3, 6, and 16 mS cm^{-1}). (Shahariar et al., 2021). The reduced CH_4 emission could be explained by the fact that higher salinity increases SO_4^{2-} availability. Methanogens are the least competitive heterotrophic microorganisms in soil (Zhang and Furman, 2021). For this reason, the availability of key electron acceptors, including SO_4^{2-}, can cause methanogens to fall out of substrate competition and eventually reduce methanogenic activity (Poffenbarger et al., 2011). Sulfate-reducing bacteria and methanogens compete for acetate and hydrogen, which are the main substrates of methanogenesis, therefore increasing sulfate-reducing activity through increased SO_4^{2-} availability can significantly decrease methanogenic activity, resulting in reduced CH_4 emission in soil. In contrast, some studies found that salinity increased soil CH_4 emissions in coastal forests (Ardn et al., 2018; Norwood et al., 2021) and tidal freshwater marshes (Weston et al., 2011). For example, in five forests along the coast of the United States, exposure to seawater increased soil salinity and soil CH_4 emissions (Norwood et al., 2021).

The increased CH_4 emission can be explained by a few different mechanisms. First, high salinity increases osmotic stress and decreases water availability, which can destroy and dehydrate cells and eventually decrease methanotrophic activity in the soil (Dalal et al., 2008). Second, dissolved Na^+ and Cl^- have a direct toxic effect on bacterial cells, including methanotrophs (Serrano-Silva et al., 2014). Displacement of NH^{4+} from binding sites (e.g. on clay minerals) by Na^+ can indirectly affect CH_4 oxidation. NH^{4+} competes with CH_4 for methane monooxygenase due to their structural similarity, hence increasing NH^{4+} inhibits CH_4 oxidation activity. Third, decreased humic matter through salt-induced occlusion of dissolved SOM can also indirectly increase CH_4 emission, since humic matter decreases methanogenic activity by providing thermodynamically favorable organic electron acceptors (Ardn et al., 2018). Although an increase in

salinity can immediately reduce soil CH_4 oxidation activity at the cellular level and offset reduced CH_4 production, most previous studies consistently show a reduction in CH_4 emissions across ecosystems, with the exception of a few studies (Weston et al., 2011; Ardn et al., 2018; Norwood et al., 2021). These results suggest that the negative effect of salinity on methanogenesis by SO_4^{2-} inhibition is greater than a negative effect on methane oxidation induced by inhibition at the cellular level. In addition, Ho et al. (2018) propose that an increase in salinity changes the composition of the soil methanotrophic community, with salt-resistant methanotrophs gradually replacing salt-sensitive methanotrophs and eventually adapting to a saline environment. This result suggests that CH_4 oxidation activity can be recovered from salinization due to the resilience of methanotrophic communities. Salinity can also affect CH_4 flux by changing the physiological properties of the plant. First, increasing salinity reduces plant species diversity and growth rate, which in turn can reduce soil CH_4 emissions by reducing organic matter inputs (Sutton-Grier and Megonigal, 2011). Second, an increase in salinity can lead to increased CH_4 emissions from tree trunks. Trees release CH_4 produced in the tree or in the surrounding soils into the atmosphere (Covey and Megonigal, 2019). Norwood et al., (2021) found that in five forests along the US coastline, exposure to seawater significantly increased CH_4 emissions from tree trunks, and it can be attributed to dying gymnosperm trees caused by contact with Seawater can accumulate higher concentrations of CH_4 (Norwood et al., 2021). Furthermore, no response of soil CH_4 emissions to soil salinity was observed in agricultural fields (Kontopoulou et al., 2015; Dang et al., 2016).

Soil microorganisms contribute to ecosystem function by driving C and nutrient cycling through the release of extracellular enzymes to meet metabolic C and nutrient needs (Dick, 1994; Sinsabaugh et al., 2002; Penton and Newman, 2007). Saltwater intrusion can alter the microbe-mediated biogeochemical cycle in coastal wetlands (Herbert et al., 2018). Enzyme activities are often suppressed when exposed to increased salinity (Frankenberger Jr. and Bingham, 1982; Jackson and Vallaire, 2009) as microbes divert resources to the production of osmolytes and consequently reduce the production of extracellular enzymes (Kempf and Bremer, 1998). Studies on phosphorus accumulation show an inverse relationship with phosphatase enzyme activities (Spiers and McGill, 1979; Wright and Reddy, 2001; Morrison et al., 2016) and positive relationships with other enzyme activities (Rejmnkov and Sirov, 2007). The effects of simultaneous exposure to osmotic stress and increased nutrient availability on microbial function are unclear.

Coastal wetlands are increasingly subject to saltwater intrusion, and the impact of salinization and land-use legacies on wetland biogeochemistry is uncertain (Green et al., 2017; Tully et al., 2019). Changes in extracellular enzyme activities associated with increased salinity and nutrients (nitrogen, N; phosphorus, P) can lead to long-term effects on C storage and nutrient removal capacity (Penton and Newman, 2007). Simultaneous increase in salinity was tested in the activities of soil microbes' extracellular enzymes, microbial biomass C, soil respiration and soil element concentrations and stoichiometric ratios using experimental manipulations of crossed gradients in added concentrations of salinity and the limiting nutrient (P). Based on recent findings (Servais et al., 2019), it has been predicted that (1) increased salinity would decrease microbial activities (EEAs, microbial biomass C, and respiration rates) in freshwater marsh soils, (2) increased P Levels that would increase microbial activity (countable with phosphatase) in both freshwater and brackish soils, (3) Brackish soils would have less sensitivity to increased salinity compared to freshwater soils due to the ecological memory of saltwater exposure and would be better at P subsidies respond in the presence of salinity, (4) brackish soils and freshwater soils exposed to increased salinity would have lower %C, C:N and C:P ratios than freshwater soils (Servais et al., 2021).

It is known that sulfate-reducing bacteria displace methanogens for various energy sources when sulfate is not limiting in the ecosystem. This is observed in marine environments where H_2 and acetate are mainly used via sulfate reduction (Oremland et al., 1982; Oremland and King, 1989). Nonetheless, methanogenesis

occurs in these environments where methanogens use methylamines, which are considered uncompetitive substrates since their use by sulfate reducers has never been described. In hypersaline ecosystems, which contain larger amounts of sulphate than marine ecosystems, competition for substrates may be increased; The main route for H_2 oxidation is via sulfate reduction. However, this does not imply the absence of *hydrogenotrophic* methanogens. For example, hydrogenotrophic methanogens belonging to the family *Methanomicrobiaceae* (Romesser et al., 1979; Widdel et al., 1988) or *Methanococcaceae* (Corder et al., 1983; Jones et al., 1983) have been isolated from marine environments. In the native Mono Lake pelagic sediment, the upper limit of NaCl concentration for H_2 utilization by methanogens has been reported as 9%. This suggests that both sulfate reducers and methanogens have similar apparent Ks values for H_2 (Oremland and King, 1989). If the NaCl concentration is above 15%, the methanogenic activity of H_2 as an electron donor is low or not pronounced. Thus, the persistence of methanogens in hypersaline environments is related to the presence of non-competing substrates such as methylamines, mainly derived from the degradation of osmoregulatory amines. This leads to the hypothesis that methanogenesis does not contribute to the mineralization of carbohydrates at a NaCl concentration of more than 15%. Above this concentration, sulfate reduction is probably the main pathway for H_2 oxidation and has a terminal function in carbohydrate degradation. However, this function decreases concomitantly with fatty acid accumulation as salt concentration increases. Therefore, the NaCl concentration in hypersaline ecosystems drastically affects the distribution and function of both methanogens and sulfate reducers. Sulfate reducers remain slightly more active with respect to H_2 metabolism, but the methanogens can also remain active by using specific organic compounds at the higher NaCl concentrations. In most ecosystems, anaerobic mineralization of organic matter leads to the production of the simplest compounds: CO_2, CH_4 and H_2S. However, this is probably not the case in hypersaline sediments, where the high salinity leads to the accumulation of VFA and H_2 (Ollivier et al., 1994).

Oremland, Marsh and Polcin (1982) reported the production of methane from H_2 plus CO_2 in a 9% NaCl lake. Although the isolation of a hydrogenotrophic halophilic methanogen has been reported (Yu and Hungate, 1983), this strain has never been mentioned again and never verified. Ahalotolerant, hydrogenotrophic, methanogenic rods growing in up to 5% NaCl have recently been isolated and characterized (Ollivier et al., 1994); it uses H_2 plus CO_2, formate and CO_2 plus 2-propanol with a doubling time of 10 h under optimal conditions. To our knowledge, the highest reported NaCl concentration for methanogens using H_2 or formate is 8.3% (Huber et al., 1982). However, given the variety of halophilic ecosystems from a physicochemical point of view, further investigations will likely lead to the isolation of hydrogenotrophic bacteria that grow at higher salt concentrations. Currently, H_2 does not appear to be an important energy source for methanogenesis in hypersaline environments. Given the high sulfate concentration in hypersaline environments, it is not surprising that sulfate reducers can outperform methanogens for H_2 (Lovley and Klug, 1983; Widdel, 1988), since marine and halophilic methanogens are not known for their ability to compete for H_2. Surprising is the inability of native SRB to consume all available H_2 under hypersaline conditions (Ollivier et al., 1994).

1.12 High-Throughput metagenomic technologies for complex microbial community analysis

Microorganisms inhabit almost every conceivable environment in the biosphere, play an integral and unique role in ecosystems, and are involved in the biogeochemical cycling of essential elements such as carbon, oxygen, nitrogen, sulfur, phosphorus, and various metals. Their structure, function, interaction, and dynamics are critical to our existence, yet their detection, identification, characterization, and quantification pose major challenges. First, microbial communities can be extremely diverse and most microorganisms in

natural settings have not yet been cultured (Quince et al., 2008; Kallmeyer et al., 2012). Second, in any ecosystem, different microorganisms interact with each other to form intricate networks whose behavior is difficult to predict (Fuhrman, 2009; Zhou et al., 2010a). Establishing mechanistic links between microbial diversity and ecosystem functions poses an additional challenge for understanding the interactions and activities of complex microbial communities (Fitter et al., 2005; Levin, 2006). Effective high-throughput technologies to analyze the structure and functions of microbial communities are crucial to advance this mechanistic understanding.

The sequencing and phylogenetic analysis of 16S rRNA genes formed the basis for the modern study of microbial communities. PCR-based 16S rRNA cloning analysis fueled the information explosion about community membership and greatly expanded the known diversity of microbial life (Pace, 1997). PCR-based analyzes of 16S rRNA genes have three main limitations: (i) PCR limits the information obtained to the sequence between primers, thereby ignoring functional information; (ii) PCR-based analysis is only somewhat quantitative, with most measurements providing only relative abundance information; and (iii) PCR primer mismatches can result in some lines being missed entirely (Zhou et al., 2010b). All three challenges have been addressed by developing metagenomic analyzes with direct sequencing or screening of unamplified environmental DNA (Rondon et al., 2000; Handelsman, 2004; Tyson et al., 2004; Tringe et al., 2005). These methods represent critical open formats that do not require prior knowledge of the community, thereby enabling unprecedented discovery of new taxa and genes and associations between them.

Analysis of cloned DNA has been largely replaced by next-generation sequencing of DNA extracted from environmental sources, transforming the field of microbial ecology by increasing the speed and throughput of DNA sequencing by orders of magnitude. Now the metagenomic databases are packed with high-quality sequence information from different habitats around the world, revolutionizing the molecular analysis of biological systems (Schena et al., 1995; Bokulich et al., 2013) and facilitating the exploration of questions that were previously not possible were approached. Although functional metagenomics, in which clones containing metagenomic DNA are screened for expressed activities, holds promise for shaping ecological theory and understanding, it lags behind shotgun sequencing due to comparatively slow advances in screening technology stayed behind. Ecological insights from the massive datasets generated by high-throughput sequencing (open formats) have been facilitated by sophisticated computational methods and by closed format methods such as microarrays, which can be used to quickly interrogate taxa, genes, or transcripts in space and time in complex communities. High-throughput sequencing and microarray technologies have been applied in different communities. The body of research using these methods has resulted in several excellent reviews (Vieites et al., 2008; Roh et al., 2010; Suenaga, 2012), particularly in relation to the human microbiome (A framework for human microbiome research, 2012; Consortium, 2012; Weinstock, 2012). Our intention here is to complement previous reviews by primarily focusing on DNA-based metagenomic technologies applied to complex environmental communities such as those found in soils.

Several high-throughput sequencing platforms have been developed and are in widespread use, including Illumina (e.g. HiSeq, MiSeq), Roche 454 GS FLX+, SOLiD 5500 series and Ion Torrent/Ion Proton platforms. The advantages and limitations of these platforms are detailed elsewhere (Metzker, 2010; Bartram et al., 2011; Caporaso et al., 2012; Kuczynski et al., 2012; Loman et al., 2012b, 2012a; Weinstock, 2012). Currently, most microbial ecology studies apply high-throughput sequencing, relying on targeted gene sequencing using either phylogenetic (e.g. 16S rRNA) (Sogin et al., 2006; Caporaso et al., 2012) or more functional (e.g .*amoA*, *nifH*) (Gubry-Rangin et al., 2011; Pester et al., 2012) gene targets or on shotgun metagenome sequencing (Figure. 1a). For targeted gene sequencing, community DNA is extracted from

environmental samples (e.g., soil, sediment, water, bioreactor, or human samples) using various extraction and purification methods (Zhou et al., 1996; Hurt et al., 2001). After high quality DNA is obtained, targeted genes can be amplified using conserved primers. Each set of primers is generally barcoded with short oligonucleotide tags (6- to 12-mer) as well as sequencing adapters, allowing multiple samples to be pooled and sequenced simultaneously (Sogin et al., 2006; Caporaso et al., 2012). After non-target DNA fragments are removed by gel electrophoresis, the target DNA is quantified, sequenced and analyzed using bioinformatic approaches, e.g., 2012).

The HiSeq platform has a unique feature of switching between a rapid output mode and a higher output but slightly slower mode, depending on the size of the study. HiSeq runs produce paired-end reads of around 250 bp. On the other hand, the MiSeq system is a more streamlined approach that combines the rapid output capacity of HiSeq with slightly longer paired-end 300 base pair reads. The MiSeq is capable of long reads, making it great for de novo assembly of small genomes, and is also great for QC tests on sequencing workflows before committing to larger batches on more expensive machines. MiSeq is also a cost-effective tool for various analyses focused on targeted gene sequencing, metagenomics, and gene expression studies (Ravi et al., 2018).

To study microbial compositions in Philippine paddy soil under long-term NaCl and seasalt treatments at intermediate salinity concentrations, using high-throughput technologies such as HiSeq and MiSeq for analyzing PCR amplicons of 16S rRNA, *dsrB*, and *mcrA* genes can provide valuable insights into the microbial community functions and compositions.

1.13 Hypothesis and aim of the study

Soil salinity is part of the natural ecosystem in arid and semi-arid regions and an increasing problem in agricultural soils the world over (Pathak and Rao, 1998; Keren, 2000; Qadir et al., 2000). The microbial communities of the soil perform a fundamental role in cycling nutrients, in the volume of organic matter in the soil and in maintaining plant productivity. Thus it is important to understand the microbial response to environmental stress such as soil salinity. Stress can be detrimental for sensitive microorganisms and decrease the activity of surviving cells, due to the metabolic load imposed by the need for stress tolerance mechanisms (Schimel et al, 2007; Yuan et al., 2007, Ibekwe et al., 2010; Chowdhury, 2011).

Saline stress can gain importance, especially in agricultural soils where the high salinity may be a result of irrigation practices and the application of chemical fertilizers. Research has been carried out on naturally saline soils, and the detrimental influence of salinity on the microbial soil communities and their activities reported in the majority of studies (Batra & Manna, 1997; Zahran, 1997; Rietz & Haynes, 2003; Sardinha et al., 2003). In addition, the effect of seawater intrusion on microbial community of paddy soil has been poorly addressed in previous investigations. Hence, this project aimed to assess how the simulation of seawater intrusion affects the structure, composition and function of microbial communities in the Philippine paddy soil at non-molecular measurements combined with molecular approaches.

To address the objectives of my PhD study, we elucidated the implications of moderate soil salinization (primarily triggered by NaCl) and saltwater intrusion on microbial communities in rice field soil from the Philippines. Both soil salinization and saltwater intrusion were simulated in rice straw-amended slurries that incubated for up to 49 days under anoxic conditions. Process measurements and molecular ecology analyses combined to depict the treatment effects. Methane production, CO_2 evolution, H_2S gas generation and the turnover of metabolites (acetate, propionate, butyrate) were determined over incubation times. The molecular ecology analyses involved quantitation of marker genes (qPCR) and their transcripts (RT-qPCR),

such as 16S rRNA, mcrA (methanogens), and dsrB (sulfate reducers). In addition, amplicon sequencing applied on gene and transcript levels to determine the effects of soil salinization and saltwater intrusion on the overall community composition (16S rRNA) and on particular functional guilds, such as methanogens, and sulfate reducers. We hypothesized that methanogenesis suppression would be more noticeable in seasalt-treated samples compared with NaCl treatment regardless of sodium and chloride ions toxicity on the taxonomic composition and diversity.

The workflow deals with the impact of differences between the two types of salt treatments (at 150 mM) on the microbial community of Philippine paddy soil in an anaerobic condition. Thus, this research studied the soil microbial syntrophy, anaerobic respirations i.e. dissimilatory sulphate reduction and methanogenesis coupled with fatty acids oxidation, taxonomic and functional analyses, and the genes expression with three primer sets (Figure. 5).
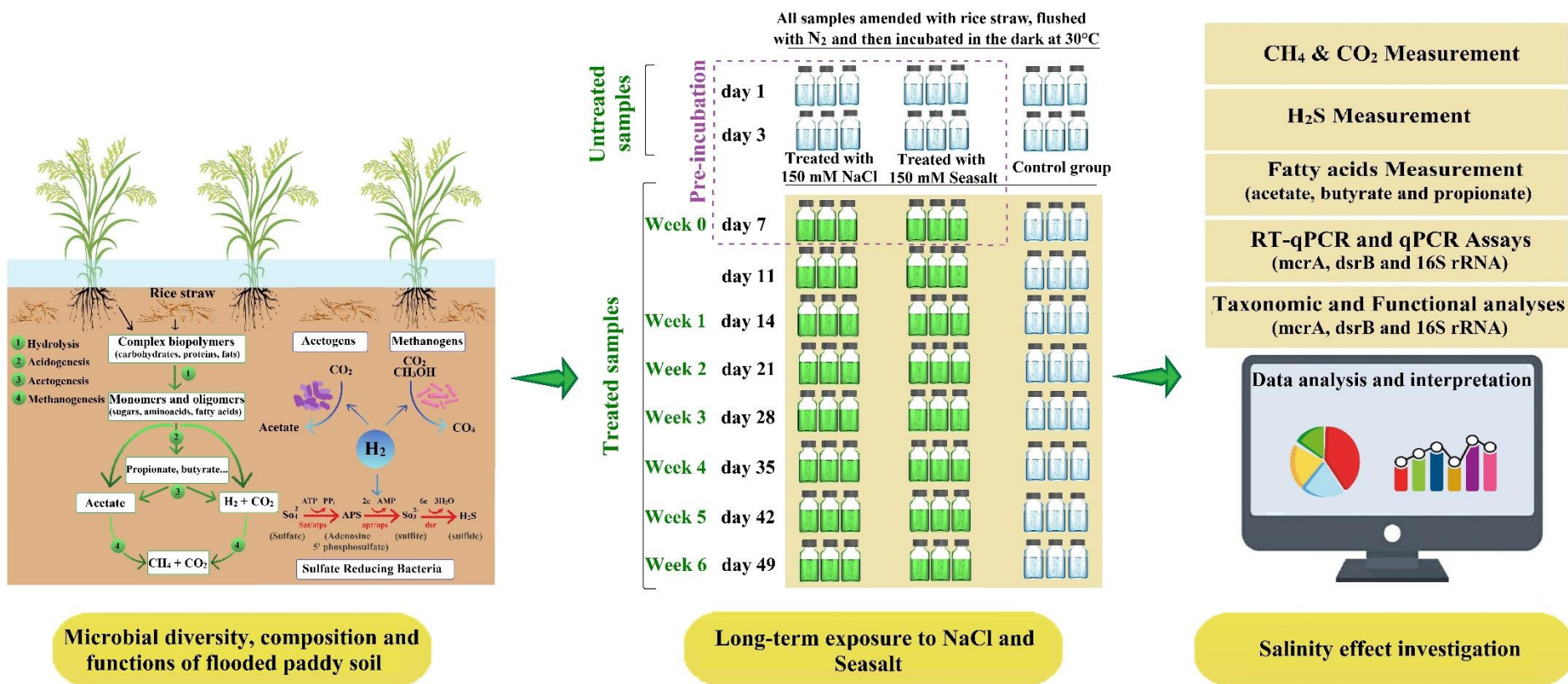
Figure. 1.5. Overview of the experimental design for 42-day salt treatment with NaCl and seasalt. The experiment was set up to stimulate the effect of moderate seawater intrusion on microbial community of the Philippine paddy soil.

2-Methods and materials

Chemicals, instruments and kits

#	Items	Manufacturer	City/Country
1	H$_2$S microsensor	Unisense	Denmark
2	UniAmp Multi Channel	Unisesne	Denmark
3	RNeasy® PowerSoil® Total RNA Kit	Qiagen	Hilden, Germany
4	Fast DNA®SPIN Kit for Soil	MP Biomedicals	Santa, CA, United States
5	GC-8A gas chromatograph	Shimadzu	Duisburg, Germany
6	FastPrep®-24 bead beater MP	Biomedicals	California, USA
7	NanoDrop® ND-1000 UV-Vis spectrophotometry	NanoDrop	Tech. Inc. USA
8	Qubit® 2.0 Fluorometer	Invitrogen	California, USA
9	Experion automated electrophoresis system	Bio-Rad	Hercules, USA
10	C1000 Touch™ Thermal Cycler	Bio-Rad	Hercules, USA
11	Magnetic stand	Invitrogen	California, USA
12	CFX Connect Real-Time PCR detection system	Bio-Rad, USA	Hercules, USA
13	Absolve™	PerkinElmer	Boston, USA
14	DEPC-treated water	Ambion	Austin, USA
15	Tris-HCl	Sigma	Steinheim, Germany
16	Polyvinylpyrrolidone K25	Fluka	Buchs, Switzerland
17	Water-saturated phenol (pH 8.0)	Carl Roth	Karlsruhe, Germany
18	Phenol-chloroform-isoamyl alcohol (pH 8.0)	Carl Roth	Karlsruhe, Germany
19	Chloroform-isoamyl alcohol [24:1 (v/v)]	Carl Roth	Karlsruhe, Germany
20	Magnesium chloride (MgCl2)	Carl Roth	Karlsruhe, Germany
21	GoScript Reverse Transcription System	Promega	Mannheim, Germany
22	Isopropanol	Carl Roth	Karlsruhe, Germany
23	Ethanol (Nuclease-free)	Applichem	Darmstadt, Germany
24	RNase-free TE buffer	Applichem	Darmstadt, Germany
25	Sodium Chloride (NaCl)	Carl Roth	Karlsruhe, Germany
26	Glass beads (0.17-0.18 mm)	Sartorius	Goettingen, Germany
27	Sodium hydroxide (NaOH) solution (10M)	Sigma	Buchs, Switzerland
28	SeaKem Agrose	Lonza	Basel, Switzerland
29	pH meter		Germany
30	FastDNA® SPIN kit for soil MP	Biomedicals	California, USA
31	GelRed(TM)	Bioswisstec	Schaffhausen, Switzerland
32	RNasin® Ribonuclease Inhibitor	Promega	Madison, USA
33	RNA Clean & Concentrator™-5	Zymo Research	California, USA
34	Qubit® RNA assay kit	Invitrogen	California, USA
34	Qubit® DNA assay kit	Invitrogen	California, USA
35	Spin and vortex		Germany
36	Refrigerated Laboratory Centrifuge	Hettich GmbH & Co	Germany
37	DNA Smart Ladder	Eurogentec	Seraing, Belgium

38	GoScript Reverse Transcription System and random primers	Promega	Madison, USA
39	Oligonucleotides (primer sets)	Eurofins	Constance, Germany
40	Sybr Green kit	Sigma-Aldrich	Missouri, USA
41	AMPure XP Beads	New England Biolabs	Ipswich, USA
42	GoTaq® G2 Green Master Mix	Promega	Germany
43	SYBR® Green JumpStart™ Taq ReadyMix™	Merck	Darmstadt, Germany
44	HyperLadder 1kb	Meridian Bioscience	Germany
45	Agarose	Biolab	USA

2.1 Microcosm Slurry Setup and Sampling

The study conducted soil sampling at the lowland farm of the International Rice Research Institute (IRRI) in Los Baños, Philippines. In 2012, the authorities at IRRI collected soil samples from the paddy field, which were then transferred to Dr. Ralf Conrad's laboratory at the Max Planck Institute for Terrestrial Microbiology (MPI) in Marburg, Germany for deeper investigation. Detailed description can be found in Heinz et al., (2013). Liu, Klose and Conrad, (2019) and Breidenbach et al., (2017).
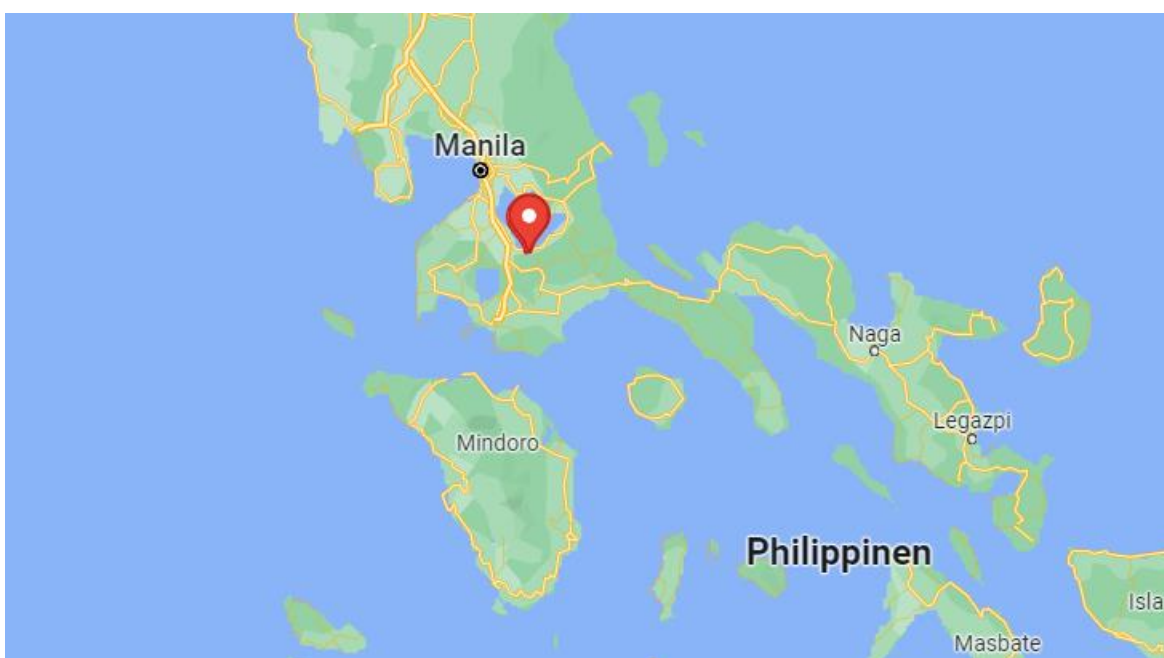
Figure. 2.1. the International Rice Research Institute (IRRI) is an international agricultural research and training organization with its headquarters in Los Baños, Laguna, in the Philippines.

Microcosm slurry, which is a mixture of microorganisms and their surrounding environment such as soil or water, is a widely-used method in microbiology research. It allows researchers to investigate microorganisms and their environment in a controlled laboratory setting. In this particular study, microcosm slurry was employed to examine the impact of two different salt treatments on microbial communities in Philippine paddy soil. By collecting soil samples and creating the microcosm slurry, we were able to simulate natural environments and analyze the interactions between microorganisms, their functions, and their environment in vitro. The use of microcosm slurry was crucial in generating reliable and accurate data on the effects of salt treatments on soil microbial communities. The Philippine paddy soil was air-dried,

crushed, and stored at room temperature until its use. Microcosms sluries were established by adding 35 mL of autoclaved water and 40 gr of dry soil to 120 mL serum bottles. A total of 0.5 gr chopped rice straw fiber was added to each bottle as nitrogen and carbon sources (Pedraza-Zapata et al., 2017; Chivenge et al., 2020; Van et al., 2022).

To create an anoxic condition, the rice straw amended slurries were completely mixed and sealed with butyl rubber stoppers and aluminum caps, flushed with N_2 for 5 min at 0.5 bar pressure. Slurries were statically pre-incubated for seven days at 30°C in the dark before seasalt and NaCl treatments. On day 7, 5.0 ml of sea salt (Sigma-Aldrich, Germany) (Table. 2.1) (Table 2.2) and NaCl (Sigma-Aldrich, Germany) solutions (1.2 M) were then adjusted with triplicate slurries to a concentration of 150 mM, equal to 13.0 and 8.7 g/L, respectively. Under this condition, all experimental and control samples incubated up to week 6 (from day 1 to day 49) (Figure. 1.5). In total, 90 serum bottles were used for this experiment. At sampling time, sampling from the triplicate slurry-containing bottles associated with each treatment and control group (from week 1 to 6) were destructively carried out for molecular analysis, and the fresh slurry pellets immediately shock-frozen under liquid nitrogen and then stored at -80°C until further experiments. Three marker genes were applied in amplicon-sequencing metagenomics and transcriptomic studies as well as RT-qPCR and qPCR assays with three primer sets (16S rRNA, mcrA and dsrB) (Table S25; Table S26; Table S27). Other analysis conducted on the experimental and control groups included gas (CH_4, CO_2 and H_2S), and measurement of fatty acids concentrations.

Table. 2.1: The quantity of commercial sea salt compositions per liter provided by Sigma Aldrich company.

TEST	Specification
Appearance (Color)	White
Appearance (Form)	Powder
Solubility (Color)	Colorless
Solubility (Turbidity)	Clear
38 mg/ml, Water	
Chloride (Cl)	Conforms
19000-20000 mg/L	
Sodium	Conforms
10700-11000mg/L	
Sulfate	Conforms
2660 mg/L	
Potassium	Conforms
300-400 mg/L	
Calcium	Conforms
400 mg/L	
Carbonate	Conforms
140-200mg/L	
Boron	Conforms
5.6 mg/L	
Magnesium (Mg)	Conforms
1320 mg/L	
Strontium (Sr)	Conforms
8.8 mg/L	
Insoluble Matter	$\leq$ 0.05 %

2.2 Calculation of NaCl and seasalt concentrations

Molarity refers to a number of moles of the compound in 1 liter of the solution and expressed in molars (abbreviated as "M"). Molarity is equal to number of moles/Volume of solution (in a liter). The prefix "milli-" indicates "1 thousandth," that is, the magnitude of 0.001. Hence, 1 mole is equal to 1 millimoles multiplied by 1,000.

To make 1.2 molar NaCl solution, the weight of NaCl multiply 1.2 by the following formula. We took 70.12 gr of NaCl and reached it to one liter. First, the "formula weight" of NaCl was determined by adding together the "atomic weights" of its elements, Na and Cl, as follows:

22.99 + 35.45 = 58.44 g NaCl/mole.

In a NaCl solution, approximately 35% of the solution is made up of cations (Na^+), while the remainder is composed of chloride anions (Cl^-). It is known that about 85-86% of sea water consists of NaCl and 15% of it is related to other elements like Mg^{2+}, Ca^{2+}, K^+, SO_4^{2-}, carbonate and so on. According to the information, we made a 1.2 molar sea salt solution as shown in the calculation below: 86.58 gr * 1.2 = 103.9 gr.

Table. 2.2: Details of seawater compositions and molarity calculation

Element	Weight	Unit	compositions amount/L	Unit	%	% in seawater	a M of seasalt	1.2 M of seasalt
Cl	35.4	g/mol	20	g/L	55.50%	86%	30.444	36.5328
Na	22.9	g/mol	11	g/L	30.50%		19.694	23.6328
Mg	24.3	g/mol	1.3	g/L	3.67%		3.402	4.0824
Ca	40	g/mol	0.4	g/L	1.11%		5.6	6.72
K	39	g/mol	0.4	g/L	1.11%	14%	5.46	6.552
Sulfate	96	g/mol	2.66	g/L	7.39%		13.44	16.128
Carbonate	61	g/mol	0.2	g/L	0.56%		8.54	10.248
			35.96	g/L			**86.58 gr**	**103.9 gr**

Table. 2.3: Details of microcosm slurry setup, incubation and salt treatments.

Bottle Volume	Salt type	Soil Weight	Rice Straw	Autoclaved water	Added saline amount	Final salt concentration in bottle	Incubation condition	Flashing with N_2	Exposure time
120 ml	seasalt	40 gr	0.5 gr	35 ml	5 ml (1.2 M)	40 ml (150 mM)	30ºC in the dark	5 min	1-6 weeks or 49 days
120 ml	NaCl	40 gr	0.5 gr	35 ml	5 ml (1.2 M)	40 ml (150 mM)	30ºC in the dark	5 min	1-6 weeks or 49 days

2.3 Gas and Fatty Acids Measurements

Bottle headspace gas were used for analyzing methane, carbon dioxide and H_2S concentrations using a gas chromatograph equipped with a Sephadex® column, a methanizer (for CH_4 and CO_2 measurement) and a special microsensor (for H_2S measurement), respectively. The H_2S concentrations of samples were analyzed with Type-I, the SULF-type, microsensor. This H_2S sensors acquired from Unisense A/S (Denmark) that functions by direct oxidation of H_2S (www. unisense.com). The UniAmp Multi Channel connected to a lab computer, and the microsensor automatically initiated the Windows service app. The short chain fatty acids (SCFAs) acetate, butyrate and propionate quantified by ion exclusion HPLC, applying an Aminex® HPX87H organic acid analysis column (Bio-Rad, Munich, Germany) (Peng et al., 2017).

2.3.1 Calculation of CH_4 and CO_2 concentrations

From day 3 to the end of the experiment, we measured the two gases with a gas chromatograph (GC) instrument, which measures the content of components in a sample. Before starting the measurement, the instrument calibration was performed with sampling three times. The values for methane and carbon dioxide were recorded on a screen. To calculate their concentrations at ppm unit, the following formula was used:

$$X = \frac{A * B}{C}$$

$$Y = X * 10000 \text{ ppm}$$

A = value of sample gained GC machine
B = CH_4 and CO_2 coefficient (0.991 for CO_2 and 0.995 for CH_4)
C = average value of gas calibration

2.3.2 Calculation of CH_4 and CO_2 pressure from Concentration

The Van der Waals equation, also known as the Van der Waals equation of state, is a formula used in chemistry and thermodynamics to account for the effects of molecule interactions and the finite size of molecules that are not considered in the ideal gas law. Unlike the ideal gas law, which assumes gas molecules as point particles that don't take up space or change kinetic energy during collisions, the Van der Waals equation considers the volume V occupied by n moles of gas at temperature T and pressure P, where R is the gas constant. The Van der Waals equation provides a more accurate representation of the behavior of real gases:

$$PV = nRT$$

To account for the volume occupied by real gas molecules, the Van der Waals equation modifies the ideal gas law by replacing V/n with (V_m/ - b), where Vm represents the molar volume of the gas, and b denotes the volume that one mole of the gas molecules occupies:

$$P(V_m-b) = RT$$

The ideal gas law is modified a second time to consider the interaction between gas molecules. This is accomplished in the Van der Waals equation by adding a term of the form a/V_m^2 to the observed pressure

P in the equation of state. The constant a is specific to the gas being measured. Therefore, the complete Van der Waals equation includes this term to account for intermolecular interaction:

P = pressure
V = volume
T = absolute temperature
R = ideal gas constant
n = number of moles of gas
a = attraction between individual gas particles
b = average volume of individual gas particles

$$P + a(n/V)^2 = nRT/(V-nb)$$

After the calculation of the gases concentrations, pressures of the two gases were calculated with the formula above at bar unit.

2.4 Nucleic Acids Extraction

Genomic DNA and total RNA were extracted from 0.5 g and 1.5 g soil using FastDNA®SPIN Kit for Soil (MP Biomedicals, Santa, CA, United States) and RNeasy® PowerSoil® Total RNA Kit (Table. 2.7) (Qiagen, Hilden, Germany) following the manufacturer's guidelines, respectively. The quality of the extracted RNA and DNA were evaluated by electrophoresis on 1% agarose gel at 100 V for 40 min in TAE buffer, and their quantity were also measured by Qubit 2.0 fluorometer using Qubit® RNA Assay Kit and Qubit™ dsDNA BR Assay Kit (Life Technologies). Co-extracted substances from soil (e.g., humic acid) with DNA and RNA extractions were measured in a NanoDrop 1000 UV–Vis Spectrophotometer (Thermo-Scientific). cDNAs synthesized from total RNA using the GoScript Reverse Transcription System (Promega, Mannheim, Germany), and cDNAs concentrations measured by a Qubit Assay kits. The nucleic acids were stored at -80°C for downstream processes.

Table. 2.7: Modified RNeasy® PowerSoil® Total RNA Kit.
RNA was extracted from Philippine paddy soil using the RNeasy® PowerSoil® Total RNA Kit. The table below displays the modifications made to the RNA extraction protocol in this project.

RNA Extraction Steps	Modification
1. Add up to 2 g of soil to the 15 ml PowerBead Tube (provided). Please refer to the troubleshooting Guide for information regarding the amount of soil to process.	1.5-gram soil used. This amount of soil was optimized in this study.
2. Add 2.5 ml of PowerBead Solution, 0.25 ml of Solution SR1 and 0.8 ml of Solution IRS.	SR1 solution heated up at 60 centigrade. 0.40 ml of SR1 used

3. Add 3.5 ml of phenol/chloroform/isoamyl alcohol (pH 6.5–8.0, [User supplied]). Cap and vortex the PowerBead Tube to mix until the biphasic layer disappears.	Not modified
4. Place the PowerBead Tube on a Vortex Adapter (cat. no. 13000-V1-15) and vortex at maximum speed for 15 min.	**used a BeadBeater for 40 seconds with speed 6**
5. Remove the PowerBead Tube and centrifuge at 2500 x g for 10 min.	**centrifuged at 2500 x g for 20 min**
6. Transfer the upper aqueous phase (avoid the interphase and lower phenol layer) to a clean 15 ml Collection Tube (provided). Discard the phenol/chloroform/isoamyl alcohol.	Not modified
7. Add 1.5 ml of Solution SR3 to the aqueous phase and vortex to mix. Incubate at 2–8°C for 10 min and then centrifuge at 2,500 x g for 10 min at room temperature.	**centrifuged at 2,500 x g for 30 min at room temperature**
8. Transfer the supernatant, without disturbing the pellet (if there is one), to a new 15 ml Collection Tube (provided).	Not modified
9. Add 5 ml of Solution SR4 to the supernatant in the Collection Tube and invert or vortex to mix. Incubate at room temperature for 30 min.	Not modified
10. Centrifuge at 2500 x g for 30 min.	**centrifuged for more than 60 min**
11. Decant the supernatant and invert the 15 ml Collection Tube on a paper towel for 5 min.	Not modified

12. Shake Solution SR5 to mix and add 1 ml to the 15 ml Collection Tube. Resuspend the pellet completely by repeatedly pipetting or vortexing.	Not modified
13. Prepare one JetStar Mini Column (provided) for each RNA isolation sample: **13a. Remove the cap of a 15 ml Collection Tube (provided) and place the JetStar Mini Columninside it. The column will hang in the Collection Tube.13b. Add 2 ml of Solution SR5 to the JetStar Mini Column. Allow it to completely gravityflow through the column and collect in the 15 ml Collection Tube.**	Not modified
14. Add the RNA isolation sample from Step 12 onto the JetStar Mini Column and allow it to gravity flow through the column into the 15 ml Collection Tube.	Not modified
15. Add 1 ml of Solution SR5 to the JetStar Mini Column and allow it to completely gravity flow into the 15 ml Collection Tube.	Not modified
16. Transfer the JetStar Mini Column to a new 15 ml Collection Tube (provided). Shake Solution SR6 to mix and then add 1 ml to the JetStar Mini Column to elute the bound RNA. Allow Solution SR6 to gravity flow into the 15 ml Collection Tube.	Not modified
17. Transfer the eluted RNA to a 2.2 ml Collection Tube (provided). Add 1 ml of Solution SR4. Invert at least once to mix and incubate at –15°C to –30°C for a minimum of 10 min.	Not modified
18. Centrifuge the 2.2 ml Collection Tube at 13,000 x g for 15 min to pellet the RNA.	**centrifuged the 2.2 ml Collection Tube at 13,000 x g for 20 min**
19. Decant the supernatant and invert the 2.2 ml Collection Tubeonto a paper towel for 10 min to air dry the pellet.	Not modified

20. Resuspend the RNA pellet in 100 µl of Solution SR7.	Re-suspended the RNA pellet in 60 µl of Solution SR7.

2.5. *Desulfovibrio vulgaris* Hildenborough growth condition

Desulfovibrio vulgaris was first isolated at Hildenborough, UK, in 1946, and has been subsequently discovered at other sites (Postgate and Campbell 1966; Voordouw et al. 1990; Javaherdashti 1999). It plays important roles in the geochemistry of sedimentary environments (Lovley et al. 1993) and *D. vulgaris* has been chosen as a model strain for SRB researches (Heidelberg et al., 2004; Zhou et al., 2011). *Desulfovibrio vulgaris* Hildenborough (DvH) is grown at 33°C in a liquid medium under anaerobic conditions in 10 ml serum tube in the role of a Hungate tube (Ramel et al., 2015). The medium preparation is described below (Table 2.8):

We dissolved the components of Solution A, brought them to a boil, and then cooled them to room temperature while purging them with 100% N_2 gas. Solutions B and C were added, pH adjusted to 7.8 with NaOH and diffused under 100% N_2 gas atmosphere in anoxic state. During distribution, the medium was vortexed continuously to keep the gray precipitate suspended. The mixed medium was autoclaved at 121°C for 15 min. Then we adjusted the pH of the whole medium to 6.8-7.0. Besides that, *Escherichia coli* str. K-12 substr. MG1655 was cultured aerobically in LB (lysogeny broth) medium for 20 hours at 37°C.

Table 2.8: Recipe of POSTGATE medium for *Desulfovibrio vulgaris* Hildenborough growth.

Solution A		Solution B		Solution C	
K_2HPO_4	0.50 g	$FeSO_4$ x 7 H_2O	0.50 g	Na-thioglycolate	0.10 g
NH_4Cl	1.00 g	Distilled water	10.00 ml	Ascorbic acid	0.10 g
Na_2SO_4	1.00 g			Distilled water	10.00 ml
$CaCl_2$ x $2H_2O$	0.10 g				
$MgSO_4$ x 7 H_2O	2.00 g				
Na-DL-lactate	2.00 g				
Yeast extract	1.00 g				
Sodium resazurin (0.1% w/v)	0.50 ml				
Distilled water	980.00 ml				

After 20 hours, we visualized black precipitate at the bottom of serum tubes. Then sampling of the newly growth bacterium was performed. Next, we extracted *Desulfovibrio vulgaris* Hildenborough and *Escherichia coli* str. K-12 substr. MG1655 gDNA by a commercial kit (Merck). The gDNA of *Desulfovibrio vulgaris* Hildenborough and *Escherichia coli* str. K-12 substr. MG1655 were used to create standard curves to run absolute qPCR assays of dsrB and 16S genes and transcripts, respectively. For mcrA standard curve, we used cloned mcrA gene in a vector (pGEM®-T Easy Vector).

2.6 RT-qPCR and qPCR Assays

Quantitative Polymerase Chain Reaction (qPCR) and Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR) are molecular biology techniques that allow the quantification of nucleic acid sequences. qPCR measures the amount of DNA, while RT-qPCR measures the amount of RNA. The amount of DNA, from week one to week six in three groups, is quantified by monitoring the accumulation of a fluorescent signal during the amplification process. In contrast, RT-qPCR measures the amount of RNA by first converting it into complementary DNA (cDNA) using reverse transcription and then quantifying the cDNA using qPCR for three groups (NaCl and seasalt treatment groups, and control). By comparing the relative amounts of DNA and RNA in a sample using qPCR and RT-qPCR, respectively, it is possible to determine whether changes in gene expression are due to changes in transcriptional regulation or changes in RNA stability. In this part, bacterial 16S rRNA, *dsrB*, *mcrA* genes and transcripts numbers per gram of dry soil were determined by qPCR and RT-qPCR assays. Additionally, the genes and transcripts numbers encoding key enzymes of sulfate reducing bacteria (SRB), the β-subunit of dissimilatory (bi) sulfite reductase (*dsrB*); of methanogens, methyl-coenzyme M Reductase A (*mcrA*); and of 16S rRNA were quantified. To create standard curves, genomic DNA from *Escherichia* coli k-12 MG1655 (bacterial 16S rRNA genes calibrated from 10 to 10^9 copies); gDNA from *Desulfovibrio vulgaris* Hildenboroug (Leibniz Institute DSMZ Websit*e*) (www.dsmz.de) (*dsrB* gene calibrated from 10 to 10^9 copies); and *mcrA* gene fragments cloned into pGEM-T Easy Vector (methanogens *mcrA* genes calibrated from 10 to 10^9 copies) were used. A CFX Connect Real-Time PCR detection system (Bio-Rad) was used for qPCR and RT-qPCR in optical 96-well reaction plates. SYBR Green-based assays were performed to the analyses (Peng et al., 2018). A CFX Connect Real-Time PCR detection system (Bio-Rad) was used for qPCR and RT-qPCR assays. Three technical replicates were used for each reaction. The efficiency of the PCR was over 90% (R^2 > 0.99), and melt curve analysis verified the absence of non-specific PCR product.

2.6.1 Creation of standard curve for absolute quantitative Real-Time PCR:

In absolute qPCR assays, the standard curve is used to determine the absolute quantity of a specific target gene in a sample by comparing the fluorescence signal of the unknown sample to the standard curve. To create standard curves for each marker gene and their transcript quantification, we followed the steps below:

Step1: Identify genome size (organism of interest)

Step2: Identify the mass of DNA per genome.
Calculate the mass of the genome by inserting the genome-size value in the formula Below.

$$m = \left[n \right] \left[1.096\text{e-}21 \ \frac{g}{bp} \right]$$

Step3: Divide the mass of the genome by the copy number of the gene of interest per haploid genome.

Step4: Calculate the mass of gDNA containing the copy of interest sample:

$$\text{Copy \# of interest} \times \text{mass of haploid genome} = \text{mass of gDNA needed}$$

Step5: Calculate the concentrations of gDNA needed to achieve the copy of interest sample. Divide the mass needed (calculated in Step 4) by the volume to be pipetted into each reaction.

Step6: Prepare a serial dilution of the gDNA. For the dilutions we will use the formula below:

$$C_1V_1 = C_2V_2$$

The stock concentration of gDNA is determined by spectrophotometric (μg/μl). The standard curves generated based on the protocol have been mentioned in the following link: https://tools.thermofisher.com/content/sfs/brochures/cms_042486.pdf

2.7 PCR amplicon libraries and High-Throughput Sequencing

In the context of DNA sequencing of each marker gene in our experiment, a library is created to allow for the simultaneous sequencing of a large number of DNA fragments from a single or multiple samples. The library preparation step is necessary before sequencing because the sequencing platforms cannot directly sequence a DNA sample in its native form. We used 12-barcoded-stagger primer sets for *mcrA*, 16S rRNA and *dsrB* genes, and performed these steps (amplification of DNA, quality control, purification, and quantity control) described below.

The PCR reactions were amplified using the barcod-staggered 16S rRNA primer set 515F/806R (Walters *et al*., 2015), the methanogen barcod-staggered primer set Mlas-F/mcrA-R (Casañas *et al*., 2015) and the barcoded dsrB primer set 2060F/Dsr4R (Wang et al., 2016). The barcode and stagger sequences for each sample are listed in the Table 2.4; Table 2.5; and Table 2.6. Each PCR reaction (50 μl volumes) consisted of (10 μl buffer; MgCl$_2$ 4 μl; dNTPs 1 μl (GoTaq® G2 Green Master Mix), (10 μM) of each primer (1 μl for *mcrA*, *dsrB*, and 16S 1RNA genes), 0.5 μl BSA, 4 μl of template and 28.5 μl sterile water. PCR amplifications for 16S rRNA gene (V4 region) (Katiraei et al., 2022) were performed on a C1000 Touch Thermal Cycler (Bio-Rad) instrument with the following cycling conditions: initial denaturation (94°C, 3 min), followed by 35 cycles of denaturation (94°C, 30 s), annealing (50°C, 30s) and elongation (72°C, 90s), and final extension (72°C, 10 min) and a 4°C hold; for *mcrA* gene were performed with the following thermal profile: initial denaturation (95°C, 30s), followed by 35 cycles of denaturation (94°C, 15 s), annealing (55°C, 30s) and elongation (72°C, 30s), and final extension (80°C, 30 min) and a 4°C hold; and The amplifications for *dsrB* gene were performed run under the following cycling conditions: 2 min initial denaturation at 95°C, followed by 25 cycles of denaturing at 95°C for 30 s; annealing at 55°C for 30 s; extension at 30°C for 72 s; and completed with a final extension for 10 min at 72°C and a 4°C hold. The quality and quantity of PCR products evaluated and quantified by running electrophoresis on 1% agarose gel and Qubit Assay kits mentioned before, respectively. Prior to sequencing, the PCR products purified using Agencourt AMPure XP beads according to the manufacturer's protocol, and DNA concentrations converted from ng/μl to nM for accurate quantification. Standard Illumina libraries, libraries having undergone PCR amplification, require the use of dsDNA-specific fluorescent dye methods (i.e., Qubit) for accurate quantification. These methods typically measure dsDNA concentration in ng/μl. To convert from ng/μl to nM for cluster generation, we followed the instructions below:
1. Determine the average size of the library by running it on the Fragment Analyzer.
2. Use the following formula to convert from ng/μl to nM.

$$\text{Concentration in nM} = \frac{(concentration\ in\ \frac{ng}{\mu l})}{(\frac{660g}{mol} * \text{average library size in bp})} \times 10^6$$

libraries prepared in sterile and nuclease-free tubes, then final DNA concentrations measured by the dsDNA Qubit Kit. The amplicon libraries of 16S rRNA and *dsrB* (week 1, 2, 4 and 6) were sequenced on

an Illumina HiSeq 2500 system using paired-end 2×250 bp sequencing at the Max Planck-Genome-Centre (Cologne, Germany). The amplicon libraries of *mcrA* (week 1, 2, 4 and 6) were sequenced on an Illumina MiSeq platform using a paired-end 2×300 bp mode by LGC group (Berlin, Germany).

2.8 Data analysis

2.7.1 QIIME2 Installation

To work with QIIME2, we installed in on Linux operation system or local computer, there are detailed installation instructions on the QIIME2 website.

2.7.2 Marker genes amplicon sequencing

The QIIME2 (version 2019.10) was used for analysis of three marker genes sequences in Illumina platform. In this study, we focused on targeted amplicon sequencing of the 16S rRNA, *dsrB* and *mcrA* genes. The 16S rRNA gene (~1500 bp) codes for a ribosomal RNA of the small ribosomal subunit of the prokaryotic ribosome (30S). Ribosomes are made up of proteins and RNAs and are important for translation (protein synthesis from mRNA). The 16S rRNA is highly conserved among bacteria and archaea due to the importance of their function. Within conserved regions of 16S rRNA, there are nine hypervariable regions (V1-V9), and these regions are used for establishing phylogenetic relationships useful for taxonomic classification. See the following figure from Fukuda et al., (2016).
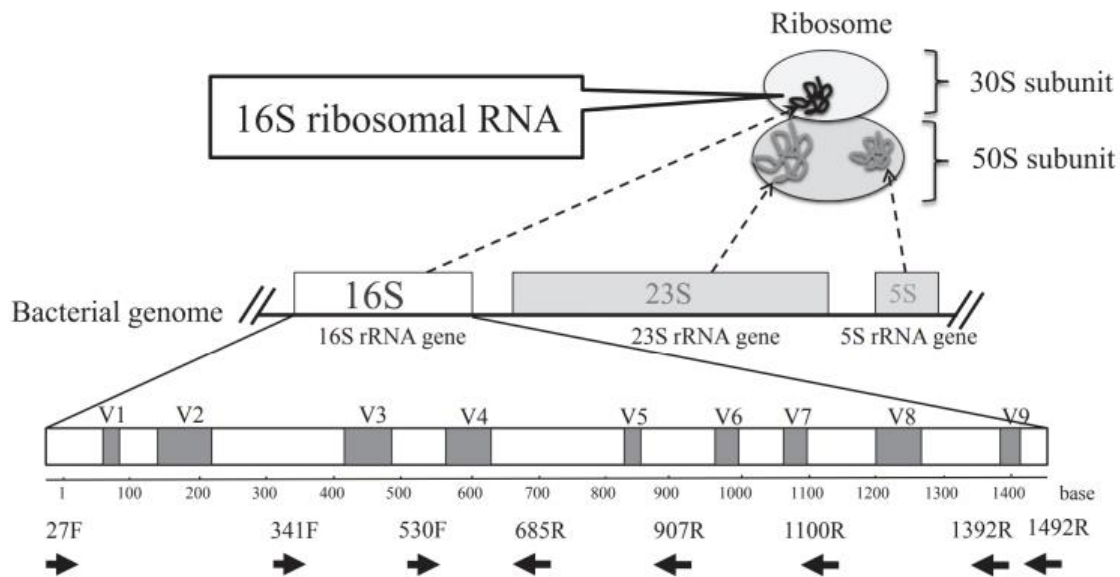
Figure. 2.2. The schema of ribosome complex and 16S rRNA gene. The white and grey boxes indicate conserved regions and hypervariable regions respectively. The bold arrows are shown approximate positions of universal primers on 16S rRNA gene sequence of *Escherichia coli*. White: conserved regions, grey: hypervariable regions (V1-V9).

The libraries created for *mcrA*, *dsrB*, and 16S rRNA genes had different lengths, with the mcrA library at 496 bp, the dsrB library at 366 bp, and the 16S rRNA library at 330 bp with barcode and stagger sequences.

We generally followed the workflows recommended by the QIIME 2 developers described. First of all, the quality of the sequence files was considered then imported the data. In this pipeline, the paired-end reads get merged, filtered by quality and then denoised using DADA2. The denoising methods generate a table of features with frequency and sequences. The pipeline created a rarefaction curve labeled based on the

metadata columns provided. In the final steps of the pipeline, the sequences will be classified by your choice of method (VSEARCH) and database (SILVA), and a barplot of taxa relative abundances generated.
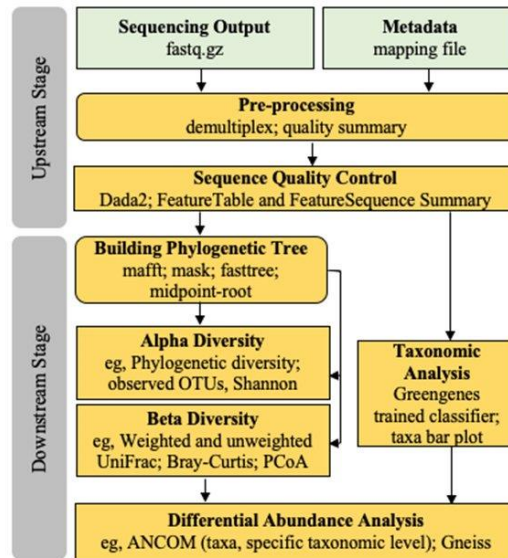
Figure. 2.3. QIIME2 workflow consists of several steps that are typically performed in the following order: 1) The first step is to import the raw sequencing data (fast1.gz file) into QIIME2 besdie a metadata file. 2) quality control on the data to remove any low-quality reads or sequences. 3) generating a feature table 4) assign taxonomic identities to each feature using classifier 5) diversity analysis, and 6) visualisation.

2.7.3 Metadata formatting

To interpret the results of any next-generation sequencing experiment, it is necessary to have sample metadata. Bioinformatics software usually requires tabular text files, which have to be formatted in a specific way, and are often created by researchers using spreadsheet software. We provided all the necessary items of the metadata to support our hypotheses and validated it using Keemei, a tool for checking bioinformatics file formats in Google Sheets (Rideout et al., 2016). To ensure a study's success, metadata is an essential element that investigators have significant control over. We started generating metadata during sample collection to facilitate effective data analysis and recorded all sample attributes that are pertinent to our hypotheses. These attributes serve as the foundation for the statistical tests and visualizations in QIIME2.

2.7.4 QIIME2 requirements for sample metadata

1. In .tsv (tab separated) format
2. Include a SampleID column as the first column.
3. Missing data is represented by empty cells, not NAs
4. Supports categorical and numeric data (may include a row with #q2:types of either categorical or numeric)
5. rows that begin with # are ignored.
6. Whitespace is ignored.

2.7.5 Data import

As mentioned previously, the first step of any QIIME 2 analysis is to import of the data. Each type of data is stored in its own QIIME2 artifact, with .qza extension file. This will make more sense as we begin to work through the data. We imported the of fastq files to demonstrate initial sequence processing steps. Then

we demultiplexd the reads, split the reads by sample. Each fastq file the forward reads, and the reverse reads containing the barcodes, which separated samples.

2.7.6 Sequence quality control and feature table construction

After sequencing, amplicon sequence variants (ASVs) were formed by denoising samples based on fixed dissimilarity thresholds: 97% for 16S rRNA, 90% for dsrB, and 80% for mcrA. In a denoising approach, the exact biological sequence is inferred and noise is removed from the dataset via error correction. Sequences were trimmed and filtered to ensure quality control. To obtain high-resolution amplicon sequence variants (ASVs), DADA2 was used instead of OTUs as it can resolve differences as small as one nucleotide. DADA2 is available through the QIIME 2 q2-dada2 plugin (Callahan et al., 2016) and is consistently more effective than clustering methods in identifying true community composition. DADA2 also includes joining paired-end reads, making it convenient to use for this purpose. Before using DADA2, we checked out the quality of our data referring back to our output from the summarize of qiime demux plugin. Based on the pipeline, all reads were truncated (according to the quality plots), trimmed (according to the non-biological sequences' lengths), denoised, filtered (Chimeras removal) where the reads (forward and reverse) drop in quality, later the forward and reverse paired-end reads were merged, and finally the reads were clustered into amplicon sequence variants (ASVs) with a 99% similarity threshold using DADA2 plugin. The length of our sequences (forward and reverse) for merging them was cautiously noticed to have a general size of the overlap (between forward and reverse for merging) before using the DADA2 plugin. The following simple formula was used to calculate approximate overlap length:

$$\text{Overlap} = (\text{forward read}) + (\text{reverse read}) - (\text{length of amplicon})$$

2.7.7 Alpha rarefaction plot

Rarefaction is a statistical method that determines species richness by sampling. This technique is often applied to operational taxonomic unit analysis (OTUs) and is very useful in microbial ecology. In this study, rarefaction was utilized to check if a sample was sequenced enough to identify it and to determine if a group of samples were from the same community. The technique involves randomly discarding reads from larger samples until the number of remaining samples reaches a specified threshold. The calculation of species richness is based on the rarefaction curve, which plotted the number of species against the number of samples.

2.7.8 Alpha diversity

Alpha diversity is within sample diversity. When exploring alpha diversity, we are interested in the distribution of microbes within a sample or metadata category. This distribution not only includes the number of different organisms (richness) but also how evenly distributed these organisms are in terms of abundance (evenness). The alpha-diversity group significance command creates boxplots of the alpha-diversity values and significant differences between groups are assessed statistically with special command in QIIME2. This software performed the statistical tests for each of the sample groupings present in the metadata file with the rarefied SampleData[AlphaDiversity] artifact.

2.7.9 Taxonomic classification

After the sequences were derived from denoising methods, it provided us with the highest possible resolution of our features given our sequencing data. Taxonomic affiliation step was started to study the microbes from which sequences were obtained. To do this, 16S rRNA ASVs were taxonomically classified with VSEARCH-based consensus taxonomy classifier with 97 % cut-off and the SILVA 132 database

(Rognes et al., 2016). Similarly, dsrB and mcrA ASVs were taxonomically classified with VSEARCH-based consensus taxonomy classifier with 80 and 90% cut-off, respectively. mcrA and dsrB databases were curated by the q2-RESCRIPt plugin in QIIME2 (Robeson et al., 2021). Taxonomic classification of the ASVs was completed using a pre-existing model that was trained on the SILVA 138 99% OTUs from the V4 region of the 16S rRNA gene (Bokulich et al. 2018). The full resulting taxonomies, from phylum to species, were transferred and further analysed in Microsoft Excel to ensure that all ASVs were properly classified.

2.7.10 QIIME2 View
QIIME2 View simplified to visualize the complex interactive data through a novel combination of modern web browser APIs within a single-page application. We opened and visualized all generated QIIME2 files (.qza and .qzv) through QIIME2 View.

2.7.11 Statistical Analysis
The Kruskal-Wallis (a non-parametric statistical test) was used to identify significant differences in alpha diversity ($P \leq 0.05$) among three groups (untreated, NaCl⁻treated, and seasalt-treated) by QIIME2. Normality of our data was assessed using both the Shapiro-Wilk test in R software and measures of kurtosis and skewness in Excel 2016. Statistical tests for gas measurements, fatty acid concentrations, qPCR assays and RT-qPCR assays data between control groups (0 mM) and salt treatments (150 mM seasalt and NaCl) were performed using the ANOVA (analysis of variance), and the Kruskal-Wallis considered significant at $P \leq 0.05$, and considered at $P > 0.05$ when the data was not statistically significant. The choice of the tests depends on the characteristics of our data and the assumptions of the test. The ANOVA and MANOVA tests assume that the data are normally distributed and have equal variances which was done in Excel and R, respectively, while the Kruskal-Wallis test does not assume normality and used for non-normally distributed data which was performed in R software. The data of the H_2S gas concentration, RT-qPCR assays, qPCR assays, CH_4 and CO_2 pressures were plotted with SigmaPlot 14.0. Heatmaps and stacked bar graphs were generated using the "heatmap" package in R.

2.7.12 Data availability
All sequencing data associated with this study have been deposited at the NCBI Sequence Read Archive (SRA) under ID BioProject number PRJNA779407 involved submissions SUB10607456 and SUB12513777.

3-Results
The results chapter of this thesis is organized into five main sections: Methane and carbon dioxide pressures, (ii) H_2S concentrations, (iii) Fatty acid concentrations, (iv) RT-qPCR and qPCR assays, and (v) Taxonomy classifications. The project began by measuring the concentrations of gases (CH_4, CO_2, and H_2S). The results investigated the salt treatments effect on the amount of gases produced in the treatment and control groups. The impact of the salt treatments on other experimental parameters were also evaluated, and will be discussed in the following sections.

3.1 Methane and carbon dioxide pressures.
Soil slinisation reduces gas emissions from soil by altering the composition and function of soil microbial communities. Methane and carbon dioxide are two of the major greenhouse gases produced in soil, and

their production is tightly linked to soil microbial communities. In this experiment, the research question arises: How does salt treatments (NaCl and seasalt) affect the functional of soil microbial communities, and what is the impact on methane and carbon dioxide production. In this regard, we measured CH_4 gas concentration was monitored and recorded by a GC machine to analyze samples during the experiment (Figure. 3.1) throught week one to week six for all groups. The groups (NaCl, seasalt treatment and control groups) had their methane levels gradually raised over time. Methane production appears to have been higher in the control group than in the NaCl and the seasalt treatment groups. Compared to the treated groups, the control group had a greater concentration (or pressure) of methane in the bottles, as shown by the higher pressure of methane (22.1 bar). Samples treated with NaCl or sea salt showed signs of reducing methane generation. Inhibitory impact of the NaCl and seasalt treatments at 150 mM on methnogens in the treated-samples account for the lower methane pressure in the NaCl treatment group (20.4, bar), and the sea salt treatment group (13.6 bar). The normality test was performed for the three group sperately. The data was noramly distributed. The three groups differed across the time points in methan production (ANOVA test: F-statistic = 12.4, df = 2, significance level = 0.05, p-value = 0.0005).
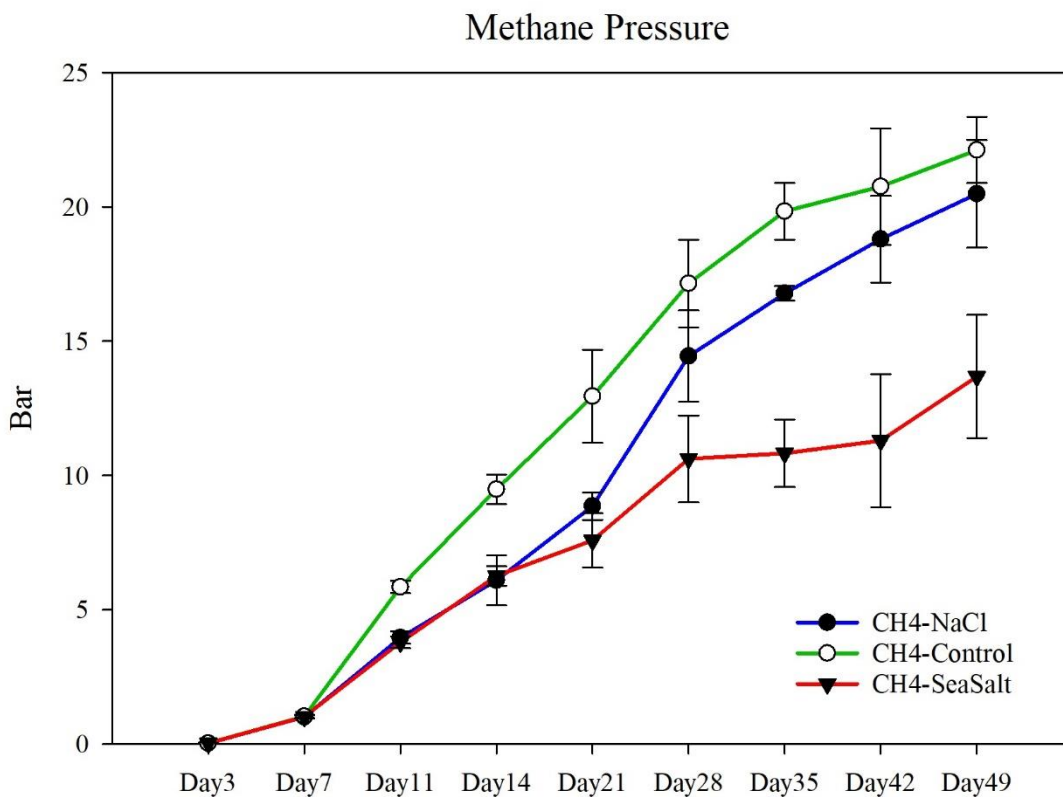
Figure 3.1: The pressures of methane (in bar) were measured in two treated samples and a control group. Three replicas (n =3) were used for each time-point sample of experimental groups. Error bar shows standard error of the mean (SEM).

According to the findings, the group control produced more carbon dioxide than both of the treatment groups that were given sodium chloride and sea salt (Fig 3.2). It may be deduced from the fact that the control group's samples had a greater pressure of carbon dioxide—which was recorded as 4.9 bar—that the

control group's sample also contained a higher concentration of carbon dioxide. The inhibitory effect that the NaCl and seasalt treatments had on the microorganisms, including fermentative bacteria, acetogenic bacteria, and methanogenic archaea, that were responsible for producing carbon dioxide in the samples may have been the cause of the lower carbon dioxide pressure that was observed in the treatment groups that received NaCl and sea salt (4.4 bar and 3.9 bar, respectively). The normality test was performed for the three group sperately. The data was noramly distributed. The three groups differed across the time points in carbon dioxide production (ANOVA: F-statistic = 14.3, df = 2, significance level = 0.05, p-value = 0.0002).
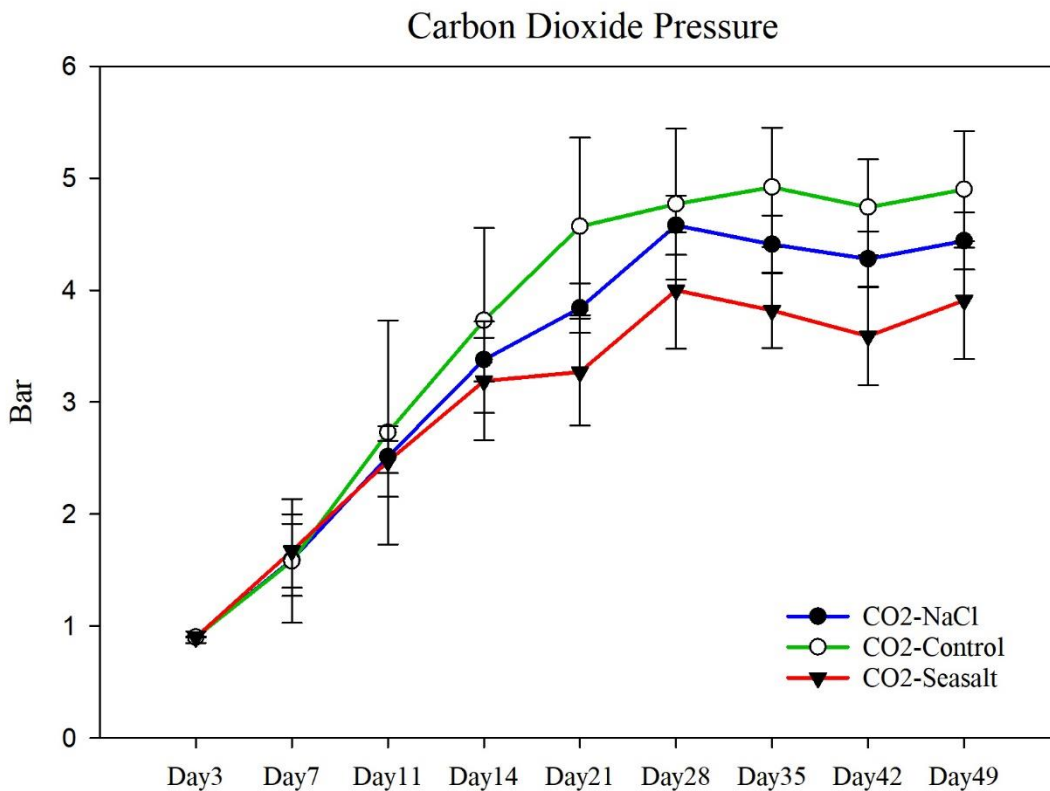
Figure 3.2: The measured methane pressures (in bar) in two treated samples and a control group. Three replicas (n =3) were used for each time-point sample of experimental groups. Error bar shows standard error of the mean (SEM).

3.2 H$_2$S concentration

Sulfate is a common component of seawater and is present in many types of salts. In our study, we investigated the effect of sulfate on the production of hydrogen sulfide (H$_2$S) gas by soil microbial communities. H$_2$S gas is produced by sulfate-reducing bacteria (SRB) in anaerobic conditions. These bacteria use sulfate as an electron acceptor to oxidize organic matter and release H$_2$S gas as a byproduct. The presence of sulfate in the seasalt provided a source of electron acceptors for SRB, leading to an increase in H$_2$S production. We measured H$_2$S concentrations in all sample (seasalt and NaCl teated- and control groups) with a special H$_2$S microsensor. We found that when soil samples were treated with seasalt

containing sulfate, the H_2S gas concentration increased significantly compared to the control group and the samples treated with NaCl that did not contain sulfate. In this experiment, concentration of H_2S is greater in the sea salt-treated sample than in the control and NaCl-treated samples, as depicted by the graph (Figure 3.3). By week four, the concentration had reached its highest point (4.6 mol/L). Following that, the quantity reduced slightly during weeks five and six. By week four, H_2S concentrations in control and NaCl samples were roughly comparable (3.9 and 3.8 mol/L, respectively). The quantity of this gas dropped between weeks 4 and 6. The result suggested that the seasalt treatment activate sulfate reducing bacteria between the other groups. The normality test was performed for the three group sperately. The data was noramly distributed. The three groups differed across the time points in terms of H_2S cocentration (ANOVA: F-statistic = 43.1, df = 2, significance level = 0.05, p-value = 1.2E-05).
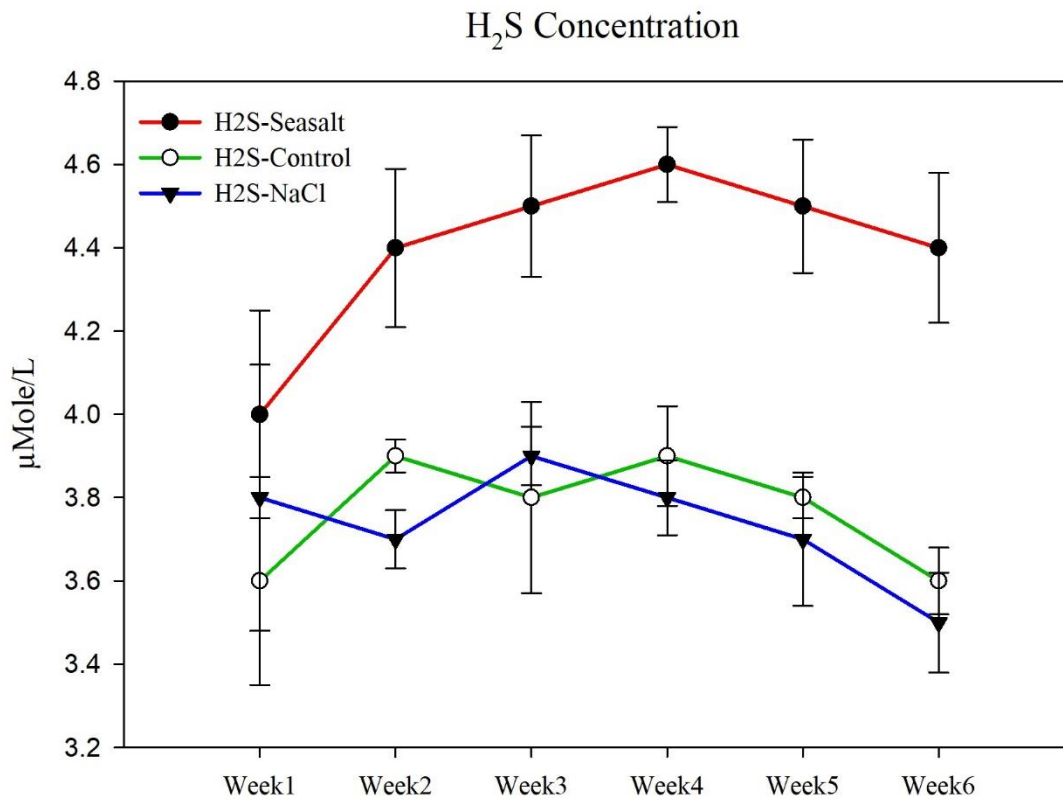
Figure 3.3: The measured carbon dioxide pressures (µmol/L) in two treated samples and a control group. Three replicas (n =3) were used for each time-point sample of experimental groups. Error bar shows standard error of the mean (SEM).

3.3 Fatty acid concentrations

The addition of NaCl and seasalt to soil samples can impact on the concentrations of fatty acids such as acetate, butyrate, and propionate. The presence of salts can affect soil microbial communities in a variety of ways, including altering their diversity, abundance, and activity levels. In this experiment, we showed that the moderate level (at 150 mM) of the two salts change and differ the amount of the fatty acids through

week one to week six. As can be seen in figure 3.4, the intake of acetate in the experimental groups dropped during the course of the study. The result of the experiment showed that the hindrance of acetate digestion was more pronounced in the soil samples treated with the seasalt treatment compared to those treated with NaCl. In the control group, it was rapidly broken down by microorganisms in the soil by day 11. In samples that had been treated with sea salt, the acetate concentration reached its minimum value by day 21 or week 2. In addition, in samples that had been treated with NaCl, the concentration dropped close to zero on day 14 or the first week. The result indicated that sulfate-reducing bacteria, methanogens, and homoacetogens probably became less active with the intermediate salt stresses. The normality test was performed for the three group sperately. The data was not noramly distributed. The three groups did not differ across the time points in terms of acetate breakdown (Kruskal-Wallis test: chi-squared = 1.31, df = 2, significance level = 0.05, p-value = 0.51).
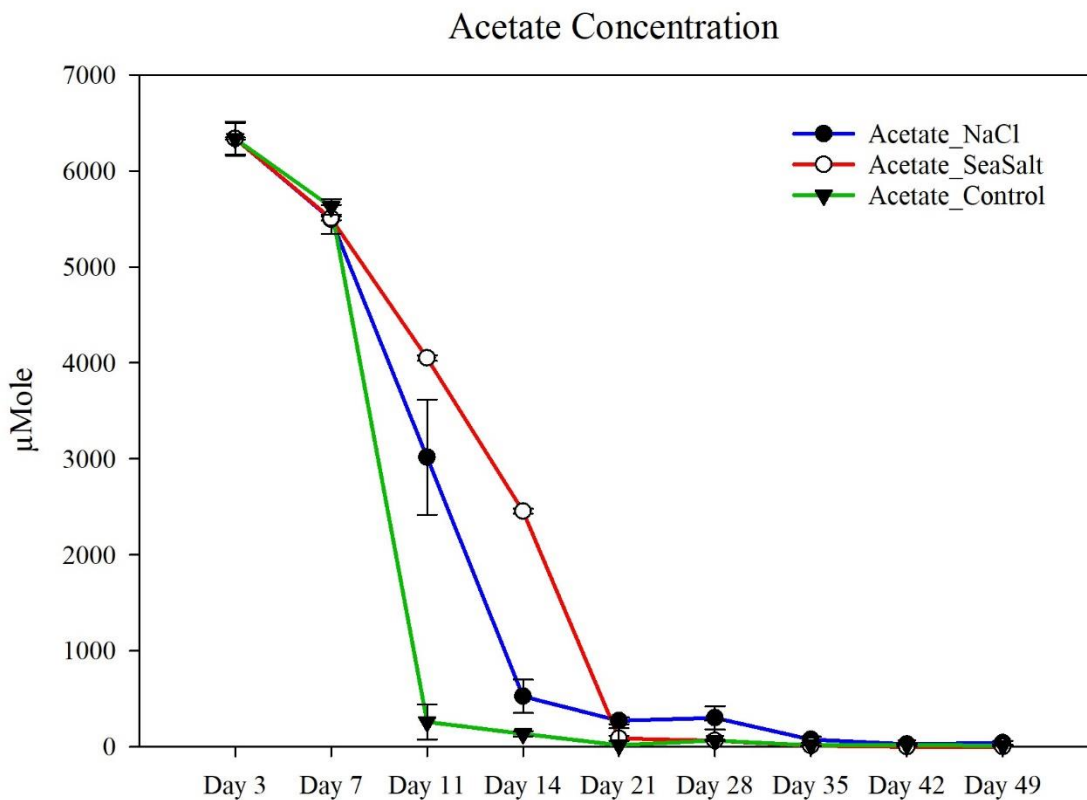
Figure 3.4: The concentrations of acetate in two treated samples and a control group. Three replicas (n =3) were used for each time-point sample of experimental groups. Error bar shows standard error of the mean (SEM).

As comparison to other butyrate concentrations (Figure 3.5), the NaCl treatment acquired the greatest amount of butyrate over time by day 21, 1240,8 μmole. On day 21 to day 28, the concentration reduced dramatically and reached zero μmole. Similarly, seasalt inhibited the consumption of butyrate by microorganisms from day 7 to day 11, and it reached 627.2 μmole. In contrast, butyrate was consumed progressively in the control group following incubation. In contrast to the seasalt treatment, the NaCl treatment exhibited an additional reduction in the intake of butyrate, indicating a pronounced impact of the NaCl treatment on butyrate intake. The result showed that sulfate-reducing bacteria, syntrophic bacteria,

and some species of methanogenic archaea were impacted by salt stresses, especially by NaCl. The normality test was performed for the three group seperately. The data was not noramly distributed. The three groups did not differ statistically across the time points in terms of butyrate breakdown (Kruskal-Wallis test: chi-squared = 1.34, df = 2, significance level = 0.05, p-value = 0.509).
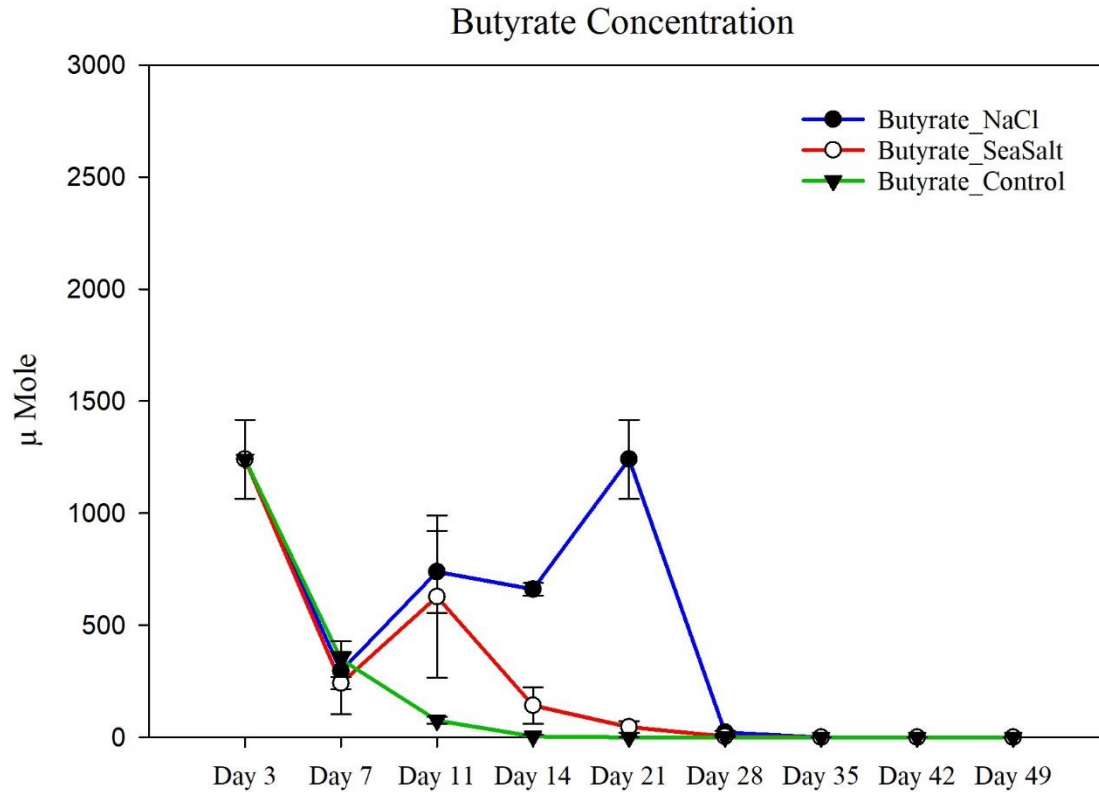
Figure 3.5: The concentrations of butyrate in two treated samples and a control group. Three replicas (n =3) were used for each time-point sample of experimental groups. Error bar shows standard error of the mean (SEM).

Over the course of the experiment, starting from the beginning and up until day 21, there was a modest increase observed in the concentration of propionate in the NaCl-treated sample. From day 21 to day 28, the concentration of propionate doubled, reaching 1040,3 µmole. The NaCl treatment accumulated significantly more propionate than the control and sea salt treatment. In the control group, this fatty acid accumulated from day 7 to day 14 (278.2, 563.7 µmole, respectively), but was completely consumed from day 14 to day 21. In the seasalt group, the fatty acid exhibited a gradual decrease as a result of microbial consumption, eventually reaching zero concentration on day 28. It demonstrated that microorganism specially propionate-oxidizing bacteria became less active to break down the organic materials. The normality test was performed for the three group seperately. The data was not noramly distributed. The three groups did not differ statistically across the time points in terms of propionate breakdown (Kruskal-Wallis test: chi-squared = 2.45, df = 2, significance level = 0.05, p-value = 0.29).
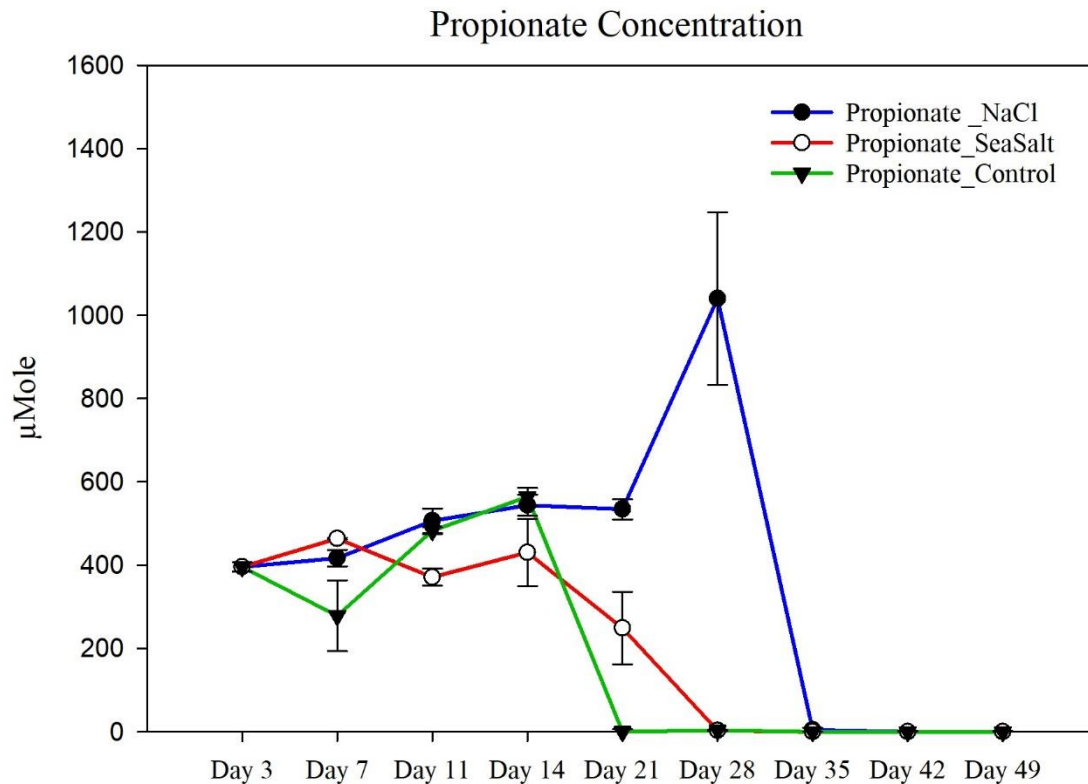
Figure 3.6: The concentrations of propionate in two treated samples and a control group. Three replicas (n =3) were used for each time-point sample of experimental groups. Error bar shows standard error of the mean (SEM).

3.4 Nucleic acids concentrations, purity and integrity

To gain a comprehensive understanding of microbial communities of the experimental groups (NaCl, seasalt treatments and control group) through the six weeks, it was necessary to study their taxonomic compositions, and microbial functions. This was achieved through the extraction and analysis of DNA and RNA from the samples. The success of these techniques depends heavily on the quality and purity of the extracted genetic material. To do so, a Qubit kit was used to determine the concentration of the extracted nucleic acids (DNA and RNA). For RNA, the range of nucleic acid concentrations was between 3 and 40 ng/µl. while for DNA, the range was between 8 and 30 ng/µl. Electrophoresis performed and used to determine the quality of the nucleic acids. High quality and high yield of the nucleic acids were verified by gel electrophoresis, containing 1% agarose. We utilized a UV-Vis Spectrophotometer-NanoDrop in order to evaluate the presence of humic acid substances in the samples. These chemicals are prevalent in soil. The mount of them were less than 0.33 in 400 nm wavelength. More details are listed in the Table S28 and Table S29. After RNA extraction, cDNAs of each week were synthesized. The cDNA concentrations were at least 700 ng/µl which quantified by a NanoDrop-1000 spectrophotometer.

3.4.1 libraries preparation and concentrations before sequencing

By converting the concentration from ng/µl to nM, we can accurately determine the number of DNA molecules in a sample, which is important for sequencing applications. To do so, all libraries' concentrations (ng/µl) of each week sample (for marker genes and their transcripts) converted to nanomolar (nM) by the formula below:

$$\text{Concentration (nM)} = \frac{concentration\ in\ \text{ng/µl}}{(660\frac{g}{mol}*library\ size\ in\ bp)} * 1000000$$

Normalizing libraries before sequencing is important to ensure that each library contributes an equal amount of DNA to the sequencing run. This helps to avoid bias towards certain libraries and improves the accuracy and reproducibility of the sequencing results. This involves diluting the DNA concentration of each library to a specific concentration, typically in nanomolar (nM) unit. In our experiment, the concentrations were normalized in 15 nM and adjusted in 20 µl with formula below:

$$C1 * V1 = V2 * V2$$

Finally, the libraries were pooled, and their concentrations were measured with a Qubit kit. The concentrations are listed as follows:

For mcrA libraries:

Concentration (nM) = (concentration in [samples] ng/µl)/ (660 g/mol*496 bp) * 1000000

Library 1: 8.8 ng/µl   Library 3: 8.06 ng/µl   Library 5: 6.58 ng/µl   Library 7: 5.1 ng/µl

Library 2: 8.62 ng/µl   Library 4: 6.39 ng/µl   Library 6: 7.33 ng/µl

For 16S rRNA libraries:

Concentration (nM) = (concentration in [samples] ng/µl)/ (660 g/mol*330 bp) * 1000000

Library 1: 7.31 ng/µl   Library 3: 6.51 ng/µl   Library 5: 4.43 ng/µl

Library 2: 6.12 ng/µl   Library 4: 4.62 ng/µl   Library 6: 5.13 ng/µl

For dsrB libraries:

Concentration (nM) = (concentration in [samples] ng/µl)/ (660 g/mol*366 bp) * 1000000

Library 1: 4.98 ng/µl   Library 3: 4.26 ng/µl   Library 5: 5.99 ng/µl

Library 2: 4.3 ng/µl   Library 4: 8.06 ng/µl   Library 6: 8.8 ng/µl

The libraries were sent to the sequencing center (Max Planck Genome Centre (MP-GC), Cologne, Germany) for further processing. The cDNA and DNA obtained at each time point were divided into aliquots for further analysis using RT-qPCR and qPCR assays.

3.5 RT-qPCR and qPCR assays

The objective of this experiment was to investigate the effects of NaCl and seasalt treatments on the expression levels of three specific genes (16S rRNA, *dsrB*, and *mcrA*) and their transcripts copies over a period of six weeks, in comparison to a control group. The selected genes are known to play a critical role in microbial function and growth. By conducting qPCR and RT-qPCR assays, we aimed to determine whether these salt treatments have any impact on the gene and transcripts copy number levels. The data generated from these assays will enable us to identify potential changes in gene expression patterns and transcript abundance that occur in response to the salt treatments.

In qPCR (quantitative PCR), the template DNA is amplified directly from the genomic DNA, without any additional processing steps. qPCR was used to measure the DNA copy numbers of the 16S rRNA, *dsrB*, and *mcrA* genes. In contrast, in RT-qPCR, the RNA was first converted to complementary DNA (cDNA) using a reverse transcription step, and the resulting cDNA is then used as a template in RT-qPCR reaction. This allows for the measurement of mRNA levels in our samples through the control and salt-treatment samples.

The absolute abundance of bacterial 16S rRNA, dsrB and mcrA transcripts were determined by a RT-qPCR (cDNA) assay. The RT-qPCR assay was used to calculate the transcript copy numbers as a proxy for metabolic activity. Similary, the absolute abundance of bacterial 16S rRNA, *dsrB* and *mcrA* genes were determined by a qPCR (DNA) assay. The qPCR assay was used to calculate the genes copy numbers as a proxy for cell biomass. Overall, in our study, we used both qPCR and RT-qPCR to gain a more complete picture of the gene and transcipts copy levels of the three genes and transcipts among the experimental groups.

3.5.1 RT-qPCR assay for 16S rRNA transcript copies
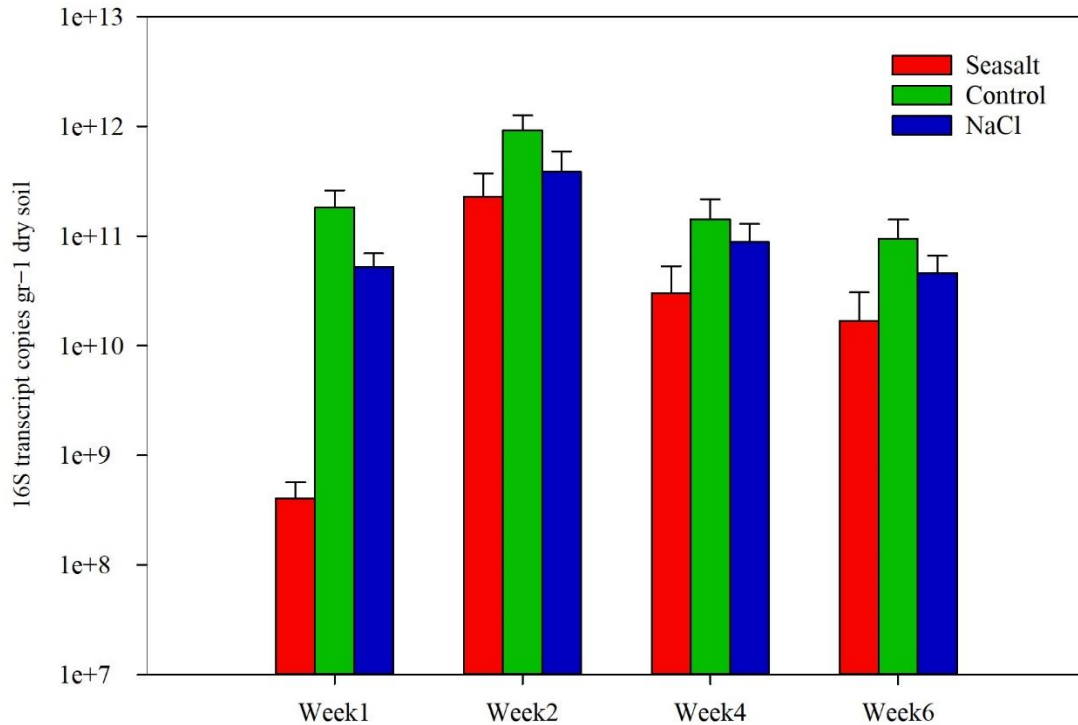
Figure 3.7: 16S rRNA transcript numbers of bacteria. Bar chart shows the number of bacterial transcript per gram dry weight of soil. Statistical test indicates significant difference ($P \leq 0.05$) within groups, but it was not significant difference ($p > 0.05$) between the groups over the time points. Three replicas (n =3) were used for each time-point sample of experimental groups. The error bar shows standard error of the mean (SEM).

The NaCl and Seasalt groups had lower transcript copy numbers in comparisons to the control group, and their responses to the salt treatments varied across the experiment (Figure 3.8). The NaCl group had the highest mean transcript copy number compared to the salt-treated group in week one, with a value of 5.24E+10 copies. However, this number declined in the subsequent weeks, reaching a minimum of 4.60E+10 copies in week six.

On the other hand, the seasalt group showed a consistently lower mean transcript copy number throughout the four weeks, ranging from 4.06E+08 copies in week one to 1.69E+10 copies in week six. This indicates that Seasalt treatment had a more severe impact on the transcript copy numbers than NaCl treatment. This study highlights the differences between the control group and the salt-treated groups, with the control group consistently showing higher 16S rRNA transcript copy numbers. In conclusion, seasalt treatment reduced 28.63%, 17%, 50%, and 30.86% the 16S rRNA transcript copy numbers compared to the NaCl treatment for week one, two, four and six, respectively. The result showed that bacterial community were less active in the seasalt treatment group compared to the NaCl-teated samples.

Statistically, the number of 16S rRNA bacterial transcripts copies were significant difference (ANOVA: F statistic = 6.19, df = 3, significance level = 0.05, p-value = 0.02) within groups, but they were not different

significantly (ANOVA: F statistic = 3.21, df = 2, significance level = 0.05, p-value = 0.1) between the groups from week 1 (day 14) to week 6 (day 49).

3.5.2 qPCR assay for 16S rRNA gene copies
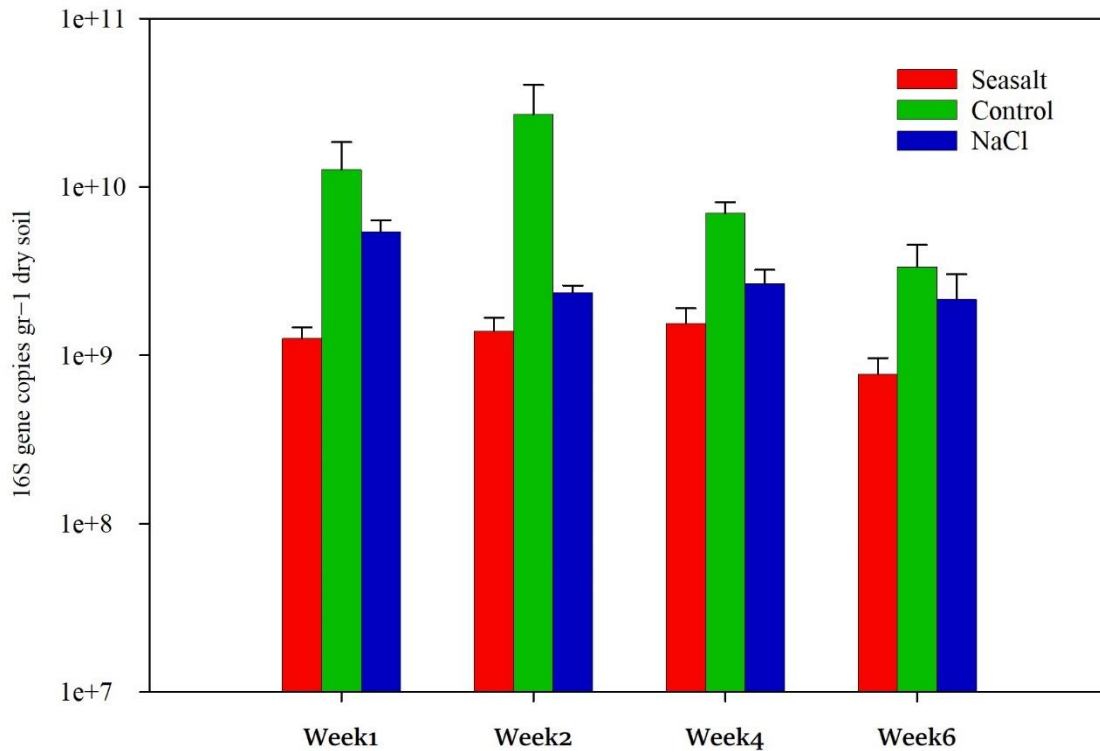
Figure 3.8: 16S rRNA gene numbers of bacteria. Bar chart shows the number of bacterial gene per gram dry weight of soil. Statistical test indicates no significant difference (P > 0.05) within groups, also, it was near to significant difference (P-value = 0.07) between the groups over the time points. Three replicas (n =3) were used for each time-point sample of experimental groups. The error bar shows standard error of the mean (SEM).

The seasalt group consistently showed the lowest mean the 16S rRNA gene copy numbers, ranging from 1.26E+09 copies in week one to 7.71E+08 copies in week six. Notably, the Seasalt group had a lower gene copy number compared to the Control and NaCl groups throughout the experiment (Figure 3.8).

The NaCl group's response to the salt treatment varied across the four weeks, with a maximum mean gene copy number of 5.39E+09 copies in week one, followed by a decline to 2.15E+09 copies in week six. This indicates that seasalt treatment had a more impact on gene copy numbers compared to the Control and NaCl groups. In conclusion, the seasalt treatment reduced 32.66%, 3.62%, 16.14%, and 41.05% the 16S rRNA gene copy numbers compared to NaCl treatment for week one, two, four, and six, respectively. The result showed that bacterial community growth in the seasalt treatment group was less than the other groups.

Statistically, the number of bacterial 16S rRNA gene copies were not significant difference (ANOVA: F statistic = 1.05, df = 3, significance level = 0.05, p-value = 0.4) within groups, but they were different

significantly (ANOVA: F statistic = 3.9, df = 2, significance level = 0.05, p-value = 0.07) between the groups from week 1 (day 14) to week 6 (day 49).

3.5.3 RT-qPCR assay for dsrB transcript copies
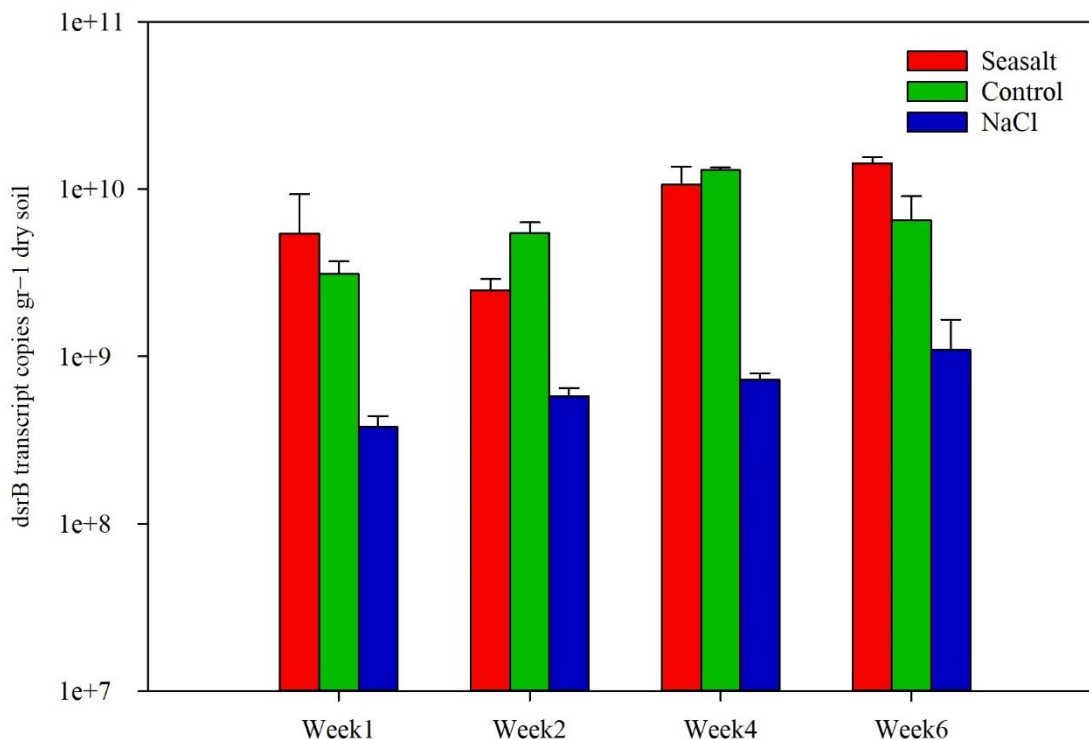
Figure 3.9: dsrB transcript copy numbers of sulfate reducing bacteria (SRB). Bar chart shows the number of dsrB transcript copies per gram dry weight of soil. Statistical test indicates significant difference (P ≤ 0.05) between groups, but it was not significant difference (P > 0.05) within the groups over the time poins. Three replicas (n =3) were used for each time-point sample of experimental groups. The error bar shows standard error of the mean (SEM).

In Figure 3.9, it can be observed that the dsrB transcript copy numbers varied among the groups. The sea salt group had the highest dsrB transcript copy number during weeks one and six, while the NaCl group had the lowest dsrB transcript copy number compared to the control and sea salt groups over the four-week period. This study highlights that in week one and six, the dsrB transcript copies in the control group were 58.07% and 45.46% lower than those in the sea salt treatment, respectively. Across the experiment, the copy number was lower in the NaCl treatment, ranging from 5.58% to 16.93%, compared to the control group. The result showed that sulfate reducing bacteria in the seasalt treatment group were active functionally rather than the NaCl-teated samples.

According to statistical analysis, the copy number of dsrB transcripts through the experiment were not significant difference (ANOVA: F statistic = 2.08, df = 3, significance level = 0.05, p-value = 0.2) within groups, but they were different significantly (ANOVA: F statistic = 5.7, df = 2, significance level = 0.05, p-value = 0.03) between the groups from week 1 (day 14) to week 6 (day 49).

3.5.4 qPCR assay for *dsrB* gene copies
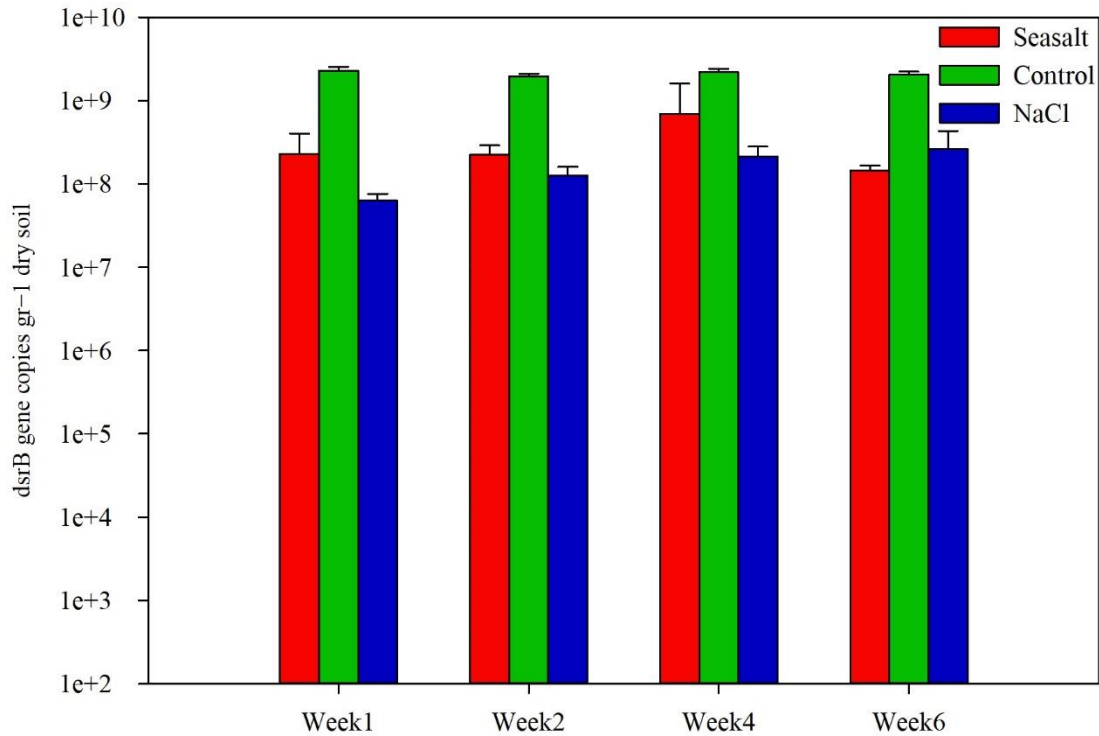
Figure 3.10: *dsrB* gene copy numbers of sulfate reducing bacteria (SRB). Bar chart shows the number of *dsrB* gene copies per gram dry weight of soil. Statistical test indicates no significant difference (P > 0.05) between and within three groups (NaCl treatment, seasalt treatment and control group) over the time points. Three replicas (n =3) were used for each time-point sample of experimental groups. The error bar shows standard error of the mean (SEM).

The analysis presented in figure 3.10 indicates that the *dsrB* gene copy number varied significantly across the groups and weeks. Specifically, in week one, two, and four, the seasalt group had a higher dsrB gene copy number than NaCl, with differences of 7.32%, 5.01%, and 21.71%, respectively. However, in week six, the *dsrB* gene copy number in the seasalt group was 5.61% lower than that of the NaCl group. In contrast, the *dsrB* gene copy number in the control group consistently exceeded that of the treated groups. Based on these results, it can be concluded that treatment with 150 mM seasalt led to an increase in the *dsrB* gene copy number compared to NaCl treatment. However, the *dsrB* gene copy number in the seasalt group was lower than that of the control group. The result showed that growth of sulfate reducing bacteria in the seasalt treatment group was more than the the NaCl-teated samples.

According to statistical analysis, the copy number of *dsrB* gene of three groups were not significant difference (ANOVA: F statistic = 1.54, df = 3, significance level = 0.05, p-value = 0.29) within groups, but

they were significantly different (ANOVA: F statistic = 184.6, df = 2, significance level = 0.05, p-value = 4.08642E-06) between the groups from week 1 (day 14) to week 6 (day 49).

3.5.5 RT-qPCR assay for mcrA transcript copies
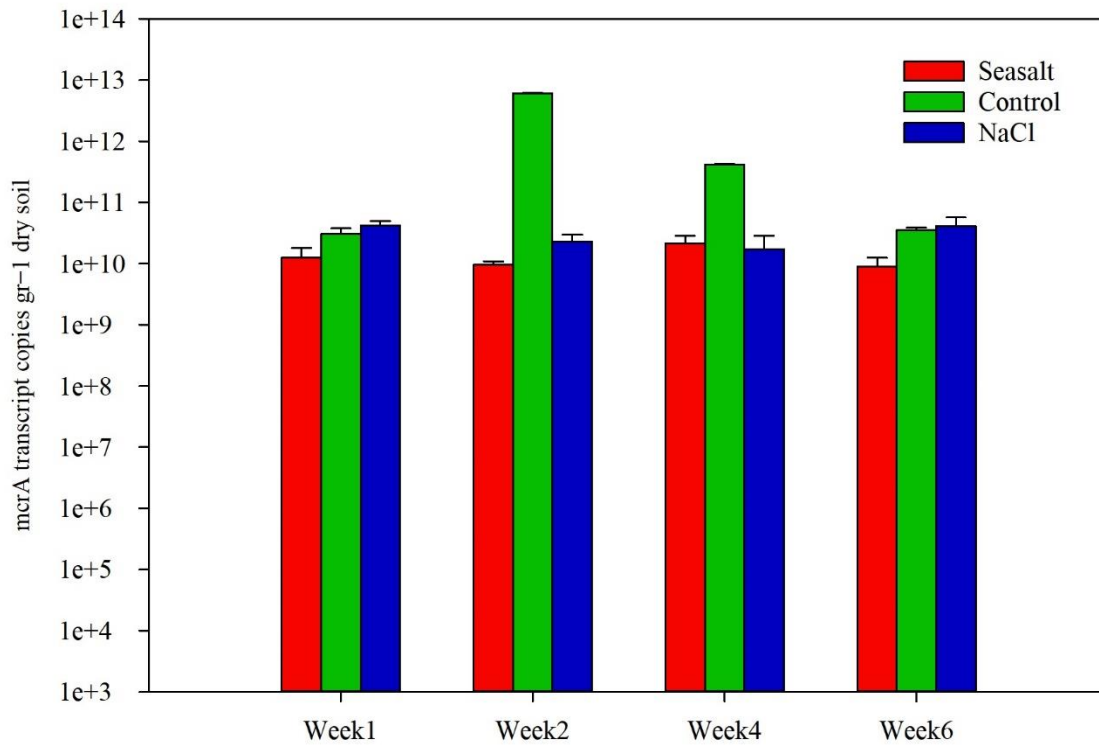
Figure 3.11: mcrA transcript copy numbers of methanogens. Bar chart shows the number of mcrA transcript copies per gram dry weight of soil. Statistical test indicates no significant difference ($P > 0.05$) between and within groups over the time points. Three replicas (n =3) were used for each time-point sample of experimental groups. The error bar shows standard error of the mean (SEM).

The findings presented in figure 3.11 indicate that the impact of applying a seasalt treatment at a concentration of 150 mM on the mcrA transcript copy number is incongruous over the weeks, as compared to the control and NaCl groups. However, the influence of the NaCl treatment (150 mM) on the mcrA transcript copy numbers was slightly greater than that of the seasalt treatment and control group. Specifically, during weeks one and six, the mcrA transcript copy numbers were 27.24% and 12.92% higher in the NaCl treatment group, respectively, compared to the control group. Nonetheless, during weeks two and four, the copy numbers were substantially higher in the control group, relevant to both the NaCl and seasalt treatment groups. Upon analysis, it was revealed that the methanogens present in the treated groups exhibited changes functionally in comparison to the control groups. Particularly, a noticeable decrease was observed in the samples treated with seasalt.

According to statistical analysis, the copy number of mcrA transcripts between the three groups were not significant difference (ANOVA: F statistic = 0.9, df = 3, significance level = 0.05, p-value = 0.4) within groups, and also they were not different significantly (ANOVA: F statistic = 1.1, df = 2, significance level = 0.05, p-value =0.3) between the groups from week 1 (day 14) to week 6 (day 49).

3.5.6 qPCR assay for *mcrA* gene copies
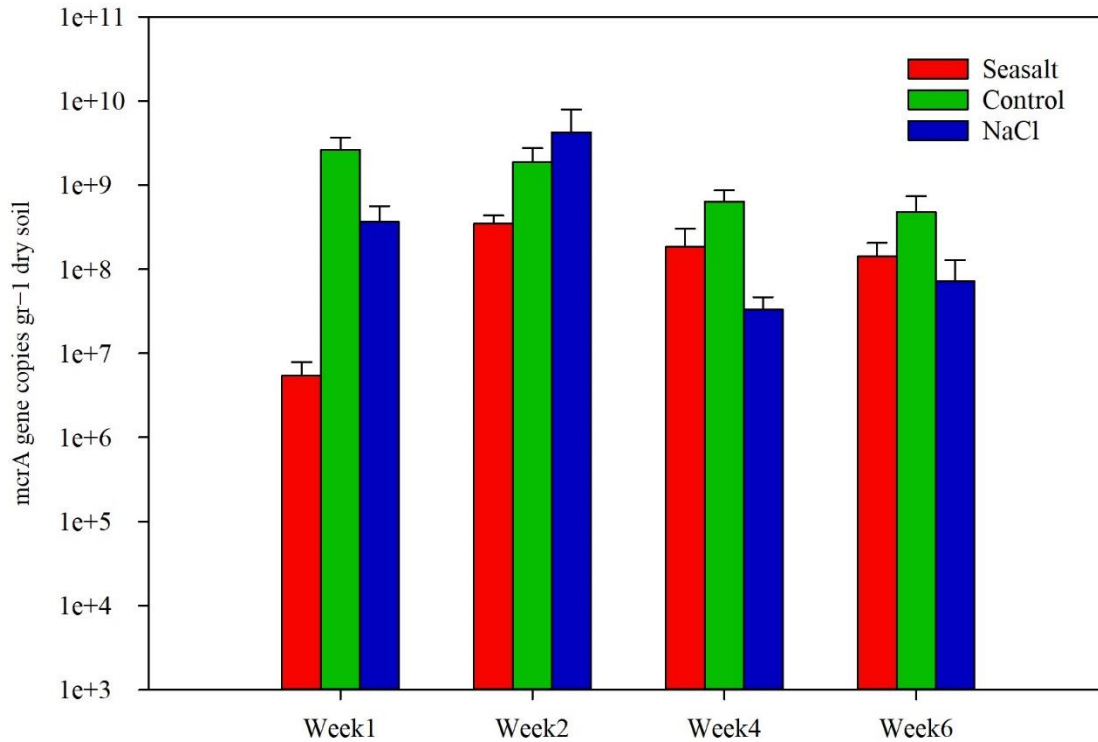
Figure 3.12: *mcrA* gene copy numbers of methanogens. Bar chart shows the number of *mcrA* gene copies per gram dry weight of soil. Statistical test indicates no significant difference (P > 0.05) between and within three groups (NaCl treatment, seasalt treatment and control group) over the time points. Three replicas (n =3) were used for each time-point sample of experimental groups. The error bar shows standard error of the mean (SEM).

The study shows that the application of seasalt treatment at 150 mM resulted in a great reduction in *mcrA* gene copy numbers during the initial week, followed by a subsequent increase in the following weeks. However, in week four and week six, there was a notable decrease in the *mcrA* gene copy numbers between the control and NaCl groups (Figure 3.12). The NaCl treatment at 150 mM had a substantial negative impact on the gene copy numbers, with a higher reduction of 94.8% in week four and 85.16% in week six, while the seasalt treatment resulted in a reduction of 71.18% and 70.57%, respectively. It became apparent that the growth of methanogens had suffered negative effects. According to statistical analysis, the copy number of *mcrA* gene between the three groups were not significant difference (ANOVA: F statistic = 1.2, df = 3, significance level = 0.05, p-value = 0.2 within groups, and also they were not different significantly

(ANOVA: F statistic = 1.6, df = 2, significance level = 0.05, p-value =0.3) between the groups from week 1 (day 14) to week 6 (day 49).

3.6 Alpha diversity

The quantity of raw reads associated with the marker genes (16S rRNA, *dsrB* and *mcrA*) and their transcripts listed in Tables 3.3, Table 3.4, and 3.5. The sample demultiplexing of distinct time points was performed with Qiime2. More than fifty percent of forward and reverse reads (R1 and R2) for the same sample were successfully merged. Tables 3.6, 3.7, and 3.8 provide the overall number and frequency of ASVs for each project.

Table 3.3: Raw read numbers of 16S rRNA PCR amplicon sequencing.

Nucleic acid-16S rRNA	Groups	Raw reads
RNA	Control	4236080
RNA	NaCl	4211920
RNA	Seasalt	4390848
DNA	Control	4341216
DNA	NaCl	4336504
DNA	Seasalt	4380418

Table 3.4: Raw read numbers of dsrB PCR amplicon sequencing.

Nucleic acid-dsrB	Groups	Raw reads
RNA	Control	767216
RNA	NaCl	758886
RNA	Seasalt	745644
DNA	Control	766174
DNA	NaCl	762936
DNA	Seasalt	762940

Table 3.5: Raw read numbers of mcrA PCR amplicon sequencing.

Nucleic acid-mcrA	Groups	Raw reads
RNA	NaCl + Control w1&2	330,068
RNA	NaCl + Control w4&6	271,856
RNA	Seasalt + Control w1&2	268,374
RNA	Seasalt + Control w4&6	232,658
DNA	Seasalt	360,716
DNA	Control	311,646
DNA	NaCl	318,452

Table 3.6: Detailed information of 16S rRNA PCR amplicon after data analysis.

DNA-16S		RNA-16S	
Number of samples	36	Number of samples	36
Number of features	5,276	Number of features	4,404
Total frequency	1,520,379	Total frequency	1,774,020

Table 3.7: Detailed information of dsrB PCR amplicon after data analysis.

DNA-dsrB		RNA-dsrB	
Number of samples	36	Number of samples	36
Number of features	2,242	Number of features	2,109
Total frequency	280,798	Total frequency	270,565

Table 3.8: Detailed information of mcrA PCR amplicon after data analysis.

DNA-mcrA		RNA-mcrA	
Number of samples	12	Number of samples	46
Number of features	804	Number of features	731
Total frequency	88,656	Total frequency	67,411

The reads were grouped into ASVs to determine the diversity index of the treated samples beside the control group. Communities' diversity was measured by Shannon index. The Shannon diversity index is a widely used measure of alpha diversity that takes into account both the number of different species present in a sample (richness) and their relative abundances. In this case, the Shannon value index is being used to compare the diversity of microbial communities in salt-treated samples (150 mM) and control group. Across the projects, the Kruskal-Wallis used for diversity analysis. Shannon diversity index of each experimental group are listed as follows:

Shannon index values of bacterial 16S rRNA transcripts diversity (Table 3.9) were 2.6, 1.92, and 0.16. The highest diversity was found between NaCl treatment and control group, and the lowest diversity was related to NaCl and seasalt treatment. The Shannon index values show that there are differences in the diversity of 16S rRNA transcripts between the three groups. Specifically, the Shannon index value between the NaCl treatment group and the seasalt treatment group is notably lower, indicating that these two groups have more similar diversity of 16S rRNA transcripts. Conversely, the diversity between the NaCl treatment group and the control group is higher. The Kruskal-Wallis test was not significant between the pairwise groups ($P > 0.05$) (Figure S1).

Bacterial 16S rRNA gene diversity (Table 3.10) were 3.6, 6.4, and 0.65. The highest diversity was between control and seasalt treatment, and the lowest diversity was between NaCl and seasalt treatments. These results suggest that the seasalt treatment had a greater impact on the diversity of bacterial 16S rRNA gene compared to NaCl treatment, although the difference between the two treatments was not particularly large. The statistical tests between the control group and NaCl treatment, as well as control group and seasalt were significant ($P \leq 0.05$), but it did not differ significantly between NaCl and seasalt treatments ($P > 0.05$) (Figure S2).

The diversity of *dsrB* gene was measured using a Shannon index of 1.61, 0.21, and 0.14 (Table 3.11). Diversity was found to be at its peak between the NaCl-treated and control group, and the lowest was between the NaCl-treated and seasalt-treated groups. These findings suggest that the NaCl treatment had a greater impact on the diversity of the *dsrB* gene compared to the seasalt treatment. The diversity was lower between the control and seasalt treatment groups, as well as between the NaCl and seasalt treatment groups. However, the diversity was higher between the NaCl treatment group and the control group. The statistical test between the pairwise groups did not differ significantly using the Kruskal-Wallis test ($P > 0.05$).

Table 3.12 displays the diversity of dsrB transcripts at 0.96, 2.08, and 0.03. The greatest value occurred between the control and seasalt groups, whereas the least variation occurred between the NaCl and seasalt groups. The Shannon index analysis revealed that the diversity of dsrB transcripts was greatly higher between the control group and the seasalt treatment group. Conversely, the diversity between the treatment groups, as well as between the control and NaCl treatment groups, was markedly lower. There was not a significant difference ($P > 0.05$) between the pairwise groups.

mcrA transcripts diversity was calculated with a Shannon index in Qiime2 (Table 3.13). The values between the groups were 0.01, 5.53, and 4.29. The highest value found between control and seasalt treatments, and the lowest was between control and NaCl-treated sample. The results of the Shannon index analysis suggest that the mcrA transcript diversity was very lower between the control and NaCl treatment groups. However, there was a noticeable increase in diversity between the control and seasalt treatment groups, as well as between the NaCl treatment and seasalt treatment groups. The highest level of diversity was observed in the seasalt treatment group. The statistical test was significant difference between seasalt treatment and control, but other group comparisons' diversity was not significant.

The Table 3.14 depicted diversity of *mcrA* gene among three groups. The values were 5.88, 3.63, and 0.27. The greatest value was for control group and NaCl treatment, and the lowest value was for NaCl and seasalt treatments. Between NaCl treatment and control group, the statistical test was significant ($P \leq 0.05$); however, the test was insignificant between the seasalt and NaCl treatments, and seasalt treatment and control group ($P > 0.05$). Based on the Shannon index values, it is observed that the diversity of the *mcrA* gene was higher between the control group and the two treatment groups. But, the diversity of the *mcrA* gene was considerably low in the comparison between the two treatment group.

Table 3.9: Shannon index details for 16S rRNA transcripts analysis between three groups (NaCl, seasalt treatments and control group).

Group 1	Group 2	H	p-value
Control (n=12)	NaCl Treatment (n=12)	2.6	0.1
Control (n=12)	Seasalt Treatment (n=12)	1.92	0.16
NaCl Treatment (n=12)	Seasalt Treatment (n=12)	0.16	0.68

Table 3.10: Shannon index details for 16S rRNA gene analysis between three groups (NaCl, seasalt treatments and control group).

Group 1	Group 2	H	p-value
Control (n=12)	NaCl Treatment (n=12)	3.6	0.05
Control (n=12)	Seasalt Treatment (n=12)	6.4	0.01
NaCl Treatment (n=12)	Seasalt Treatment (n=12)	0.65	0.41

Table 3.11: Shannon index details for *dsrB* gene analysis between three groups (NaCl, seasalt treatments and control group).

Group 1	Group 2	H	p-value
Control (n=12)	NaCl Treatment (n=12)	1.61	0.20
Control (n=12)	Seasalt Treatment (n=12)	0.21	0.64
NaCl Treatment (n=12)	Seasalt Treatment (n=12)	0.14	0.71

Table 3.12: Shannon index details for dsrB transcripts analysis between three groups (NaCl, seasalt treatments and control group).

Group 1	Group 2	H	p-value
Control (n=12)	NaCl Treatment (n=12)	0.96	0.33
Control (n=12)	Seasalt Treatment (n=12)	2.08	0.15
NaCl Treatment (n=12)	Seasalt Treatment (n=12)	0.03	0.86

Table 3.13: Shannon index details for mcrA transcripts analysis between three groups (NaCl, seasalt treatments and control group).

Group 1	Group 2	H	p-value
Control (n=24)	NaCl Treatment (n=9)	0.01	0.92
Control (n=24)	Seasalt Treatment (n=8)	5.53	0.02
NaCl Treatment (n=9)	Seasalt Treatment (n=8)	4.29	0.04

Table 3.14: Shannon index details for *mcrA* gene analysis between three groups (NaCl, seasalt treatments and control group).

Group 1	Group 2	H	p-value
Control (n=12)	NaCl Treatment (n=12)	5.88	0.02
Control (n=12)	Seasalt Treatment (n=12)	3.63	0.06
NaCl Treatment (n=12)	Seasalt Treatment (n=12)	0.27	0.60

On the whole, the effect of 150 mM NaCl and seasalt on the diversity of 16S rRNA gene, 16S rRNA transcripts, mcrA gene were higher than the control groups. Conversely, the salt at 150 mM reduced the diversity of dsrB gene, dsrB transcripts and mcrA transcripts compared to the control groups. The statistical anlsysis result (the Kruskal-Wallis test) and the Shanoon box plots of the experimental groups of each study have been brought in the supplementary section (from figure S1 to figure S6). It was statistically significant only in the 16S rRNA and *mcrA* gene diversities.

3.7 Relative abundance of the soil microbial communities

The present study aims to investigate the relative abundances of major microbial species within untreated- and salt-treated (150 mM; NaCl and seasalt) soil from the Philippins. To achieve this, we employed high-throughput sequencing techniques, including 16S rRNA, *mcrA*, and *dsrB* genes and their trtanscripts sequencing, utilizing both HiSeq and MiSeq platforms. The 16S rRNA gene sequencing technique is widely used in microbial ecology research, enabling the identification and quantification of bacterial species. The *mcrA* and *dsrB* genes serve as important markers for methanogenic and sulfate-reducing archaea, respectively. The utilization of these advanced sequencing technologies, namely the HiSeq and MiSeq platforms, allowed us to analyze the microbial communities in greater depth and accuracy, generating a more comprehensive dataset for further analysis. To analyze the obtained sequencing data, we utilized QIIME2, a powerful bioinformatics tool for analyzing and visualizing microbiome data. Through the use

of QIIME2, we were able to perform detailed analyses on the relative abundances of major microbial species within our samples. This approach allowed us to uncover valuable insights into the microbial ecology of the salt- treated and untreated samples from week one to week six, and understand the compositions and functional roles of these microbial communities within the soil ecosystem. To evaluate the relative abundance of microbial communities, taxonomic profiles were generated at the phylum and family levels for 16S rRNA and dsrB projects, and at the order and family levels for mcrA projects. Heatmaps (see supplementary information section) and from Tables S1 to Table S24 were used to identify dominant microbial taxa with high counts among others. The Shapiro-Wilks test is used to check if the data (from week one to six between the two salt-treated and control groups) follows a normal distribution. If the p-value from the test is less than the significance level (0.05), it suggests that the data does not follow a normal distribution. Therefore, if the data from any of the groups fail the normality test, non-parametric test meaning the Kruskal-Wallis was used; otherwise, the MANOVA was used (a parametric test). In this section, for each major composition, the repeated measurs ANOVA test was used for actual counts (relative frequency) of the three groups.

3.7.1 Relative abundance of 16S rRNA gene at phylum level
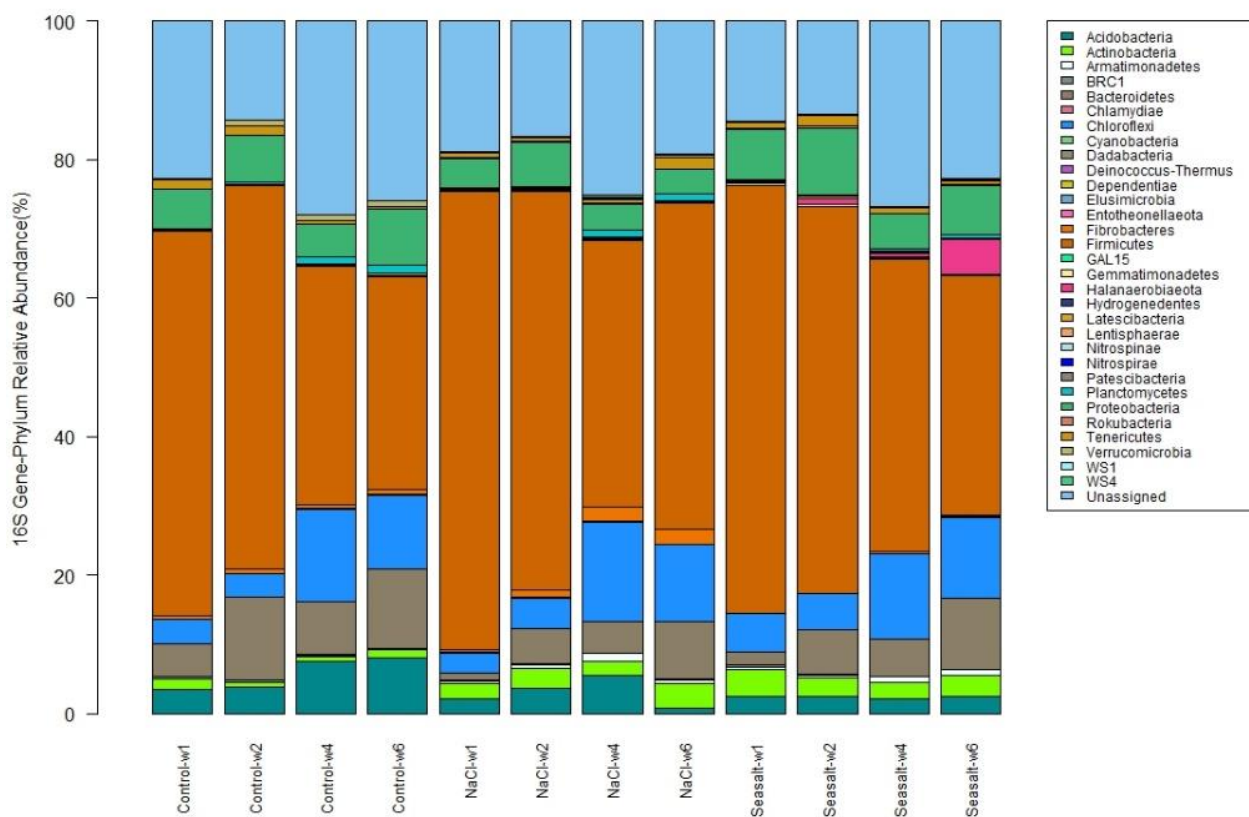
Figure. 3.13: Stacked bar-plot representation of bacterial 16S rRNA gene relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of phylum.

Table 3.15: Statistical analysis of 16S rRNA gene data at phylum level.

Kruskal-Wallis	Week 1	Week 2	Week 4	Week 6
chi-squared	0.9542	0.50963	0.52872	0.53188
df	2	2	2	2
p-value	0.6206	0.7751	0.7677	0.7665

At phylum level in bacterial 16S rRNA gene (Figure. 3.13), *Acidobacteria, Chloroflexi, Bacteroidetes, Actinobacteria, Fibrobacteres, Firmicutes, Proteobacteria, Armatimonadetes,* BRC1, *Halanaerobiaeota,* and *Verrucomicrobia* were major compositions between the groups (Table S1; Table S2). Table 3.15 shows the statistical analysis of major compositions between the groups for each week. A substantial proportion of *Firmicutes* was found in all groups and weeks. From week one to six, the relative abundance of *Firmicutes* for the NaCl and seasalt treatments were higher than control group. Relative frequency of *Firmicutes* between the three groups through the time points showed a statistically significant difference (ANOVA test: P ≤ 0.05; Panel S1).

3.7.2 Relative abundance of 16S rRNA gene at family level

Table 3.16: Statistical analysis of 16S rRNA gene data at family level.

Kruskal-Wallis	Week 1	Week 2	Week 4	Week 6
chi-squared	16.441	16.083	8.2829	10.398
df	2	2	2	2
p-value	0.0002691	0.0003219	0.0159	0.005523

The Table S3 and Table S4 comprise the relative abundances of microbial taxa from the 16S rRNA gene at family level through the three groups over six weeks: control, and salt-treated groups. The stacked bar chart shows that the microbial compostions vary between the three groups, with differences in the relative abundance. *Chitinophagaceae*, *Anaerolineaceae*, *Bacillaceae*, *Christensenellaceae*, *Clostridiaceae 1*, *Gracilibacteraceae*, *Heliobacteriaceae*, *Lachnospiraceae*, *Ruminococcaceae*, *Veillonellaceae*, *Halobacteroidaceae*, and *Archangiaceae* were the major compositions between the experimental groups. Table 3.16 shows the statistical analysis of major compositions between the groups for each week. *Clostridiaceae* 1 indicated higher relative abundance compared to the control group in two treated groups (NaCl and seasalt treatments) through the experiment. Notably, the relative abundance of *Ruminococcaceae* in the NaCl-treated group was higher than in the control group in both weeks four and six of the study. Additionally, the relative abundance of *Halobacteroidaceae* was found to be higher in week six in the seasalt treatment group. Results from ANOVA tests (P ≤ 0.05; Panel S2) showed a significant difference in the relative frequency of *Clostridiaceae* 1 and *Ruminococcaceae* between the three groups throughout the experiment, but not (P > 0.05) in the case of *Halobacteroidaceae*.

3.7.3 Relative abundance of 16S rRNA transcripts at phylum level
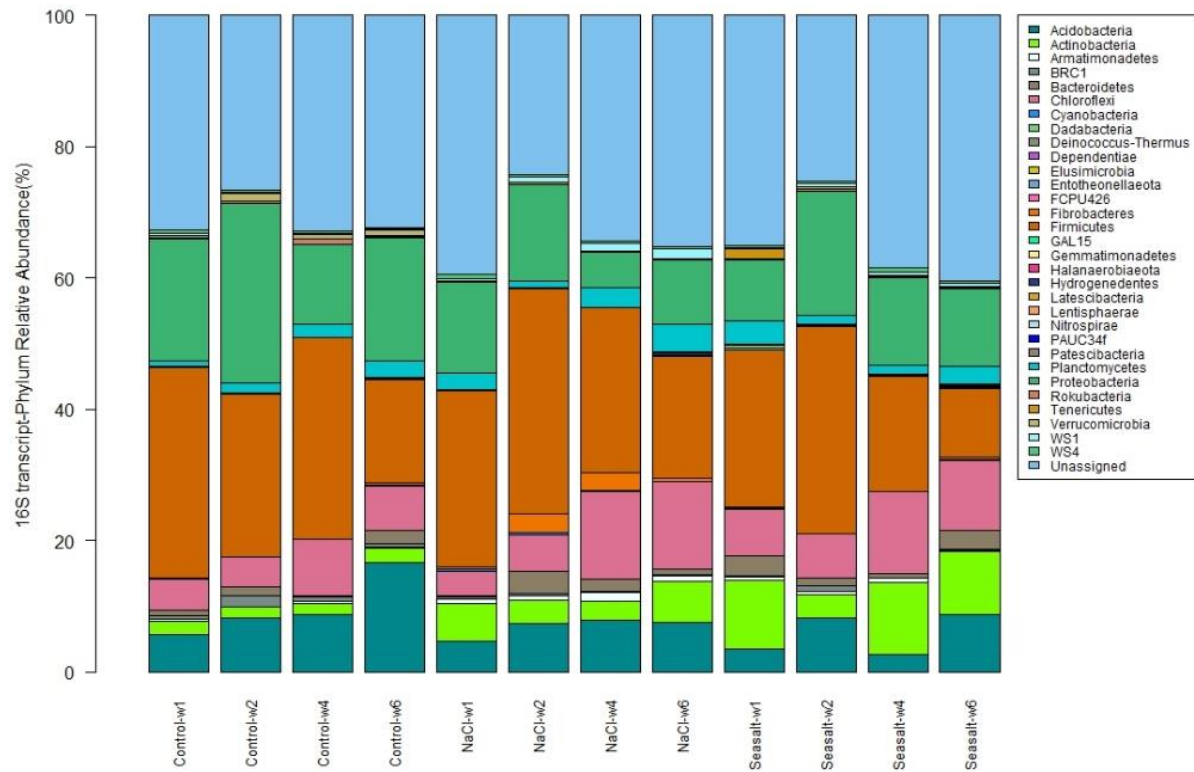
Figure. 3.14: Stacked bar-plot representation of bacterial 16S rRNA transcripts relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of phylum.

Table 3.17: Statistical analysis of 16S rRNA transcripts data at phylum level.

Kruskal-Wallis	Week 1	Week 2	Week 4	Week 6
chi-squared	0.19348	0.27923	0.43256	0.40279
df	2	2	2	2
p-value	0.9078	0.8697	0.8055	0.8176

At phylum level in bacterial 16S rRNA transcripts (Figure. 3.14), *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Proteobacteria*, and *Planctomycetes* were the seven most abundant compositions between the groups in this study (Table S5; Table S6). Table 3.17 shows the statistical analysis of major compositions between the groups for each week. The application of NaCl and seasalt treatment at a concentration of 150 mM resulted in a sustained elevation in the relative abundance of *Actinobacteria*. This increase was observed from the first week through to the sixth week of the study. Furthermore, a similar trend was observed for *Chloroflexi*, where the relative abundance remained high from week two to week six. However, the application of these treatments resulted in a lower relative abundance of *Proteobacteria* compared to the control group throughout the weeks. Relative frequency of *Actinobacteria* and *Proteobacteria* between the three groups through the time points were significant

difference (ANOVA test: P ≤ 0.05; Panel S3), while it was not singnificant (P > 0.05) for *Chloroflexi* statistically.

3.7.4 Relative abundance of 16S rRNA transcripts at family level

Table 3.18: Statistical analysis of 16S rRNA transcripts data at family level.

Kruskal-Wallis	Week 1	Week 2	Week 4	Week 6
chi-squared	17.426	2.8413	3.3383	8.5641
df	2	2	2	2
p-value	0.0001644	0.2416	0.1884	0.01381

The Table S7 and Table S8 are related to the relative abundances of microbial taxa for the 16S rRNA transcripts at family level of the three groups (control, and two salt-treated groups) over six weeks. Table 3.18 shows the statistical analysis of major compositions between the groups for each week. When examining the 16S rRNA transcripts at the family level, the most prevalent families were *Solibacteraceae* (Subgroup 3), *Roseiflexaceae*, *Clostridiaceae* 1, Family XVIII, *Heliobacteriaceae*, *Limnochordaceae*, *Geobacteraceae*, *Archangiaceae*, *Haliangiaceae*, and *Pedosphaeraceae*. Throughout the course of the experiment, it was observed that the relative abundance of *Geobacteraceae* was higher in the samples treated with salts when compared to the control group. The result from ANOVA test (P > 0.05; Panel S4) showed no significant difference (P > 0.05) in the relative frequency of *Geobacteraceae* between the three groups throughout the experiment.

3.7.5 Relative abundance of *dsrB* gene at phylum level
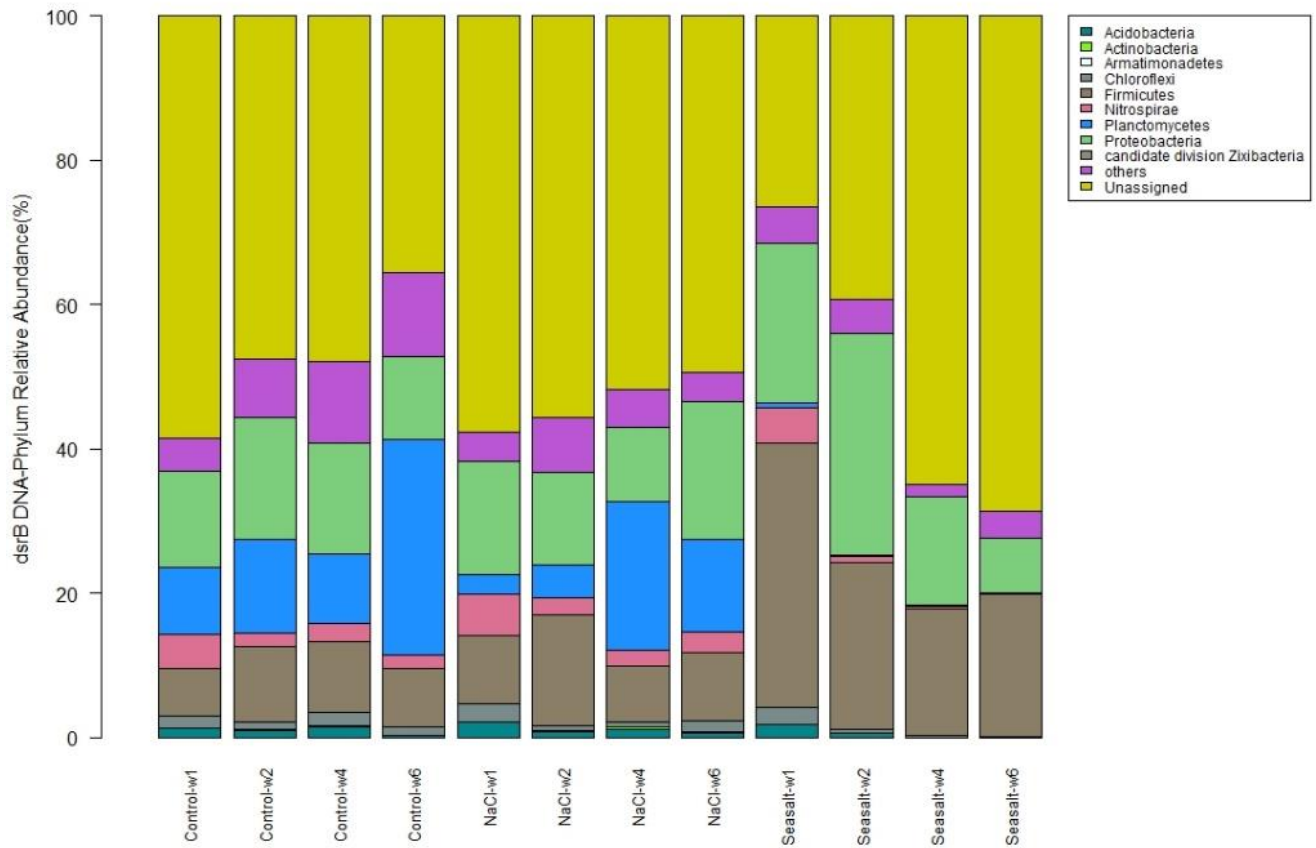
Figure. 3.15: Stacked bar-plot represents of *dsrB* gene relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of phylum.

Table 3.19: Statistical analysis of *dsrB* gene data at phylum level.

MANOVA test	Week 1	Week 2	Week 4	Week 6
approx F	5.3	12.05	46.6	33.4
df	2	2	2	2
p-value	0.033	0.0038	3.905e-05	0.0001
Significat at:	0.05	0.01	0.001	0.001

At phylum level in *dsrB* gene (Figure. 3.15), *Chloroflexi*, *Firmicutes*, *Nitrospirae*, *Planctomycetes*, and *Proteobacteria* had higher relative abundances in this study (Table S9; Table S10). Table 3.19 shows the statistical analysis of the major compositions between the groups for each week. During the initial two weeks, there was an increase in the relative abundance of *Firmicutes* in the two treated groups when compared to the control group. From the second week until the sixth week, there was an observable elevation in the relative abundance of *Proteobacteria* in the seasalt-treated group as compared to the control group. The ANOVA test revealed a statistically significant difference in the relative frequency of *Firmicutes* among the three groups at different time points ($P \leq 0.05$; Panel S5). However, there was no statistically significant difference ($P > 0.05$) observed for *Proteobacteria*.

3.7.6 Relative abundance of *dsrB* gene at family level

Figure. 3.16: Stacked bar-plot represents of *dsrB* gene relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of family.

Table 3.20: Statistical analysis of *dsrB* gene data at family level.

MANOVA test	Week 1	Week 2	Week 4	Week 6
F-statistic (approx F)	21.8	75.9	95.01	75.9
df	2	2	2	2
p-value	3.235e-06	2.395e-11	2.081e-12	2.392e-11
Significant at:	0.001	0.001	0.001	0.001

The Table S11 and Table S12 are related to the relative abundances of microbial taxa for the *dsrB* gene at family level of the three groups (control, and two salt-treated groups) over six weeks. The families of *Acidobacteria*, *Chloroflexi*, *Peptococcaceae*, *Nitrospirae*, *Planctomycetes* and *Desulfovibrionaceae* were the higher relative abundances. Table 3.20 shows the statistical analysis of the major compositions between the groups for each week. Over the course of the first to sixth week, the relative abundances of *Peptococcaceae* were observed to be significantly greater in the two treated samples as compared to the control group. Similarly, the relative abundance of *Desulfovibrionaceae* was observed to be higher than both the control and NaCl-treated groups during the second and fourth weeks. The ANOVA test conducted (P ≤ 0.05; Panel S6) yielded significant differences in the relative frequency of *Peptococcaceae* among the

three groups throughout the experiment, whereas no significant difference (P > 0.05) was observed for *Desulfovibrionaceae*.

.

3.7.7 Relative abundance of dsrB transcripts at phylum level

Figure. 3.17: Stacked bar-plot represents of dsrB transcripts relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of phylum.

Table 3.21: Statistical analysis of dsrB transcripts data at phylum level.

MANOVA test	Week 1	Week 2	Week 4	Week 6
F-statistic (approx F)	34.33	101.5	450.33	450.15
df	2	2	2	2
p-value	6.14e-05	6.692e-07	9.531e-10	9.548e-10
Significant at:	0.001	0.001	0.001	0.001

dsrB transcripts at phylum level (Figure. 3.17), *Acidobacteria*, *Chloroflexi*, *Firmicutes*, Nitrospirae, and *Proteobacteria* were the highest relative abundance in this study (Table S13; Table S14). Table 3.21 shows the statistical analysis of the major compositions between the groups for each week. Throughout the experiment, *Proteobacteria* exhibited consistently higher relative abundance when compared to the control group. Notably, during the second and fourth weeks, the NaCl treatment group displayed a higher relative abundance of *Proteobacteria* as compared to the seasalt treated group; however, during the first and sixth

weeks, this trend was reversed. The ANOVA test revealed no significant difference ($P > 0.05$) in the relative frequency of *Proteobacteria* among the three groups at different time points ($P \leq 0.05$; Panel S7).

3.7.8 Relative abundance of dsrB transcripts at family level
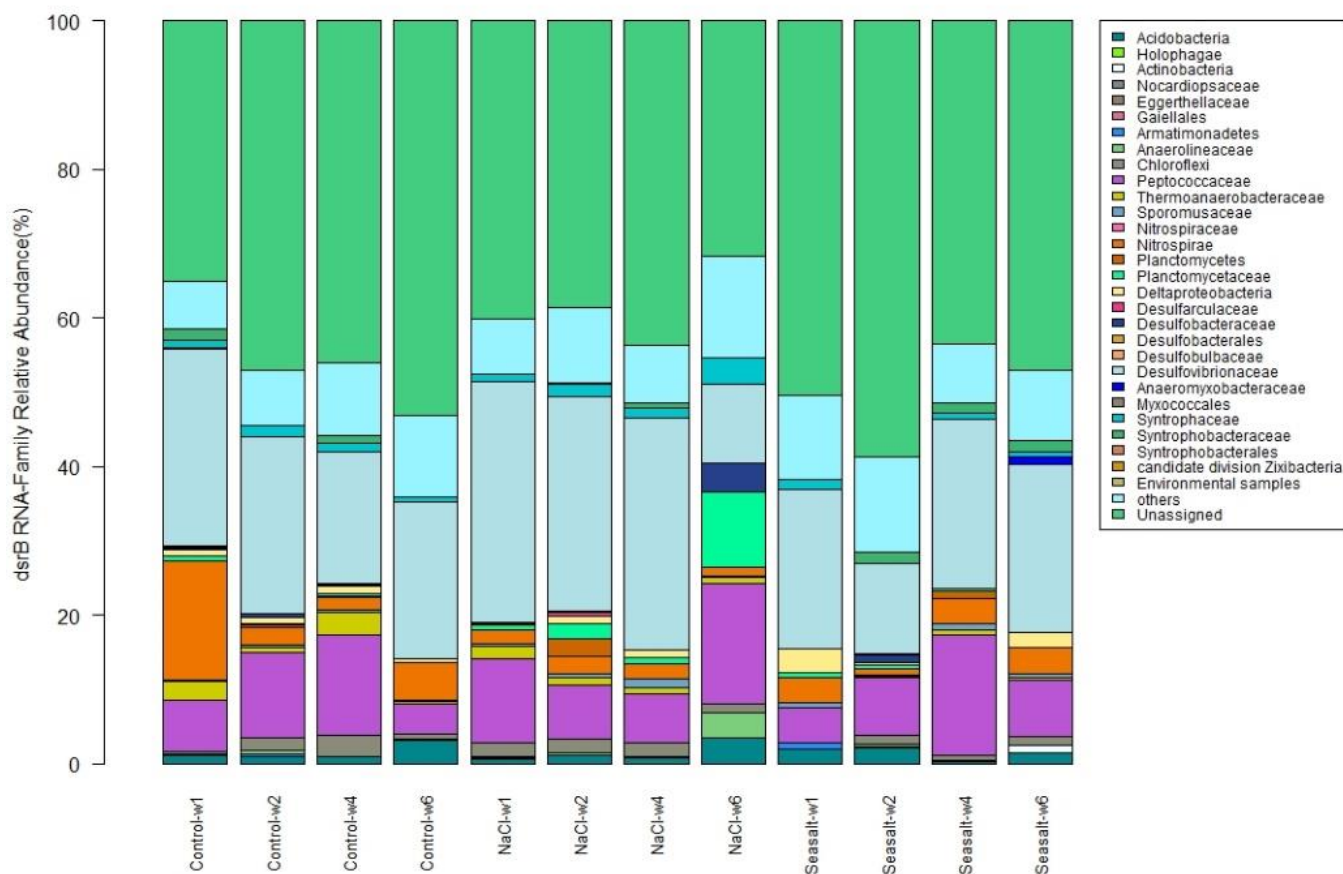
Figure. 3.18: Stacked bar-plot represents of dsrB transcripts relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of family.

Table 3.22: Statistical analysis of *dsrB* transcripts data at family level.

MANOVA test	Week 1	Week 2	Week 4	Week 6
F-statistic (approx F)	127.12	236.56	810.69	1026.6
df	2	2	2	2
p-value	8.94e-15	2.2e-16	2.2e-16	2.2e-16
Significant at:	0.001	0.001	0.001	0.001

The Table S15 and Table S16 are related to the relative abundances of microbial taxa for the dsrB transcripts at family level of the three groups (control, and two salt-treated groups) over six weeks. The families of *Peptococcaceae*, *Thermoanaerobacteraceae*, *Nitrospirae*, *Planctomycetaceae*, and *Desulfovibrionaceae* were the higher relative abundances. Table 3.22 shows the statistical analysis of the major compositions between the groups for each week. In this study, the relative abundance of *Peptococcaceae* was observed

to be higher in the seasalt group as compared to the control group, while it was found to be even higher in the NaCl treatment group in comparison to the seasalt-treated group. Moreover, during the first and fourth weeks, the relative abundance of *Desulfovibrionaceae* was observed to be higher. The ANOVA test, conducted with a p-value greater than 0.05 (Panel S8), did not yield any significant differences (P > 0.05) in the relative frequency of both *Peptococcaceae* and *Desulfovibrionaceae* among the three groups throughout the experiment.

3.7.9 Relative abundance of *mcrA* gene at order level
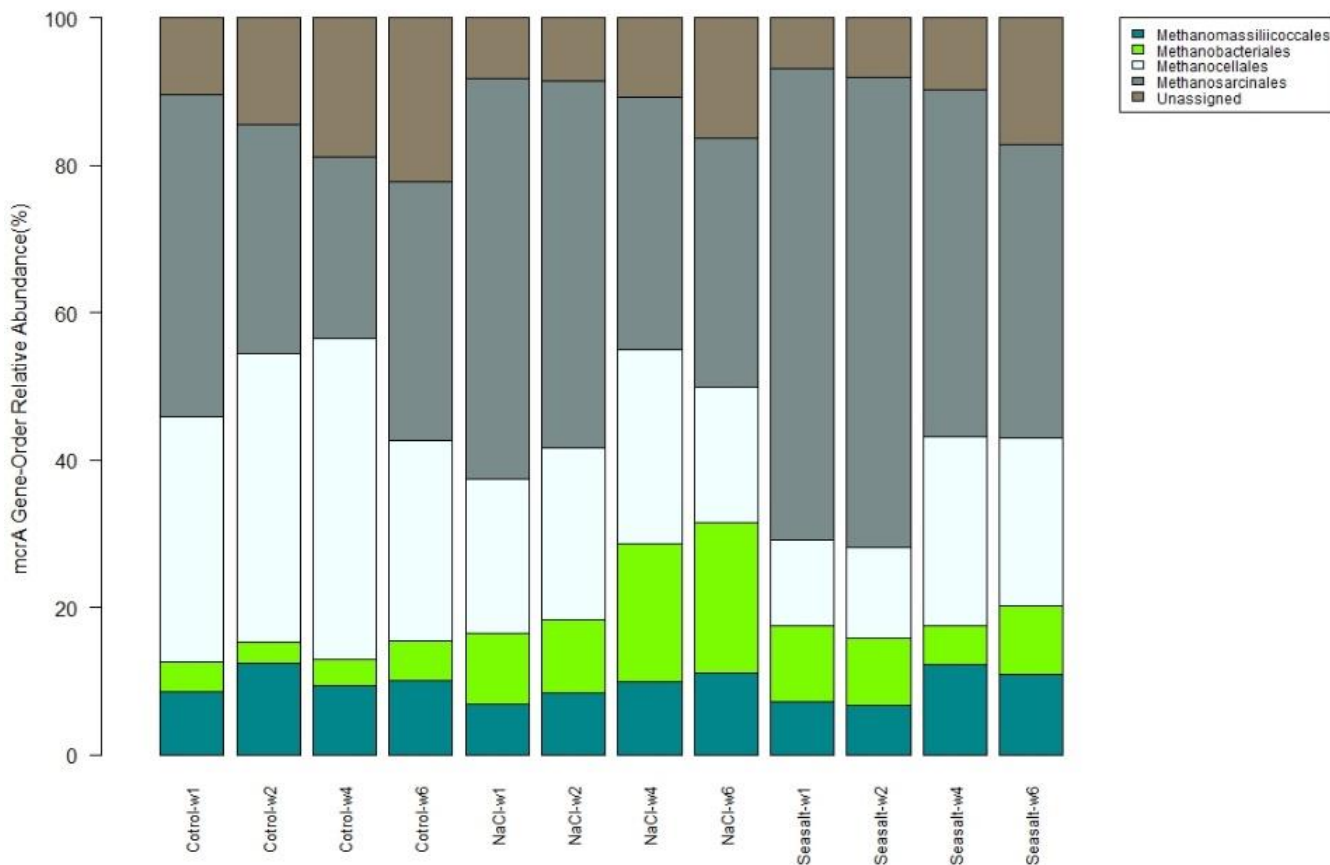
Figure. 3.19: Stacked bar-plot represents of *mcrA* gene relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of order.

Table 3.23: Statistical analysis of *mcrA* gene data at order level.

MANOVA test	Week 1	Week 2	Week 4	Week 6
F-statistic (approx F)	41.7	8.09	0.5	16.3
df	2	2	2	2
p-value	0.023	0.11	0.66	0.057

Stacked bar chart (Figure. 3.19) shows the *mcrA* gene relative abundance at order level. *Methanomassiliicoccales*, *Methanobacteriales*, *Methanocellales*, and *Methanosarcinales* orders were found in this study (Table S17; Table S18). Table 3.23 shows the statistical analysis of the compositions

between the groups for each week. Throughout the duration of the experiment, it was observed that the implementation of a seasalt treatment with a concentration of 150 mM resulted in an increase in the relative abundance of *Methanosarcinales*. Additionally, it was noted that the application of NaCl treatment (150 mM) led to a notable increase in relative abundance, up to the fourth week. However, in sharp contrast, the relative abundance of *Methanocellales* witnessed a marked decrease in response to both of the aforementioned treatments in the weeks compared to the control group. The ANOVA test revealed a significant difference in the relative frequency of *Methanosarcinales* and *Methanocellales* among the three groups at different time points ($P \leq 0.05$; Panel S9).

3.7.10 Relative abundance of *mcrA* gene at family level
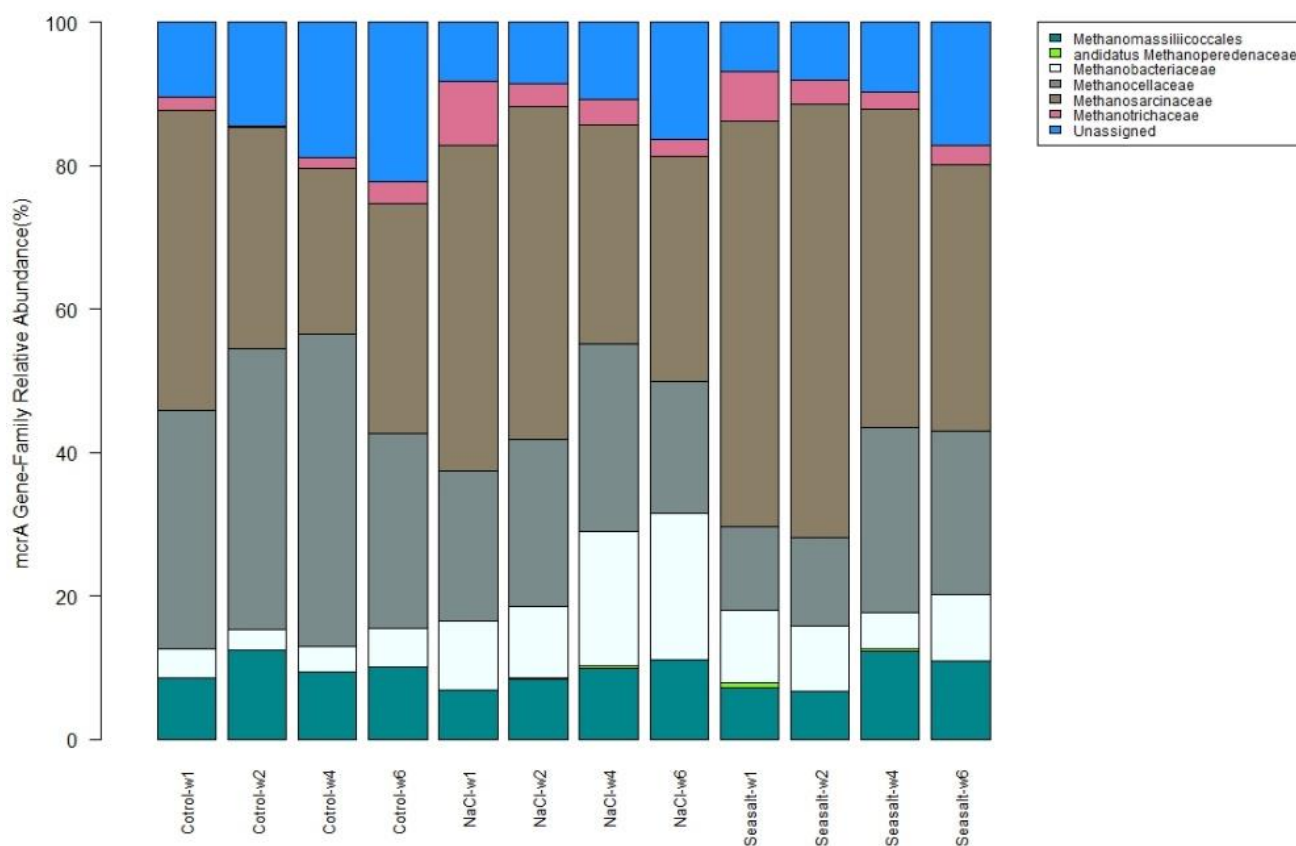
Figure. 3.20: Stacked bar-plot represents of *mcrA* gene relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of family.

Table 3.24: Statistical analysis of *mcrA* gene data at family level.

MANOVA test	Week 1	Week 2	Week 4	Week 6
F-statistic (approx F)	25.03	26.2	2.61	28.65
df	2	2	2	2
p-value	0.005	0.005	0.18	0.004
Significant at:	0.01	0.01		0.01

Figure 3.20 is related to the relative abundance of microbial taxa for the *mcrA* gene at family level for the three groups (control, and two salt-treated groups) over six weeks. The *Methanomassiliicoccales*, *Methanobacteriaceae*, *Methanocellaceae*, *Methanosarcinaceae*, and *Methanotrichaceae* were the methanogenic families were detected in this study (Table S19; Table S20). Table 3.24 shows the statistical analysis of compositions between the groups for each week. The *Methanosarcinaceae* family had a greater presence in the treated groups up to week four, while the *Methanocellaceae* family had a lower presence throughout the experiment. Surprisingly, the salt treatments made no big changes in the relative abundance of *Methanomassiliicoccales* between the three groups. Remarkably, the NaCl treatment resulted in the highest relative abundance of *Methanobacteriaceae* among the three groups. The results of the ANOVA tests (Panel S10) indicated significant diference ($P \leq 0.05$) in the relative frequency of *Methanosarcinaceae*, *Methanocellaceae* and *Methanobacteriaceae* among the three groups during weeks one to six. However, there was no significant difference statistically ($P > 0.05$) observed for *Methanomassiliicoccales* during the same period.

3.7.11 Relative abundance of mcrA transcripts at order level
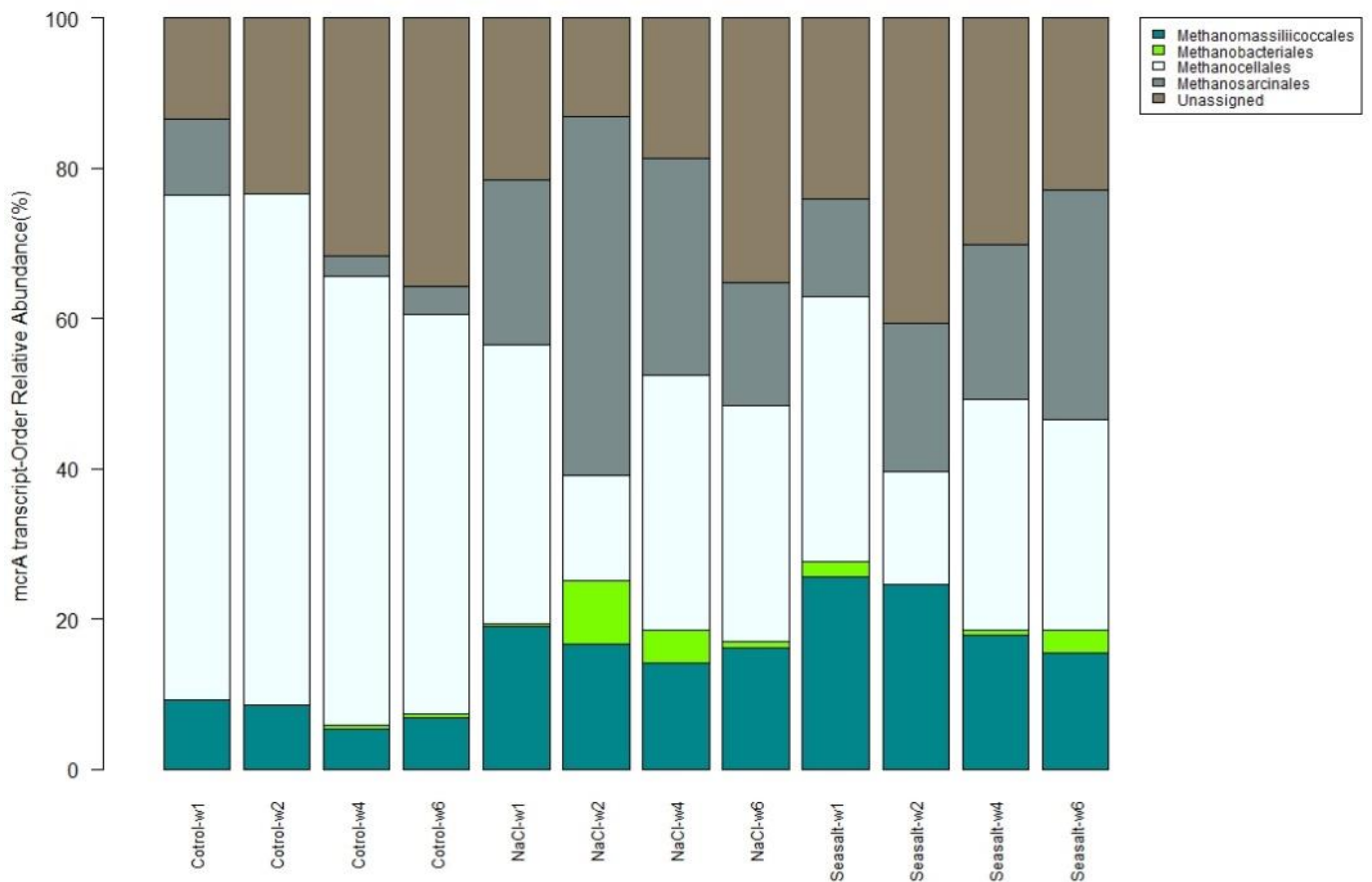
Figure. 3.21: Stacked bar-plot represents of mcrA transcripts relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of order.

Table 3.25: Statistical analysis of *mcrA* transcripts data at order level.

MANOVA test	Week 1	Week 2	Week 4	Week 6
F-statistic (approx F)	2.4	0.148	1.3	3.17
df	2	2	2	2
p-value	0.28	0.87	0.42	0.23

Stacked bar chart (Figure. 3.21) shows the mcrA transcripts relative abundance at order level. *Methanomassiliicoccales*, *Methanobacteriales*, *Methanocellales* and *Methanosarcinales* orders were found in this study (Table S21; Table S22). Table 3.25 shows the statistical analysis of the compositions between the groups for each week. Throughout the experiment, it was observed that the *Methanomassiliicoccales* and *Methanosarcinales* showed an increase in relative abundance in both treatment groups. On the other hand, the *Methanocellales* decreased in the treatment groups when compared to the control group. The ANOVA test revealed a significant difference in the relative frequency of *Methanomassiliicoccales*, *Methanosarcinales*, and *Methanocellales* among the three groups at different time points (P ≤ 0.05; Panel S9).

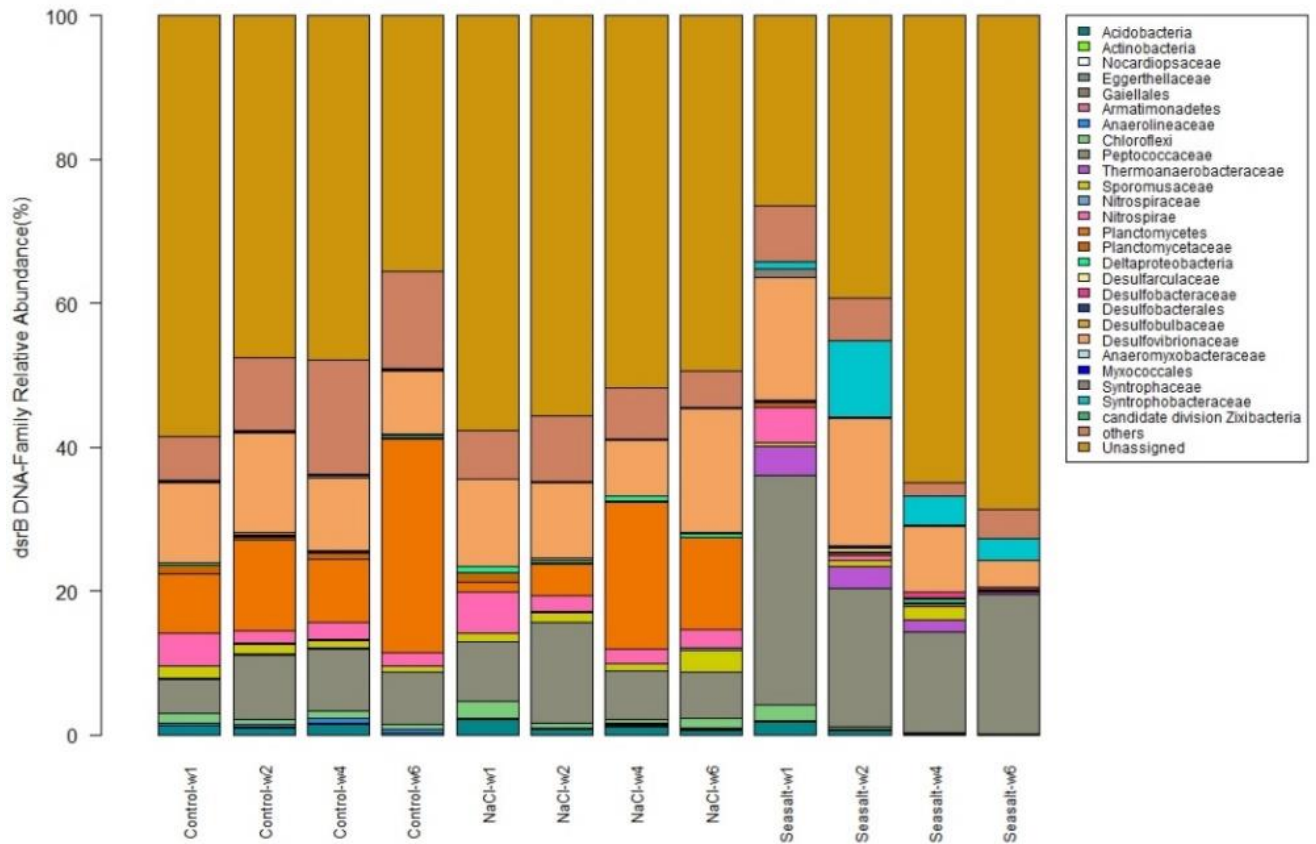## 3.7.12 Relative abundance of mcrA transcripts at family level
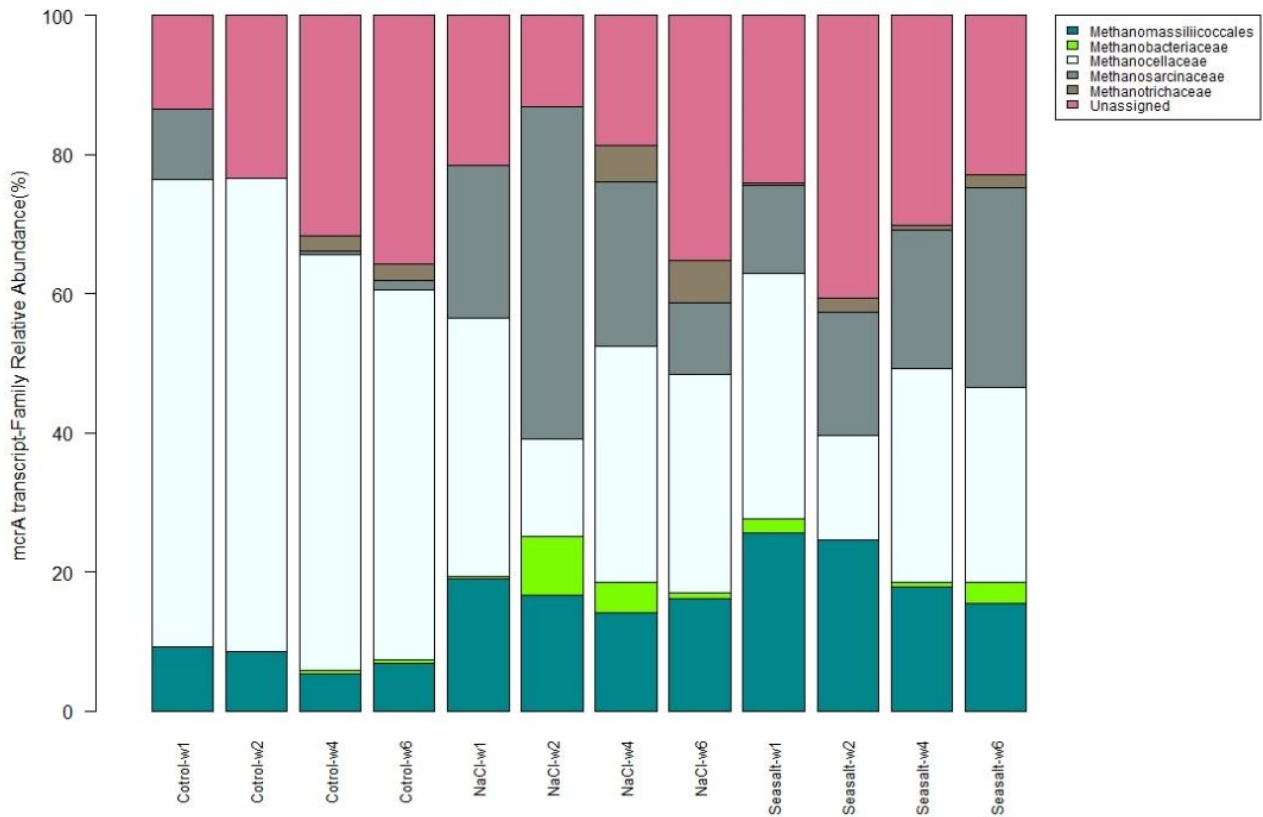
Figure. 3.22: Stacked bar-plot represents of mcrA transcripts relative abundance for two salt-treated groups and control group through week one to week six, with taxonomic features collapsed at the level of family.
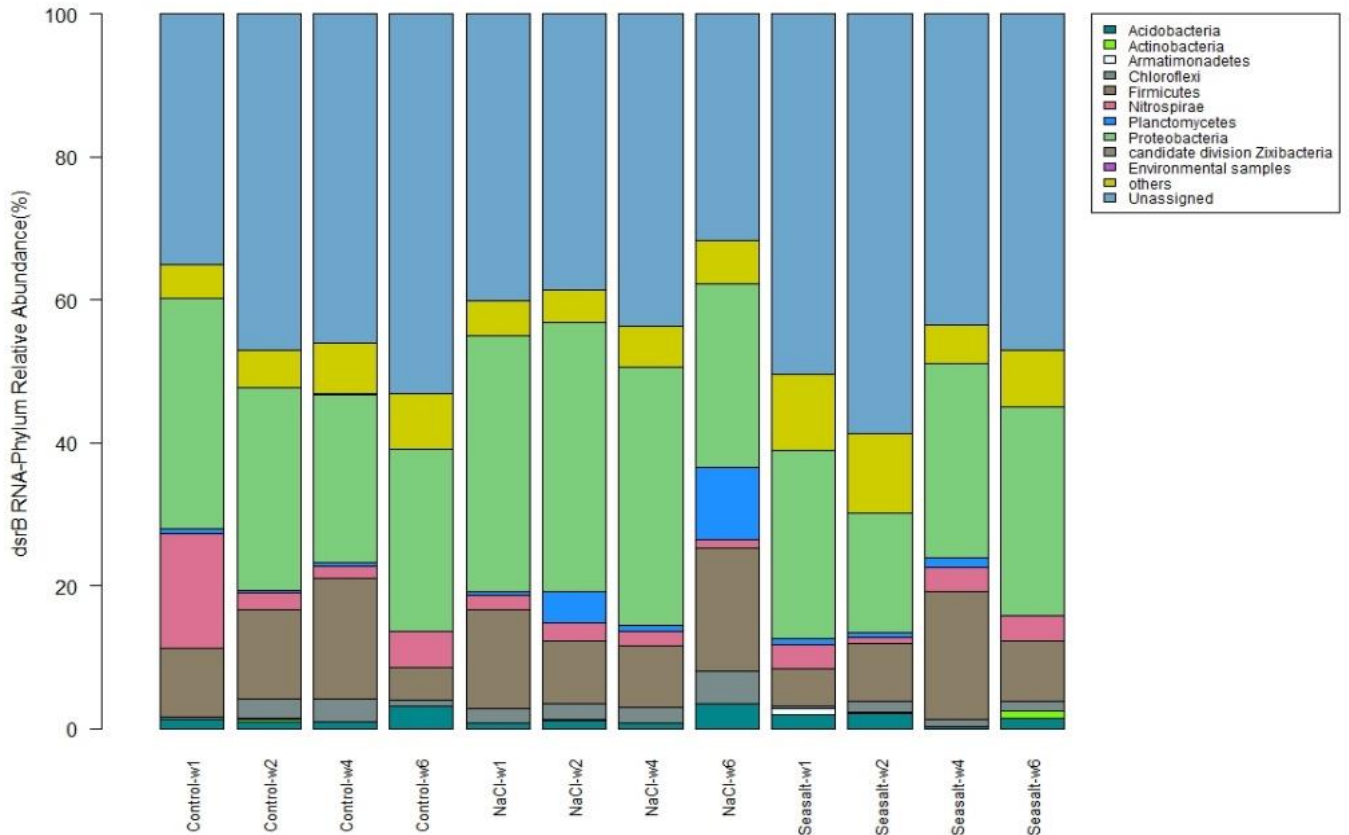
Table 3.26: Statistical analysis of *mcrA* transcripts data at family level.

MANOVA test	Week 1	Week 2	Week 4	Week 6
F-statistic (approx F)	3.56	0.17	2.46	6.7
df	2	2	2	2
p-value	0.16	0.84	0.23	0.077

Figure 3.22 is related to the relative abundance of microbial taxa for the mcrA transcipts at family level for the three groups (control, and two salt-treated groups) over six weeks. The families of *Methanomassiliicoccales*, *Methanobacteriaceae*, *Methanocellaceae*, *Methanosarcinaceae*, and *Methanotrichaceae* were found in this study (Table S23; Table S24). Table 3.26 shows the statistical analysis of compositions between the groups for each week. Over the course of six weeks, the *Methanosarcinaceae* family exhibited a greater relative abundance in the two treatment groups compared to the control group. Notably, from the first to the fourth week, the NaCl-treated group displayed a higher relative abundance of this family compared to the seasalt-treated group; however, this trend was reversed in week six, with the seasalt-treated group exhibiting a higher relative abundance. Throughout the experiment, the *Methanocellaceae* family consistently exhibited a lower relative abundance compared to the control group. The ANOVA tests (Panel S10) revealed a significant difference ($P \leq 0.05$) in the relative frequency of *Methanocellaceae* across the three groups throughout the six-week period. Conversely, there was no significant difference ($P > 0.05$) detected for *Methanosarcinaceae* during this timeframe.

4-Discussion

Rice, which serves as a primary staple food worldwide, is cultivated on extensive acreage in paddy systems that are frequently subjected to soil salinity. This salinity not only affects the growth of plants but also has a considerable impact on soil microorganisms that play a vital role in nutrient cycling. The adverse effects of soil salinity on living components of ecosystems are well-documented, as it increases osmotic pressure on cells and reduces the availability of water, creating a severe stressor (Wichern et al., 2020). Additioanlly, soil salinity can lead to a reduction in soil respiration, enzyme activity, soil microbial biomass, and bacterial growth rate, all of which can have a significant impact on biogeochemical cycling (Zhang et al., 2019). Furthermore, it can impede carbohydrate metabolism and microbial carbon metabolic functions, resulting in decreased carbon emissions (Yang et al., 2021). Ahmad Ali and his colleagues conducted a study in 2019 to investigate the potential impact of different planting systems on the abundance, community composition, and functional diversity of soil microbes in the rhizosphere. To achieve this, the team employed high-throughput sequencing (Illumina HiSeq) to generate paired-end reads of the 16S rRNA gene and analyze the structure and diversity of microbial communities across different cropping systems (Ali et al., 2019). During my PhD research, my primary goal was to investigate the effects of two types of salts at an intermediate concentration of 150 mM on bacterial and archaeal communities in paddy soils over time. To achieve this objective, we conducted an extensive analysis of gas measurements (including CH_4, CO_2, and H_2S) and fatty acid concentrations (such as acetate, butyrate, and propionate), as well as running RT-qPCR and qPCR assays, and studying the microbial community profiles of Philippine paddy soil treated with NaCl and seasalt salts using three primer sets (16S rRNA, mcrA, and dsrB) with high-throghput technologies, HiSeq and MiSeq. These efforts have the potential to provide valuable insights into the compostions and dynamics of soil microbial ecology.

4.1 Intermediate saltwater reduces potential CO_2 and CH_4 production in the paddy soil

Our study investigated the effects of NaCl and seasalt treatments on methane and carbon dioxide production in soil organic matter anaerobic decomposition. We observed a decrease of 8% and 11% in methane and carbon dioxide production, respectively, with NaCl treatment, and a greater reduction of 38% and 21% with seasalt treatment. This suggests that seasalt may have a more pronounced inhibitory effect on methanogens. Based on the findings of the previous study, it appears that elevated NaCl concentrations may have induced a significant increase in osmotic pressure, subsequently resulting in the loss of intracellular water within methanogenic organisms, thus decreasing the activities of key enzymes (e.g., dehydrogenase) (Zhang et al., 2020). Previous study has shown that sulfate has the potential to inhibit CH_4 production, likely due to competition with methanogens for substrates by sulfate reducing bacteria (SRB) such as *desulfovibrio desulfuricans* (Zhao et al., 2020).

Moreover, it agrees with existing knowledge in the literature, which suggests that high salinity can reduce soil CH_4 emissions. In fact, their findings showed that methane emissions decreased with increasing salinity, which is in line with 31 observations in the tidal marsh (Poffenbarger et al., 2011). In addition, enhanced CH_4 production was also inversely correlated ($r^2 = 0.81$) to the salinity of sampled soils (Brigham et al., 2018).

Another study found that Na^+ concentration of 2 to 10 g/L inhibited methanogenic activity moderately, while a concentration exceeding 10 g/L inhibited strongly (Gourdon et al., 1989). It was reported that methanogenesis began to be impaired at a NaCl concentration of 5 g/L, while acidogenesis was significantly damaged (Lefebvre et al., 2007). On the other hand, certain studies have suggested that methanogen growth is promoted by low salinity concentrations, specifically at around 350 mg Na^+/L (~0.8 g/L NaCl), while concentrations of 8-13 g/L NaCl can result in moderate inhibition, and values exceeding 20 g/L NaCl can lead to severe impairment (Omil Mendez, Ramon & Lema, Juan M, 1996; Appels et al., 2008; Chen et al., 2008). These findings are consistent with our own results. Anaerobic microorganisms are known to be sensitive to the presence of high sodium/or chloride concentrations, in particular the methanogenic archaea. These microorganisms can be more sensitive than acidogenic bacteria to high salinity, and their activity can typically be inhibited under salt concentrations around 20 g L^{-1} (Duarte et al., 2021).

4.2 Seasalt induces H_2S gas production compared to NaCl salt in the Philippine paddy soil

According to our findings, the analysis of H_2S concentrations among the experimental groups indicated that the seasalt treatment led to an increase of approximately 14% in gas production compared to the control group. On the other hand, the NaCl treatment resulted in a decrease of around 1% of H_2S gas production. Sulfate-reducing bacteria play a key role in H_2S production, as they are capable of absorbing sulfate in the absence of oxygen, obtaining energy through the oxidation of organic compounds, and releasing H_2S as a byproduct of sulfate reduction (Deng et al., 2018). There are two main pathways that govern microbial sulfur (or sulfate) reduction: assimilatory and dissimilatory processes. The assimilatory pathway involves the use of reduced sulfur for the biosynthesis of amino acids and proteins. In contrast, the dissimilatory pathway involves the reduction of sulfate (or sulfur) to inorganic sulfide by obligatory anaerobic sulfate reducers. This metabolic process is known as bacterial sulfate reduction and contributes significantly to the production of H_2S in various environments (Kumar et al., 2018). Saltwater is known to have a significant impact on both the dissimilatory pathway and the assimilatory process. In wetland soils, particularly in

brackish and marine wetlands, the reduction of SO_4^{2-} is an essential anaerobic process (Cornwell, 2013). Therefore, it is highly probable that the presence of saltwater affects this process. This impact may occur through a variety of mechanisms, including changes in microbial community composition, alterations in soil chemistry, and shifts in redox potentials. Based on previous evidence of the impact of salinity, it has been found that sulfate reduction activity is significantly inhibited when the total Na^+ concentration exceeds 2 M. This finding is consistent with the results obtained from previous studies on hypersaline chloride-sulfate lakes with a neutral pH, as reported by Brandt et al., (2001) and Sørensen et al., (2004). Sulfate reducing microorganisms can produce toxic hydrogen sulfide as a byproduct of anaerobic carbon mineralization in sediment, leading to its accumulation in many coastal ecosystems (Hu et al., 2018). Lefebvre et al.,(2007) found that increasing salinity from 0 to 10 gNaCl/L enhanced the acidogenic specificbiogas production rate. This suggests that the degradation of organic carbon in brackish wetlands is affected by multiple factors, including salinity levels, frequency of flooding, and sulfate concentrations. These conditions can result in increased ionic and osmotic stress, which can further impact the process of mineralization (Luo et al., 2019). Sulfate-reducing bacteria are widely known to produce hydrogen sulfide (H_2S) as a metabolic byproduct in marine environments. The intrusion of seawater into coastal regions introduces high amounts of sulfate, which can directly impact H_2S production. Our findings of this study on the Philippine paddy soil revealed that even a small amount of sulfate combined with seasalt can increase sulfate levels, leading to a corresponding increase in H_2S production. This suggests that sulfate may be a key factor in regulating H_2S levels in marine environments, and highlights the importance of understanding the mechanisms that drive sulfate reduction and H_2S production in these ecosystems.

4.3 Effect of different salts on fatty acid concentratios during six weeks

Our findings regarding the fatty acid concentrations through the six weeks demonstrated that the NaCl treatment accumulated more concentrations of three fatty acids (butyarate, and propionate) compared with others, while in the case of the seasalt treatment it was vice versa. In other words, propionate concentration was 55% and 50%, and butyrate 54% and 39% in the seasalt and control group relevant to the NaCl treatment from week one to week six. Acetate concentration was 86% and 67% for the NaCl-treated and control groups relevant to the seasalt-treated group.

Based on the results obtained from our experimentation (as depicted in Figure 3.4, Figure 3.5, and Figure 3.6), it was observed that the concentration of acetate was initially higher as compared to butyrate and propionate. Furthermore, the rate of depletion of acetate was observed to be faster in the NaCl treated sample, as opposed to the sample treated with sea salt. These observations were consistent with the data presented in Figure 3.1, which suggested that methanogens utilized acetate more efficiently in the NaCl treated sample, while the consumption rate of acetate was comparatively slower in the sea salt treated sample. To elaborate, the consumption of acetate was notably delayed in both treated groups, particularly in response to the application of seal salt treatment. The findings indicated that the NaCl treatment had an impact on butyrate oxidizer bacteria, while acidogenesis remained unaffected. Similarly, the seasalt treatment did not affect acidogenesis, but led to an increase in the utilization of fatty acid by butyrate oxidizer bacteria. The experimental results demonstrated that the propionate concentration gradually increased in the NaCl treatment group from the onset of the experiment until day 21, at which point there was a sharp increase. These findings suggest that acidogenesis was temporarily surpassed. In contrast, the seasalt treatment group showed a higher likelihood of propionate consumption by propionate oxidizer bacteria. To summarize, the NaCl treated sample experienced a decrease in pH resulting from the accumulation of fatty acids and toxic ions. In addition to ion toxicity, the consumption of fatty acids

(butyrate and propionate) by oxidizer bacteria likely contributed to a reduction in methanogenesis in the seasalt-treated sample.

During anaerobic digestion, complex organic matter undergoes hydrolysis, breaking down into simpler organic compounds. The resulting fermentation intermediates, primarily volatile fatty acids (VFAs) such as acetate, propionate, and butyrate, are then metabolized by acidogens through acidogenesis. Obligate hydrogen-producing acetogens further degrade these intermediates into acetate and hydrogen gas. Finally, methane producing archaea (MPA) convert the acetate and hydrogen gas into biogas, predominantly composed of methane and carbon dioxide (Speece, 1996; Tchobanoglous et al., 2003; Xu et al., 2003). NaCl had inhibitory effects on the production of methane and a high dosage of NaCl could severely suppress the growth of methanogens, which decreased the consumption of the VFAs. Consequently, the production of VFAs was significantly enhanced by the addition of NaCl (Su et al., 2016). Acidogenic microorganisms are able to produce intermediate volatile fatty acids (VFAs) and other products. VFAs constitute a class of organic acids such as acetates, and larger organic acids such as propionate and butyrate, typically in a ratio varying from 75:15:10 to 40:40:20 (Argenzio and Hintz, 1971). A decrease in pH levels below the optimal range for methanogens can result in system disruption. Conversely, increasing the pH can provide significant protection for methanogen activity and facilitate stable anaerobic digestion processes (Liu et al., 2020). This finding supports our result.

With regards to the environmental needs of methanogenesis, methanogenic microorganisms tend to require a higher pH than previous stages of anaerobic digestion, in addition to a lower redox potential, the latter requisite having caused significant trouble for laboratory cultivation (Wolfe, 2011). With the production of acetate through acidogenesis throught anaerobic digestion, a portion of the original substrate has already been rendered into a substrate suitable for acetoclastic methanogenesis (Santos and Da Costa, 2002). However, other produced higher VFAs have yet to be made accessible to methanogenic microorganisms. Acetogenesis is the process by which these higher VFAs and other intermediates are converted into acetate, with hydrogen also being produced (Watkins et al., 2014). Due to variations in the membrane structures of fermentative bacteria, acidogenic bacteria, and acetogenic bacteria, methanogenic archaea exhibit varying levels of sensitivity to high salinity conditions. Previous studies exploring salt inhibition and its mitigation through the use of osmoprotectants have primarily focused on the overall efficiency of the anaerobic process or specifically on the methanogenic stage (Zhang et al., 2016). Sulfate reducing bacteria (SRB) play a crucial role in the degradation of propionate, achieved through the oxidation of propionate and butyrate. SRB may directly oxidize propionate or engage in syntrophy with acetogens by utilizing hydrogen. Additionally, SRB have the capacity to oxidize propionate incompletely or completely. It is suggested that incompletely oxidizing SRB outcompete completely oxidizing species, owing to their faster growth rate when metabolizing propionate (Lopes and Lens, 2011). In line with the hypothesis, sulfate reduction was stimulated by acetate, hydrogen, and acetate plus hydrogen. Oremland showed that that sulfate-reducing bacteria will outcompete methanogens for hydrogen, acetate. It sounds that sulfate reducing bcateria reduced sufate in the seasalt treated group (Oremland and Polcin, 1982). Oremland and Polcin, (1982) found that low levels of NaCl improved the process of hydrolysis and acidification, but inhibited the production of methane, while high levels of NaCl inhibited both steps. The high salinity could moreover cause an imbalance of cell osmotic stress, resulting in plasmolysis or loss activity of cells (Jung et al., 2013; Mottet et al., 2014), which would further cause inhibition or even failure of the anaerobic digestion process. Zhang et al., (2010) showed that the activity of methanogens was severely inhibited at salinity from 65 to 85 g L^{-1}, while the hydrolytic, acidogenic and acetogenic bacteria normally metabolized under high saline conditions. Therefore, it resulted in VFAs accumulation and again led to a decrease in pH, which ultimately caused the failure of gas (methane, hydrogen and carbon dioxide) production. In addition, the hydrogen,

carbon dioxide and methane were observed in the early phase of anaerobic digestion at all the tested salinities, suggesting that the hydrogenotrophic methanogens tolerated the high saline environment.

4.4 Responses of the paddy soil microbial community to salt stresses

As part of my PhD research, I aimed to not only identify the relative abundance of microbial compositions in response to soil salinity, but also to quantify the effects of two salt treatments on the microbial community copy numbers in two levels. To achieve this, I analyzed the absolute Real-Time PCR of 16S rRNA bacterial, methanogens, and sulfate-reducing bacteria genes and transcripts in addition to control groups. By examining these information, I sought to gain a comprehensive understanding of the underlying changes in microbial community relative abundances and functions in response to soil salinity at the intermediate concentration.

The results of qPCR and RT-qPCR assays showed that the seasalt treatment reduced the 16S rRNA transcripts (48.44%), 16S rRNA gene (39.51%), mcrA trasncripts (42.30%) and *mcrA* gene (14.44%) more than the NaCl treatment, and the NaCl decreased the dsrB transcripts (8.48%) and *gene* (51.60%) relevant to the seasalt treatment over time. Based on the brief review, significant observations reveal that the sulfate reducing bacteria (SRB) were more active in the seasalt treatment group compared to the NaCl treatment group. This suggests that the presence of sulfate in the seasalt led to a higher occurrence of sulfate reduction by SRB than in the NaCl treatment. Furthermore, the decrease in mcrA transcripts and gene in the seasalt treatment group compared to the NaCl treatment group suggests that SRB potentially competed with methanogens for hydrogen and acetate.

Abdallah et al, (2016) reported that showed that 16S rRNA gene copy numbers of bacteria was 5×10^6 DNA copies g^{-1} was in a Tunisian Salt Lake, while our result indicated that the average copy numbers of the NaCl-treated and seasalt-treated groups were 3.14E+09 and 1.24E+09 (Abdallah et al., 2016). Timmusk et al. (2011) found that in "unstressed" soils, the number of microbial cells per gram of soil can reach up to 10^8 or 10^9. However, in soils subjected to stress, the microbial population can decrease dramatically to approximately 10^4 cells per gram of soil (del Carmen Orozco-Mosqueda et al., 2020). A study was conducted to analyze the abundance of the *mcrA* gene in mangrove surface layers and intertidal mudflat. The results showed a similar abundance of the *mcrA* gene in both sediment types' surface layers, ranging from $3.4 \sim 3.9 \times 10^6$ copies per gram dry weight in mangroves and $5.5 \sim 5.8 \times 10^6$ copies per gram dry weight in intertidal mudflat. However, the subsurface samples from both sediment types showed a higher abundance, ranging from 6.9×10^6 to 1.02×10^8 copies per gram dry weight (Zhou et al., 2015). Methanogens are essential in the global greenhouse gas budget and carbon cycle due to their methane production. In a study conducted by Zhang et al. (2020), it was revealed that the abundance of *mcrA* gene copies varied from 10^4 to 10^8 per gram of sediment and differed according to habitats and seasons in the River-bay system. This system serves as a transitional zone connecting land and ocean and is a significant natural source of methane emission (Zhang et al., 2020). In addition, according to Jingjing Peng's research, exposure to salt stress resulted in a significant reduction in both the mcrA gene and transcript numbers. Specifically, when exposed to 600 mM NaCl, there was a decrease in copy numbers by 34% (gene) and 59% (transcripts) compared to the control, with statistical significance ($P < 0.05$) (Peng et al., 2017).

Regarding the sulfate reducing bacteria, some experiments has been conducted in terms of *dsrB* gene and transcripts copy numbers. the creation of sulfides such as hydrogen sulfide as a result of injecting the sulfate-containing seawater into hydrocarbon reservoirs in order to maintain the required reservoir pressure,

leads to produce and growth of Sulfate Reducing Bacteria (SRB) approximately near the injection wells, turning the reservoir into sour, however SRB is not considered as the only microbial process stimulating the formation of sulfides (Haratian and Meybodi, 2021). During the analysis of sediment samples from various soda lakes, including the most saline lake, using a standard curve, a significant presence of SRB was observed. The results of the experiment indicated that the lowest copy numbers (10^5 and 10^4) were present in some of the sediment samples, while the highest dsr gene copy numbers per ml sediment (10^8) were detected in lake 3KL, which was the focus of this study (Haratian and Meybodi, 2021). A culture-independent technique was used to estimate the number of sulfate reducers, quantitative real-time PCR on genomic DNA. The highest number of dsr gene copies was found in Lake Tanatar I, with 10^8 cells per ml sediment. In another study conducted in Mono Lake, a moderate-salt soda lake, the number of SRBs ranged from 0.5×10^7 to 6×10^7 cells per ml. However, it should be noted that some Desulfovibrio species have been found to possess more than one *dsrB* gene copy, and hence, qPCR analysis may result in an overestimation of the SRB population (Kondo et al., 2004).

The study, conducted by Lovely, in 1993, revealed that methanogens and SRB were present at different depths in methane-rich water. The highest number of *mcrA* genes (4.6×10^2 copies mL^{-1}), while the number of *dsrB* genes was much lower (6.5×10^1 copies mL^{-1}) at the same depth, and no dsrB transcripts were detected through qPCR. Interestingly, *dsrB* genes were abundant at shallower (1.6×10^4 copies mL^{-1}) and deeper (2.2×10^3 dsrB copies mL^{-1}) depths, despite the low sulphate concentration in the water (0.5-1.4 mg L^{-1}) (Dalla Vecchia et al., 2014). Liu and Conrad, (2017) conducted a comprehensive investigation into the effect of gypsum application on microbial communities and acetate degradation, using three marker genes: 16S rRNA, *dsrB, and mcrA*. The study utilized quantification of bacterial 16S rRNA genes, *dsrB* genes, and *mcrA* genes, as well as their respective transcripts, to assess the absolute abundance (gene copies) and potential activity (transcript copies) of total bacteria, SRBs, and methanogenic archaea.

The average copy numbers for genes and transcripts of the three gene between the two treatments of our experiment were: 16S rRNA transcripts (5.71E+11) and 16S rRNA genes (1.26E+10) for the NaCl treatment; 16S rRNA transcripts (2.76E+11) and 16S rRNA genes (4.96E+09) for the seasalt treatment; dsrB transcripts (2.78E+09) and *dsrB* genes (6.67E+08) for the NaCl treatment; dsrB transcripts (3.28E+10) and *dsrB* genes (1.29E+09) for the seasalt treatment; mcrA transcripts (1.24E+11) and *mcrA* genes (4.73E+09) for the NaCl treatment; and mcrA transcripts (5.24E+10) and *mcrA* genes (6.82E+08) for the seasalt treatment. Our results are relatively in accordance with Liu's findings, 2018.

 4.5 Changes in soil microbial compositions linked to moderate salinity

In the gene level, hroughout the six weeks of the study, all groups showed a significant proportion of *Firmicutes*, with the NaCl and seasalt treatments demonstrating higher relative abundance than the control group. Moreover, *Clostridiaceae* 1 exhibited a higher relative abundance in both NaCl and seasalt treated groups compared to the control group, and the NaCl treatment group also showed higher relative abundance of *Ruminococcaceae* in weeks four and six. Furthermore, in week six, the seasalt treatment group showed a higher relative abundance of *Halobacteroidaceae*. Throughout the six-week study period, the application of 150 mM of NaCl and seasalt treatment led to a sustained increase in the relative abundance of *Actinobacteria*. This elevation was observed in all weeks, from the first to the sixth. Moreover, a similar trend was observed for *Chloroflexi*, where the relative abundance remained high from week two to week

six. However, the application of these treatments resulted in a lower relative abundance of *Proteobacteria* compared to the control group throughout all weeks. Notably, the relative abundance of *Geobacteraceae* was found to be higher in the samples treated with salts when compared to the control group throughout the course of the experiment at the transcript level.

According to studies utilizing 16S rRNA gene tag sequencing and metagenomics, the classes *Deltaproteobacteria* and *Gammaproteobacteria* were found to be dominant in both pristine and anthropogenically-influenced mangrove sediments (Dos Santos et al., 2011; Andreote et al., 2012). In the tropical mangrove sediments located in the Sao Paulo state of Brazil, Andreote et al., (2012) detected a higher abundance of these proteobacterial classes using metagenomic analysis. Similarly, Dos Santos et al., (2011) used pyrosequencing of 16S rDNA tags to show the dominance of these classes in the sediments of the "Restinga da Marambaia" mangrove in Rio de Janeiro, Brazil, under natural conditions and after simulated oil spills. This indicates that the proteobacterial classes *Deltaproteobacteria* and *Gammaproteobacteria* are universally dominant in tropical mangrove sediments, regardless of anthropogenic interference. The prevalence of *Deltaproteobacteria* in mangrove ecosystems can be attributed to frequent anaerobic conditions, which facilitate the selection of microaerophilic/anaerobic sulfate-reducing organisms belonging to this class, similar to those found in sea water and marine sediments (Taketani et al., 2010).

The bacterial phyla *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* and the *Methanobacterium*, *Methanosaeta*, and *Methanosarcina* genera in archaea were predominant at different salinities. Hydrogenotrophic methanogens such as *Methanobacterium* can tolerate salinity up to 85 g L^{-1}, whereas acetoclastic methanogens, *Methanosaeta* and *Methanosarcina* were severely inhibited at salinity greater than 65 g L^{-1} (Zhang et al., 2017). Ben Abdallah et al., (2018) showed that bacterial sequences were distributed into *Proteobacteria* (*Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria*), *Firmicutes*, *Actinobacteria* and *Bacteroidetes* phyla. The majority of sequences were affiliated with members of Ralstonia, in accordance with results obtained by Illumina Miseq-based analysis using the prokaryotic universal primers.

In the gene level, from the initial two weeks of the study, the two treated groups showed an increase in the relative abundance of *Firmicutes* when compared to the control group. However, from the second week until the sixth week, an observable elevation in the relative abundance of *Proteobacteria* was noted in the seasalt-treated group compared to the control group. Additionally, the relative abundances of *Peptococcaceae* were significantly greater in both treated samples than in the control group. Likewise, during the second and fourth weeks, the relative abundance of *Desulfovibrionaceae* was found to be higher than both the control and NaCl-treated groups. At the transcript level, *Proteobacteria* consistently exhibited a higher relative abundance throughout the study when compared to the control group. Interestingly, during the second and fourth weeks, the NaCl treatment group showed a higher relative abundance of *Proteobacteria* as compared to the seasalt-treated group, but this trend was reversed during the first and sixth weeks. The relative abundance of *Peptococcaceae* was found to be higher in the seasalt group compared to the control group, but it was even higher in the NaCl treatment group when compared to the seasalt-treated group. Furthermore, during the first and fourth weeks, a higher relative abundance of *Desulfovibrionaceae* was observed at the transcript level.

Regarding the sulfate reducing bacteria, most of the SRB fell into two major groups, i.e., the *Desulfovibrionales* and *Desulfobacteraceae*. Concerning the first group, sequences were affiliated with *Desulfonatronovibrio* hydrogenovorans, which belongs to the *Desulfohalobiaceae* family and to *Desulfonatronum lacustre,* the only member of the *Desulfonatronumaceae* family (Loy et al., 2002). Both these strains are low-salt-tolerant alkaliphiles isolated from soda lakes. They are incomplete oxidizers and

are able to use hydrogen and a few organic compounds as an electron donor, which is a common feature of the members of the *Desulfovibrionales* order (Foti et al., 2007).

With respect to sulfate reducing bacteria, *Desulfobacterales* dominated in soils of all vegetations (52.2%–72.3%), and families *Desulfovibrionales* and *Clostridiales* were observed at high tidal elevation, while *Syntrophobacterales* and *Desulfarculales* were only present at low tidal elevation. While in the sulfide oxidizing bacteria community, the phylum Chlorobia was only detected and even dominated at low tidal zone (Zheng et al., 2017). In a study, the sulfate-reducing Bacteria (SRB) belonging to the class *Deltaproteobacteria* were affiliated with the orders *Desulfobacterales*, *Desulfarculales* and *Desulfovibrionales*. At the family level, the analysis showed a high occurence of deltaproteobacterial representatives of *Desulfohalobiaceae*, *Desulfobacteraceae*, *Desulfarculaceae* and *Desulfobulbaceae* in ephemeral hypersaline lake (Zheng et al., 2017).

The implementation of a seasalt treatment with a concentration of 150 mM resulted in an increase in the relative abundance of *Methanosarcinales*. Similarly, the application of NaCl treatment (150 mM) led to a notable increase in relative abundance up to the fourth week. However, in contrast, the relative abundance of *Methanocellales* showed a marked decrease in response to both treatments compared to the control group. The *Methanosarcinaceae* family had a greater presence in the treated groups up to week four, while the *Methanocellaceae* family had a lower presence throughout the experiment. Interestingly, the relative abundance of *Methanomassiliicoccales* did not show significant changes among the three groups after salt treatments. Remarkably, the NaCl treatment resulted in the highest relative abundance of Methanobacteriaceae among the three groups. At transcript level, Throughout the experiment, it was observed that *Methanomassiliicoccales* and *Methanosarcinales* showed an increase in relative abundance in both treatment groups, while Methanocellales decreased in the treated groups compared to the control group. The *Methanosarcinaceae* family exhibited a greater relative abundance in the two treatment groups compared to the control group. Interestingly, from the first to the fourth week, the NaCl-treated group displayed a higher relative abundance of this family compared to the seasalt-treated group; however, this trend was reversed in week six, with the seasalt-treated group exhibiting a higher relative abundance. Throughout the experiment, the Methanocellaceae family consistently exhibited a lower relative abundance compared to the control group.

According to Zhang et al., (2020), the proportion of *Methanosaeta* remained stable, but increased from 17.01% to 36.77% as the NaCl concentration increased from 2.0 to 4.0 g/L. However, during the acclimation phase to 4.0 g NaCl/L, the proportion of *Methanosaeta* decreased, indicating a decreased resistance to higher NaCl concentrations. Nonetheless, *Methanosaeta* exhibited higher resistance to high salinity conditions than hydrogenotrophic methanogens. Other methanogens, such as *Methanosarcina*, *Methanospirillum*, and *Methanoculleus*, were also detected, with *Methanosarcina* accounting for 8.77% and 10.91%, respectively. *Methanosarcina's* ability to grow in aggregates and form irregular cell clumps may increase its tolerance to high concentrations of toxic ionic agents, suggesting that the proportion of *Methanosarcina* with high NaCl levels was higher, indicating that *Methanosarcina* may also be able to adapt to high-salinity environments. Another study found that, the shift in the methanogenic community from the acetoclastic *Methanosaeta* to the hydrogenotrophic *Methanobrevibacter* and *Methanocorpusculum* is also related with the increased salinity (Walter et al., 2016). Hydrogenotrophic methanogens such as *Methanobacterium* can tolerate salinity up to 85 g L^{-1}, whereas acetoclastic methanogens, *Methanosaeta* and *Methanosarcina* were severely inhibited at salinity greater than 65 g L^{-1} (Zhang et al., 2017).

In ephemeral hypersaline lake, Abdallah et al, (2018) dectected only one methanogen-related sequence was detected by archaeal-specific DGGE and was affiliated with *Methanomassiliicoccaceae*. As observed in

Illumina Miseq analysis using archaeal-specific primers, and they found that using prokaryotic universal primers showed low relative abundance of Archaea dominated by few OTUs related to *Methanosarcinaceae* and *Methanomassiliicoccaceae* families and the presence of sulfate-reducing Archaea affiliated with Archaeoglobus (Ben Abdallah et al., 2018).

5-Conclusions and outlook

In this study, we examined the impact of NaCl and seasalt (both at 150 mM) on microbial communities (including methanogens and bacteria) in Philippine paddy soil. Our results demonstrate that moderate changes in salinity can affect the abundance of *mcrA*, *dsrB*, and 16S rRNA genes and transcripts over time, and also alter the composition of soil microbial communities. Initial investigations were conducted by measuring gas production (CH_4, CO_2, and H_2S) in the experimental groups. This study employed high-throughput technologies (HiSeq and MiSeq), as well as RT-qPCR and qPCR assays, to comprehensively analyze bacterial and methanogenic communities in anoxic conditions in paddy soil. Our findings shed light on the ways in which salinity levels shape microbial communities in Philippine paddy soil.

Given that seasalt contains other ions (such as Mg^{2+}, Ca^{2+}, and K^+), it would be useful to investigate their impact on soil microbial communities in future studies. Additionally, performing shotgun metagenomics and metatranscriptomics would provide a more in-depth understanding of the effects of salinity on soil microorganisms.

6-Supplementary information

Table S1: Taxonomy table of bacterial 16S rRNA gene collapsed at phylum level achieved paired-end reads from HiSeq platform using QIIME2.

Phylum	Control-w1	Control-w2	Control-w4	Control-w6	NaCl⁻-w1	NaCl⁻-w2	NaCl⁻-w4	NaCl⁻-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Acidobacteria	1413.3	685.0	1532.0	2216.7	1236.0	1126.0	1972.7	328.3	1618.3	835.3	696.3	1125.7
Actinobacteria	580.0	119.3	134.3	297.7	1175.0	881.0	753.3	1455.3	2375.0	853.0	703.7	1364.0
Armatimonadetes	47.3	15.0	64.7	36.7	214.3	124.3	402.0	217.7	292.7	156.0	271.3	316.0
BRC1	131.3	62.3	34.3	20.7	115.3	66.0	36.0	20.7	140.7	45.7	37.7	23.7
Bacteroidetes	1856.7	2148.3	1535.3	3135.3	543.0	1511.7	1613.0	3435.0	1221.3	2077.7	1694.3	4583.3
Chlamydiae	0.0	0.0	0.7	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloroflexi	1463.0	611.7	2725.3	2916.7	1581.3	1377.3	5212.7	4500.0	3498.0	1778.7	3869.0	5226.0
Cyanobacteria	5.7	4.3	6.0	1.0	84.0	14.3	46.7	5.3	16.7	0.7	13.7	30.0
Dadabacteria	0.0	0.7	0.0	0.0	0.7	0.0	0.0	0.0	1.0	0.0	0.7	2.3
Deinococcus-Thermus	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	1.0	0.0
Dependentiae	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.0	3.0	0.0	0.0	0.0
Elusimicrobia	2.0	6.7	16.3	51.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Entotheonellaeota	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	5.7	0.0	0.0	0.0
Fibrobacteres	197.3	124.3	124.0	175.0	207.0	308.7	702.0	902.7	52.0	1.3	102.0	117.0
Christensenellaceae	22375.3	9991.7	7039.3	8418.0	36866.3	17527.3	13948.7	19275.3	39229.0	18402.0	13354.7	15373.3
GAL15	15.3	0.0	1.7	1.3	28.7	6.3	27.0	10.7	49.0	9.3	34.0	28.3
Gemmatimonadetes	23.7	4.3	6.3	31.3	68.0	53.0	23.0	29.3	150.0	98.0	32.0	89.7
Halanaerobiaeota	0.0	0.0	0.0	0.0	14.7	22.0	11.0	13.0	66.3	324.7	178.3	2251.7
Hydrogenedentes	0.0	0.0	2.3	11.0	0.0	0.0	0.0	13.7	0.0	0.0	0.0	12.3
Latescibacteria	27.3	14.3	23.0	71.7	37.0	54.3	61.7	30.0	97.3	68.3	81.7	80.0
Lentisphaerae	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrospinae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0
Nitrospirae	5.0	0.7	1.3	14.0	14.0	18.3	10.3	7.0	29.3	14.3	5.0	17.7
Patescibacteria	5.7	16.7	17.0	5.7	50.3	0.0	0.7	0.0	7.0	6.0	7.0	0.7
Christensenellaceae	86.7	46.3	209.3	293.0	77.3	60.0	414.0	458.3	119.7	42.0	134.7	216.0
Proteobacteria	2278.0	1219.3	965.0	2221.3	2415.0	1940.0	1328.7	1424.7	4671.7	3209.0	1564.7	3137.0
Rokubacteria	9.3	5.3	25.3	28.3	24.7	17.7	52.0	9.0	60.0	64.3	32.3	80.7
Tenericutes	547.7	244.7	73.3	82.7	388.0	179.3	207.7	684.0	588.3	541.0	235.7	202.7
Verrucomicrobia	108.3	147.7	184.0	237.3	11.7	7.7	62.0	116.0	2.7	12.0	37.3	112.7
WS1	4.7	1.3	2.0	0.0	5.0	15.3	33.0	37.7	7.0	7.3	16.7	0.0
WS4	7.0	0.0	18.3	0.0	56.3	18.0	112.0	42.7	32.3	9.0	42.0	31.0
Unassigned	9144.3	2597.3	5707.3	7089.0	10569.7	5072.7	9083.0	7865.3	9241.0	4458.7	8447.3	10164.0

Table S2: Relative abundance (%) of dominant phyla for 16S rRNA gene.

Phylum- 16S gene	Control-w1	NaCl-w1	Seasalt-w1
Acidobacteria	4.61%	2.77%	3.04%
Actinobacteria	1.89%	2.64%	4.46%
Armatimonadetes	0.15%	0.48%	0.55%
BRC1	0.43%	0.26%	0.26%
Bacteroidetes	6.06%	1.22%	2.29%
Chloroflexi	4.77%	3.55%	6.57%
Fibrobacteres	0.64%	0.46%	0.10%
Christensenellaceae	73.01%	82.68%	73.71%
Halanaerobiaeota	0.00%	0.03%	0.12%
Proteobacteria	7.43%	5.42%	8.78%
Verrucomicrobia	0.35%	0.03%	0.01%
Fibrobacteres	0.64%	0.46%	0.10%

Phylum- 16S gene	Control-w2	NaCl-w2	Seasalt-w2
Acidobacteria	4.49%	4.47%	3.02%
Actinobacteria	0.78%	3.50%	3.08%
Armatimonadetes	0.10%	0.49%	0.56%
BRC1	0.41%	0.26%	0.16%
Bacteroidetes	14.09%	6.00%	7.50%
Chloroflexi	4.01%	5.47%	6.42%
Fibrobacteres	0.82%	1.22%	0.00%
Christensenellaceae	65.52%	69.55%	66.44%
Halanaerobiaeota	0.00%	0.09%	1.17%
Proteobacteria	8.00%	7.70%	11.59%
Verrucomicrobia	0.97%	0.03%	0.04%
Fibrobacteres	0.82%	1.22%	0.00%

Phylum- 16S gene	Control-w4	NaCl-w4	Seasalt-w4
Acidobacteria	10.59%	7.38%	3.08%
Actinobacteria	0.93%	2.82%	3.11%
Armatimonadetes	0.45%	1.50%	1.20%
BRC1	0.24%	0.13%	0.17%
Bacteroidetes	10.62%	6.03%	7.49%
Chloroflexi	18.84%	19.49%	17.11%
Fibrobacteres	0.86%	2.62%	0.45%
Christensenellaceae	48.67%	52.16%	59.06%
Halanaerobiaeota	0.00%	0.04%	0.79%
Proteobacteria	6.67%	4.97%	6.92%
Verrucomicrobia	1.27%	0.23%	0.17%
Fibrobacteres	0.86%	2.62%	0.45%

Phylum- 16S gene	Control-w6	NaCl-w6	Seasalt-w6
Acidobacteria	11.17%	1.01%	3.34%
Actinobacteria	1.50%	4.47%	4.04%
Armatimonadetes	0.18%	0.67%	0.94%
BRC1	0.10%	0.06%	0.07%
Bacteroidetes	15.79%	10.54%	13.58%
Chloroflexi	14.69%	13.81%	15.49%
Fibrobacteres	0.88%	2.77%	0.35%
Christensenellaceae	42.41%	59.14%	45.55%
Halanaerobiaeota	0.00%	0.04%	6.67%
Proteobacteria	11.19%	4.37%	9.30%
Verrucomicrobia	1.20%	0.36%	0.33%
Fibrobacteres	0.88%	2.77%	0.35%

Table S3: Taxonomy table of bacterial 16S rRNA gene collapsed at family level achieved paired-end reads from HiSeq platform using QIIME2.

Family	control-w1	control-w2	control-w4	control-w6	NaCl⁻-w1	NaCl⁻-w2	NaCl⁻-w4	NaCl⁻-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Acidobacteriaceae (Subgroup 1)	2.0	0.0	0.0	0.0	0.0	0.0	4.7	0.0	6.0	0.0	0.0	0.0
Koribacteraceae	8.0	5.3	24.0	62.0	28.7	26.7	16.7	8.0	73.3	56.0	42.0	74.0
Solibacteraceae (Subgroup 3)	610.0	512.7	802.0	1850.0	374.7	452.7	268.7	114.7	301.3	341.3	115.3	477.3
uncultured bacterium	2112.0	801.3	2603.3	4728.7	4527.3	2571.3	5464.0	6090.7	6112.7	2759.3	3396.0	5039.3
Blastocatellaceae	0.0	0.0	0.0	2.7	0.0	0.0	14.0	0.0	34.7	12.0	34.7	17.3
Pyrinomonadaceae	9.3	0.0	3.3	13.3	54.0	22.7	87.3	26.0	116.0	63.3	110.0	106.0
uncultured Acidobacteria bacterium	108.0	61.3	473.3	375.3	72.0	239.3	652.0	42.0	178.7	182.7	194.0	56.7
uncultured Acidobacteriales bacterium	0.0	0.0	10.7	0.0	0.0	7.3	18.7	0.0	18.7	0.0	0.0	0.0
uncultured Holophaga sp.	0.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0
Thermoanaerobaculaceae	0.0	0.0	0.0	2.0	3.3	6.7	2.7	0.0	11.3	0.0	0.0	5.3
Iamiaceae	0.0	0.0	0.0	0.0	10.0	14.7	0.0	0.0	16.7	13.3	0.0	4.7
Ilumatobacteraceae	0.0	0.0	0.0	4.0	5.3	12.0	8.0	8.7	12.7	0.0	0.0	3.3
Microtrichaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0	2.0
Mycobacteriaceae	3.3	0.0	0.0	0.0	21.3	8.7	5.3	4.0	34.7	7.3	0.0	5.3
Cellulomonadaceae	0.0	0.0	0.0	0.0	0.0	0.0	226.0	1699.3	207.3	169.3	516.7	1226.0
Intrasporangiaceae	12.0	0.0	0.0	18.7	59.3	25.3	17.3	0.0	103.3	0.0	0.0	29.3
Microbacteriaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.3	0.0	0.0	0.0
Micrococcaceae	0.0	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Micromonosporaceae	57.3	26.7	6.0	12.7	165.3	157.3	86.0	149.3	300.7	196.7	56.0	122.7
Nocardioidaceae	88.7	9.3	0.0	4.7	264.0	70.0	66.0	69.3	388.0	34.0	46.0	62.0
Propionibacteriaceae	9.3	4.7	7.3	8.7	60.7	41.3	8.7	6.7	130.7	68.0	11.3	52.0
Pseudonocardiaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3	0.0	0.0	0.0
Streptomycetaceae	120.7	16.7	0.0	60.0	215.3	218.0	152.7	178.0	432.0	119.3	50.0	133.3

Nocardiopsaceae	0.0	0.0	0.0	0.0	0.0	8.0	0.0	0.0	3.3	4.0	0.0	0.0
Streptosporangiaceae	0.0	0.0	0.0	0.0	2.0	0.0	4.7	0.0	0.0	0.0	0.0	0.0
Thermomonosporaceae	0.0	0.0	0.0	0.0	8.7	0.0	2.0	2.7	18.0	6.0	0.0	0.0
Actinobacteria bacterium RBG_19FT_COMBO_70_19	16.0	13.3	31.3	0.0	87.3	36.7	52.7	57.3	160.0	25.3	47.3	32.7
Gaiellaceae	67.3	4.7	4.0	54.7	120.0	194.7	112.7	56.0	271.3	125.3	82.0	232.7
67-14	30.0	0.0	4.0	20.7	94.7	59.3	44.7	6.0	160.0	28.0	17.3	52.0
Solirubrobacteraceae	0.0	2.7	0.0	0.0	10.7	3.3	0.0	0.0	41.3	10.7	0.0	0.0
Chthonomonadaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.3	2.7	0.0	1.3
uncultured Armatimonadetes bacterium	0.0	0.0	16.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	11.3	0.0
uncultured Lutispora sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0
Fimbriimonadaceae	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	3.3	3.3	0.0	2.0
Bacteroidetes vadinHA17	1435.3	2158.7	1134.0	2292.0	197.3	1207.3	1295.3	2142.7	116.7	273.3	663.3	3700.7
Paludibacteraceae	501.3	644.7	604.0	589.3	274.0	1058.7	862.0	1948.7	492.0	2488.7	794.0	1084.7
Prolixibacteraceae	840.0	496.7	165.3	246.0	72.0	83.3	127.3	589.3	72.0	149.3	75.3	262.7
SB-5	12.7	37.3	331.3	778.7	0.0	0.0	22.0	65.3	0.0	0.0	30.7	171.3
Chitinophagaceae	679.3	274.7	210.0	368.0	359.3	332.0	352.7	393.3	1750.0	992.7	664.7	1605.3
Hymenobacteraceae	0.0	0.0	0.0	0.0	4.7	0.0	0.0	0.0	2.7	0.0	2.0	6.7
AKYH767	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
Lentimicrobiaceae	136.0	523.3	602.0	1749.3	32.0	48.7	156.0	1090.7	6.0	224.0	1130.0	2010.7
BSV40	108.7	161.3	24.0	247.3	146.7	293.3	359.3	422.7	3.3	27.3	28.7	177.3
Ignavibacteriaceae	0.0	0.0	0.0	0.0	0.0	0.0	27.3	152.7	0.0	0.0	0.0	144.0
BSV26	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
uncultured Clostridiales bacterium	30.0	24.0	54.0	22.0	18.0	42.7	20.7	16.7	6.7	21.3	10.0	12.7
uncultured bacterium SJA-68	0.0	0.0	0.0	3.3	2.0	0.0	3.3	0.0	0.0	9.3	4.0	0.0
Anaerolineaceae	2446.0	1035.3	3812.0	2899.3	1870.0	1873.3	7720.0	4374.7	4729.3	2412.0	5664.0	5882.7
Caldilineaceae	21.3	0.0	6.0	6.0	55.3	26.7	40.7	40.0	89.3	33.3	27.3	78.7
A4b	7.3	0.0	2.7	2.0	40.7	7.3	6.0	2.7	98.7	12.0	46.7	117.3
uncultured Caldilineaceae bacterium	0.0	0.0	0.0	0.0	0.0	2.7	1.3	0.0	0.0	0.0	0.0	0.0
uncultured Chloroflexi bacterium	44.7	28.0	130.7	176.0	100.7	71.3	259.3	296.7	140.7	68.7	418.0	841.3
uncultured soil bacterium	7.3	0.0	8.7	5.3	7.3	8.7	10.7	58.7	17.3	32.7	1.3	11.3
uncultured Bellilinea sp.	0.0	0.0	0.0	4.7	0.0	1.3	0.0	0.0	2.7	2.7	0.0	0.0
Chloroflexaceae	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	3.3	0.0	4.7
Roseiflexaceae	40.7	26.7	15.3	27.3	157.3	74.0	13.3	12.0	224.0	156.7	22.0	49.3
AKYG1722	8.0	4.7	1.3	2.7	26.7	8.7	9.3	12.0	34.7	15.3	6.0	8.7
JG30-KF-CM45	54.0	3.3	0.0	10.7	83.3	100.7	107.3	98.7	120.7	43.3	64.7	86.0
Chroococcidiopsaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3
Nostocaceae	6.7	0.0	4.0	0.0	18.7	0.0	48.0	0.0	22.0	0.0	18.7	58.7
Unknown Family	0.0	2.7	0.0	0.0	6.0	3.3	0.0	0.0	0.0	1.3	0.0	10.0
Deinococcaceae	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	2.0	0.0
Endomicrobiaceae	4.0	13.3	32.7	102.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Entotheonellaceae	0.0	0.0	0.0	0.0	4.7	0.0	0.0	0.0	11.3	0.0	0.0	0.0
possible family 01	394.7	248.7	248.0	350.0	414.0	617.3	1404.0	1805.3	104.0	2.7	204.0	234.0

Alicyclobacillaceae	7.3	3.3	0.0	0.0	50.0	0.0	0.0	8.0	18.0	11.3	3.3	10.0
Bacillaceae	2374.7	533.3	195.3	168.7	5777.3	1013.3	475.3	428.0	15327.3	4776.7	726.7	712.0
Paenibacillaceae	252.0	111.3	22.0	14.0	812.0	406.0	181.3	946.7	1536.0	238.0	94.7	60.0
Planococcaceae	0.0	0.0	0.0	0.0	22.0	0.0	7.3	10.0	18.7	0.0	16.0	4.0
Thermoactinomycetaceae	0.0	0.0	0.0	0.0	26.7	0.0	2.0	2.7	2.7	2.0	36.7	0.0
Caldicoprobacteraceae	0.0	12.0	0.0	0.0	245.3	231.3	67.3	23.3	552.0	373.3	718.0	192.7
Christensenellaceae	1447.3	1906.0	1048.0	2992.7	2126.7	3544.0	2867.3	5284.0	2544.7	3325.3	2748.7	4451.3
Clostridiaceae 1	3013.3	955.3	258.7	486.7	9645.3	4055.3	2564.7	4428.0	8948.7	2759.3	1617.3	2300.7
Clostridiaceae 3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	172.0	0.0	828.7
Clostridiaceae 4	25.3	0.0	0.0	1.3	28.0	19.3	8.0	19.3	309.3	239.3	15.3	50.7
Clostridiales vadinBB60 group	362.0	404.0	355.3	708.0	521.3	636.0	374.7	648.0	437.3	485.3	218.7	460.0
Defluviitaleaceae	0.0	0.0	0.0	0.0	0.0	0.0	11.3	0.0	12.0	0.0	0.0	0.0
Eubacteriaceae	38.7	5.3	9.3	38.0	82.0	71.3	49.3	88.7	117.3	74.7	72.0	133.3
Family XI	348.0	107.3	30.0	56.0	1467.3	633.3	297.3	672.0	2669.3	1614.7	1572.7	1519.3
Family XIII	849.3	302.0	317.3	464.7	1275.3	757.3	344.7	700.7	1745.3	969.3	610.7	795.3
Family XVIII	5866.7	659.3	1268.0	642.7	3210.0	167.3	110.0	4.0	1959.3	172.7	110.7	35.3
Gracilibacteraceae	2937.3	1440.0	478.7	1372.7	4170.7	4328.0	2118.0	3985.3	4192.7	2754.0	970.0	1278.7
Heliobacteriaceae	3057.3	2036.7	4436.7	2176.0	1904.0	1280.7	3127.3	3115.3	2436.7	1540.0	3215.3	1379.3
Lachnospiraceae	7383.3	2594.7	740.7	912.7	13642.7	3386.7	1964.7	2363.3	13553.3	4108.7	2213.3	1634.0
Peptococcaceae	1888.7	1000.0	939.3	1301.3	3914.0	2334.7	2080.0	1754.7	3955.3	2601.3	2828.0	4447.3
Peptostreptococcaceae	42.7	3.3	0.0	6.0	122.0	74.7	39.3	61.3	185.3	30.7	6.0	47.3
Ruminococcaceae	11234.7	6430.7	1948.7	3504.0	15726.0	8754.7	7016.0	11355.3	12619.3	7782.7	4698.0	6503.3
Syntrophomonadaceae	496.0	255.3	54.0	425.3	705.3	1143.3	467.3	735.3	474.7	627.3	215.3	335.3
uncultured Christensenellaceae bacterium	0.0	2.0	50.0	82.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
uncultured Bacilli bacterium	94.0	31.3	85.3	76.7	219.3	68.7	56.0	20.0	0.0	0.0	66.0	94.7
SRB2	46.7	45.3	0.0	42.0	131.3	198.7	225.3	284.0	264.7	478.7	508.7	704.7
Erysipelotrichaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0
Limnochordaceae	44.0	12.0	24.0	2.7	260.7	38.7	255.3	204.0	98.7	40.7	343.3	315.3
Veillonellaceae	2407.3	1004.0	1496.0	648.7	6460.0	1448.0	2601.3	1218.0	3896.7	1507.3	2794.0	2038.0
Gemmatimonadaceae	24.7	8.7	1.3	27.3	100.0	78.7	29.3	45.3	212.7	103.3	25.3	109.3
uncultured Gemmatimonadetes bacterium	4.7	0.0	0.0	8.0	8.7	0.0	0.0	0.0	10.0	17.3	6.0	0.0
Halobacteroidaceae	0.0	0.0	0.0	0.0	29.3	44.0	22.0	26.0	132.7	649.3	356.7	4503.3
Hydrogenedensaceae	0.0	0.0	4.7	22.0	0.0	0.0	0.0	27.3	0.0	0.0	0.0	24.7
Latescibacteraceae	34.0	28.7	32.7	132.0	32.0	78.0	48.0	34.7	15.3	44.0	13.3	40.7
uncultured Latescibacteria bacterium	4.7	0.0	0.0	0.0	0.0	0.0	10.0	2.0	6.7	0.0	0.0	12.0
uncultured Pelobacter sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	2.0	0.0	0.0
uncultured prokaryote	0.0	0.0	0.0	0.0	2.0	2.7	4.7	0.0	13.3	7.3	8.0	3.3
Nitrospiraceae	4.7	0.0	2.7	28.0	15.3	24.7	14.7	14.0	28.7	28.7	10.0	24.0
uncultured Clostridium sp.	0.0	4.0	0.0	0.0	86.0	0.0	0.0	0.0	14.0	0.0	0.0	0.0
uncultured microorganism	2.7	6.7	24.7	6.0	0.0	0.0	1.3	0.0	0.0	0.0	14.0	0.0
4572-13	16.0	26.0	76.7	176.7	0.0	38.0	145.3	206.7	0.0	24.0	78.0	112.0

SG8-4	20.7	22.0	147.3	286.0	19.3	17.3	124.7	300.0	17.3	6.0	55.3	206.0
CPla-3 termite group	0.0	4.7	8.0	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WD2101 soil group	127.3	35.3	128.7	40.0	86.0	38.0	62.7	10.0	54.0	29.3	75.3	34.0
uncultured planctomycete	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0
Gemmataceae	2.0	0.0	2.7	0.0	14.0	0.0	59.3	14.7	68.0	15.3	2.7	11.3
Isosphaeraceae	2.7	0.0	0.0	1.3	0.0	4.0	6.0	3.3	30.0	3.3	0.0	3.3
Pirellulaceae	3.3	0.0	0.0	0.0	30.0	15.3	184.7	14.0	60.0	4.0	22.0	21.3
Rubinisphaeraceae	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetobacteraceae	0.0	0.0	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0	2.0
Caulobacteraceae	0.0	0.0	0.0	0.0	12.7	0.0	0.0	2.0	12.0	19.3	3.3	3.3
Hyphomonadaceae	1.3	0.0	0.0	0.0	10.0	0.0	0.0	0.0	6.7	7.3	0.0	12.0
Dongiaceae	19.3	6.0	1.3	4.0	71.3	50.7	39.3	51.3	112.0	46.0	26.0	59.3
Beijerinckiaceae	69.3	6.0	0.0	0.0	130.0	86.0	85.3	91.3	226.7	78.7	32.0	112.0
Hyphomicrobiaceae	0.0	2.7	0.0	0.0	24.0	0.0	0.0	10.0	44.7	5.3	0.0	0.0
KF-JG30-B3	0.0	0.0	0.0	0.0	11.3	4.7	0.0	0.0	8.0	0.0	0.0	7.3
Methyloligellaceae	36.0	7.3	5.3	38.7	138.0	122.0	96.7	93.3	214.0	170.0	50.0	124.0
Pleomorphomonadaceae	0.0	0.0	0.0	0.0	20.0	5.3	4.0	0.0	21.3	15.3	0.0	0.0
Rhizobiaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0
Rhizobiales Incertae Sedis	0.0	0.0	0.0	0.0	5.3	6.7	0.0	0.0	12.7	0.0	0.0	0.0
Xanthobacteraceae	0.0	0.0	0.0	2.0	19.3	18.7	0.0	0.0	44.7	8.7	0.0	11.3
Magnetospirillaceae	3.3	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhodopirillaceae	0.0	0.0	0.0	0.0	4.0	3.3	2.0	2.7	4.0	4.7	4.7	5.3
Sphingomonadaceae	53.3	12.0	12.0	66.7	128.7	104.7	64.0	28.0	190.0	118.7	63.3	143.3
Geminicoccaceae	0.0	0.0	0.0	0.0	5.3	1.3	0.0	0.0	2.7	3.3	0.0	0.0
Bdellovibrionaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0
Syntrophorhabdaceae	3.3	10.0	73.3	420.0	6.7	8.7	42.0	236.0	5.3	10.7	16.7	169.3
Desulfarculaceae	14.0	6.7	9.3	46.0	69.3	72.7	8.0	25.3	90.7	58.0	35.3	74.0
Desulfobacteraceae	0.0	0.0	0.0	4.7	0.0	0.0	0.0	2.7	4.7	0.0	0.0	1.3
Desulfovibrionaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	10.0	0.0	0.0	7.3
Desulfuromonadaceae	70.0	22.7	20.7	22.0	0.0	5.3	9.3	15.3	66.0	22.0	16.7	47.3
Geobacteraceae	962.0	495.3	315.3	518.7	817.3	766.7	751.3	496.0	2068.0	883.3	437.3	893.3
Archangiaceae	1873.3	1073.3	849.3	1418.7	1387.3	1169.3	698.0	918.0	2240.7	2418.7	1275.3	1944.0
BIrii41	2.7	0.0	50.7	38.7	20.7	3.3	0.0	0.0	11.3	8.7	0.0	6.0
Haliangiaceae	440.7	405.3	203.3	401.3	266.7	161.3	90.7	88.0	463.3	237.3	94.7	248.7
Myxococcaceae	64.0	16.0	16.7	0.0	52.7	8.0	20.0	0.0	31.3	19.3	0.0	5.3
Nannocystaceae	1.3	0.0	0.0	0.0	3.3	2.0	1.3	2.7	8.7	17.3	2.0	0.0
P3OB-42	191.3	90.7	97.3	207.3	167.3	136.7	105.3	130.7	316.7	203.3	151.3	326.0
Phaselicystidaceae	204.0	49.3	24.7	154.0	44.0	5.3	0.0	0.0	0.0	0.0	0.0	7.3
Polyangiaceae	5.3	0.0	0.0	0.0	48.7	26.0	13.3	24.7	75.3	14.7	6.0	0.0
Sandaracinaceae	1.3	0.0	0.0	0.0	48.0	23.3	9.3	18.0	40.0	4.7	0.0	10.0
UASB-TL25	0.0	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Vulgatibacteraceae	4.7	0.0	0.0	0.0	11.3	0.0	0.0	0.0	10.7	4.7	0.0	0.0
bacteriap25	0.0	0.0	0.0	0.0	32.0	30.7	6.7	0.0	47.3	14.7	0.0	5.3

mle1-27	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	22.0	3.3	0.0	1.3
0319-6G20	0.0	3.3	4.0	14.7	0.0	8.7	0.0	3.3	11.3	0.0	0.0	3.3
Syntrophaceae	4.0	0.0	43.3	215.3	0.0	0.0	0.0	0.0	8.7	2.7	2.7	29.3
Syntrophobacteraceae	14.0	0.0	2.7	8.0	13.3	4.0	15.3	11.3	211.3	592.7	256.7	462.7
A21b	0.0	0.0	0.0	3.3	3.3	2.7	0.0	6.7	21.3	0.0	0.0	0.0
B1-7BS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7	12.7
Burkholderiaceae	56.0	38.7	22.7	76.0	188.7	139.3	101.3	96.7	226.7	198.0	110.7	251.3
Nitrosomonadaceae	37.3	34.7	26.0	76.7	142.0	191.3	97.3	86.0	329.3	319.3	116.7	266.0
Rhodocyclaceae	0.0	0.0	0.0	0.0	6.7	0.0	0.0	0.0	5.3	6.0	0.0	0.0
SC-I-84	8.7	8.7	0.0	0.0	22.0	22.0	2.7	0.0	58.0	20.0	4.0	6.7
TRA3-20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.7	8.7	0.0	10.0
Enterobacteriaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	773.3	0.0	0.0	0.0
Methylomonaceae	20.7	6.0	0.0	5.3	46.0	35.3	19.3	22.0	67.3	29.3	0.0	29.3
Pseudomonadaceae	0.0	0.0	2.0	0.0	16.7	14.7	3.3	2.7	20.7	11.3	0.0	19.3
Steroidobacteraceae	62.0	39.3	56.0	164.7	186.7	189.3	124.7	98.7	302.7	265.3	167.3	312.0
Rhodanobacteraceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7
Xanthomonadaceae	2.0	0.0	0.0	4.7	17.3	18.0	4.7	2.0	9.3	6.7	9.3	16.0
Methylomirabilaceae	0.0	2.0	40.0	12.0	26.7	8.7	25.3	3.3	40.7	16.7	20.7	31.3
Haloplasmataceae	1018.0	262.7	104.0	94.0	558.7	299.3	379.3	1350.0	1138.0	1004.7	385.3	390.0
Chthoniobacteraceae	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	2.7	0.0	0.0	8.7
Opitutaceae	108.7	239.3	251.3	428.7	12.0	14.0	114.7	230.0	0.0	8.7	66.0	177.3
Pedosphaeraceae	108.0	56.0	116.7	46.0	11.3	0.0	9.3	2.0	2.7	15.3	6.7	39.3
Unassigned	18288.7	5194.7	11414.7	14178.0	21139.3	10145.3	18166.0	15730.7	18482.0	8917.3	16894.7	20328.0

Table S4: Relative abundance (%) of dominant families for 16S rRNA gene.

Family-16S gene	**control-w1**	**NaCl-w1**	**Seasalt-w1**
Chitinophagaceae	1.75%	0.57%	2.42%
Anaerolineaceae	6.30%	2.96%	6.53%
Bacillaceae	6.11%	9.16%	21.18%
Christensenellaceae	3.73%	3.37%	3.52%
Clostridiaceae 1	7.76%	15.29%	12.36%
Gracilibacteraceae	7.56%	6.61%	5.79%
Heliobacteriaceae	7.87%	3.02%	3.37%
Lachnospiraceae	19.00%	21.62%	18.73%
Ruminococcaceae	28.92%	24.92%	17.44%
Veillonellaceae	6.20%	10.24%	5.38%
Halobacteroidaceae	0.00%	0.05%	0.18%
Archangiaceae	4.82%	2.20%	3.10%
Family-16S gene	**control-w2**	**NaCl-w2**	**Seasalt-w2**
Chitinophagaceae	1.42%	1.06%	2.83%
Anaerolineaceae	5.37%	6.00%	6.89%

Bacillaceae	2.77%	3.24%	13.64%
Christensenellaceae	9.88%	11.35%	9.49%
Clostridiaceae 1	4.95%	12.99%	7.88%
Gracilibacteraceae	7.47%	13.86%	7.86%
Heliobacteriaceae	10.56%	4.10%	4.40%
Lachnospiraceae	13.46%	10.84%	11.73%
Ruminococcaceae	33.35%	28.03%	22.22%
Veillonellaceae	5.21%	4.64%	4.30%
Halobacteroidaceae	0.00%	0.14%	1.85%
Archangiaceae	5.57%	3.74%	6.91%

Family-16S gene	**control-w4**	**NaCl-w4**	**Seasalt-w4**
Chitinophagaceae	1.36%	1.12%	2.47%
Anaerolineaceae	24.63%	24.49%	21.02%
Bacillaceae	1.26%	1.51%	2.70%
Christensenellaceae	6.77%	9.09%	10.20%
Clostridiaceae 1	1.67%	8.13%	6.00%
Gracilibacteraceae	3.09%	6.72%	3.60%
Heliobacteriaceae	28.67%	9.92%	11.93%
Lachnospiraceae	4.79%	6.23%	8.21%
Ruminococcaceae	12.59%	22.25%	17.44%
Veillonellaceae	9.67%	8.25%	10.37%
Halobacteroidaceae	0.00%	0.07%	1.32%
Archangiaceae	5.49%	2.21%	4.73%

Family-16S gene	**control-w6**	**NaCl-w6**	**Seasalt-w6**
Chitinophagaceae	2.17%	1.04%	4.69%
Anaerolineaceae	17.11%	11.55%	17.18%
Bacillaceae	1.00%	1.13%	2.08%
Christensenellaceae	17.66%	13.95%	13.00%
Clostridiaceae 1	2.87%	11.69%	6.72%
Gracilibacteraceae	8.10%	10.52%	3.74%
Heliobacteriaceae	12.84%	8.22%	4.03%
Lachnospiraceae	5.39%	6.24%	4.77%
Ruminococcaceae	20.68%	29.97%	19.00%
Veillonellaceae	3.83%	3.21%	5.95%
Halobacteroidaceae	0.00%	0.07%	13.16%
Archangiaceae	8.37%	2.42%	5.68%

Table S5: Taxonomy table of bacterial 16S rRNA transcripts collapsed at phylum level achieved paired-end reads from HiSeq platform using QIIME2.

Phylum	Control-w1	Control-w2	Control-w4	Control-w6	NaCl⁻-w1	NaCl⁻-w2	NaCl⁻-w4	NaCl⁻-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Acidobacteria	2468.3	2195.3	2358.3	4607.0	1467.7	2170.0	2150.0	2580.3	1292.0	2071.7	621.3	2676.7
Actinobacteria	902.0	456.0	463.7	638.7	1795.7	1083.3	790.0	2158.3	3852.3	893.0	2542.3	2939.7
Armatimonadetes	187.7	33.0	81.7	22.0	203.3	181.0	364.3	289.3	147.3	116.0	148.3	77.7
BRC1	232.7	450.0	179.0	152.0	102.0	76.7	59.7	48.3	72.0	207.3	11.7	43.3
Bacteroidetes	298.3	365.0	39.0	533.3	86.7	1001.7	476.0	318.7	1107.3	316.3	142.3	844.3
Chloroflexi	2145.0	1211.0	2336.0	1909.0	1160.3	1665.7	3667.3	4576.0	2590.7	1662.7	2892.7	3295.3
Cyanobacteria	9.0	1.0	0.0	2.3	91.3	105.0	44.7	5.0	106.3	1.0	0.0	22.7
Dadabacteria	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0
Deinococcus-Thermus	4.7	0.0	0.0	0.0	0.0	8.3	0.0	13.0	4.7	1.0	0.0	1.3
Dependentiae	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	1.0	0.0	0.0	0.0
Elusimicrobia	0.0	0.0	0.0	24.7	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0
Entotheonellaeota	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
FCPU426	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fibrobacteres	2.7	4.0	0.0	89.0	84.0	849.7	714.0	171.7	32.3	2.0	14.7	101.7
Christensenellaceae	14119.0	6691.3	8258.7	4356.7	8441.3	10125.3	6875.7	6384.3	8801.7	7958.0	4083.0	3225.7
GAL15	0.0	1.7	0.0	0.0	7.7	0.0	0.0	0.0	89.3	0.0	0.0	20.3
Gemmatimonadetes	12.7	18.7	7.7	10.3	30.0	21.3	3.3	37.3	179.3	23.3	32.0	57.0
Halanaerobiaeota	0.0	0.0	0.0	0.0	6.7	12.0	0.0	0.0	28.7	48.0	28.3	79.7
Hydrogenedentes	6.0	12.0	16.3	46.3	6.3	12.7	23.3	135.7	3.3	0.0	26.3	31.7
Latescibacteria	21.0	17.3	1.7	43.3	11.3	14.7	8.3	28.0	0.0	8.3	0.0	12.7
Lentisphaerae	1.7	7.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrospirae	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0	2.0	0.0	0.0	1.3
PAUC34f	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Patescibacteria	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Christensenellaceae	418.0	402.0	539.7	713.3	756.7	314.0	797.0	1476.7	1332.3	317.0	306.0	807.0
Proteobacteria	8143.7	7357.7	3264.3	5195.0	4368.7	4349.0	1479.3	3333.0	3380.3	4763.0	3089.0	3624.0
Rokubacteria	54.3	69.7	206.7	62.0	27.3	3.7	9.3	18.0	66.3	13.3	58.3	62.3
Tenericutes	23.3	37.3	0.0	2.0	12.3	77.0	15.3	4.0	573.3	76.3	6.3	20.0
Verrucomicrobia	102.7	302.0	202.3	261.3	18.0	5.3	23.7	73.7	2.7	73.7	22.3	50.3
WS1	163.3	46.3	41.3	34.7	81.7	236.3	314.3	526.3	46.3	145.3	119.7	124.3
WS4	211.3	92.0	73.0	48.7	247.7	104.3	129.7	115.7	127.0	60.3	137.3	135.7
Unassigned	14387.7	7175.0	8878.3	8984.0	12362.3	7194.0	9381.3	12100.3	12856.0	6372.7	8941.0	12374.0

Table S6: Relative abundance (%) of dominant phyla for 16S rRNA transcripts.

Phylum-16S transcripts	Control-w1	NaCl-w1	Seasalt-w1
Acidobacteria	8.66%	8.12%	5.78%
Actinobacteria	3.17%	9.93%	17.23%
Bacteroidetes	1.05%	0.48%	4.95%

Chloroflexi	7.53%	6.42%	11.59%
Christensenellaceae	49.55%	46.70%	39.37%
Christensenellaceae	1.47%	4.19%	5.96%
Proteobacteria	28.58%	24.17%	15.12%

Phylum-16S transcripts	**Control-w2**	**NaCl-w2**	**Seasalt-w2**
Acidobacteria	11.75%	10.48%	11.52%
Actinobacteria	2.44%	5.23%	4.97%
Bacteroidetes	1.95%	4.84%	1.76%
Chloroflexi	6.48%	8.04%	9.25%
Christensenellaceae	35.82%	48.89%	44.26%
Christensenellaceae	2.15%	1.52%	1.76%
Proteobacteria	39.39%	21.00%	26.49%

Phylum-16S transcripts	**Control-w4**	**NaCl-w4**	**Seasalt-w4**
Acidobacteria	13.66%	13.24%	4.54%
Actinobacteria	2.69%	4.87%	18.59%
Bacteroidetes	0.23%	2.93%	1.04%
Chloroflexi	13.53%	22.59%	21.15%
Christensenellaceae	47.85%	42.35%	29.85%
Christensenellaceae	3.13%	4.91%	2.24%
Proteobacteria	18.91%	9.11%	22.59%

Phylum-16S transcripts	**Control-w6**	**NaCl-w6**	**Seasalt-w6**
Acidobacteria	25.66%	12.39%	15.37%
Actinobacteria	3.56%	10.36%	16.88%
Bacteroidetes	2.97%	1.53%	4.85%
Chloroflexi	10.63%	21.97%	18.92%
Christensenellaceae	24.27%	30.65%	18.52%
Christensenellaceae	3.97%	7.09%	4.63%
Proteobacteria	28.94%	16.00%	20.81%

Table S7: Taxonomy table of bacterial 16S rRNA transcripts collapsed at family level achieved paired-end reads from HiSeq platform using QIIME2.

Family	control-w1	control-w2	control-w4	control-w6	NaCl-w1	NaCl-w2	NaCl-w4	NaCl-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Nitrososphaeraceae	22.67	40.67	33.00	71.33	80.33	70.67	39.67	105.67	178.00	36.67	31.00	59.33
Koribacteraceae	9.00	0.00	0.00	54.00	0.00	9.00	10.00	5.67	34.00	9.00	0.00	4.67
Solibacteraceae (Subgroup 3)	1712.00	1374.00	521.00	2233.67	1019.67	1776.00	871.33	1692.33	912.33	1786.67	261.00	821.33
uncultured bacterium	1995.67	1590.33	1963.33	2999.33	2489.00	1680.33	2321.67	3950.67	3514.67	1200.00	1941.00	2980.00
uncultured Acidobacteria **bacterium**	115.33	39.00	300.00	107.00	67.00	26.33	90.33	9.00	48.67	72.00	100.67	95.00

Thermoanaerobaculaceae	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.00	2.00
Iamiaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.33	0.00	0.00	4.33
Ilumatobacteraceae	2.00	2.33	0.00	0.00	1.33	8.00	1.33	3.33	26.67	4.67	6.67	2.00
Microtrichaceae	0.00	0.00	0.00	1.33	2.67	0.00	0.00	0.00	1.33	0.00	0.00	0.00
Corynebacteriaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.33	0.00	0.00	6.67
Cellulomonadaceae	0.00	0.00	0.00	6.67	11.00	53.33	248.33	802.33	135.67	140.00	914.67	1680.33
Intrasporangiaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00
Micromonosporaceae	39.00	69.67	50.33	80.00	114.00	165.67	32.00	87.67	159.00	105.00	111.00	57.67
Nocardioidaceae	14.00	4.67	2.00	2.33	18.67	30.67	6.33	27.00	64.33	2.33	6.67	14.00
Propionibacteriaceae	14.00	7.33	1.67	0.00	9.67	18.00	0.00	18.33	46.67	11.33	13.00	15.00
Streptomycetaceae	0.00	0.00	0.00	0.00	8.33	15.33	0.00	3.33	11.33	0.00	0.00	0.00
Streptosporangiaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00
Coriobacteriaceae bacterium EMTCatB1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00
uncultured Coriobacteriales bacterium	0.00	0.00	0.00	4.33	0.00	2.67	0.00	5.67	15.67	0.00	3.67	11.00
Actinobacteria bacterium RBG_19FT_COMBO_70_19	2.00	8.33	5.33	0.00	7.00	0.00	0.00	0.00	50.00	0.00	4.00	22.67
Euzebyaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	0.00	0.00	0.00
Rubrobacteriaceae	1.33	0.00	0.00	0.00	1.00	0.00	0.00	0.00	3.33	0.00	0.00	1.67
Gaiellaceae	0.00	0.00	1.00	3.33	7.67	4.33	0.00	0.00	15.00	0.00	2.33	10.00
67-14	0.00	2.33	0.00	2.33	7.67	0.00	1.00	2.33	67.00	0.00	2.00	9.33
Solirubrobacteraceae	27.00	13.67	6.67	20.00	38.33	51.67	0.00	31.67	133.67	18.33	25.67	26.67
uncultured Armatimonadetes bacterium	0.00	0.00	0.00	0.00	0.00	0.00	6.67	0.00	0.00	0.00	0.00	0.00
Bacteroidetes vadinHA17	23.33	65.67	12.00	101.67	18.33	153.33	129.67	43.67	47.67	80.33	8.00	219.00
Paludibacteraceae	38.00	82.67	7.00	33.00	27.67	199.00	86.00	77.00	538.67	137.67	48.67	62.33
Prolixibacteraceae	219.33	153.33	12.67	60.00	26.33	418.67	28.67	39.67	47.67	57.67	17.00	58.00
SB-5	0.00	5.33	6.00	111.00	0.00	1.00	19.33	72.67	0.00	0.00	10.33	207.67
Chitinophagaceae	16.00	29.00	0.00	82.00	12.67	148.33	98.33	21.33	440.33	31.00	23.33	87.00
Hymenobacteraceae	0.00	0.00	0.00	2.33	0.00	3.00	0.00	0.00	1.33	1.33	0.00	2.33
Weeksellaceae	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lentimicrobiaceae	1.67	23.00	1.33	94.67	0.00	21.00	38.00	43.67	28.00	8.33	27.33	169.00
BSV40	0.00	6.00	0.00	47.67	1.67	57.33	56.33	12.67	0.00	0.00	6.67	21.00
Ignavibacteriaceae	0.00	0.00	0.00	0.00	0.00	0.00	12.67	8.00	0.00	0.00	0.00	16.00
UA-50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.67	0.00	0.00	2.00
uncultured bacterium SJA-68	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Anaerolineaceae	1851.33	616.00	1907.33	1186.00	925.00	1237.67	2970.33	3102.33	1336.33	1346.67	2157.00	2196.00
Caldilineaceae	1.33	0.00	0.00	0.00	2.00	0.00	1.33	0.00	6.33	0.00	0.00	0.00
uncultured Chloroflexi bacterium	82.33	64.33	62.00	97.00	40.33	93.33	87.33	208.33	56.33	72.00	137.67	211.33
uncultured soil bacterium	10.67	2.67	15.00	12.67	17.00	18.33	2.00	12.67	12.67	2.67	7.33	21.67
A4b	3.67	0.67	0.00	2.67	4.00	4.33	3.33	2.67	7.00	4.00	15.00	24.33
Chloroflexaceae	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	25.67	0.00	0.00	3.67
Herpetosiphonaceae	0.00	8.67	0.00	1.00	0.00	0.00	0.00	0.00	2.00	0.67	0.00	0.00
Roseiflexaceae	95.33	355.33	109.00	96.00	90.33	167.00	22.00	31.00	812.33	155.33	78.00	307.00
AKIW781	0.00	3.33	2.00	0.00	0.00	0.00	0.00	0.00	11.33	0.00	0.00	0.00

AKYG1722	0.00	1.33	0.00	0.00	0.00	0.00	0.00	2.67	12.00	0.67	1.67	0.00
JG30-KF-CM45	5.00	36.33	18.00	24.67	10.67	17.33	10.33	4.67	138.33	3.33	15.00	36.33
metagenome	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00
Leptolyngbyaceae	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	8.33
Nostocaceae	0.67	0.00	0.00	0.00	7.00	17.33	22.67	2.00	90.00	0.00	0.00	13.67
Deinococcaceae	4.67	0.00	0.00	0.00	0.00	8.33	0.00	13.00	4.67	1.00	0.00	1.33
Endomicrobiaceae	0.00	0.00	0.00	21.33	0.00	0.00	1.67	0.00	0.00	0.00	0.00	0.00
Entotheonellaceae	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
uncultured Syntrophaceae bacterium	0.00	0.00	3.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
possible family 01	2.67	4.00	0.00	89.00	84.00	849.67	714.00	171.67	32.33	2.00	14.67	101.67
Alicyclobacillaceae	16.00	9.33	0.00	1.67	10.00	0.00	0.00	0.00	2.67	8.00	1.00	5.00
Bacillaceae	465.33	340.00	13.67	56.67	407.00	541.33	69.33	93.00	730.33	276.00	61.00	73.00
Paenibacillaceae	92.33	40.67	1.00	65.00	93.00	193.00	7.67	148.33	35.67	25.33	83.00	2.00
Planococcaceae	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00
Thermoactinomycetaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.33	0.00	26.33	1.00
Caldicoprobacteraceae	0.00	0.00	0.00	0.00	0.00	17.67	0.00	0.00	16.00	0.00	0.00	0.00
Christensenellaceae	22.00	105.67	5.67	174.00	8.00	411.67	103.67	32.00	235.33	136.33	3.33	33.00
Clostridiaceae 1	160.67	183.00	4.00	385.00	598.33	1832.33	308.33	158.00	1245.00	203.00	309.67	317.33
Clostridiaceae 3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	4.00	26.67
Clostridiaceae 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.33	0.00	0.00	0.00
Clostridiales vadinBB60 group	0.00	9.33	0.00	24.67	0.00	25.67	7.33	4.67	20.00	9.33	0.00	8.67
Defluviitaleaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67
Family XI	0.00	0.00	0.00	5.67	18.00	60.67	21.33	8.00	296.33	14.67	0.00	17.33
Family XIII	11.33	16.33	0.00	36.67	25.67	36.67	0.00	2.67	38.00	6.33	1.67	10.33
Family XVIII	5647.00	436.67	616.67	245.67	3390.00	316.67	225.00	26.33	832.67	214.00	32.33	63.33
Gracilibacteraceae	81.67	103.00	0.00	98.33	84.00	975.00	202.00	103.33	329.33	80.33	31.33	16.33
Heliobacteriaceae	5962.67	4392.67	7271.67	2220.67	1311.00	1781.33	4317.00	4671.33	602.67	5165.00	2218.67	966.33
Lachnospiraceae	661.33	450.67	38.00	299.00	873.00	1184.00	230.00	94.00	2164.33	714.00	110.67	166.33
Peptococcaceae	142.00	165.67	19.67	134.33	99.67	334.00	272.33	96.67	237.00	420.00	395.33	339.00
Peptostreptococcaceae	7.67	1.67	0.00	0.00	0.00	4.67	0.00	0.00	2.33	2.67	4.00	11.00
Ruminococcaceae	168.67	200.00	7.67	146.00	195.67	1429.00	272.33	75.33	1046.00	179.33	35.00	120.00
Syntrophomonadaceae	0.00	2.33	0.00	5.67	0.00	61.33	17.00	7.33	11.33	1.67	3.33	2.33
uncultured Clostridiales bacterium	2.67	1.33	1.67	3.33	1.00	12.33	3.67	0.00	0.00	4.00	11.67	0.00
uncultured Christensenellaceae bacterium	16.33	6.33	52.00	42.67	12.33	11.33	16.67	0.00	8.33	5.67	0.00	12.33
uncultured Bacilli bacterium	92.00	51.67	65.33	69.67	224.67	30.00	21.67	24.33	29.00	19.33	49.67	39.67
SRB2	34.67	25.67	0.00	25.00	69.33	375.00	114.00	146.67	55.00	170.33	154.00	230.00
Limnochordaceae	197.33	64.33	71.00	60.33	596.67	181.33	338.67	365.33	194.33	158.67	432.67	536.33
Veillonellaceae	58.33	31.33	19.33	11.67	8.67	114.00	77.67	37.00	290.33	43.67	13.00	21.33
Gemmatimonadaceae	11.00	14.67	6.33	4.67	20.00	16.00	2.00	24.33	135.33	17.33	19.00	37.00
Longimicrobiaceae	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
uncultured Gemmatimonadetes bacterium	1.67	0.00	1.33	5.67	7.00	4.00	1.33	2.00	19.33	4.67	13.00	17.33

Halobacteroidaceae	0.00	0.00	0.00	0.00	6.67	12.00	0.00	0.00	28.67	48.00	28.33	79.67
Hydrogenedensaceae	6.00	12.00	16.33	46.33	6.33	12.67	23.33	135.67	3.33	0.00	26.33	31.67
Latescibacteraceae	19.33	17.33	1.00	41.33	11.33	14.67	8.33	28.00	0.00	8.33	0.00	6.00
uncultured Latescibacteria bacterium	0.00	0.00	0.67	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.33
uncultured microorganism	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
4572-13	0.00	4.00	1.67	13.00	0.00	2.00	17.00	20.33	0.00	0.00	0.00	56.67
SG8-4	11.67	118.67	157.33	482.00	8.00	142.67	369.33	1097.33	5.33	85.33	27.67	332.33
AKAU3564 sediment group	0.00	0.00	9.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.67
CPla-3 termite group	7.00	19.33	28.67	25.67	0.00	2.00	0.00	0.00	0.00	17.67	0.00	2.00
WD2101 soil group	368.00	101.00	106.67	53.33	572.00	73.33	100.67	78.67	330.67	170.67	154.33	85.00
Gemmataceae	3.33	89.33	63.00	14.33	94.00	3.00	27.33	11.67	687.00	4.33	64.67	103.67
Isosphaeraceae	1.67	55.67	13.00	0.00	43.00	2.67	11.67	10.33	195.00	4.67	28.33	48.33
Pirellulaceae	4.00	4.33	33.00	7.33	13.00	4.33	33.33	0.00	76.67	3.67	2.67	9.33
Azospirillaceae	2.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00
Caulobacteraceae	3.33	1.33	0.00	3.00	2.00	3.00	0.00	1.67	3.00	0.00	1.33	7.67
Hyphomonadaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00
Dongiaceae	1.00	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	1.33
Reyranellaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.00	0.00	0.00	0.00
Beijerinckiaceae	7.00	7.67	2.00	20.00	9.00	24.00	3.00	4.33	22.33	10.67	3.00	21.00
Hyphomicrobiaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	14.67	0.00	0.00	0.00
Methyloligellaceae	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	11.33	0.00	0.00	0.00
Rhizobiales Incertae Sedis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Rhodomicrobiaceae	0.00	0.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Xanthobacteraceae	1.67	2.33	4.67	3.33	0.00	2.00	0.67	0.00	5.33	0.00	1.00	10.00
Rhodobacteraceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.67
Magnetospirillaceae	11.33	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhodopirillaceae	18.00	7.67	9.00	24.33	11.67	28.00	3.00	14.33	61.33	15.67	17.00	20.67
Mitochondria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20.67
Sphingomonadaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	3.00
Geminicoccaceae	1.00	0.00	1.00	0.00	0.00	5.33	0.00	0.00	23.67	0.00	1.33	14.00
Bdellovibrionaceae	0.67	0.00	0.00	1.33	1.33	0.00	0.00	0.00	1.33	0.00	0.00	0.00
Syntrophorhabdaceae	3.67	44.67	34.33	209.33	0.00	2.67	7.00	110.67	1.00	9.33	17.33	40.00
Desulfarculaceae	12.67	10.33	4.33	36.67	1.67	2.67	0.00	1.67	12.00	6.00	2.33	9.33
Desulfobacteraceae	0.00	0.67	0.00	1.33	0.00	1.33	0.00	0.00	0.00	0.67	0.00	0.00
Desulfovibrionaceae	1.33	1.33	8.67	0.00	20.00	0.00	2.00	8.00	164.67	12.33	26.33	66.00
Desulfuromonadaceae	90.00	25.00	12.67	7.67	18.00	56.33	10.67	30.33	46.00	61.33	56.33	48.33
Geobacteraceae	1256.00	759.33	221.33	1339.00	379.33	2467.67	886.00	1833.00	512.00	789.00	538.67	1616.67
Archangiaceae	4916.00	4596.67	2189.67	1756.00	3202.00	1177.00	400.33	895.67	1633.33	2350.67	1920.67	1026.00
BIrii41	0.00	4.00	36.00	26.00	0.00	4.00	0.00	0.00	11.00	4.00	0.00	2.00
Haliangiaceae	780.33	1217.00	448.33	647.67	114.33	120.00	22.67	33.00	67.00	705.67	24.33	33.00
MSB-4B10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.67	0.00	0.00	0.00
Myxococcaceae	24.67	16.67	0.00	16.00	17.00	24.00	0.00	19.67	54.67	13.67	5.67	0.00
Nannocystaceae	5.00	1.33	4.67	6.00	5.00	2.33	0.67	0.00	21.33	0.00	7.33	6.00

P3OB-42	290.33	233.00	172.00	420.33	259.00	85.33	47.00	74.00	135.33	176.67	123.33	104.33
PS-B29	0.00	0.00	0.00	14.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phaselicystidaceae	155.33	114.00	0.00	81.33	14.00	22.33	8.00	0.00	0.00	54.67	0.00	12.00
Polyangiaceae	13.67	8.00	6.33	5.33	12.33	55.67	18.00	13.33	49.33	0.00	27.33	43.67
Sandaracinaceae	1.67	3.33	0.00	14.33	1.00	8.00	0.00	4.00	10.67	0.00	1.00	4.00
UASB-TL25	0.00	0.00	0.00	2.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VHS-B3-70	0.00	6.67	0.00	6.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Vulgatibacteraceae	4.67	4.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
bacteriap25	0.00	0.00	0.67	0.00	1.00	0.00	0.00	1.33	3.33	0.00	3.33	13.00
mle1-27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.67	0.00	0.00	0.00
0319-6G20	0.00	6.67	0.00	1.00	0.00	0.00	0.00	0.00	1.33	0.00	0.00	0.00
Syntrophaceae	0.00	5.33	0.00	102.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
Syntrophobacteraceae	39.67	8.33	6.00	24.67	2.00	19.67	5.33	10.67	70.33	374.67	167.00	199.67
Burkholderiaceae	11.00	6.67	0.00	6.33	0.00	26.67	4.67	2.33	45.00	4.67	0.00	16.00
Nitrosomonadaceae	2.33	3.67	0.00	2.33	2.67	10.33	1.00	2.67	1.67	0.00	0.00	0.00
Rhodocyclaceae	1.33	0.00	0.00	8.67	0.00	7.33	0.00	1.33	11.00	0.00	0.00	11.00
Enterobacteriaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	31.33	0.00	0.00	0.00
Unknown Family	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67
Halomonadaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.67	0.00	0.00	0.00
Moraxellaceae	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonadaceae	0.00	0.00	0.00	8.00	0.00	9.33	0.00	0.00	4.33	0.00	0.00	2.00
Steroidobacteraceae	5.00	5.00	0.00	6.33	1.67	6.00	0.67	10.00	19.00	2.67	0.00	7.67
Xanthomonadaceae	2.00	1.33	0.00	0.00	1.00	0.00	0.00	0.00	0.67	0.00	0.00	6.67
Methylomirabilaceae	54.33	64.33	206.67	62.00	27.33	3.67	9.33	18.00	66.33	12.00	56.33	44.67
Haloplasmataceae	23.33	37.33	0.00	2.00	10.67	50.00	15.33	4.00	571.67	75.33	6.33	20.00
Opitutaceae	100.00	296.67	199.33	258.67	18.00	5.33	18.67	73.67	0.00	70.67	22.33	41.33
Pedosphaeraceae	2.67	5.33	3.00	2.67	0.00	0.00	5.00	0.00	2.67	3.00	0.00	9.00
Unassigned	14387.67	7175.00	8878.33	8984.00	12362.33	7194.00	9381.33	12100.33	12856.00	6372.67	8941.00	12374.00

Table S8: Relative abundance (%) of dominant families for 16S rRNA transcripts.

Family-16S transcripts	control-w1	NaCl-w1	Seasalt-w1
Solibacteraceae (Subgroup 3)	7.53%	7.73%	8.83%
uncultured bacterium	8.78%	18.87%	34.03%
Roseiflexaceae	0.42%	0.68%	7.86%
Clostridiaceae 1	0.71%	4.54%	12.05%
Family XVIII	24.85%	25.70%	8.06%
Heliobacteriaceae	26.24%	9.94%	5.83%
Limnochordaceae	0.87%	4.52%	1.88%
Geobacteraceae	5.53%	2.88%	4.96%
Archangiaceae	21.63%	24.27%	15.81%
Haliangiaceae	3.43%	0.87%	0.65%
Pedosphaeraceae	0.01%	0.00%	0.03%

Family-16S transcripts	control-w2	NaCl-w2	Seasalt-w2
Solibacteraceae (Subgroup 3)	9.18%	15.44%	14.03%
uncultured bacterium	10.62%	14.61%	9.43%
Roseiflexaceae	2.37%	1.45%	1.22%
Clostridiaceae 1	1.22%	15.93%	1.59%
Family XVIII	2.92%	2.75%	1.68%
Heliobacteriaceae	29.33%	15.49%	40.57%
Limnochordaceae	0.43%	1.58%	1.25%
Geobacteraceae	5.07%	21.46%	6.20%
Archangiaceae	30.70%	10.24%	18.46%
Haliangiaceae	8.13%	1.04%	5.54%
Pedosphaeraceae	0.04%	0.00%	0.02%

Family-16S transcripts	control-w4	NaCl-w4	Seasalt-w4
Solibacteraceae (Subgroup 3)	3.88%	8.97%	3.36%
uncultured bacterium	14.63%	23.89%	25.02%
Roseiflexaceae	0.81%	0.23%	1.01%
Clostridiaceae 1	0.03%	3.17%	3.99%
Family XVIII	4.60%	2.32%	0.42%
Heliobacteriaceae	54.19%	44.42%	28.60%
Limnochordaceae	0.53%	3.48%	5.58%
Geobacteraceae	1.65%	9.12%	6.94%
Archangiaceae	16.32%	4.12%	24.76%
Haliangiaceae	3.34%	0.23%	0.31%
Pedosphaeraceae	0.02%	0.05%	0.00%

Family-16S transcripts	control-w6	NaCl-w6	Seasalt-w6
Solibacteraceae (Subgroup 3)	18.64%	12.39%	9.47%
uncultured bacterium	25.02%	28.93%	34.35%
Roseiflexaceae	0.80%	0.23%	3.54%
Clostridiaceae 1	3.21%	1.16%	3.66%
Family XVIII	2.05%	0.19%	0.73%
Heliobacteriaceae	18.53%	34.21%	11.14%
Limnochordaceae	0.50%	2.68%	6.18%
Geobacteraceae	11.17%	13.42%	18.63%
Archangiaceae	14.65%	6.56%	11.83%
Haliangiaceae	5.40%	0.24%	0.38%
Pedosphaeraceae	0.02%	0.00%	0.10%

Table S9: Taxonomy table of *dsrB* gene collapsed at phylum level achieved paired-end reads from HiSeq platform using QIIME2.

Phylum	Control-w1	Control-w2	Control-w4	Control-w6	NaCl⁻ w1	NaCl⁻ w2	NaCl⁻ w4	NaCl⁻ w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Acidobacteria	78.00	98.67	145.00	24.33	51.00	47.67	61.00	24.33	145.00	95.33	0.67	5.67
Actinobacteria	3.00	5.67	19.00	0.00	0.00	0.00	14.67	3.33	0.00	4.00	0.00	0.00
Armatimonadetes	0.00	7.00	7.33	0.00	1.33	2.67	1.67	3.33	0.00	6.00	0.00	0.67
Chloroflexi	114.33	110.33	191.33	87.67	63.33	44.67	34.67	60.67	209.33	66.33	7.33	5.00
Christensenellaceae	419.67	1095.33	1005.67	587.00	234.00	875.67	391.00	375.00	3087.33	3550.67	669.00	1960.67
Nitrospirae	297.67	198.33	259.33	136.33	142.67	133.00	106.33	112.00	418.00	119.00	13.00	11.33
Christensenellaceae	598.67	1355.33	975.00	2196.67	66.33	254.33	1043.33	503.67	54.67	38.33	8.00	5.00
Proteobacteria	846.00	1767.67	1578.67	832.33	391.67	730.67	523.67	750.67	1867.67	4730.67	568.67	764.33
candidate division Zixibacteria	0.67	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
others	296.33	852.33	1173.67	854.33	98.33	437.67	267.00	163.33	424.00	720.00	64.00	365.67
Unassigned	3739.00	4973.67	4907.33	2614.00	1433.67	3170.00	2623.00	1945.67	2237.33	6030.00	2467.00	6836.67

Table S10: Relative abundance (%) of dominant phyla for *dsrB* gene.

Phylum-dsrB gene	Control-w1	NaCl-w1	Seasalt-w1
Chloroflexi	5.02%	7.05%	3.71%
Christensenellaceae	18.44%	26.06%	54.77%
Nitrospirae	13.08%	15.89%	7.42%
Christensenellaceae	26.30%	7.39%	0.97%
Proteobacteria	37.17%	43.62%	33.13%

Phylum-dsrB gene	Control-w2	NaCl-w2	Seasalt-w2
Chloroflexi	2.44%	2.19%	0.78%
Christensenellaceae	24.20%	42.96%	41.75%
Nitrospirae	4.38%	6.52%	1.40%
Christensenellaceae	29.94%	12.48%	0.45%
Proteobacteria	39.05%	35.85%	55.62%

Phylum-dsrB gene	Control-w4	NaCl-w4	Seasalt-w4
Chloroflexi	4.77%	1.65%	0.58%
Christensenellaceae	25.08%	18.63%	52.84%
Nitrospirae	6.47%	5.07%	1.03%
Christensenellaceae	24.31%	49.71%	0.63%
Proteobacteria	39.37%	24.95%	44.92%

Phylum-dsrB gene	Control-w6	NaCl-w6	Seasalt-w6
Chloroflexi	2.28%	3.37%	0.18%

Christensenellaceae	15.29%	20.81%	71.39%
Nitrospirae	3.55%	6.22%	0.41%
Christensenellaceae	57.20%	27.95%	0.18%
Proteobacteria	21.68%	41.66%	27.83%

Table S11: Taxonomy table of *dsrB* gene collapsed at family level achieved paired-end reads from HiSeq platform using QIIME2.

Family	Control-w1	Control-w2	Control-w4	Control-w6	NaCl⁻ w1	NaCl⁻ w2	NaCl⁻ w4	NaCl⁻ w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Acidobacteria	78.00	98.67	145.00	24.33	51.00	47.67	61.00	24.33	145.00	95.33	0.67	5.67
Actinobacteria	0.67	0.67	0.67	0.00	0.00	0.00	1.67	2.33	0.00	1.33	0.00	0.00
Nocardiopsaceae	1.33	2.67	14.00	0.00	0.00	0.00	5.67	1.00	0.00	0.00	0.00	0.00
Eggerthellaceae	0.00	0.00	0.00	0.00	0.00	0.00	7.33	0.00	0.00	0.00	0.00	0.00
Gaiellales	1.00	2.33	4.33	0.00	0.00	0.00	0.00	0.00	0.00	2.67	0.00	0.00
Armatimonadetes	0.00	7.00	7.33	0.00	1.33	2.67	1.67	3.33	0.00	6.00	0.00	0.67
Anaerolineaceae	19.67	34.33	67.67	30.00	5.00	4.00	5.00	5.33	27.67	9.00	3.00	0.00
Chloroflexi	86.00	71.67	107.33	57.67	58.33	40.67	28.67	55.33	171.67	51.67	4.33	5.00
Peptococcaceae	308.67	942.67	873.33	528.33	206.00	797.00	336.00	254.33	2694.33	2967.33	535.67	1930.00
Thermoanaerobacteraceae	9.33	20.00	19.67	2.33	0.00	2.33	0.00	0.00	348.33	462.67	66.00	30.67
Sporomusaceae	101.67	132.67	112.67	56.33	28.00	76.33	55.00	120.67	44.67	120.67	65.33	0.00
Nitrospiraceae	0.67	16.00	8.33	6.00	0.00	10.67	2.67	7.33	1.00	20.67	0.00	0.00
Nitrospirae	297.00	181.00	251.00	130.33	142.67	122.33	103.67	104.67	417.00	98.33	13.00	11.33
Christensenellaceae	530.33	1332.00	886.33	2185.00	33.33	249.67	1032.00	501.00	0.00	0.00	0.00	0.00
Planctomycetaceae	68.33	23.33	88.67	11.67	33.00	4.67	11.33	2.67	54.67	38.33	8.00	5.00
DeltaProteobacteria	24.33	16.00	24.00	17.33	23.00	22.33	29.33	20.67	2.33	44.67	22.33	17.33
Desulfarculaceae	0.00	7.33	0.67	0.00	0.00	3.33	0.00	0.67	8.33	56.33	1.00	0.00
Desulfobacteraceae	0.00	19.00	3.33	1.33	0.00	0.00	0.00	0.00	17.00	45.33	33.00	38.67
Desulfobacterales	0.00	2.33	0.00	3.33	0.00	0.00	0.00	0.00	0.00	6.33	1.33	0.00
Desulfobulbaceae	0.67	33.33	8.33	13.67	0.00	16.00	1.67	2.33	0.00	0.00	0.00	0.00
Desulfovibrionaceae	716.67	1443.00	1047.33	640.00	299.33	597.67	396.67	681.33	1437.33	2722.33	348.67	366.33
Anaeromyxobacteraceae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.67	0.00	0.00
Myxococcales	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00
Syntrophaceae	4.33	18.67	34.67	14.00	2.67	7.33	2.67	3.00	103.33	41.00	6.33	4.00
Syntrophobacteraceae	8.00	19.33	19.33	8.33	0.00	6.33	2.67	1.00	81.00	1628.67	152.33	308.33
candidate division Zixibacteria	0.67	1.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
others	397.00	1066.67	1631.00	988.67	165.00	515.33	358.00	205.00	652.33	910.00	69.67	395.33
Unassigned	3739.00	4973.67	4907.33	2614.00	1433.67	3170.00	2623.00	1945.67	2237.33	6030.00	2467.00	6836.67

Table S12: Relative abundance (%) of dominant families for *dsrB* gene.

Family-dsrB gene	Control-w1	NaCl-w1	Seasalt-w1
Acidobacteria	3.87%	6.45%	2.98%
Chloroflexi	4.26%	7.38%	3.53%
Peptococcaceae	15.31%	26.05%	55.38%
Nitrospirae	14.73%	18.04%	8.57%
Christensenellaceae	26.30%	4.22%	0.00%
Desulfovibrionaceae	35.54%	37.86%	29.54%

Family-dsrB gene	Control-w2	NaCl-w2	Seasalt-w2
Acidobacteria	2.42%	2.57%	1.61%
Chloroflexi	1.76%	2.19%	0.87%
Peptococcaceae	23.17%	42.96%	50.00%
Nitrospirae	4.45%	6.59%	1.66%
Christensenellaceae	32.74%	13.46%	0.00%
Desulfovibrionaceae	35.46%	32.22%	45.87%

Family-dsrB gene	Control-w4	NaCl-w4	Seasalt-w4
Acidobacteria	4.38%	3.12%	0.07%
Chloroflexi	3.24%	1.46%	0.48%
Peptococcaceae	26.38%	17.16%	59.36%
Nitrospirae	7.58%	5.29%	1.44%
Christensenellaceae	26.77%	52.71%	0.00%
Desulfovibrionaceae	31.64%	20.26%	38.64%

Family-dsrB gene	Control-w6	NaCl-w6	Seasalt-w6
Acidobacteria	0.68%	1.50%	0.24%
Chloroflexi	1.62%	3.41%	0.22%
Peptococcaceae	14.82%	15.69%	83.25%
Nitrospirae	3.66%	6.46%	0.49%
Christensenellaceae	61.28%	30.91%	0.00%
Desulfovibrionaceae	17.95%	42.03%	15.80%

Table S13: Taxonomy table of dsrB transcripts collapsed at phylum level achieved paired-end reads from HiSeq platform using QIIME2.

Phylum	Control-w1	Control-w2	Control-w4	Control-w6	NaCl-w1	NaCl-w2	NaCl-w4	NaCl-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Acidobacteria	65.0	96.7	116.0	307.0	34.7	82.0	79.0	199.3	111.3	198.3	17.7	89.7
Actinobacteria	5.3	20.7	0.0	1.3	1.3	3.7	11.0	2.7	0.0	13.0	1.0	65.7
Armatimonadetes	0.0	23.3	0.0	0.0	0.0	0.0	0.0	0.0	43.7	7.3	0.0	0.0
Chloroflexi	17.0	250.0	387.0	78.0	99.7	155.0	225.0	257.3	12.7	148.7	96.7	87.7

Christensenellaceae	512.0	1193.0	2011.5	446.0	664.3	597.7	938.0	994.7	289.3	771.0	1495.0	524.7
Nitrospirae	842.0	217.0	206.5	484.3	90.3	163.7	216.0	62.0	185.7	78.3	284.3	217.7
Christensenellaceae	36.0	46.0	67.5	8.7	28.3	301.3	92.7	585.0	37.7	52.0	109.0	4.3
Proteobacteria	1710.0	2684.0	2807.5	2470.3	1705.0	2557.3	3929.3	1476.3	1435.3	1585.7	2290.3	1815.7
candidate division Zixibacteria	0.0	0.0	11.5	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0
Environmental samples	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
others	255.0	501.7	843.0	749.3	237.3	307.0	621.3	348.3	577.0	1070.3	447.0	495.7
Unassigned	1855.0	4469.3	5521.0	5153.3	1919.3	2625.3	4731.7	1821.0	2739.3	5564.0	3651.7	2928.7

Table S14: Relative abundance (%) of dominant phyla for dsrB transcripts.

Phylum-dsrB transcripts	Control-w1	NaCl-w1	Seasalt-w1
Acidobacteria	2.07%	1.34%	5.47%
Chloroflexi	0.54%	3.84%	0.62%
Christensenellaceae	16.27%	25.61%	14.22%
Nitrospirae	26.76%	3.48%	9.13%
Proteobacteria	54.35%	65.73%	70.56%

Phylum-dsrB transcripts	Control-w2	NaCl-w2	Seasalt-w2
Acidobacteria	2.18%	2.31%	7.13%
Chloroflexi	5.63%	4.36%	5.34%
Christensenellaceae	26.87%	16.81%	27.71%
Nitrospirae	4.89%	4.60%	2.82%
Proteobacteria	60.44%	71.92%	57.00%

Phylum-dsrB transcripts	Control-w4	NaCl-w4	Seasalt-w4
Acidobacteria	2.10%	1.47%	0.42%
Chloroflexi	7.00%	4.18%	2.31%
Christensenellaceae	36.38%	17.41%	35.73%
Nitrospirae	3.74%	4.01%	6.80%
Proteobacteria	50.78%	72.94%	54.74%

Phylum-dsrB transcripts	Control-w6	NaCl-w6	Seasalt-w6
Acidobacteria	8.11%	6.67%	3.28%
Chloroflexi	2.06%	8.61%	3.20%
Christensenellaceae	11.78%	33.27%	19.18%
Nitrospirae	12.79%	2.07%	7.96%
Proteobacteria	65.25%	49.38%	66.38%

Table S15: Taxonomy table of dsrB transcripts collapsed at family level achieved paired-end reads from HiSeq platform using QIIME2.

Family	Control-w1	Control-w2	Control-w4	Control-w6	NaCl⁻w1	NaCl⁻w2	NaCl⁻w4	NaCl⁻w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Acidobacteria	60.7	96.7	77.3	307.0	33.3	76.0	79.0	199.3	111.3	195.3	17.7	89.7
Holophagae	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0
Actinobacteria	0.0	0.0	0.0	0.0	0.0	3.7	1.0	0.0	0.0	0.0	1.0	65.7
Nocardiopsaceae	0.0	0.0	0.0	1.3	0.0	0.0	0.0	2.7	0.0	9.7	0.0	0.0
Eggerthellaceae	0.0	0.0	0.0	0.0	1.3	0.0	6.7	0.0	0.0	3.3	0.0	0.0
Gaiellales	5.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Armatimonadetes	0.0	23.3	0.0	0.0	0.0	0.0	0.0	0.0	43.7	7.3	0.0	0.0
Anaerolineaceae	0.0	47.3	0.0	8.0	10.7	15.7	23.7	191.3	0.0	39.3	13.0	0.0
Chloroflexi	17.0	160.3	229.3	70.0	85.7	126.3	194.0	66.0	0.0	106.0	56.3	74.0
Peptococcaceae	363.3	1087.0	1079.7	390.3	546.7	493.7	708.3	935.0	252.3	741.0	1372.3	474.0
Thermoanaerobacteraceae	135.3	68.0	239.7	36.0	80.7	74.0	106.3	50.3	0.0	6.0	56.7	16.0
Sporomusaceae	13.3	38.0	21.7	19.7	14.0	30.0	123.3	9.3	37.0	24.0	66.0	34.7
Nitrospiraceae	0.0	0.0	0.0	0.0	0.0	1.3	0.0	1.0	0.0	0.0	1.3	0.0
Nitrospirae	842.0	217.0	137.7	484.3	90.3	157.3	216.0	61.0	185.7	78.3	283.0	217.7
Christensenellaceae	0.0	30.7	20.3	0.0	0.0	165.3	2.3	0.0	0.0	0.0	83.0	0.0
Planctomycetaceae	36.0	15.3	24.7	8.7	28.3	136.0	90.3	585.0	37.7	52.0	26.0	4.3
DeltaProteobacteria	52.3	90.0	73.7	46.7	11.7	69.3	101.3	0.0	170.7	21.0	0.0	123.3
Desulfarculaceae	1.3	4.3	23.3	1.3	1.0	31.0	8.3	0.0	0.0	6.0	0.0	0.0
Desulfobacteraceae	9.3	46.7	1.7	0.7	1.3	8.0	4.0	226.3	0.0	93.0	0.0	0.0
Desulfobacterales	15.0	0.0	0.0	1.3	1.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
Desulfobulbaceae	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	17.7	0.0	0.0
Desulfovibrionaceae	1398.0	2251.0	1422.0	2036.7	1552.7	1966.3	3373.0	605.7	1170.0	1157.0	1915.7	1407.3
Anaeromyxobacteraceae	8.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	62.7
Myxococcales	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	2.7	0.0	0.0
Syntrophaceae	57.0	145.3	99.0	63.7	44.0	106.3	151.7	211.0	73.7	2.3	67.0	46.3
Syntrophobacteraceae	78.0	10.3	70.0	1.7	2.0	11.3	85.0	0.0	0.0	133.3	119.0	97.0
Syntrophobacterales	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.7	0.0
candidate division Zixibacteria	0.0	0.0	7.7	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0
Environmental samples	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
others	345.3	701.0	772.3	1067.7	355.0	689.3	838.0	781.7	610.7	1226.3	661.3	588.3
Unassigned	1855.0	4469.3	3680.7	5153.3	1919.3	2625.3	4731.7	1821.0	2739.3	5564.0	3651.7	2928.7

Table S16: Relative abundance (%) of dominant families for dsrB transcripts.

Family-dsrB transcripts	Control-w1	NaCl-w1	Seasalt-w1
Peptococcaceae	13.09%	23.78%	15.33%
Thermoanaerobacteraceae	4.88%	3.51%	0.00%
Nitrospirae	30.35%	3.93%	11.28%
Planctomycetaceae	1.30%	1.23%	2.29%

Desulfovibrionaceae	50.38%	67.55%	71.10%

Family-dsrB transcripts	Control-w2	NaCl-w2	Seasalt-w2
Peptococcaceae	29.88%	17.46%	36.42%
Thermoanaerobacteraceae	1.87%	2.62%	0.29%
Nitrospirae	5.96%	5.56%	3.85%
Planctomycetaceae	0.42%	4.81%	2.56%
Desulfovibrionaceae	61.87%	69.55%	56.87%

Family-dsrB transcripts	Control-w4	NaCl-w4	Seasalt-w4
Peptococcaceae	37.18%	15.76%	37.56%
Thermoanaerobacteraceae	8.25%	2.37%	1.55%
Nitrospirae	4.74%	4.81%	7.75%
Planctomycetaceae	0.85%	2.01%	0.71%
Desulfovibrionaceae	48.97%	75.06%	52.43%

Family-dsrB transcripts	Control-w6	NaCl-w6	Seasalt-w6
Peptococcaceae	13.20%	41.80%	22.37%
Thermoanaerobacteraceae	1.22%	2.25%	0.75%
Nitrospirae	16.38%	2.73%	10.27%
Planctomycetaceae	0.29%	26.15%	0.20%
Desulfovibrionaceae	68.90%	27.07%	66.40%

Table S17: Taxonomy table of *mcrA* gene collapsed at order level achieved paired-end reads from MiSeq platform using QIIME2.

Order	Control-w1	Control-w2	Control-w4	Control-w6	NaCl-w1	NaCl-w2	NaCl-w4	NaCl-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Methanomassiliicoccales	722	852	660	461	608	636	875	677	686	440	1101	608
Methanobacteriales	332	192	242	242	848	746	1655	1240	967	612	460	517
Methanocellales	2801	2691	3050	1239	1834	1751	2311	1111	1109	814	2296	1266
Methanosarcinales	3672	2126	1730	1607	4790	3744	3023	2055	6069	4245	4206	2221
Unassigned	881	995	1322	1013	717	642	951	987	645	536	870	957

Table S18: Relative abundance (%) of dominant orders for *mcrA* gene.

Order-mcrA gene	Control-w1	NaCl-w1	Seasalt-w1
Methanomassiliicoccales	9.59%	7.52%	7.77%
Methanobacteriales	4.41%	10.50%	10.95%
Methanocellales	37.21%	22.70%	12.56%
Methanosarcinales	48.78%	59.28%	68.72%

Order-mcrA gene	Control-w2	NaCl-w2	Seasalt-w2
Methanomassiliicoccales	14.54%	9.25%	7.20%
Methanobacteriales	3.28%	10.85%	10.01%
Methanocellales	45.91%	25.46%	13.32%
Methanosarcinales	36.27%	54.44%	69.46%

Order-mcrA gene	Control-w4	NaCl-w4	Seasalt-w4
Methanomassiliicoccales	11.62%	11.13%	13.65%
Methanobacteriales	4.26%	21.05%	5.71%
Methanocellales	53.68%	29.39%	28.48%
Methanosarcinales	30.45%	38.44%	52.16%

Order-mcrA gene	Control-w6	NaCl-w6	Seasalt-w6
Methanomassiliicoccales	12.99%	13.32%	13.18%
Methanobacteriales	6.82%	24.40%	11.21%
Methanocellales	34.91%	21.86%	27.45%
Methanosarcinales	45.28%	40.43%	48.16%

Table S19: Taxonomy table of *mcrA* gene collapsed at family level achieved paired-end reads from MiSeq platform using QIIME2.

Family	Control-w1	Control-w2	Control-w4	Control-w6	NaCl-w1	NaCl-w2	NaCl-w4	NaCl-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Methanomassiliicoccales	722	852	660	461	608	636	875	677	686	440	1101	608
andidatus Methanoperedenaceae	0	0	0	0	0	12	26	0	53	0	21	0
Methanobacteriaceae	332	192	242	242	848	746	1655	1240	967	612	460	517
Methanocellaceae	2801	2691	3050	1239	1834	1751	2311	1111	1109	814	2296	1266
Methanosarcinaceae	3522	2116	1626	1470	3993	3493	2680	1910	5359	4024	3968	2069
Methanotrichaceae	150	10	104	137	797	239	317	145	657	221	217	152
Unassigned	881	995	1322	1013	717	642	951	987	645	536	870	957

Table S20: Relative abundance (%) of dominant families for *mcrA* gene.

Family-mcrA gene	Control-w1	NaCl-w1	Seasalt-w1
Methanomassiliicoccales	9.59%	7.52%	7.81%
Methanobacteriaceae	4.41%	10.50%	11.02%
Methanocellaceae	37.21%	22.70%	12.63%
Methanosarcinaceae	46.79%	49.42%	61.05%
Methanotrichaceae	1.99%	9.86%	7.48%

Family-mcrA gene	Control-w2	NaCl-w2	Seasalt-w2
Methanomassiliicoccales	14.54%	9.26%	7.20%
Methanobacteriaceae	3.28%	10.87%	10.01%
Methanocellaceae	45.91%	25.51%	13.32%
Methanosarcinaceae	36.10%	50.88%	65.85%
Methanotrichaceae	0.17%	3.48%	3.62%

Family-mcrA gene	Control-w4	NaCl-w4	Seasalt-w4
Methanomassiliicoccales	11.62%	11.16%	13.69%
Methanobacteriaceae	4.26%	21.12%	5.72%
Methanocellaceae	53.68%	29.48%	28.55%
Methanosarcinaceae	28.62%	34.19%	49.34%
Methanotrichaceae	1.83%	4.04%	2.70%

Family-mcrA gene	Control-w6	NaCl-w6	Seasalt-w6
Methanomassiliicoccales	12.99%	13.32%	13.18%
Methanobacteriaceae	6.82%	24.40%	11.21%
Methanocellaceae	34.91%	21.86%	27.45%
Methanosarcinaceae	41.42%	37.58%	44.86%
Methanotrichaceae	3.86%	2.85%	3.30%

Table S21: Taxonomy table of mcrA transcripts collapsed at order level achieved paired-end reads from MiSeq platform using QIIME2.

Order	Control-w1	Control-w2	Control-w4	Control-w6	NaCl-w1	NaCl-w2	NaCl-w4	NaCl-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Methanomassiliicoccales	582	394	244	200	1154	524	726	463	1188	338	776	313
Methanobacteriales	0	0	26	16	18	265	229	20	99	0	33	65
Methanocellales	4228	3129	2743	1556	2264	444	1753	897	1631	207	1339	569
Methanosarcinales	642	0	124	113	1328	1501	1488	468	606	272	896	620
Unassigned	846	1080	1461	1046	1313	415	968	1005	1121	558	1318	467

Table S22: Relative abundance (%) of dominant orders for mcrA transcripts.

Order-mcrA transcripts	Control-w1	NaCl-w1	Seasalt-w1
Methanomassiliicoccales	10.67%	24.22%	33.71%
Methanobacteriales	0.00%	0.38%	2.81%
Methanocellales	77.55%	47.52%	46.28%
Methanosarcinales	11.78%	27.88%	17.20%

Order-mcrA transcripts	Control-w2	NaCl-w2	Seasalt-w2
Methanomassiliicoccales	11.18%	19.17%	41.37%

	0.00%	9.69%	0.00%
Methanocellales	88.82%	16.24%	25.34%
Methanosarcinales	0.00%	54.90%	33.29%

Order-mcrA transcripts	**Control-w4**	**NaCl-w4**	**Seasalt-w4**
Methanomassiliicoccales	7.78%	17.30%	25.49%
Methanobacteriales	0.83%	5.46%	1.08%
Methanocellales	87.44%	41.78%	43.99%
Methanosarcinales	3.95%	35.46%	29.43%

Order-mcrA transcripts	**Control-w6**	**NaCl-w6**	**Seasalt-w6**
Methanomassiliicoccales	10.61%	25.05%	19.97%
Methanobacteriales	0.85%	1.08%	4.15%
Methanocellales	82.55%	48.54%	36.31%
Methanosarcinales	5.99%	25.32%	39.57%

Table S23: Taxonomy table of mcrA transcripts collapsed at family level achieved paired-end reads from MiSeq platform using QIIME2.

Family	Control-w1	Control-w2	Control-w4	Control-w6	NaCl-w1	NaCl-w2	NaCl-w4	NaCl-w6	Seasalt-w1	Seasalt-w2	Seasalt-w4	Seasalt-w6
Methanomassiliicoccales	582	394	244	200	1154	524	726	463	1188	338	776	313
Methanobacteriaceae	0	0	26	16	18	265	229	20	99	0	33	65
Methanocellaceae	4228	3129	2743	1556	2264	444	1753	897	1631	207	1339	569
Methanosarcinaceae	642	0	27	42	1328	1501	1222	292	592	244	865	583
Methanotrichaceae	0	0	97	71	0	0	266	176	14	28	31	37
Unassigned	846	1080	1461	1046	1313	415	968	1005	1121	558	1318	467

Table S24: Relative abundance (%) of dominant families for mcrA transcripts.

Family-mcrA transcripts	**Control-w1**	**NaCl-w1**	**Seasalt-w1**
Methanomassiliicoccales	10.67%	24.22%	33.71%
Methanobacteriaceae	0.00%	0.38%	2.81%
Methanocellaceae	77.55%	47.52%	46.28%
Methanosarcinaceae	11.78%	27.88%	16.80%
Methanotrichaceae	0.00%	0.00%	0.40%

Family-mcrA transcripts	**Control-w2**	**NaCl-w2**	**Seasalt-w2**
Methanomassiliicoccales	11.18%	19.17%	41.37%
Methanobacteriaceae	0.00%	9.69%	0.00%
Methanocellaceae	88.82%	16.24%	25.34%
Methanosarcinaceae	0.00%	54.90%	29.87%

Methanotrichaceae	0.00%	0.00%	3.43%

Family-mcrA transcripts	Control-w4	NaCl-w4	Seasalt-w4
Methanomassiliicoccales	7.78%	17.30%	25.49%
Methanobacteriaceae	0.83%	5.46%	1.08%
Methanocellaceae	87.44%	41.78%	43.99%
Methanosarcinaceae	0.86%	29.12%	28.42%
Methanotrichaceae	3.09%	6.34%	1.02%

Family-mcrA transcripts	Control-w6	NaCl-w6	Seasalt-w6
Methanomassiliicoccales	10.61%	25.05%	19.97%
Methanobacteriaceae	0.85%	1.08%	4.15%
Methanocellaceae	82.55%	48.54%	36.31%
Methanosarcinaceae	2.23%	15.80%	37.20%
Methanotrichaceae	3.77%	9.52%	2.36%

Table S25: 12 16S rRNA primer set with stagger and barcode sequences.

#	Stagger	8 mer Barcode	Stagger+Barcode	Primer Forward of 16SrRNA: 515F	Combined Seq.
F1	CTT	CACGTGTT	CTTCACGTGTT	GTGCCAGCMGCCGCGGTAA	CTTCACGTGTTGTGCCAGCMGCCGCGGTAA
F2	CTA	GTGGGATA	CTAGTGGGATA	GTGCCAGCMGCCGCGGTAA	CTAGTGGGATAGTGCCAGCMGCCGCGGTAA
F3	ATT	ATCGCCAG	ATTATCGCCAG	GTGCCAGCMGCCGCGGTAA	ATTATCGCCAGGTGCCAGCMGCCGCGGTAA
F4	GGC	CGAACTGT	GGCCGAACTGT	GTGCCAGCMGCCGCGGTAA	GGCCGAACTGTGTGCCAGCMGCCGCGGTAA
F5	GAA	AACACCTA	GAAAACACCTA	GTGCCAGCMGCCGCGGTAA	GAAAACACCTAGTGCCAGCMGCCGCGGTAA
F6	AAA	TGAGAGTG	AAATGAGAGTG	GTGCCAGCMGCCGCGGTAA	AAATGAGAGTGGTGCCAGCMGCCGCGGTAA
F7	TAT	AGTAGTGG	TATAGTAGTGG	GTGCCAGCMGCCGCGGTAA	TATAGTAGTGGGTGCCAGCMGCCGCGGTAA
F8	CGC	CTCTGTCT	CGCCTCTGTCT	GTGCCAGCMGCCGCGGTAA	CGCCTCTGTCTGTGCCAGCMGCCGCGGTAA
F9	CAT	ACTAACTG	CATACTAACTG	GTGCCAGCMGCCGCGGTAA	CATACTAACTGGTGCCAGCMGCCGCGGTAA
F10	TCA	CGCATTAA	TCACGCATTAA	GTGCCAGCMGCCGCGGTAA	TCACGCATTAAGTGCCAGCMGCCGCGGTAA
F11	ACA	GGATATGG	ACAGGATATGG	GTGCCAGCMGCCGCGGTAA	ACAGGATATGGGTGCCAGCMGCCGCGGTAA
F12	GAAA	ATCCTATT	GAAAATCCTATT	GTGCCAGCMGCCGCGGTAA	GAAAATCCTATTGTGCCAGCMGCCGCGGTAA

#	Stagger	8 mer Barcode	Stagger+Barcode	Primer Reverse of 16SrRNA: 806R	Combined Seq.
R1	GGG	GCAAGTAG	GGGGCAAGTAG	GGACTACHVGGGTWTCTAAT	GGGGCAAGTAGGGACTACHVGGGTWTCTAAT
R2	ATA	CGGTTGAT	ATACGGTTGAT	GGACTACHVGGGTWTCTAAT	ATACGGTTGATGGACTACHVGGGTWTCTAAT
R3	TTT	GCGGCGAA	TTTGCGGCGAA	GGACTACHVGGGTWTCTAAT	TTTGCGGCGAAGGACTACHVGGGTWTCTAAT
R4	TCC	AGTCAACA	TCCAGTCAACA	GGACTACHVGGGTWTCTAAT	TCCAGTCAACAGGACTACHVGGGTWTCTAAT
R5	TGC	AGTTCCGT	TGCAGTTCCGT	GGACTACHVGGGTWTCTAAT	TGCAGTTCCGTGGACTACHVGGGTWTCTAAT
R6	GCA	ATGTCAGA	GCAATGTCAGA	GGACTACHVGGGTWTCTAAT	GCAATGTCAGAGGACTACHVGGGTWTCTAAT
R7	GAT	ATATAGGA	GATATATAGGA	GGACTACHVGGGTWTCTAAT	GATATATAGGAGGACTACHVGGGTWTCTAAT
R8	TTA	GTGAAACG	TTAGTGAAACG	GGACTACHVGGGTWTCTAAT	TTAGTGAAACGGGACTACHVGGGTWTCTAAT
R9	GCC	GTGGCCTT	GCCGTGGCCTT	GGACTACHVGGGTWTCTAAT	GCCGTGGCCTTGGACTACHVGGGTWTCTAAT
R10	TAA	GAGTGGAT	TAAGAGTGGAT	GGACTACHVGGGTWTCTAAT	TAAGAGTGGATGGACTACHVGGGTWTCTAAT
R11	CGG	ACTGATAT	CGGACTGATAT	GGACTACHVGGGTWTCTAAT	CGGACTGATATGGACTACHVGGGTWTCTAAT
R12	ATTAA	ACCGTTAT	ATTAAACCGTTAT	GGACTACHVGGGTWTCTAAT	ATTAAACCGTTATGGACTACHVGGGTWTCTAAT

Table S26: 12 mcrA primer sets with stagger and barcode sequences. sequences.

#	mcrAsequencing	Primer	Primer Name	Stagger+Barcode	mcrA Primer Seq.	Combined Seq.
1	mcrAsequencing	F	Mlas-mod	GGGCTAACACCTIA	GGYGGTGTMGGDTTCACMCARTA	GGGCTAACACCTIAGGYGGTGTMGGDTTCACMCARTA
2	mcrAsequencing	F	Mlas-mod	TTTAGACGTIAGCT	GGYGGTGTMGGDTTCACMCARTA	TTTAGACGTIAGCTGGYGGTGTMGGDTTCACMCARTA
3	mcrAsequencing	F	Mlas-mod	TGGAGATATAGGA	GGYGGTGTMGGDTTCACMCARTA	TGGAGATATAGGAGGYGGTGTMGGDTTCACMCARTA
4	mcrAsequencing	F	Mlas-mod	GAACACAGTTG	GGYGGTGTMGGDTTCACMCARTA	GAACACAGTTGGGYGGTGTMGGDTTCACMCARTA
5	mcrAsequencing	F	Mlas-mod	CCACCTACAAC	GGYGGTGTMGGDTTCACMCARTA	CCACCTACAACGGYGGTGTMGGDTTCACMCARTA
6	mcrAsequencing	F	Mlas-mod	AAAGTCGGGCT	GGYGGTGTMGGDTTCACMCARTA	AAAGTCGGGCTGGYGGTGTMGGDTTCACMCARTA
7	mcrAsequencing	F	Mlas-mod	TAACGACGTCAA	GGYGGTGTMGGDTTCACMCARTA	TAACGACGTCAAGGYGGTGTMGGDTTCACMCARTA
8	mcrAsequencing	F	Mlas-mod	ACCCGCGTTTCG	GGYGGTGTMGGDTTCACMCARTA	ACCCGCGTTTCGGGYGGTGTMGGDTTCACMCARTA
9	mcrAsequencing	F	Mlas-mod	TGGAAGGTCTGAC	GGYGGTGTMGGDTTCACMCARTA	TGGAAGGTCTGACGGYGGTGTMGGDTTCACMCARTA
10	mcrAsequencing	F	Mlas-mod	CATGTTTCACT	GGYGGTGTMGGDTTCACMCARTA	CATGTTTCACTGGYGGTGTMGGDTTCACMCARTA
11	mcrAsequencing	F	Mlas-mod	CCCGCTCCAGCCT	GGYGGTGTMGGDTTCACMCARTA	CCCGCTCCAGCCTGGYGGTGTMGGDTTCACMCARTA
12	mcrAsequencing	F	Mlas-mod	GCCGGTGCGGTTA	GGYGGTGTMGGDTTCACMCARTA	GCCGGTGCGGTTAGGYGGTGTMGGDTTCACMCARTA
#	**mcrAsequencing**	**Primer**	**Primer Name**	**Stagger+Barcode**	**mcrA Primer Seq.**	**Combined Seq.**
1	mcrAsequencing	R	McrA-rev	CAATTGCAGCCTC	CGTTCATBGCGTAGTTVGGRTAGT	CAATTGCAGCCTCCGTTCATBGCGTAGTTVGGRTAGT
2	mcrAsequencing	R	McrA-rev	CCCGGGGCGAGGA	CGTTCATBGCGTAGTTVGGRTAGT	CCCGGGGCGAGGACGTTCATBGCGTAGTTVGGRTAGT
3	mcrAsequencing	R	McrA-rev	CTAGTGGGATA	CGTTCATBGCGTAGTTVGGRTAGT	CTAGTGGGATACGTTCATBGCGTAGTTVGGRTAGT
4	mcrAsequencing	R	McrA-rev	GAATTTATCTCCG	CGTTCATBGCGTAGTTVGGRTAGT	GAATTTATCTCCGCGTTCATBGCGTAGTTVGGRTAGT
5	mcrAsequencing	R	McrA-rev	CAATGACTAACTG	CGTTCATBGCGTAGTTVGGRTAGT	CAATGACTAACTGCGTTCATBGCGTAGTTVGGRTAGT
6	mcrAsequencing	R	McrA-rev	GAAAATCCTATT	CGTTCATBGCGTAGTTVGGRTAGT	GAAAATCCTATTCGTTCATBGCGTAGTTVGGRTAGT
7	mcrAsequencing	R	McrA-rev	CTTCACGTGTT	CGTTCATBGCGTAGTTVGGRTAGT	CTTCACGTGTTCGTTCATBGCGTAGTTVGGRTAGT
8	mcrAsequencing	R	McrA-rev	TCACCTTTACA	CGTTCATBGCGTAGTTVGGRTAGT	TCACCTTTACACGTTCATBGCGTAGTTVGGRTAGT
9	mcrAsequencing	R	McrA-rev	TCCGGCTAGATTC	CGTTCATBGCGTAGTTVGGRTAGT	TCCGGCTAGATTCCGTTCATBGCGTAGTTVGGRTAGT
10	mcrAsequencing	R	McrA-rev	GGGCAGAGAACTC	CGTTCATBGCGTAGTTVGGRTAGT	GGGCAGAGAACTCCGTTCATBGCGTAGTTVGGRTAGT
11	mcrAsequencing	R	McrA-rev	GTTAGCTCAGTT	CGTTCATBGCGTAGTTVGGRTAGT	GTTAGCTCAGTTCGTTCATBGCGTAGTTVGGRTAGT
12	mcrAsequencing	R	McrA-rev	GAATGGTACTTGC	CGTTCATBGCGTAGTTVGGRTAGT	GAATGGTACTTGCCGTTCATBGCGTAGTTVGGRTAGT

Table S27: 12 dsrB primer sets with stagger and barcode sequences.

Target Gene	Primer Type	Barcode Seq.	Primer Seq.	Combined Seq.
dsrB	F1	ACTA ACTG	CAAACATCGTYCAYACCCAGGG	ACTAACTGCAAACATCGTYCAYACCCAGGG
dsrB	F2	TGAA TTCG	CAAACATCGTYCAYACCCAGGG	TGAATTCGCAAACATCGTYCAYACCCAGGG
dsrB	F3	CCGG ATAG	CAAACATCGTYCAYACCCAGGG	CCGGATAGCAAACATCGTYCAYACCCAGGG
dsrB	F4	GTGA CTAC	CAAACATCGTYCAYACCCAGGG	GTGACTACCAAACATCGTYCAYACCCAGGG
dsrB	F5	TATA CCGT	CAAACATCGTYCAYACCCAGGG	TATACCGTCAAACATCGTYCAYACCCAGGG
dsrB	F6	TGAC GCAT	CAAACATCGTYCAYACCCAGGG	TGACGCATCAAACATCGTYCAYACCCAGGG
dsrB	F7	TTCC TCAC	CAAACATCGTYCAYACCCAGGG	TTCCTCACCAAACATCGTYCAYACCCAGGG
dsrB	F8	AATG CTGA	CAAACATCGTYCAYACCCAGGG	AATGCTGACAAACATCGTYCAYACCCAGGG
dsrB	F9	AGGT CAGT	CAAACATCGTYCAYACCCAGGG	AGGTCAGTCAAACATCGTYCAYACCCAGGG
dsrB	F10	ATTG CATC	CAAACATCGTYCAYACCCAGGG	ATTGCATCCAAACATCGTYCAYACCCAGGG
dsrB	F11	CCAT ACAC	CAAACATCGTYCAYACCCAGGG	CCATACACCAAACATCGTYCAYACCCAGGG
dsrB	F12	CGCT TCTG	CAAACATCGTYCAYACCCAGGG	CGCTTCTGCAAACATCGTYCAYACCCAGGG

Target Gene	Primer Type	Barcode Seq.	Primer Seq.	Combined Seq.
dsrB	R1	ATAT AGGA	GTGTAGCAGTTACCGCA	ATATAGGAGTGTAGCAGTTACCGCA
dsrB	R2	ATTC CTTT	GTGTAGCAGTTACCGCA	ATTCCTTTGTGTAGCAGTTACCGCA
dsrB	R3	GCTC AGTT	GTGTAGCAGTTACCGCA	GCTCAGTTGTGTAGCAGTTACCGCA
dsrB	R4	AGTC AACA	GTGTAGCAGTTACCGCA	AGTCAACAGTGTAGCAGTTACCGCA
dsrB	R5	AGTT CCGT	GTGTAGCAGTTACCGCA	AGTTCCGTGTGTAGCAGTTACCGCA
dsrB	R6	ATGT CAGA	GTGTAGCAGTTACCGCA	ATGTCAGAGTGTAGCAGTTACCGCA
dsrB	R7	CACA GTTG	GTGTAGCAGTTACCGCA	CACAGTTGGTGTAGCAGTTACCGCA
dsrB	R8	GTCC GCAC	GTGTAGCAGTTACCGCA	GTCCGCACGTGTAGCAGTTACCGCA
dsrB	R9	GAGA ACTC	GTGTAGCAGTTACCGCA	GAGAACTCGTGTAGCAGTTACCGCA
dsrB	R10	GTGG CCTT	GTGTAGCAGTTACCGCA	GTGGCCTTGTGTAGCAGTTACCGCA
dsrB	R11	TCCA GCCT	GTGTAGCAGTTACCGCA	TCCAGCCTGTGTAGCAGTTACCGCA
dsrB	R12	CGTA CGTA	GTGTAGCAGTTACCGCA	CGTACGTAGTGTAGCAGTTACCGCA

Table S28: RNA concentration of three different samples per 1.5 g wet weight of soil (from week one to week six).
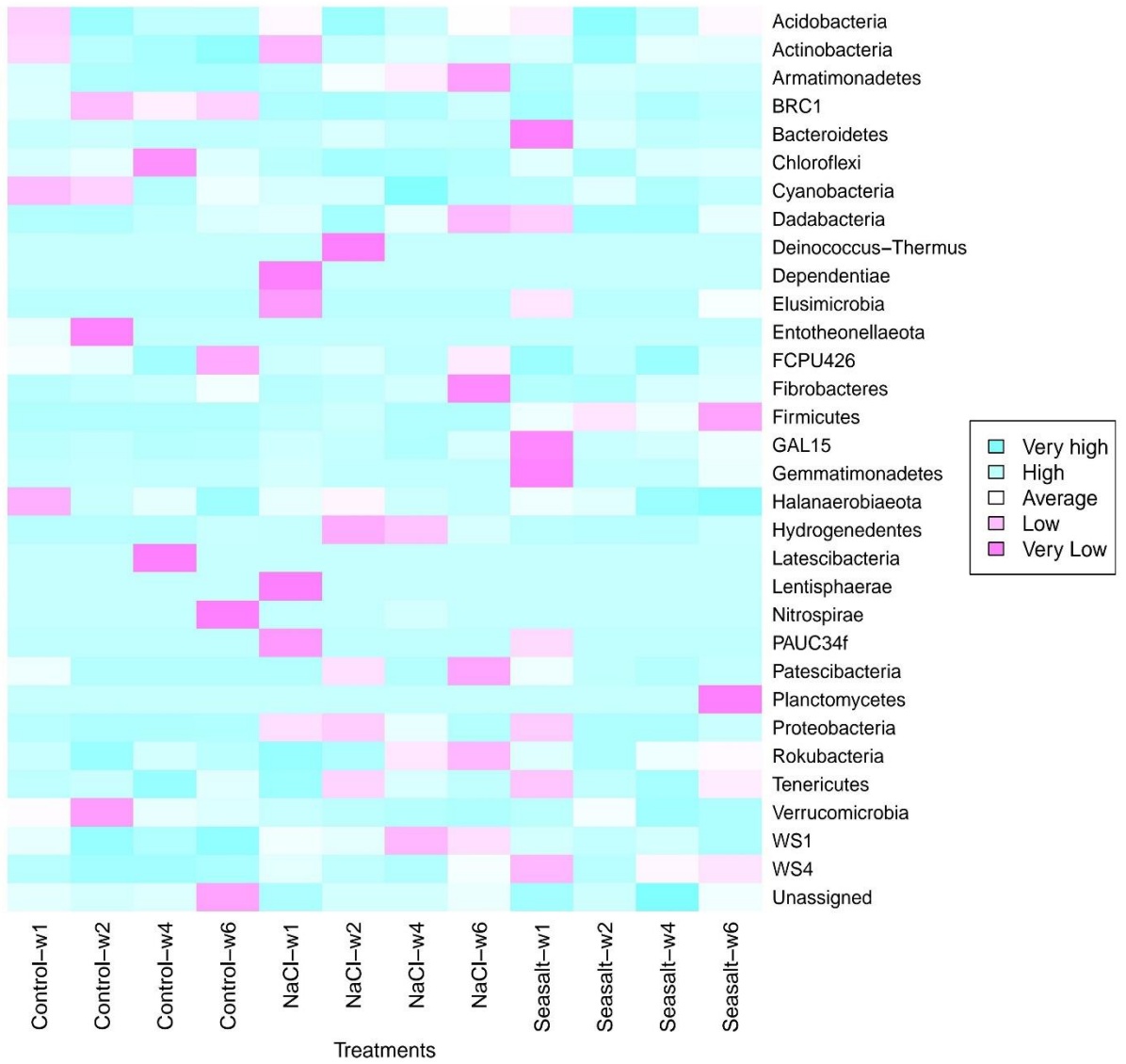
NaCl-treated samples	Concentration	UV absorbance at 400 nm	Seasalt-treated samples	Concentration	UV absorbance at 400 nm	Control	Concentration	UV absorbance at 400 nm	RNA integrity
S 1-1	6.93 ng/μl	0.007	S 1-1	10.3 ng/μl	0.008	C 1-1	15.8 ng/μl	0.009	Intact
S 1-2	11.4 ng/μl	0.009	S 1-2	7.87 ng/μl	0.018	C 1-2	11.9 ng/μl	0.017	Intact
S 1-3	4.56 ng/μl	0.016	S 1-3	19.4 ng/μl	0.019	C 1-3	19.5 ng/μl	0.017	Intact
S 2-2	41.5 ng/μl	0.018	S 2-2	8.73 ng/μl	0.015	C 2-1	7.13 ng/μl	0.03	Intact
S 2-3	9.4 ng/μl	0.02	S 2-3	26.3 ng/μl	0.017	C 2-2	12.6 ng/μl	0.011	Intact
S 2-4	15.2 ng/μl	0.013	S 2-4	11.3 ng/μl	0.02	C 2-3	5.79 ng/μl	0.016	Intact
S 4-1	8.73 ng/μl	0.004	S 4-1	7.53 ng/μl	0.016	C 4-1	14 ng/μl	0.009	Intact
S 4-2	27.8 ng/μl	0.031	S 4-2	3.27 ng/μl	0.016	C 4-2	27.8 ng/μl	0.01	Intact
S 4-3	6.80 ng/μl	0.09	S 4-3	6.64 ng/μl	0.012	C 4-3	10.8 ng/μl	0.013	Intact
S 6-1	8.60 ng/μl	0.002	S 6-1	3.73 ng/μl	0.014	C 6-1	23.1 ng/μl	0.019	Intact
S 6-2	6.42 ng/μl	0.009	S 6-2	6.80 ng/μl	0.019	C 6-2	12.1 ng/μl	0.016	Intact
S 6-3	5.05 ng/μl	0.02	S 6-3	3.45 ng/μl	0.017	C 6-3	26.1 ng/μl	0.015	Intact

Table S29: DNA concentration of three different samples per 0.5 g wet weight of soil (from week one to week six).

NaCl-treated samples	Concentration	UV absorbance at 400 nm	Seasalt-treated samples	Concentration	UV absorbance at 400 nm	Control	Concentration	UV absorbance at 400 nm	DNA integrity
S 1-1	10.12 ng/μl	0.012	S 1-1	11 ng/μl	0.017	C 1-1	8.7 ng/μl	0.032	Intact
S 1-2	11.33 ng/μl	0.014	S 1-2	14.2 ng/μl	0.008	C 1-2	9.8 ng/μl	0.01	Intact
S 1-3	14.8 ng/μl	0.008	S 1-3	25 ng/μl	0.019	C 1-3	20 ng/μl	0.021	Intact
S 2-2	12.2 ng/μl	0.009	S 2-2	18.1 ng/μl	0.003	C 2-1	12.5 ng/μl	0.025	Intact
S 2-3	17.4 ng/μl	0.004	S 2-3	19.4 ng/μl	0.007	C 2-2	14.3 ng/μl	0.011	Intact
S 2-4	18.9 ng/μl	0.015	S 2-4	17.5 ng/μl	0.009	C 2-3	30 ng/μl	0.008	Intact
S 4-1	16.9 ng/μl	0.006	S 4-1	18.33 ng/μl	0.013	C 4-1	12.9 ng/μl	0.002	Intact
S 4-2	20.8 ng/μl	0.013	S 4-2	17.7 ng/μl	0.014	C 4-2	19.6 ng/μl	0.014	Intact
S 4-3	16.8 ng/μl	0.019	S 4-3	18.9 ng/μl	0.019	C 4-3	17.9 ng/μl	0.019	Intact
S 6-1	13.87 ng/μl	0.015	S 6-1	10 ng/μl	0.015	C 6-1	13.7 ng/μl	0.026	Intact
S 6-2	13.76 ng/μl	0.018	S 6-2	12.1 ng/μl	0.012	C 6-2	11.3 ng/μl	0.028	Intact
S 6-3	15.65 ng/μl	0.009	S 6-3	19.35 ng/μl	0.03	C 6-3	11.8 ng/μl	0.019	Intact

Heatmaps:

Heatmap−Phylum(16S−Gene)

Heatmap–Phylum(16S–transcript)

Treatments

Legend:
- Very high
- High
- Average
- Low
- Very Low

Row labels (top to bottom): Acidobacteria, Actinobacteria, Armatimonadetes, BRC1, Bacteroidetes, Chloroflexi, Cyanobacteria, Dadabacteria, Deinococcus–Thermus, Dependentiae, Elusimicrobia, Entotheonellaeota, FCPU426, Fibrobacteres, Firmicutes, GAL15, Gemmatimonadetes, Halanaerobiaeota, Hydrogenedentes, Latescibacteria, Lentisphaerae, Nitrospirae, PAUC34f, Patescibacteria, Planctomycetes, Proteobacteria, Rokubacteria, Tenericutes, Verrucomicrobia, WS1, WS4, Unassigned

Column labels: Control–w1, Control–w2, Control–w4, Control–w6, NaCl–w1, NaCl–w2, NaCl–w4, NaCl–w6, Seasalt–w1, Seasalt–w2, Seasalt–w4, Seasalt–w6

Heatmap−Phylum(dsrB−Gene)

■	Very high
■	High
■	Average
■	Low
■	Very Low

Heatmap−Phylum(dsrB−transcript)

Acidobacteria
Actinobacteria
Armatimonadetes
Chloroflexi
Firmicutes
Nitrospirae
Planctomycetes
Proteobacteria
candidate division Zixibacteria
Environmental samples
others
Unassigned

Control−w1
Control−w2
Control−w4
Control−w6
NaCl−w1
NaCl−w2
NaCl−w4
NaCl−w6
Seasalt−w1
Seasalt−w2
Seasalt−w4
Seasalt−w6

Treatments

Legend:
- Very high
- High
- Average
- Low
- Very Low

Heatmap–Family(dsrB–Gene)

Acidobacteria
Actinobacteria
Nocardiopsaceae
Eggerthellaceae
Gaiellales
Armatimonadetes
Anaerolineaceae
Chloroflexi
Peptococcaceae
Thermoanaerobacteraceae
Sporomusaceae
Nitrospiraceae
Nitrospirae
Planctomycetes
Planctomycetaceae
Deltaproteobacteria
Desulfarculaceae
Desulfobacteraceae
Desulfobacterales
Desulfobulbaceae
Desulfovibrionaceae
Anaeromyxobacteraceae
Myxococcales
Syntrophaceae
Syntrophobacteraceae
candidate division Zixibacteria
others
Unassigned

Control–w1
Control–w2
Control–w4
Control–w6
NaCl–w1
NaCl–w2
NaCl–w4
NaCl–w6
Seasalt–w1
Seasalt–w2
Seasalt–w4
Seasalt–w6

Treatments

Legend:
- Very high
- High
- Average
- Low
- Very Low

Heatmap−Family(dsrB−transcript)

Heatmap−Order(mcrA−Gene)

Methanomassiliicoccales

Methanobacteriales

Methanocellales

Methanosarcinales

Unassigned

	Very high
	High
	Average
	Low
	Very Low

Control−w1
Control−w2
Control−w4
Control−w6
NaCl−w1
NaCl−w2
NaCl−w4
NaCl−w6
Seasalt−w1
Seasalt−w2
Seasalt−w4
Seasalt−w6

Treatments

Heatmap−Order(mcrA−transcript)

Legend:
- Very high
- High
- Average
- Low
- Very Low

Rows (top to bottom): Methanomassiliicoccales, Methanobacteriales, Methanocellales, Methanosarcinales, Unassigned

Columns (Treatments): Control−w1, Control−w2, Control−w4, Control−w6, NaCl−w1, NaCl−w2, NaCl−w4, NaCl−w6, Seasalt−w1, Seasalt−w2, Seasalt−w4, Seasalt−w6

Heatmap–Family(mcrA–Gene)

Methanomassiliicoccales

andidatus Methanoperedenaceae

Methanobacteriaceae

Methanocellaceae

Methanosarcinaceae

Methanotrichaceae

Unassigned

	Very high
	High
	Average
	Low
	Very Low

Control-w1
Control-w2
Control-w4
Control-w6
NaCl-w1
NaCl-w2
NaCl-w4
NaCl-w6
Seasalt-w1
Seasalt-w2
Seasalt-w4
Seasalt-w6

Treatments

Heatmap–Family(mcrA–transcript)

Panel S1: 16S rRNA gene compositions at phylum level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.
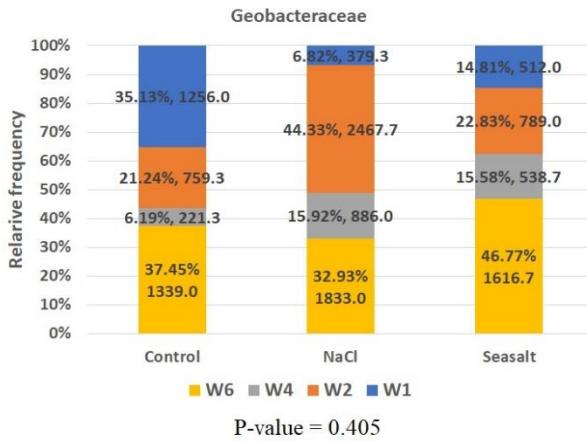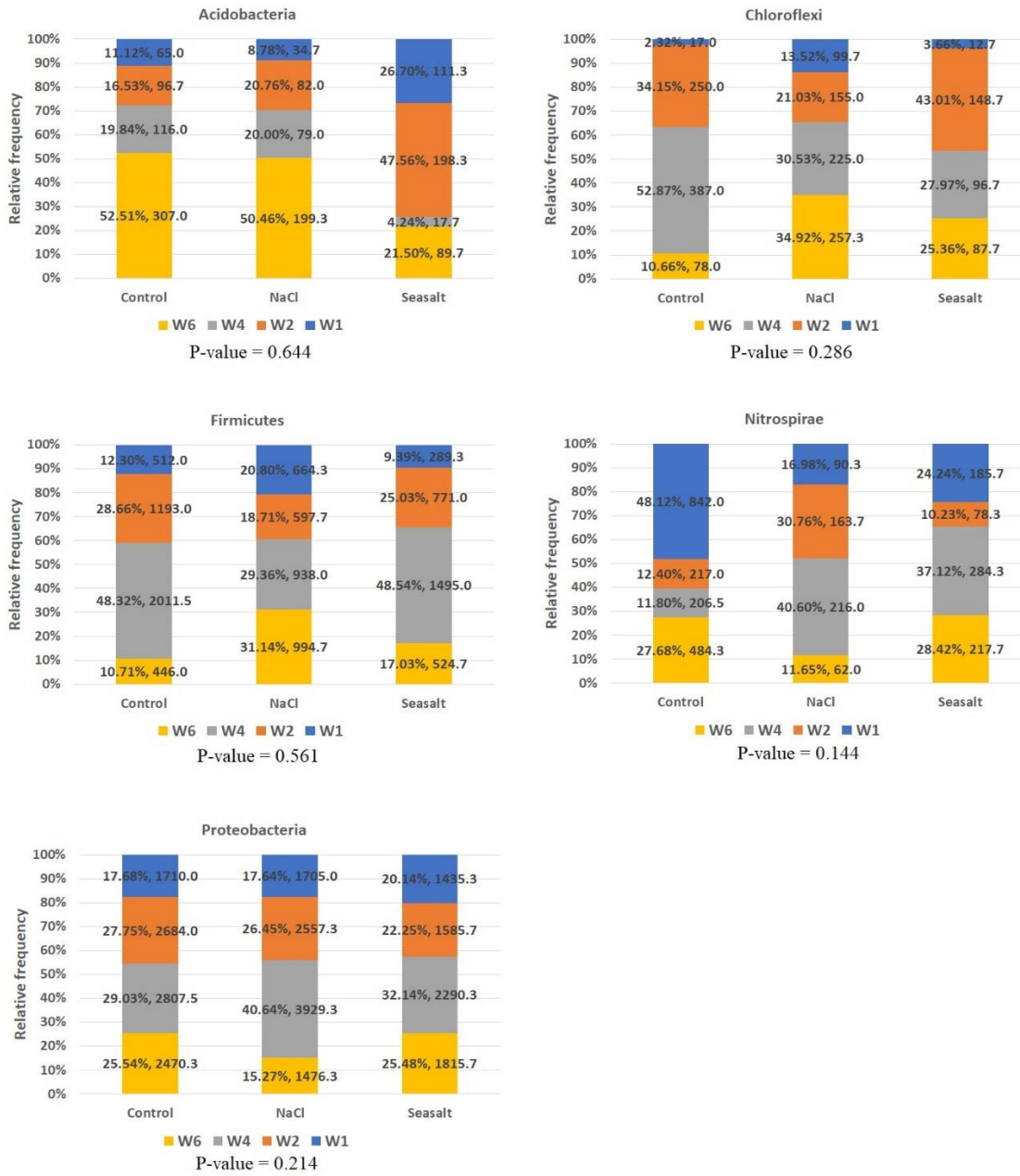

Acidobacteria
P-value = 0.683

Bacteroidetes
P-value = 0.284

Chloroflexi
P-value = 0.043

Fibrobacteres
P-value = 0.024

Firmicutes
P-value = 0.003

Proteobacteria
P-value = 0.017

Actinobacteria

- 51.27%, 580.0
- 27.55%, 1175.0
- 44.85%, 2375.0
- 10.55%, 119.3
- 17.66%, 753.3
- 16.11%, 853.0
- 11.87%, 134.3
- 13.29%, 703.7
- 26.31%, 297.7
- 34.13%, 1455.3
- 25.76%, 1364.0

P-value = 0.014

Armatimonadetes

- 28.92%, 47.3
- 22.37%, 214.3
- 28.25%, 292.7
- 9.16%, 15.0
- 12.97%, 124.3
- 15.06%, 156.0
- 39.51%, 64.7
- 41.95%, 402.0
- 26.19%, 271.3
- 22.40%, 36.7
- 22.71%, 217.7
- 30.50%, 316.0

P-value = 0.004

BRC1

- 52.82% 131.3
- 48.46% 115.3
- 56.80% 140.7
- 25.07% 62.3
- 27.73% 66.0
- 18.44% 45.7
- 13.81%, 34.3
- 15.13%, 36.0
- 15.21%, 37.7
- 8.31%, 20.7
- 8.68%, 20.7
- 9.56%, 23.7

P-value = 0.912

Halanaerobiaeota

- 2.35%, 66.3
- 11.51%, 324.7
- 6.32%, 178.3
- 24.18%, 14.7
- 36.26%, 22.0
- 79.82%, 2251.7
- 18.13%, 11.0
- 21.43%, 13.0
- 0, 0.0

Axis Title

P-value = 0.242

Verrucomicrobia

- 15.99%, 108.3
- 5.91%, 11.7
- 1.62%, 2.7
- 3.89%, 7.7
- 7.29%, 12.0
- 21.80%, 147.7
- 22.67%, 37.3
- 31.42%, 62.0
- 27.17%, 184.0
- 35.04%, 237.3
- 58.78%, 116.0
- 68.42%, 112.7

P-value = 6.25649E-06

Panel S2: 16S rRNA gene compositions at family level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.

Heliobacteriaceae

	Control	NaCl	Seasalt
W1	26.12%, 3057.3	20.20%, 1904.0	28.43%, 2436.7
W2	17.40%, 2036.7	13.58%, 1280.7	17.97%, 1540.0
W4	37.90%, 4436.7	33.17%, 3127.3	37.51%, 3215.3
W6	18.59%, 2176.0	33.05%, 3115.3	16.09%, 1379.3

Relative frequency

W6 ■ W4 ■ W2 ■ W1

P-value = 0.249

Lachnospiraceae

	Control	NaCl	Seasalt
W1	63.48% 7383.3	63.88% 13642.7	63.01% 13553.3
W2	22.31%, 2594.7	15.86%, 3386.7	19.10%, 4108.7
W4	6.37%, 740.7	9.20%, 1964.7	10.29%, 2213.3
W6	7.85%, 912.7	11.07%, 2363.3	7.60%, 1634.0

Relative frequency

W6 ■ W4 ■ W2 ■ W1

P-value = 0.091

Ruminococcaceae

	Control	NaCl	Seasalt
W1	48.6% 11234.7	36.7% 15726.0	39.9% 12619.3
W2	27.8% 6430.7	20.4% 8754.7	24.6% 7782.7
W4	8.4%, 1948.7	16.4%, 7016.0	14.9%, 4698.0
W6	15.2% 3504.0	26.5% 11355.3	20.6% 6503.3

Relative frequency

W6 ■ W4 ■ W2 ■ W1

P-value = 0.003

Veillonellaceae

	Control	NaCl	Seasalt
W1	43.33% 2407.3	55.08% 6460.0	38.07% 3896.7
W2	18.07% 1004.0	12.35% 1448.0	14.73% 1507.3
W4	26.93% 1496.0	22.18% 2601.3	27.30% 2794.0
W6	11.68% 648.7	10.39% 1218.0	19.91% 2038.0

Relative frequency

W6 ■ W4 ■ W2 ■ W1

P-value = 0.130

Halobacteroidaceae

	Control	NaCl	Seasalt
W1		24.18%, 29.3	2.35%, 132.7
			11.51%, 649.3
			6.32%, 356.7
W2		36.26%, 44.0	
W4		18.13%, 22.0	79.82% 4503.3
W6	0, 0.0	21.43% 26.0	
	0.0		

Relative frequency

W6 ■ W4 ■ W2 ■ W1

P-value = 0.242

Archangiaceae

	Control	NaCl	Seasalt
W1	35.92% 1873.3	33.25% 1387.3	28.44% 2240.7
W2	20.58% 1073.3	28.02% 1169.3	30.70% 2418.7
W4	16.29% 849.3	16.73% 698.0	16.19% 1275.3
W6	27.21% 1418.7	22.00% 918.0	24.67% 1944.0

Relative frequency

W6 ■ W4 ■ W2 ■ W1

P-value = 0.004

117

Panel S3: 16S rRNA transcript compositions at phylum level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.


P-value = 0.059

P-value = 0.014

P-value = 0.603

P-value =0.340

P-value = 0.328

P-value = 0.465

Proteobacteria

P-value = 0.018

Panel S4: 16S rRNA transcript compositions at family level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.

Geobacteraceae

- 35.13%, 1256.0 (Control, W1)
- 6.82%, 379.3 (NaCl, W1)
- 14.81%, 512.0 (Seasalt, W1)
- 44.33%, 2467.7 (NaCl, W2)
- 22.83%, 789.0 (Seasalt, W2)
- 21.24%, 759.3 (Control, W2)
- 6.19%, 221.3 (Control, W4)
- 15.92%, 886.0 (NaCl, W4)
- 15.58%, 538.7 (Seasalt, W4)
- 37.45% 1339.0 (Control, W6)
- 32.93% 1833.0 (NaCl, W6)
- 46.77% 1616.7 (Seasalt, W6)

■ W6 ■ W4 ■ W2 ■ W1

P-value = 0.405

Archangiaceae

- 36.53% 4916.0 (Control, W1)
- 56.42% 3202.0 (NaCl, W1)
- 23.57% 1633.3 (Seasalt, W1)
- 34.15% 4596.7 (Control, W2)
- 20.74% 1177.0 (NaCl, W2)
- 33.92% 2350.7 (Seasalt, W2)
- 16.27% 2189.7 (Control, W4)
- 7.05% 400.3 (NaCl, W4)
- 27.71% 1920.7 (Seasalt, W4)
- 13.05%, 1756.0 (Control, W6)
- 15.78%, 895.7 (NaCl, W6)
- 14.80%, 1026.0 (Seasalt, W6)

■ W6 ■ W4 ■ W2 ■ W1

P-value = 0.048

Haliangiaceae

- 25.23%, 780.3 (Control, W1)
- 39.43%, 114.3 (NaCl, W1)
- 8.07%, 67.0 (Seasalt, W1)
- 39.34% 1217.0 (Control, W2)
- 41.38% 120.0 (NaCl, W2)
- 85.02% 705.7 (Seasalt, W2)
- 14.49% 448.3 (Control, W4)
- 7.82%, 22.7 (NaCl, W4)
- 2.93%, 24.3 (Seasalt, W4)
- 20.94%, 647.7 (Control, W6)
- 11.38%, 33.0 (NaCl, W6)
- 3.98%, 33.0 (Seasalt, W6)

■ W6 ■ W4 ■ W2 ■ W1

P-value = 0.003

Panel S5: *dsrB* gene compositions at phylum level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.
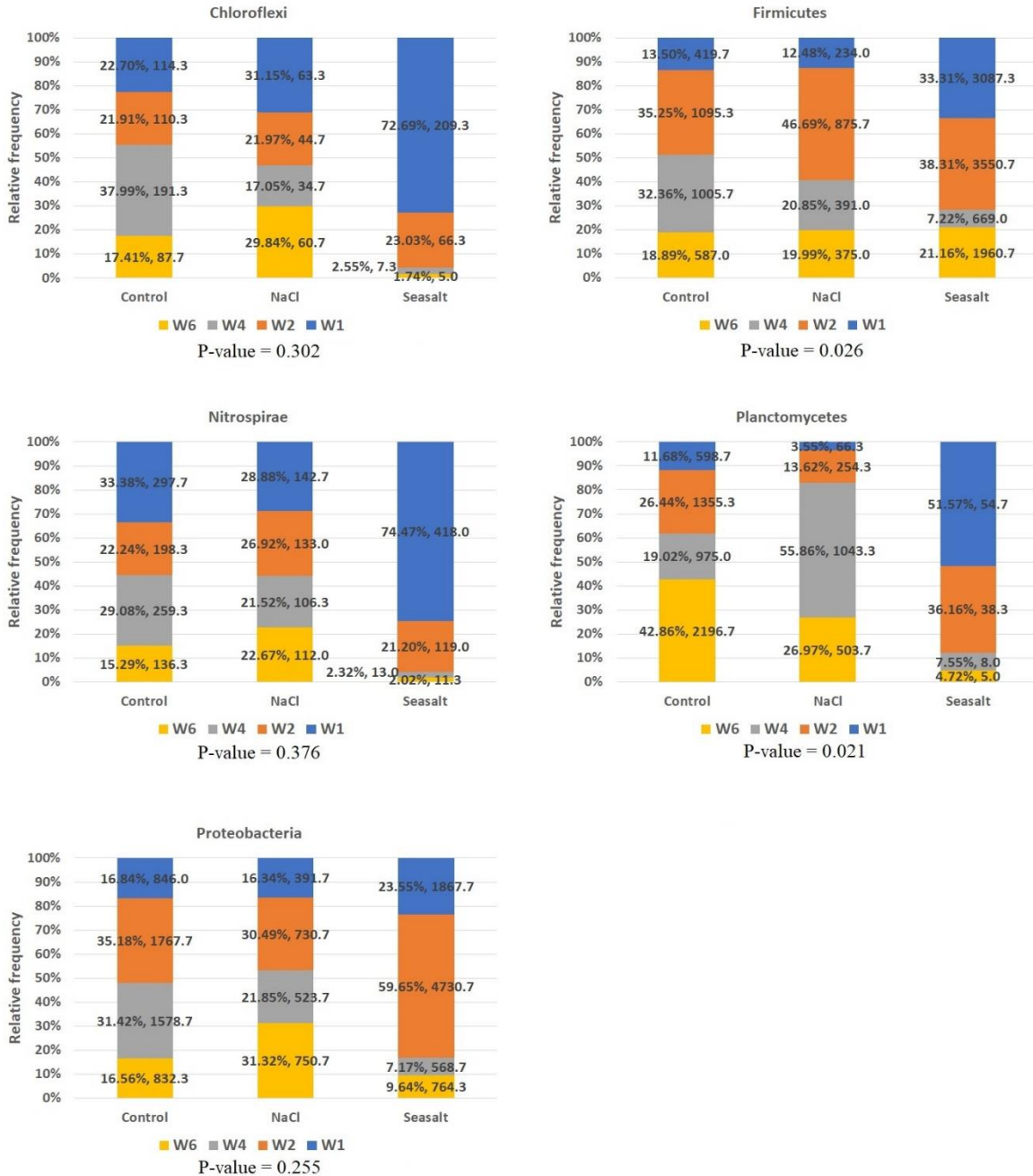
Panel S6: *dsrB* gene compositions at family level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.
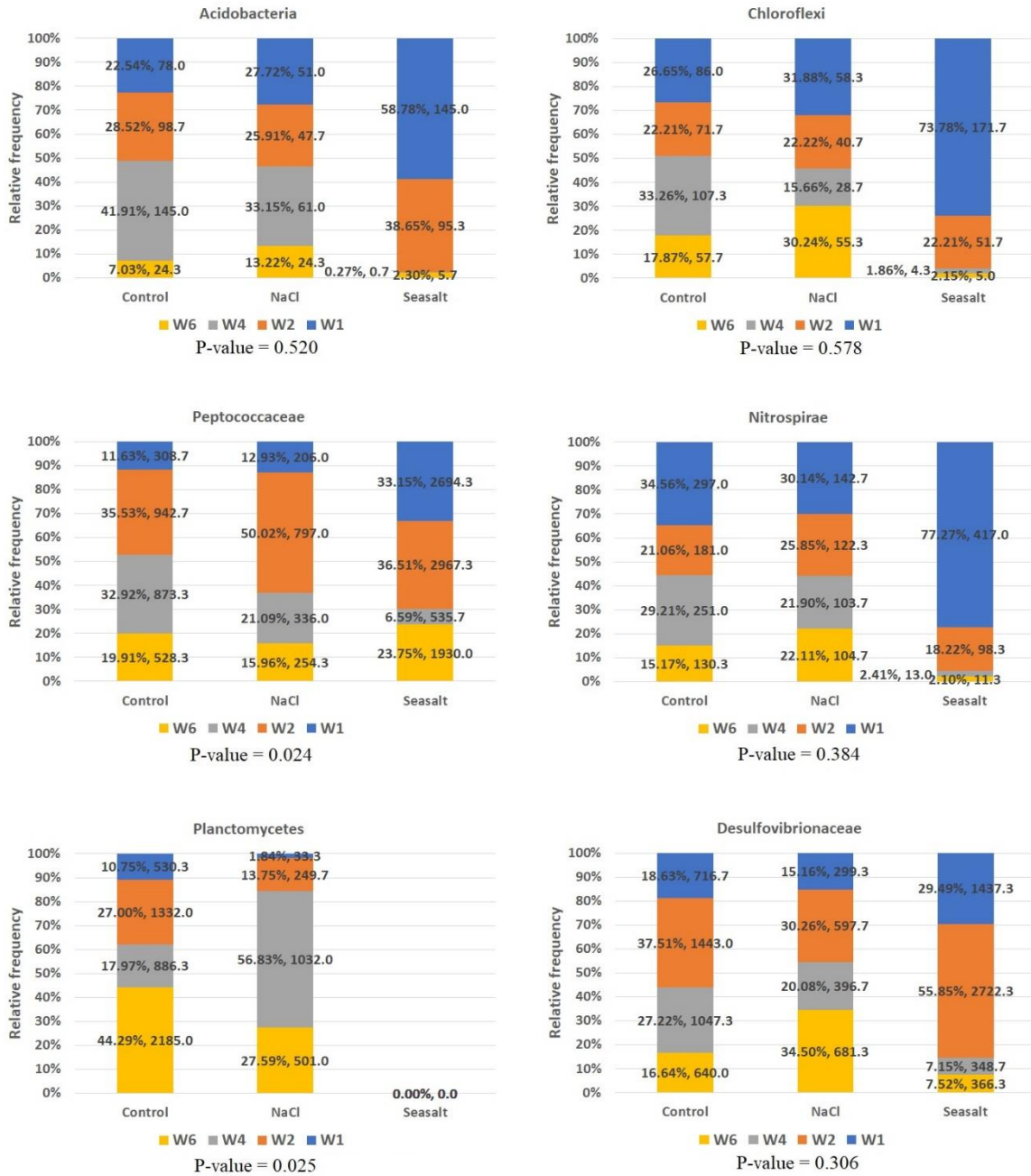
Panel S7: dsrB transcript compositions at phylum level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.

Acidobacteria — W6, W4, W2, W1. P-value = 0.644

Chloroflexi — W6, W4, W2, W1. P-value = 0.286

Firmicutes — W6, W4, W2, W1. P-value = 0.561

Nitrospirae — W6, W4, W2, W1. P-value = 0.144

Proteobacteria — W6, W4, W2, W1. P-value = 0.214

Panel S8: dsrB transcript compositions at family level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.
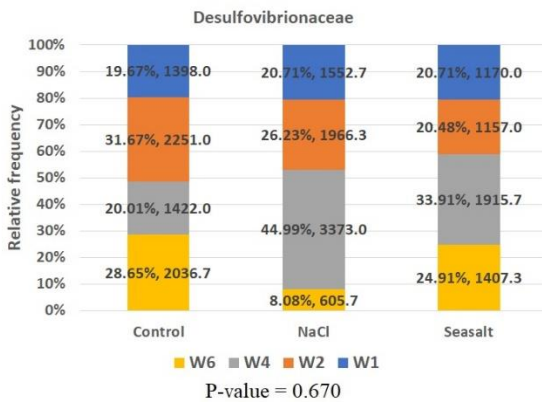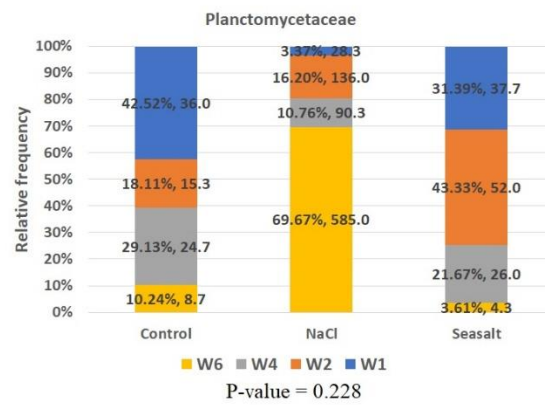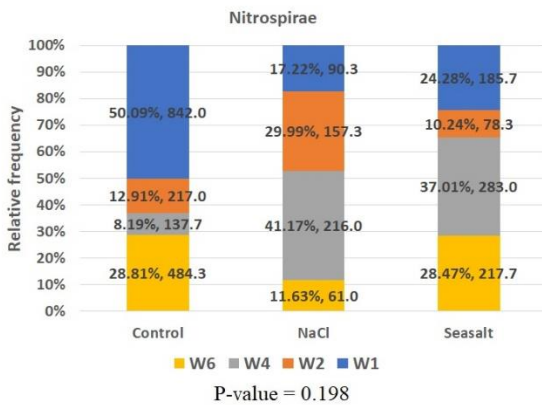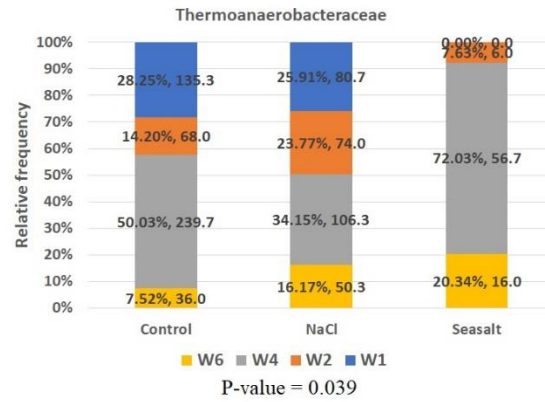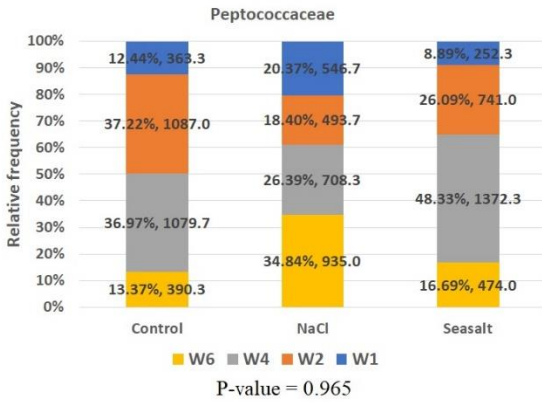
Panel S9: *mcrA* gene compositions at order level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.
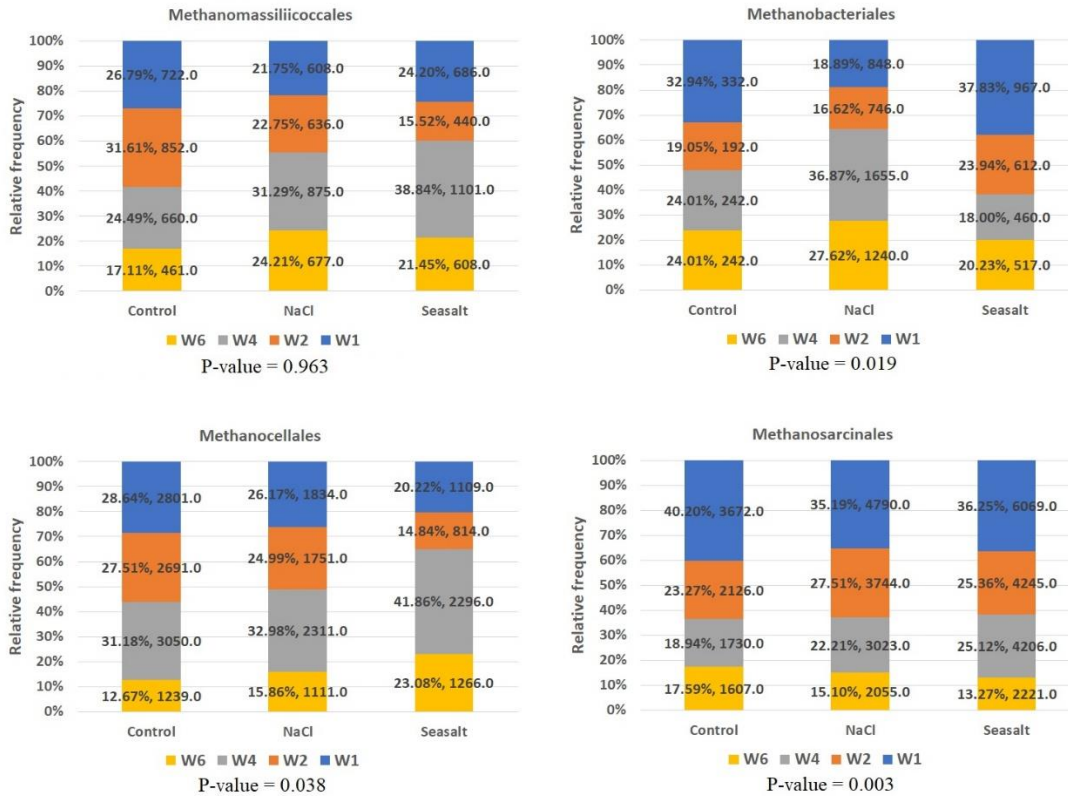
Panel S10: *mcrA* gene compositions at family level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.
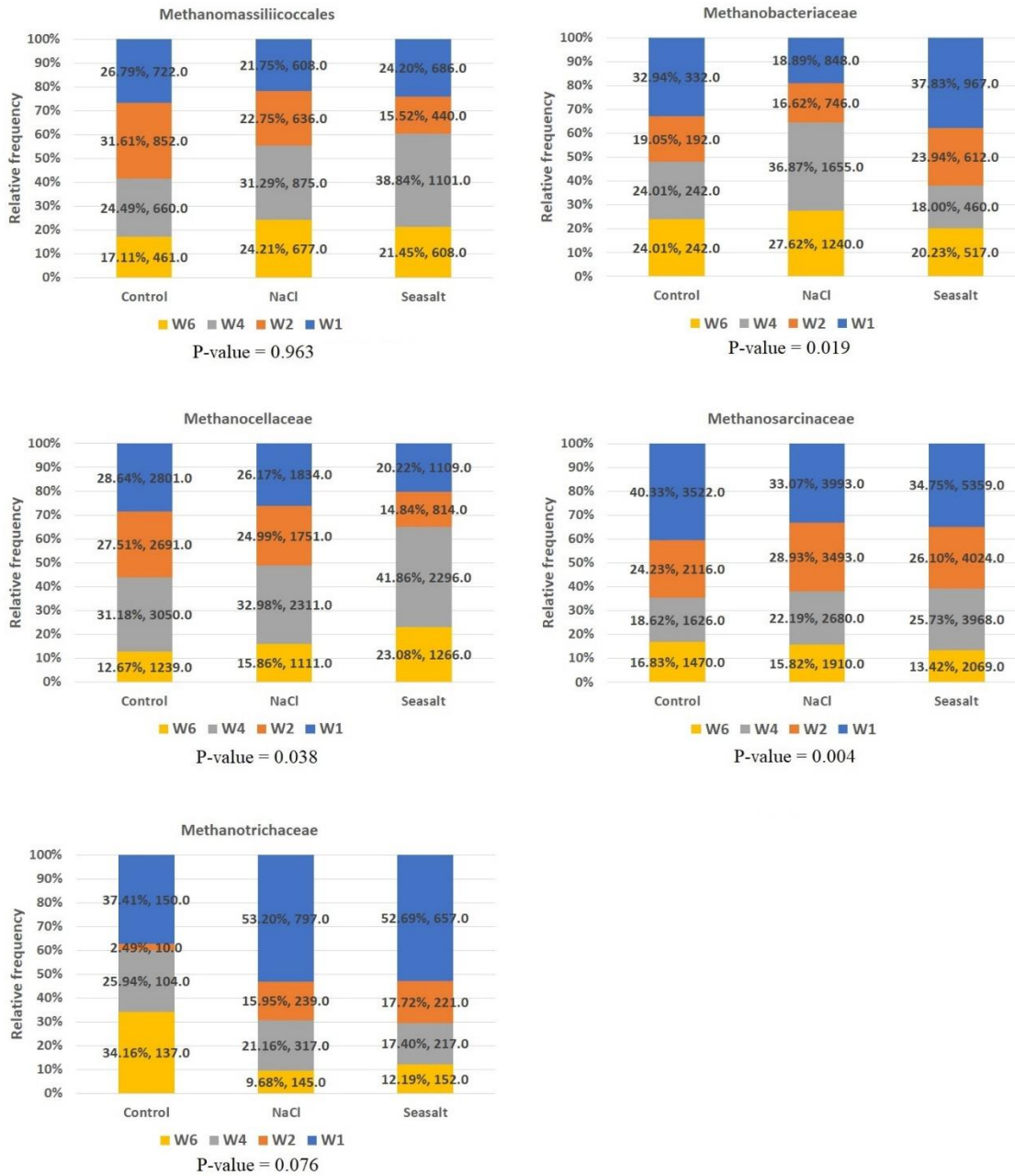
Panel S11: mcrA transcript compositions at order level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.

P-value = 0.041

P-value = 0.226

P-value = 0.004

P-value = 0.021
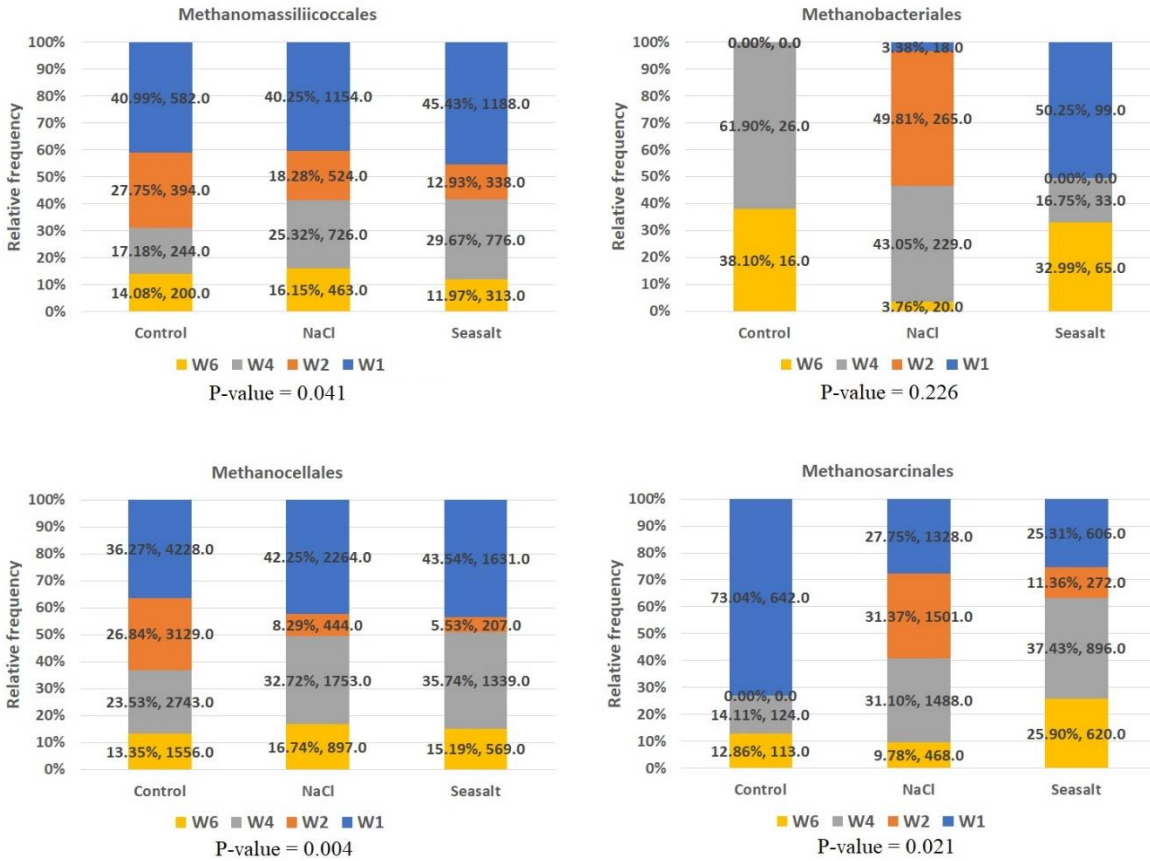
Panel S12: mcrA transcript compositions at family level.

Relative frequency between three groups (NaCl and seasalt treatments plus control) accompanied with percentages, counts and significance values.
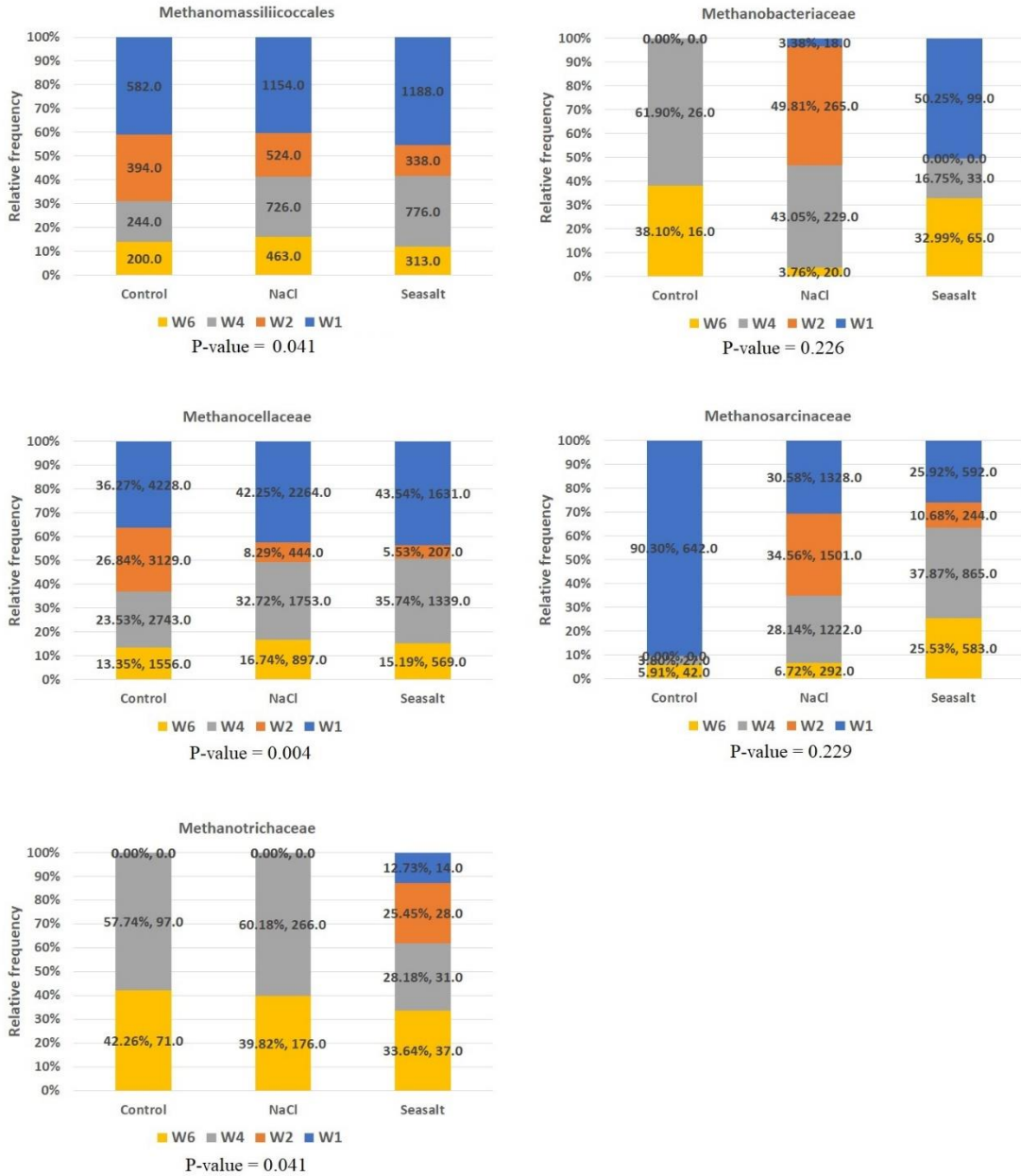
Shannon Index values and statistical anlysis between the three groups:

Figure S1: Shannon Index for 16S rRNA Tanscripts:
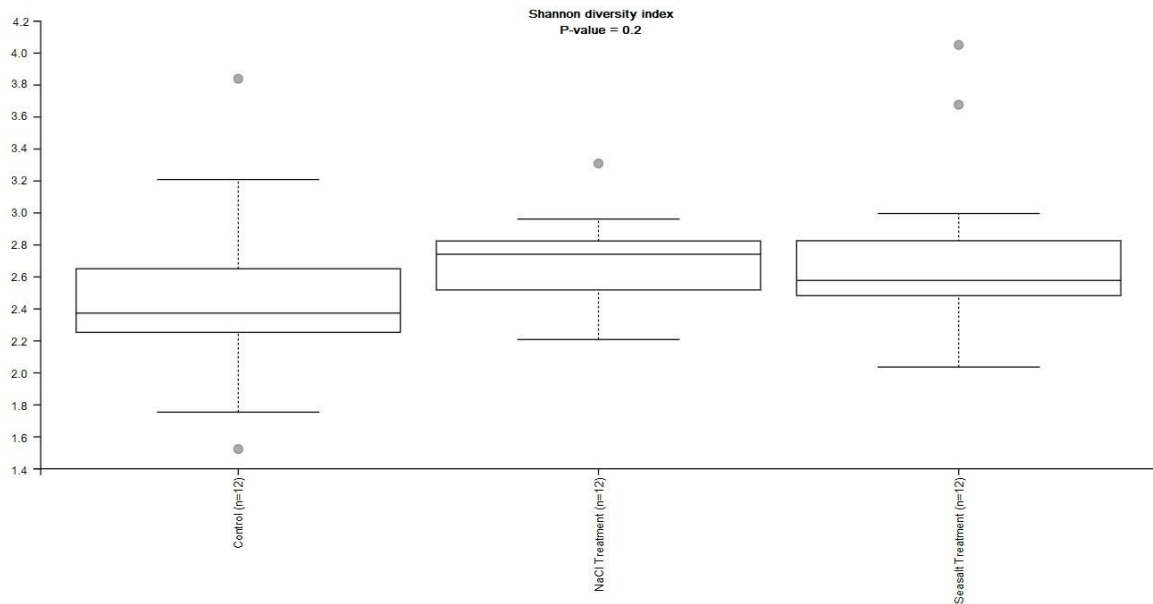
Figure S2: Shannon Index for 16S rRNA gene:
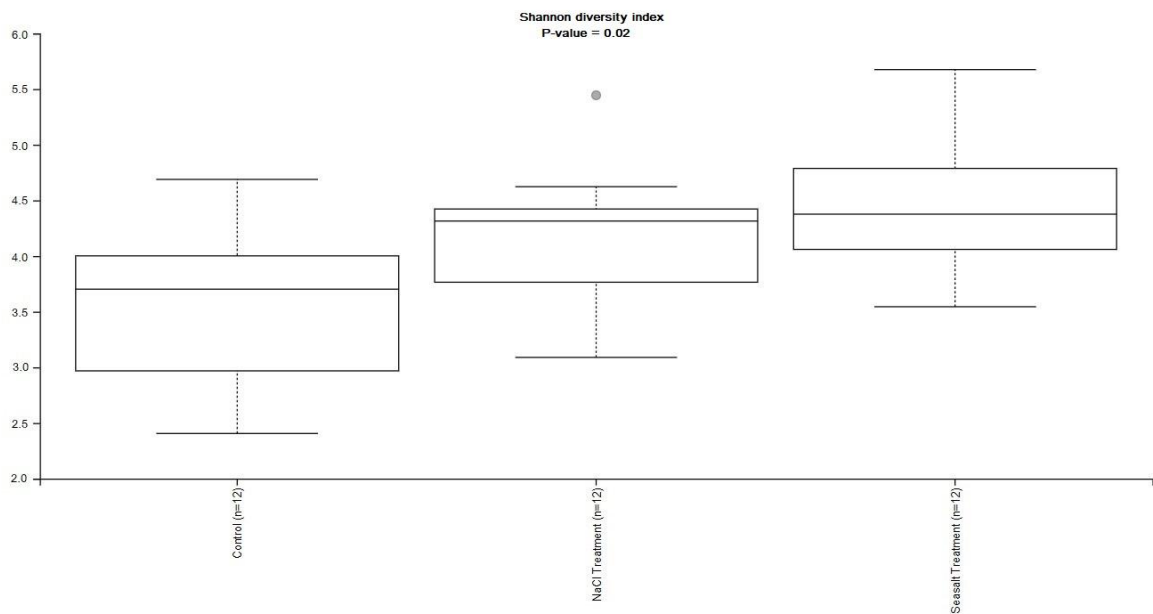
Figure S3: Shannon Index for dsrB Transcripts:
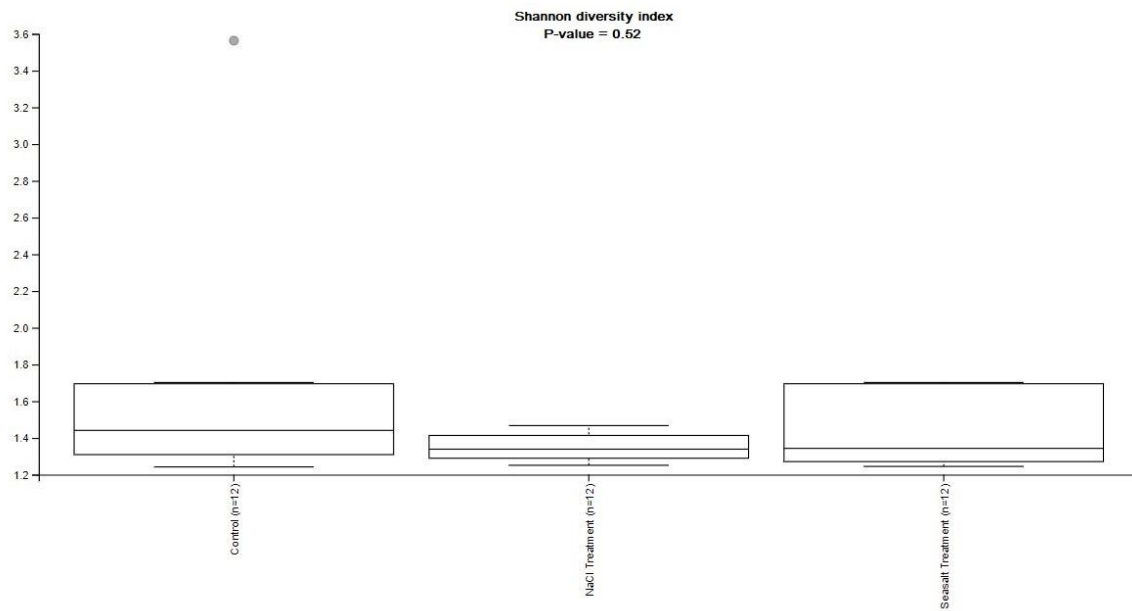
Figure S4: Shannon Index for *dsrB* Gene:
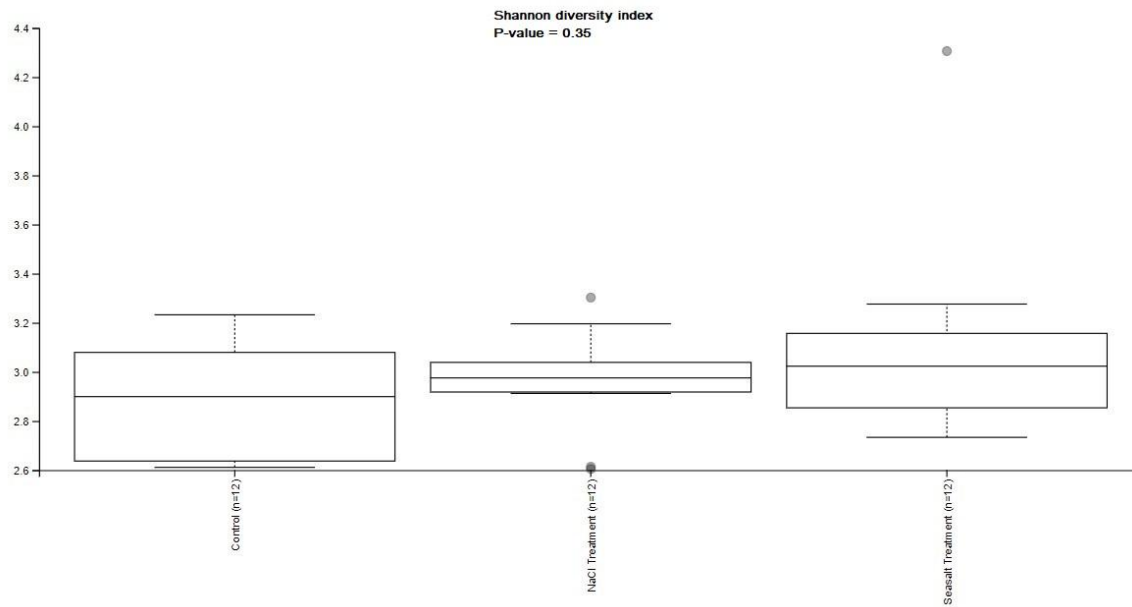
Figure S5: Shannon Index for mcrA transcripts:
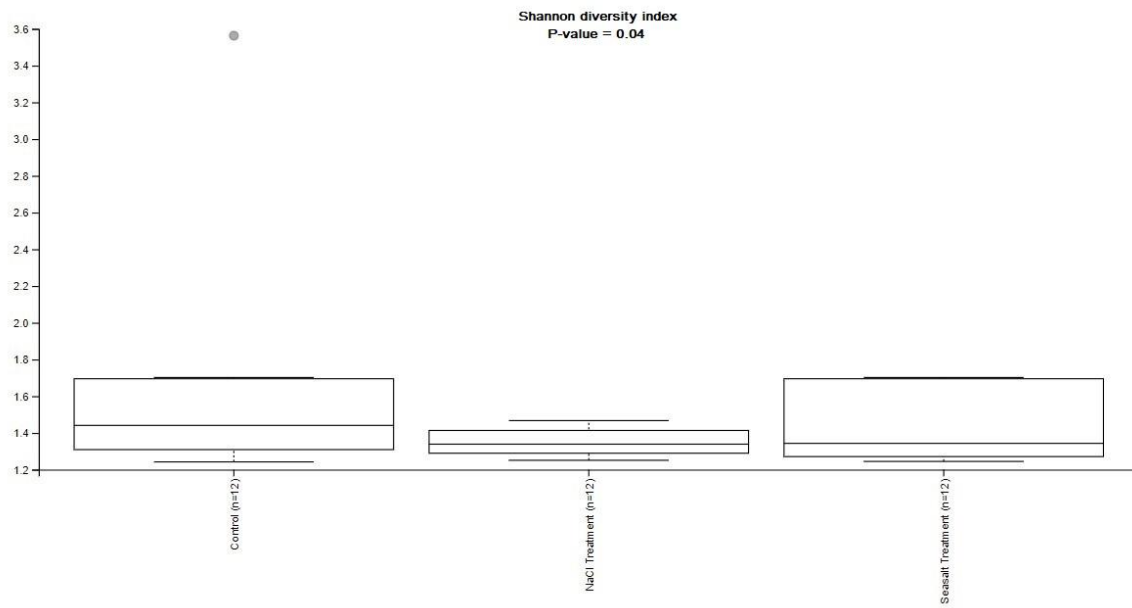
Figure S6: Shannon Index for *mcrA* gene:
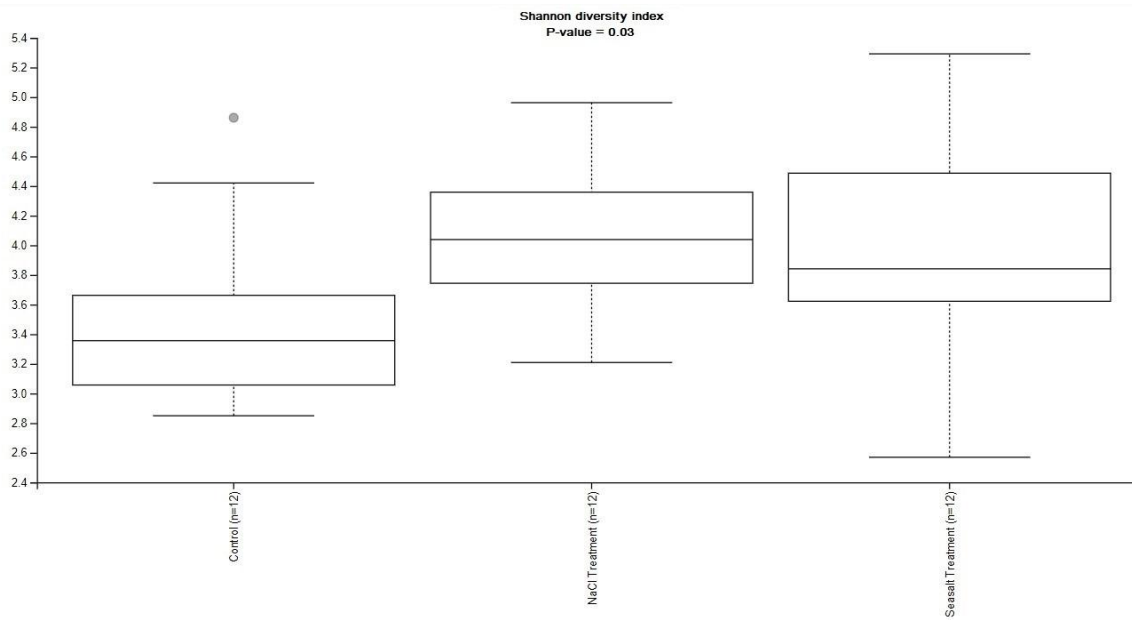
7-References

A framework for human microbiome research (2012). Nature 486, 215–221.

Abdallah, M. Ben, Karray, F., Mhiri, N., Mei, N., Quéméneur, M., Cayol, J.-L., et al. (2016). Prokaryotic diversity in a Tunisian hypersaline lake, Chott El Jerid. Extremophiles 20, 125–138.

Adviento-Borbe JW, M. A. A. D., and Drijber A, R. A. D. (2006). TECHNICAL REPORTS-Atmospheric Pollutants and Trace Gases-Soil Electrical Conductivity and Water Content Affect Nitrous Oxide and Carbon Dioxide Emissions in Intensively Managed Soils. J. Environ. Qual. 35, 1999–2010.

Alexander, M. (1964). Biochemical ecology of soil microorganisms. Annu. Rev. Microbiol. 18, 217–250.

Alhraishawi, A. A., and Alani, W. K. (2018). The co-fermentation of organic substrates: A review performance of biogas production under different salt content. in Journal of Physics: Conference Series (IOP Publishing), 12041.

Ali, Y., Aslam, Z., Ashraf, M. Y., and Tahir, G. R. (2004). Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. Int. J. Environ. Sci. Technol. 1, 221–225. doi: 10.1007/BF03325836.

Ammari, T. G., Tahhan, R., Abubaker, S., Al-Zu'bi, Y., Tahboub, A., Ta'Any, R., et al. (2013). Soil salinity changes in the Jordan Valley potentially threaten sustainable irrigated agriculture. Pedosphere 23, 376–384.

Anderson, T. R., Hawkins, E., and Jones, P. D. (2016). CO2, the greenhouse effect and global warming: from the pioneering work of Arrhenius and Callendar to today's Earth System Models. Endeavour 40, 178–187.

Andreote, F. D., Jiménez, D. J., Chaves, D., Dias, A. C. F., Luvizotto, D. M., Dini-Andreote, F., et al. (2012). The microbiome of Brazilian mangrove sediments as revealed by metagenomics. PLoS One 7, e38600.

Andronov, E. E., Petrova, S. N., Pinaev, A. G., Pershina, E. V., Rakhimgalieva, S. Z., Akhmedenov, K. M., et al. (2012). Analysis of the structure of microbial community in soils with different degrees of salinization using T-RFLP and real-time PCR techniques. Eurasian Soil Sci. 45, 147–156. doi: 10.1134/S1064229312020044.

Appels, L., Baeyens, J., Degrève, J., and Dewil, R. (2008). Principles and potential of the anaerobic digestion of waste-activated sludge. Prog. energy Combust. Sci. 34, 755–781.

Ardón, M., Helton, A. M., and Bernhardt, E. S. (2018). Salinity effects on greenhouse gas emissions from wetland soils are contingent upon hydrologic setting: a microcosm experiment. Biogeochemistry 140, 217–232.

Argenzio, R. A., and Hintz, H. F. (1971). Volatile fatty acid tolerance and effect of glucose and VFA on plasma insulin levels in ponies. J. Nutr. 101, 723–729.

Asakawa, S., and Kimura, M. (2008). Comparison of bacterial community structures at main habitats in paddy field ecosystem based on DGGE analysis. Soil Biol. Biochem. 40, 1322–1329.

Asghar, H. N., Setia, R., and Marschner, P. (2012). Community composition and activity of microbes from saline soils and non-saline soils respond similarly to changes in salinity. Soil Biol. Biochem. 47, 175–178. doi: 10.1016/j.soilbio.2012.01.002.

Ashraf, M., and Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166, 3–16.

Baldwin, D. S., Rees, G. N., Mitchell, A. M., Watson, G., and Williams, J. (2006). The short-term effects of salinization on anaerobic nutrient cycling and microbial community structure in sediment from a freshwater wetland. Wetlands 26, 455–464.

Barich, D. (n.d.). MicrobeWiki. Available at: https://microbewiki.kenyon.edu.

Barmina, I., Lickrastina, A., Valdmanis, R., Zake, M., Arshanitsa, A., Solodovnik, V., et al. (2013). Effects of biomass composition variations on gasification and combustion characteristics. Eng. Rural Dev. 5, 23–24.

Bartram, A. K., Lynch, M. D. J., Stearns, J. C., Moreno-Hagelsieb, G., and Neufeld, J. D. (2011). Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. Appl. Environ. Microbiol. 77, 3846–3852.

Batra, L., and Manna, M. C. (1997). Dehydrogenase activity and microbial biomass carbon in salt-affected soils of semiarid and arid regions. Arid Soil Res. Rehabil. 11, 295–303. doi: 10.1080/15324989709381481.

Ben Abdallah, M., Karray, F., Kallel, N., Armougom, F., Mhiri, N., Quéméneur, M., et al. (2018). Abundance and diversity of prokaryotes in ephemeral hypersaline lake Chott El Jerid using Illumina Miseq sequencing, DGGE and qPCR assays. Extremophiles 22, 811–823.

Bernhard, A. E., Colbert, D., McManus, J., and Field, K. G. (2005). Microbial community dynamics based on 16S rRNA gene profiles in a Pacific Northwest estuary and its tributaries. FEMS Microbiol. Ecol. 52, 115–128.

Boer, W. de, Folman, L. B., Summerbell, R. C., and Boddy, L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol. Rev. 29, 795–811.

Bohn, H. L. (1971). Redox potentials. Soil Sci. 112, 39–45.

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., et al. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59.

Brandt, K. K., Vester, F., Jensen, A. N., and Ingvorsen, K. (2001). Sulfate reduction dynamics and enumeration of sulfate-reducing bacteria in hypersaline sediments of the Great Salt Lake (Utah, USA). Microb. Ecol. 41, 1–11.

Breidenbach, B., and Conrad, R. (2015). Seasonal dynamics of bacterial and archaeal methanogenic communities in flooded rice fields and effect of drainage. Front. Microbiol. 5, 752.

Breidenbach, B., Blaser, M. B., Klose, M., and Conrad, R. (2016). Crop rotation of flooded rice with upland maize impacts the resident and active methanogenic microbial community. Environ. Microbiol. 18, 2868–2885.

Breidenbach, B., Brenzinger, K., Brandt, F. B., Blaser, M. B., and Conrad, R. (2017). The effect of crop rotation between wetland rice and upland maize on the microbial communities associated with roots. Plant Soil 419, 435–445.

Brigham, B. A., Montero, A. D., O'Mullan, G. D., and Bird, J. A. (2018). Acetate additions stimulate CO2 and CH4 production from urban wetland soils. Soil Sci. Soc. Am. J. 82, 1147–1159.

Brzezińska, M. (2004). Aeration status of soil and enzyme activity. Soil–plant–atmosph. AERATION Environ. Probl., 55.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., et al. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624.

Casañas, M. A. A., Rangkasenee, N., Krattenmacher, N., Thaller, G., Metges, C. C., and Kuhla, B. (2015). Methyl-coenzyme M reductase A as an indicator to estimate methane production from dairy cows. J. Dairy Sci. 98, 4074–4083.

Chambers, L. G., Davis, S. E., Troxler, T., Boyer, J. N., Downey-Wall, A., and Scinto, L. J. (2014). Biogeochemical effects of simulated sea level rise on carbon loss in an Everglades mangrove peat soil. Hydrobiologia 726, 195–211.

Change, W. G. I. I. I. of the I. P. on C. (2014). Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, eds Edenhofer O, et al. ed. Cambridge Univ Press. Cambridge, UK.

Che, R., Wang, Y., Li, K., Xu, Z., Hu, J., Wang, F., et al. (2019). Degraded patch formation significantly changed microbial community composition in alpine meadow soils. Soil Tillage Res. 195, 104426. doi: 10.1016/j.still.2019.104426.

Chen, F. H., Dong, G. H., Zhang, D. J., Liu, X. Y., Jia, X., An, C. B., et al. (2015). Agriculture facilitated permanent human occupation of the Tibetan Plateau after 3600 B.P. Science (80-.). 347, 248–250. doi: 10.1126/science.1259172.

Chen, L., Li, C., Feng, Q., Wei, Y., Zheng, H., Zhao, Y., et al. (2017). Shifts in soil microbial metabolic activities and community structures along a salinity gradient of irrigation water in a typical arid region of China. Sci. Total Environ. 598, 64–70.

Chen, Y., Cheng, J. J., and Creamer, K. S. (2008). Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, 4044–4064.

Chen, Y., Cheng, J. J., and Creamer, K. S. (2008). Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, 4044–4064.

Chin, K.-J., Hahn, D., Hengstmann, U. L. F., Liesack, W., and Janssen, P. H. (1999). Characterization and identification of numerically abundant culturable bacteria from the anoxic bulk soil of rice paddy microcosms. Appl. Environ. Microbiol. 65, 5042–5049.

Chivenge, P., Rubianes, F., Van Chin, D., Van Thach, T., Khang, V. T., Romasanta, R. R., et al. (2020). Rice straw incorporation influences nutrient cycling and soil organic matter. Sustain. rice straw Manag., 131–144.

Chou, C.-S., Lin, S.-H., and Lu, W.-C. (2009). Preparation and characterization of solid biomass fuel made from rice straw and rice bran. Fuel Process. Technol. 90, 980–987.

Chowdhury, N., Marschner, P., and Burns, R. G. (2011a). Soil microbial activity and community composition: impact of changes in matric and osmotic potential. Soil Biol. Biochem. 43, 1229–1236.

Chowdhury, N., Nakatani, A. S., Setia, R., and Marschner, P. (2011b). Microbial activity and community composition in saline and non-saline soils exposed to multiple drying and rewetting events. Plant Soil 348, 103–113.

Chu, H., Wang, S., Yue, H., Lin, Q., Hu, Y., Li, X., et al. (2014). Contrasting soil microbial community functional structures in two major landscapes of the <scp>T</scp> ibetan alpine meadow. Microbiologyopen 3, 585–594. doi: 10.1002/mbo3.190.

Consortium, H. M. P. (2012). Structure, function and diversity of the healthy human microbiome. Nature 486, 207–214.

Corder, R. E., Hook, L. A., Larkin, J. M., and Frea, J. I. (1983). Isolation and characterization of two new methane-producing cocci: Methanogenium olentangi, sp. nov., and Methanococcus deltae, sp. nov. Arch. Microbiol. 134, 28–32.

Cornwell, J. C. (2013). Measurement of Sulfate Reduction in Wetland Soils. Methods Biogeochem. Wetl. 10, 765–773.

Cornwell, W. K., Cornelissen, J. H. C., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., et al. (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol. Lett. 11, 1065–1071.

Corwin, D. L., and Yemoto, K. (2017). Salinity: Electrical Conductivity and Total Dissolved Solids. Methods Soil Anal. 2. doi: 10.2136/msa2015.0039.

Covey, K. R., and Megonigal, J. P. (2019). Methane production and emissions in trees and forests. New Phytol. 222, 35–51.

Csonka, L. N. (1989). Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev. 53, 121–147. doi: 10.1128/mr.53.1.121-147.1989.

Dalal, R. C., Allen, D. E., Livesley, S. J., and Richards, G. (2008). Magnitude and biophysical regulators of methane emission and consumption in the Australian agricultural, forest, and submerged landscapes: a review. Plant Soil 309, 43–76.

Daliakopoulos, I. N., Tsanis, I. K., Koutroulis, A., Kourgialas, N. N., Varouchakis, A. E., Karatzas, G. P., et al. (2016a). The threat of soil salinity: A European scale review. Sci. Total Environ. 573, 727–739.

Daliakopoulos, I. N., Tsanis, I. K., Koutroulis, A., Kourgialas, N. N., Varouchakis, A. E., Karatzas, G. P., et al. (2016b). The threat of soil salinity: A European scale review. Sci. Total Environ. 573, 727–739. doi: 10.1016/j.scitotenv.2016.08.177.

Dalla Vecchia, E., Suvorova, E. I., Maillard, J., and Bernier-Latmani, R. (2014). Fe (III) reduction during pyruvate fermentation by D esulfotomaculum reducens strain MI-1. Geobiology 12, 48–61.

Dang, D. M., Macdonald, B., Warneke, S., and White, I. (2016). Available carbon and nitrate increase greenhouse gas emissions from soils affected by salinity. Soil Res. 55, 47–57.

Davenport, R., James, R. A., Zakrisson-Plogander, A., Tester, M., and Munns, R. (2005). Control of sodium transport in durum wheat. Plant Physiol. 137, 807–818.

De Gara, L., Locato, V., Dipierro, S., and de Pinto, M. C. (2010). Redox homeostasis in plants. The challenge of living with endogenous oxygen production. Respir. Physiol. Neurobiol. 173, S13–S19.

del Carmen Orozco-Mosqueda, M., Glick, B. R., and Santoyo, G. (2020). ACC deaminase in plant growth-promoting bacteria (PGPB): an efficient mechanism to counter salt stress in crops. Microbiol. Res. 235, 126439.

Del Moral, A., Quesada, E., and Ramos-Cormenzana, A. (1987). Distribution and types of bacteria isolated from an inland saltern. in Annales de l'Institut Pasteur/Microbiologie (Elsevier), 59–66.

Deng, Q., Wu, X., Wang, Y., and Liu, M. (2018). Activity characteristics of sulfate reducing bacteria and formation mechanism of hydrogen sulfide. Appl. Ecol. Environ. Res. 16, 6369–6383.

Dessaux, Y., Hinsinger, P., and Lemanceau, P. (2009). Rhizosphere: so many achievements and even more challenges. Plant Soil 321, 1–3.

Deublein, D., and Steinhauser, A. (2011). Biogas from waste and renewable resources: an introduction. John Wiley & Sons.

Dick, R. P. (1994). Soil enzyme activities as indicators of soil quality. Defin. soil Qual. a Sustain. Environ. 35, 107–124.

Dietz, K.-J. (2003). Redox control, redox signaling, and redox homeostasis in plant cells. Int Rev Cytol 228, 141–193.

Domingues, C. M., Church, J. A., White, N. J., Gleckler, P. J., Wijffels, S. E., Barker, P. M., et al. (2008). Improved estimates of upper-ocean warming and multi-decadal sea-level rise. Nature 453, 1090–1093. doi: 10.1038/nature07080.

Dos Santos, H. F., Cury, J. C., Do Carmo, F. L., Dos Santos, A. L., Tiedje, J., van Elsas, J. D., et al. (2011).Mangrove bacterial diversity and the impact of oil contamination revealed by pyrosequencing: bacterial proxies for oil pollution. PLoS One 6, e16943.

Duan, F., Chyang, C.-S., Zhang, L., and Yin, S.-F. (2015). Bed agglomeration characteristics of rice straw combustion in a vortexing fluidized-bed combustor. Bioresour. Technol. 183, 195–202.

Duarte, M. S., Oliveira, J. V, Pereira, C., Carvalho, M., Mesquita, D. P., and Alves, M. M. (2021). Volatile Fatty Acids (VFA) Production from Wastewaters with High Salinity—Influence of pH, Salinity and Reactor Configuration. Fermentation 7, 303.

Eastman, J. A., and Ferguson, J. F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. J. (Water Pollut. Control Fed., 352–366.

Falkowski, P. G., Fenchel, T., and Delong, E. F. (2008). The microbial engines that drive Earth's biogeochemical cycles. Science (80-.). 320, 1034–1039.

Fang, C., Boe, K., and Angelidaki, I. (2011). Anaerobic co-digestion of desugared molasses with cow manure; focusing on sodium and potassium inhibition. Bioresour. Technol. 102, 1005–1011.

Fischer, H., Mille-Lindblom, C., Zwirnmann, E., and Tranvik, L. J. (2006). Contribution of fungi and bacteria to the formation of dissolved organic carbon from decaying common reed (Phragmites australis). Arch. für Hydrobiol. 166, 79–97.

Fitter, A. H., Gilligan, C. A., Hollingworth, K., Kleczkowski, A., Twyman, R. M., Pitchford, J. W., et al. (2005). Biodiversity and ecosystem function in soil. Funct. Ecol. 19, 369–377.

Flowers, T. J., and Flowers, S. A. (2005). Why does salinity pose such a difficult problem for plant breeders? Agric. water Manag. 78, 15–24.

Foti, M., Sorokin, D. Y., Lomans, B., Mussman, M., Zacharova, E. E., Pimenov, N. V, et al. (2007). Diversity, activity, and abundance of sulfate-reducing bacteria in saline and hypersaline soda lakes. Appl. Environ. Microbiol. 73, 2093–2100.

Foyer, C. H., and Noctor, G. (2005). Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17, 1866–1875.

Frankenberger Jr, Wt., and Bingham, F. T. (1982). Influence of salinity on soil enzyme activities. Soil Sci. Soc. Am. J. 46, 1173–1177.

Fuhrman, J. A. (2009). Microbial community structure and its functional implications. Nature 459, 193–199.

Fukuda, K., Ogawa, M., Taniguchi, H., and Saito, M. (2016). Molecular approaches to studying microbial communities: targeting the 16S ribosomal RNA gene. J. UOEH 38, 223–232.

Garcia, C., and Hernandez, T. (1996). Influence of salinity on the biological and biochemical activity of a calciorthird soil. Plant Soil 178, 255–263.

Gerardi, M. H. (2003). The microbiology of anaerobic digesters. John Wiley & Sons.

Giller, K. E., and Cadisch, G. (1995). Future benefits from biological nitrogen fixation: an ecological approach to agriculture. in Management of Biological Nitrogen Fixation for the Development of More Productive and Sustainable Agricultural Systems: Extended versions of papers presented at the Symposium on Biological Nitrogen Fixation for Sustainable Agriculture at the 15th Congress (Springer), 255–277.

Gkiougkis, I., Kallioras, A., Pliakas, F., Pechtelidis, A., Diamantis, V., Diamantis, I., et al. (2015). Assessment of soil salinization at the eastern Nestos River Delta, N.E. Greece. CATENA 128, 238–251. doi: 10.1016/j.catena.2014.06.024.

Gourdon, R., Comel, C., Vermande, P., and Veron, J. (1989). Fractionation of the organic matter of a landfill leachate before and after aerobic or anaerobic biological treatment. Water Res. 23, 167–173.

Green, A. J., Alcorlo, P., Peeters, E. T. H. M., Morris, E. P., Espinar, J. L., Bravo-Utrera, M. A., et al. (2017). Creating a safe operating space for wetlands in a changing climate. Front. Ecol. Environ. 15, 99–107.

Gros, R., Poly, F., Jocteur Monrozier, L., and Faivre, P. (2003). Plant and soil microbial community responses to solid waste leachates diffusion on grassland. Plant Soil 255, 445–455.

Großkopf, R., Stubner, S., and Liesack, W. (1998). Novel euryarchaeotal lineages detected on rice roots and in the anoxic bulk soil of flooded rice microcosms. Appl. Environ. Microbiol. 64, 4983–4989.

Gubry-Rangin, C., Hai, B., Quince, C., Engel, M., Thomson, B. C., James, P., et al. (2011). Niche specialization of terrestrial archaeal ammonia oxidizers. Proc. Natl. Acad. Sci. 108, 21206–21211.

Guérin, B. (2004). Bioénergétique. EDP sciences Les Ulis, France.

Hairmansis, A., Berger, B., Tester, M., and Roy, S. J. (2014). Image-based phenotyping for non-destructive screening of different salinity tolerance traits in rice. Rice 7, 1–10.

Handelsman, J. (2004). Metagenomics: application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev. 68, 669–685.

HARATIAN, A., and MEYBODI, S. E. (2021). Review on Microbial Enhanced Oil Recovery and Controlling Its Produced Hydrogen Sulfide Effects on Reservoir and Transporting Pipelines. Eurasia Proc. Sci. Technol. Eng. Math. 15, 88–98.

Hariadi, Y. C., Nurhayati, A. Y., Soeparjono, S., and Arif, I. (2015). Screening Six Varieties of Rice (Oryzasativa) for Salinity Tolerance. Procedia Environ. Sci. 28, 78–87. doi: 10.1016/j.proenv.2015.07.012.

Hasbullah, H., and Marschner, P. (2015). Residue properties influence the impact of salinity on soil respiration. Biol. Fertil. Soils 51, 99–111.

Hättenschwiler, S., Tiunov, A. V, and Scheu, S. (2005). Biodiversity and litter decomposition in terrestrial ecosystems. Annu. Rev. Ecol. Evol. Syst. 36, 191–218.

Heidelberg, J. F., Seshadri, R., Haveman, S. A., Hemme, C. L., Paulsen, I. T., Kolonay, J. F., et al. (2004). The genome sequence of the anaerobic, sulfate-reducing bacterium Desulfovibrio vulgaris Hildenborough. Nat. Biotechnol. 22, 554–559.

Heintze, S. G. (1934). The use of the glass electrode in soil reaction and oxidation-reduction potential measurements. J. Agric. Sci. 24, 28–41.

Heinz, E., Kraft, P., Buchen, C., Frede, H.-G., Aquino, E., and Breuer, L. (2013). Set up of an automatic water quality sampling system in irrigation agriculture. Sensors 14, 212–228.

Henckel, T., Friedrich, M., and Conrad, R. (1999). Molecular analyses of the methane-oxidizing microbial community in rice field soil by targeting the genes of the 16S rRNA, particulate methane monooxygenase, and methanol dehydrogenase. Appl. Environ. Microbiol. 65, 1980–1990.

Herbert, E. R., Schubauer-Berigan, J., and Craft, C. B. (2018). Differential effects of chronic and acute simulated seawater intrusion on tidal freshwater marsh carbon cycling. Biogeochemistry 138, 137–154.

Ho, A., Mo, Y., Lee, H. J., Sauheitl, L., Jia, Z., and Horn, M. A. (2018). Effect of salt stress on aerobic methane oxidation and associated methanotrophs; a microcosm study of a natural community from a non-saline environment. Soil Biol. Biochem. 125, 210–214.

Horie, T., Karahara, I., and Katsuhara, M. (2012). Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. Rice 5, 1–18.

Hu, X., Liu, J., Liu, H., Zhuang, G., and Xun, L. (2018). Sulfur metabolism by marine heterotrophic bacteria involved in sulfur cycling in the ocean. Sci. China Earth Sci. 61, 1369–1378.

Huber, H., Thomm, M., König, H., Thies, G., and Stetter, K. O. (1982). Methanococcus thermolithotrophicus, a novel thermophilic lithotrophic methanogen. Arch. Microbiol. 132, 47–50.

Hurt, R. A., Qiu, X., Wu, L., Roh, Y., Palumbo, A. V, Tiedje, J. M., et al. (2001). Simultaneous recovery of RNA and DNA from soils and sediments. Appl. Environ. Microbiol. 67, 4495–4503.

Hussain, M., Jang, K. H., Farooq, M., and Lee, D. J. (2012). Morphological and physiological evaluation of Korean rice genotypes for salt resistance. Int J Agric Biol 14, 970–974.

Hussain, M., Park, H.-W., Farooq, M., Jabran, K., and Lee, D.-J. (2013). Morphological and Physiological Basis of Salt Resistance in Different Rice Genotypes. Int. J. Agric. Biol. 15.

Ibekwe, A. M., Poss, J. A., Grattan, S. R., Grieve, C. M., and Suarez, D. (2010). Bacterial diversity in cucumber (Cucumis sativus) rhizosphere in response to salinity, soil pH, and boron. Soil Biol. Biochem. 42, 567–575.

Ishii, M., and Kimoto, M. (2009). Reevaluation of historical ocean heat content variations with time-varying XBT and MBT depth bias corrections. J. Oceanogr. 65, 287–299. doi: 10.1007/s10872-009-0027-7.

Ito, T., Nielsen, J. L., Okabe, S., Watanabe, Y., and Nielsen, P. H. (2002). Phylogenetic identification and substrate uptake patterns of sulfate-reducing bacteria inhabiting an oxic-anoxic sewer biofilm determined by combining microautoradiography and fluorescent in situ hybridization. Appl. Environ. Microbiol. 68, 356–364.

Itoh, H., Ishii, S., Shiratori, Y., Oshima, K., Otsuka, S., Hattori, M., et al. (2013). Seasonal transition of active bacterial and archaeal communities in relation to water management in paddy soils. Microbes Environ. 28, 370–380.

Jackson, C. R., and Vallaire, S. C. (2009). Effects of salinity and nutrients on microbial assemblages in Louisiana wetland sediments. Wetlands 29, 277–287.

Jackson, R. B., Solomon, E. I., Canadell, J. G., Cargnello, M., Field, C. B., and Abernethy, S. (2020b). Reply to: Practical constraints on atmospheric methane removal. Nat. Sustain. 3, 358–359. doi: 10.1038/s41893-020-0498-5.

Janssen, P. H., Schuhmann, A., Mörschel, E., and Rainey, F. A. (1997). Novel anaerobic ultramicrobacteria belonging to the Verrucomicrobiales lineage of bacterial descent isolated by dilution culture from anoxic rice paddy soil. Appl. Environ. Microbiol. 63, 1382–1388.

Jenkins, B. M., Bakker, R. R., and Wei, J. B. (1996). On the properties of washed straw. Biomass and bioenergy 10, 177–200.

Johnson, J. B., Peat, S. M., and Adams, B. J. (2009). Where's the ecology in molecular ecology? Oikos 118, 1601–1609.

Jones, W. J., Paynter, M. J. B., and Gupta, R. (1983). Characterization of Methanococcus maripaludis sp. nov., a new methanogen isolated from salt marsh sediment. Arch. Microbiol. 135, 91–97.

Jördening, H.-J., and Winter, J. (2005). Environmental biotechnology: concepts and applications.

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

Jung, K. A., Lim, S.-R., Kim, Y., and Park, J. M. (2013). Potentials of macroalgae as feedstocks for biorefinery. Bioresour. Technol. 135, 182–190.

Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C., and D'Hondt, S. (2012). Global distribution of microbial abundance and biomass in subseafloor sediment. Proc. Natl. Acad. Sci. 109, 16213–16216.

Kargbo, F. R., Xing, J., and Zhang, Y. (2010). Property analysis and pretreatment of rice straw for energy use in grain drying: A review. Agric. Biol. JN Am 1, 195–200.

Kargi, F., and Dincer, A. R. (1996). Effect of salt concentration on biological treatment of saline wastewater by fed-batch operation. Enzyme Microb. Technol. 19, 529–537.

Katiraei, S., Anvar, Y., Hoving, L., Berbée, J. F. P., van Harmelen, V., and Willems van Dijk, K. (2022). Evaluation of full-length versus V4-region 16S rRNA sequencing for phylogenetic analysis of mouse intestinal microbiota after a dietary intervention. Curr. Microbiol. 79, 276.

Keller, J. K., Bauers, A. K., Bridgham, S. D., Kellogg, L. E., and Iversen, C. M. (2006b). Nutrient control of microbial carbon cycling along an ombrotrophic-minerotrophic peatland gradient. J. Geophys. Res. Biogeosciences 111.

Kempf, B., and Bremer, E. (1998). Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. Arch. Microbiol. 170, 319–330.

Keren, R. (2000). Salinity, p. G3-G25. Handb. soil Sci. CRC Press. Boca Raton, USA.

Khan, K. S., Mack, R., Castillo, X., Kaiser, M., and Joergensen, R. G. (2016). Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. Geoderma 271, 115–123. doi: 10.1016/j.geoderma.2016.02.019.

Khandekar, M. L., Murty, T. S., and Chittibabu, P. (2005). The global warming debate: A review of the state of science. Pure Appl. Geophys. 162, 1557–1586.

Killham, K. (1994). Soil ecology. Cambridge University Press.

Kimbrough, D. E., Kouame, Y., Moheban, P., and Springthorpe, S. (2006). The effect of electrolysis and oxidation–reduction potential on microbial survival, growth, and disinfection. Int. J. Environ. Pollut. 27, 211–221.

Kimura, M., Miyaki, M., Fujinaka, K.-I., and Maie, N. (2001). Microbiota responsible for the decomposition of rice straw in a submerged paddy soil estimated from phospholipid fatty acid composition. Soil Sci. Plant Nutr. 47, 569–578.

Koeck, D. E., Pechtl, A., Zverlov, V. V, and Schwarz, W. H. (2014). Genomics of cellulolytic bacteria. Curr. Opin. Biotechnol. 29, 171–183.

Kögel-Knabner, I., Amelung, W., Cao, Z., Fiedler, S., Frenzel, P., Jahn, R., et al. (2010). Biogeochemistry of paddy soils. Geoderma 157, 1–14.

Kondo, R., Nedwell, D. B., Purdy, K. J., and Silva, S. Q. (2004). Detection and enumeration of sulphate-reducing bacteria in estuarine sediments by competitive PCR. Geomicrobiol. J. 21, 145–157.

Kontopoulou, C.-K., Bilalis, D., Pappa, V. A., Rees, R. M., and Savvas, D. (2015). Effects of organic farming practices and salinity on yield and greenhouse gas emissions from a common bean crop. Sci. Hortic. (Amsterdam). 183, 48–57.

Kováčová, V., and Velísková, Y. (2012). The Risk of the Soil Salinization of the Eastern Part of Žitný Ostrov. J. Hydrol. Hydromechanics 60. doi: 10.2478/v10098-012-0005-4.

Kralova, M., Masscheleyn, P. H., and Patrick Jr, W. H. (1992). Redox potential as an indicator of electron availability for microbial activity and nitrogen transformations in aerobic soil. Zentralbl. Mikrobiol. 147, 388–399.

Kuczynski, J., Lauber, C. L., Walters, W. A., Parfrey, L. W., Clemente, J. C., Gevers, D., et al. (2012). Experimental and analytical tools for studying the human microbiome. Nat. Rev. Genet. 13, 47–58.

Kumar, U., Panneerselvam, P., Gupta, V. V. S. R., Manjunath, M., Priyadarshinee, P., Sahoo, A., et al. (2018). Diversity of sulfur-oxidizing and sulfur-reducing microbes in diverse ecosystems. Adv. Soil Microbiol. Recent Trends Futur. Prospect. Vol. 1 Soil-Microbe Interact., 65–89.

Lambers, H., Chapin III, F. S., and Pons, T. L. (2008). Plant physiological ecology.,(Springer: New York).

Lan, X., Thoning, K. W., and Dlugokencky, E. J. (2022). Trends in globally-averaged CH4, N2O, and SF6 determined from NOAA Global Monitoring Laboratory measurements.

Laura, R. D. (1974). Effects of neutral salts on carbon and nitrogen mineralisation of organic matter in soil. Plant Soil 41, 113–127.

Lefebvre, O., Quentin, S., Torrijos, M., Godon, J.-J., Delgenes, J. P., and Moletta, R. (2007). Impact of increasing NaCl concentrations on the performance and community composition of two anaerobic reactors. Appl. Microbiol. Biotechnol. 75, 61–69.

Leibniz Institute DSMZ Website (n.d.). Available at: www.dsmz.de.

Levin, S. A. (2006). Fundamental questions in biology. PLoS Biol. 4, e300.

Levitus, S., Antonov, J. I., Boyer, T. P., Locarnini, R. A., Garcia, H. E., and Mishonov, A. V. (2009). Global ocean heat content 1955-2008 in light of recently revealed instrumentation problems. Geophys. Res. Lett. 36, n/a-n/a. doi: 10.1029/2008GL037155.

Li, H. Y., Webster, R., and Shi, Z. (2015). Mapping soil salinity in the Yangtze delta: REML and universal kriging (E-BLUP) revisited. Geoderma 237–238, 71–77. doi: 10.1016/j.geoderma.2014.08.008.

Li, S., Song, L., Gao, X., Jin, Y., Liu, S., Shen, Q., et al. (2017). Microbial abundances predict methane and nitrous oxide fluxes from a windrow composting system. Front. Microbiol. 8, 409.

Li, X., Sun, M., Zhang, H., Xu, N., and Sun, G. (2016). Use of mulberry–soybean intercropping in salt–alkali soil impacts the diversity of the soil bacterial community. Microb. Biotechnol. 9, 293–304. doi: 10.1111/1751-7915.12342.

Li, X., Wan, W., Luo, X., Zheng, L., He, G., Huang, D., et al. (2021). High salinity inhibits soil bacterial community mediating nitrogen cycling. Appl. Environ. Microbiol. 87, e01366-21.

Lindroth, A., Lund, M., Nilsson, M., Aurela, M., Christensen, T. R., Laurila, T., et al. (2007). Environmental controls on the CO2 exchange in north European mires. Tellus B Chem. Phys. Meteorol. 59, 812. doi: 10.1111/j.1600-0889.2007.00310.x.

Liu, P., and Conrad, R. (2017). Syntrophobacteraceae-affiliated species are major propionate-degrading sulfate reducers in paddy soil. Environ. Microbiol. 19, 1669–1686.

Liu, P., Klose, M., and Conrad, R. (2019). Temperature-dependent network modules of soil methanogenic bacterial and archaeal communities. Front. Microbiol. 10, 496.

Liu, P., Pommerenke, B., and Conrad, R. (2018). Identification of Syntrophobacteraceae as major acetate-degrading sulfate reducing bacteria in Italian paddy soil. Environ. Microbiol. 20, 337–354.

Liu, X., Liu, H., Peng, L., and Su, H. (2020). Influence of ammonium acetate and betaine supplements on the anaerobic digestion under high salinity conditions. Energy Sci. Eng. 8, 2621–2627.

Liu, Z., Xu, A., and Long, B. (2011). Energy from combustion of rice straw: status and challenges to China. Energy Power Eng. 3, 325.

Loman, N. J., Constantinidou, C., Chan, J. Z. M., Halachev, M., Sergeant, M., Penn, C. W., et al. (2012a). High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. Nat. Rev. Microbiol. 10, 599–606.

Loman, N. J., Misra, R. V, Dallman, T. J., Constantinidou, C., Gharbia, S. E., Wain, J., et al. (2012b). Performance comparison of benchtop high-throughput sequencing platforms. Nat. Biotechnol. 30, 434–439.

Lopes, A. R., Manaia, C. M., and Nunes, O. C. (2014). Bacterial community variations in an alfalfa-rice rotation system revealed by 16S rRNA gene 454-pyrosequencing. FEMS Microbiol. Ecol. 87, 650–663.

Lopes, S. I. C., and Lens, P. N. L. (2011). "Anaerobic Treatment of Organic Sulfate-Rich Wastewaters," in Comprehensive Biotechnology (Elsevier), 399–418. doi: 10.1016/B978-0-08-088504-9.00469-4.

Lopez-Cortes, A., Fardeau, M.-L., Fauque, G., Joulian, C., and Ollivier, B. (2006). Reclassification of the sulfate-and nitrate-reducing bacterium Desulfovibrio vulgaris subsp. oxamicus as Desulfovibrio oxamicus sp. nov., comb. nov. Int. J. Syst. Evol. Microbiol. 56, 1495–1499.

Lovley, D. R., and Klug, M. J. (1983). Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations. Appl. Environ. Microbiol. 45, 187–192.

Lovley, D. R., Roden, E. E., Phillips, E. J. P., and Woodward, J. C. (1993). Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar. Geol. 113, 41–53.

Loy, A., Lehner, A., Lee, N., Adamczyk, J., Meier, H., Ernst, J., et al. (2002). Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. Appl. Environ. Microbiol. 68, 5064–5081.

Lu, Y., Rosencrantz, D., Liesack, W., and Conrad, R. (2006). Structure and activity of bacterial community inhabiting rice roots and the rhizosphere. Environ. Microbiol. 8, 1351–1360.

Lu, Y., Watanabe, A., and Kimura, M. (2003). Carbon dynamics of rhizodeposits, root-and shoot-residues in a rice soil. Soil Biol. Biochem. 35, 1223–1230.

Luo, M., Huang, J.-F., Zhu, W.-F., and Tong, C. (2019). Impacts of increasing salinity and inundation on rates and pathways of organic carbon mineralization in tidal wetlands: a review. Hydrobiologia 827, 31–49.

Ma, L., Ma, F., Li, J., Gu, Q., Yang, S., Wu, D., et al. (2017). Characterizing and modeling regional-scale variations in soil salinity in the arid oasis of Tarim Basin, China. Geoderma 305, 1–11. doi: 10.1016/j.geoderma.2017.05.016.

Mahabubur Rahman, M., and Yamamoto, A. (2020). "Methane Cycling in Paddy Field: A Global Warming Issue," in Agrometeorology (IntechOpen). doi: 10.5772/intechopen.94200.

Mansaray, K. G., and Ghaly, A. E. (1997). Physical and thermochemical properties of rice husk. Energy sources 19, 989–1004.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., and Ågren, G. I. (2012). Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196, 79–91.

Marino, D., Pucciariello, C., Puppo, A., and Frendo, P. (2009). The redox state, a referee of the legume–rhizobia symbiotic game. Adv. Bot. Res. 52, 115–151.

Martinez-Romero, E. (2006). Dinitrogen-fixing prokaryotes. The prokaryotes 2, 793.

Marton, J. M., Herbert, E. R., and Craft, C. B. (2012). Effects of salinity on denitrification and greenhouse gas production from laboratory-incubated tidal forest soils. Wetlands 32, 347–357.

Mathis, P. (1995). Photosynthesis: From Light to Biosphere: Proceedings of the Xth International Photosynthesis Congress, Montpellier, France, 20-25 August 1995. Kluwer Academic Pub.

Matsuyama, T., Nakajima, Y., Matsuya, K., Ikenaga, M., Asakawa, S., and Kimura, M. (2007). Bacterial community in plant residues in a Japanese paddy field estimated by RFLP and DGGE analyses. Soil Biol. Biochem. 39, 463–472.

Maucieri, C., Zhang, Y., McDaniel, M. D., Borin, M., and Adams, M. A. (2017). Short-term effects of biochar and salinity on soil greenhouse gas emissions from a semi-arid Australian soil after re-wetting. Geoderma 307, 267–276.

Meadows, P. S., Reichelt, A. C., Meadows, A., and Waterworth, J. S. (1994). Microbial and meiofaunal abundance, redox potential, pH and shear strength profiles in deep sea Pacific sediments. J. Geol. Soc. London. 151, 377–390.

Megonigal, J. P., and Neubauer, S. C. (2019). "Biogeochemistry of tidal freshwater wetlands," in Coastal Wetlands (Elsevier), 641–683.

Meng, X., Zhou, J., and Sui, N. (2018). Mechanisms of salt tolerance in halophytes: Current understanding and recent advances. Open life Sci. 13, 149–154.

Metzker, M. L. (2010). Sequencing technologies—the next generation. Nat. Rev. Genet. 11, 31–46.

Migo, M. V. P. (2019). Optimization and life cycle assessment of the direct combustion of rice straw using a small scale, stationary grate furnace for heat generation. Unpubl. Masters thesis. Univ. Philipp. Los Baños.

Miller, F. C., Macauley, B. J., and Harper, E. R. (1991). Investigation of various gases, pH and redox potential in mushroom composting phase I stacks. Aust. J. Exp. Agric. 31, 415–423.

Mitra, S., Wassmann, R., and Vlek, P. L. G. (2005). An appraisal of global wetland area and its organic carbon stock. Curr. Sci. 88, 25–35.

Morrison, E., Newman, S., Bae, H. S., He, Z., Zhou, J., Reddy, K. R., et al. (2016). Microbial genetic and enzymatic responses to an anthropogenic phosphorus gradient within a subtropical peatland. Geoderma 268, 119–127.

Morrissey, E. M., Gillespie, J. L., Morina, J. C., and Franklin, R. B. (2014). Salinity affects microbial activity and soil organic matter content in tidal wetlands. Glob. Chang. Biol. 20, 1351–1362.

Mottet, A., Habouzit, F., and Steyer, J. P. (2014). Anaerobic digestion of marine microalgae in different salinity levels. Bioresour. Technol. 158, 300–306.

Munder, S., Karaj, S., Gummert, M., Haefele, S. M., and Müller, J. (2013). Improving thermal conversion properties of rice straw by briquetting.

Nakamura, A., Tun, C. C., Asakawa, S., and Kimura, M. (2003). Microbial community responsible for the decomposition of rice straw in a paddy field: estimation by phospholipid fatty acid analysis. Biol. Fertil. Soils 38, 288–295.

Ngan, N. V. C., Chan, F. M. S., Nam, T. S., Van Thao, H., Maguyon-Detras, M. C., Hung, D. V., et al. (2020). Anaerobic digestion of rice straw for biogas production. Sustain. rice straw Manag., 65–92.

Nguyen, V. H., Nguyen, T. N., Le, Q. V., Le, M. A., and Gummert, M. (2018). Developing densified products to reduce transportation costs and improve the quality of rice straw feedstocks for cattle feeding. J. Vietnamese Environ. 10, 11–15.

Niklaus, P. A., Wardle, D. A., and Tate, K. R. (2006). Effects of plant species diversity and composition on nitrogen cycling and the trace gas balance of soils. Plant Soil 282, 83–98.

Norwood, M. J., Ward, N. D., McDowell, N. G., Myers-Pigg, A. N., Bond-Lamberty, B., Indivero, J., et al. (2021). Coastal forest seawater exposure increases stem methane concentration. J. Geophys. Res. Biogeosciences 126, e2020JG005915.

Odum, W. E. (1988). Comparative ecology of tidal freshwater and salt marshes. Annu. Rev. Ecol. Syst. 19, 147–176.

Oktyabrskiy, V. P. (2016). A new opinion of the greenhouse effect. St. Petersbg. Polytech. Univ. J. Phys. Math. 2, 124–126.

Ollivier, B., Caumette, P., Garcia, J.-L., and Mah, R. A. (1994). Anaerobic bacteria from hypersaline environments. Microbiol. Rev. 58, 27–38.

Omil Mendez, Ramon & Lema, Juan M, F. (1996). Anaerobic treatment of seafood processing waste waters in an industrial anaerobic pilot plant. Water Sa 22, 173–181.

Oremland, R. S., and King, G. M. (1989). Methanogenesis in hypersaline environments.

Oremland, R. S., and Polcin, S. (1982). Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. Appl. Environ. Microbiol. 44, 1270–1276.

Oremland, R. S., Marsh, L. M., and Polcin, S. (1982). Methane production and simultaneous sulphate reduction in anoxic, salt marsh sediments. Nature 296, 143–145.

Oren, A. (2001). The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implications for the functioning of salt lake ecosystems. in Saline Lakes: Publications from

the 7th International Conference on Salt Lakes, held in Death Valley National Park, California, USA, September 1999 (Springer), 61–72.

Pace, N. R. (1997). A molecular view of microbial diversity and the biosphere. Science (80-.). 276, 734–740.

Pan, C., Liu, C., Zhao, H., and Wang, Y. (2013). Changes of soil physico-chemical properties and enzyme activities in relation to grassland salinization. Eur. J. Soil Biol. 55, 13–19.

Pankhurst, C. E., Yu, S., Hawke, B. G., and Harch, B. D. (2001). Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biol. Fertil. Soils 33, 204–217.

Paterson, E., Osler, G., Dawson, L. A., Gebbing, T., Sim, A., and Ord, B. (2008). Labile and recalcitrant plant fractions are utilised by distinct microbial communities in soil: independent of the presence of roots and mycorrhizal fungi. Soil Biol. Biochem. 40, 1103–1113.

Pathak, H., and Rao, D. L. N. (1998). Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. Soil Biol. Biochem. 30, 695–702. doi: 10.1016/S0038-0717(97)00208-3.

Pearsall, W. H., and Mortimer, C. H. (1939). Oxidation-reduction potentials in waterlogged soils, natural waters and muds. J. Ecol., 483–501.

Pedraza-Zapata, D. C., Sánchez-Garibello, A. M., Quevedo-Hidalgo, B., Moreno-Sarmiento, N., and Gutiérrez-Rojas, I. (2017). Promising cellulolytic fungi isolates for rice straw degradation. J. Microbiol. 55, 711–719.

Peng, J., Wegner, C.-E., and Liesack, W. (2017). Short-term exposure of paddy soil microbial communities to salt stress triggers different transcriptional responses of key taxonomic groups. Front. Microbiol. 8, 400.

Peng, J., Wegner, C.-E., and Liesack, W. (2017). Short-term exposure of paddy soil microbial communities to salt stress triggers different transcriptional responses of key taxonomic groups. Front. Microbiol. 8, 400.

Peng, J., Wegner, C.-E., Bei, Q., Liu, P., and Liesack, W. (2018). Metatranscriptomics reveals a differential temperature effect on the structural and functional organization of the anaerobic food web in rice field soil. Microbiome 6, 1–16.

Penton, C. R., and Newman, S. (2007). Enzyme activity responses to nutrient loading in subtropical wetlands. Biogeochemistry 84, 83–98.

Pester, M., Rattei, T., Flechl, S., Gröngröft, A., Richter, A., Overmann, J., et al. (2012). amoA-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of amoA genes from soils of four different geographic regions. Environ. Microbiol. 14, 525–539.

Pezeshki, S. R. (2001). Wetland plant responses to soil flooding. Environ. Exp. Bot. 46, 299–312.

Poffenbarger, H. J., Needelman, B. A., and Megonigal, J. P. (2011). Salinity influence on methane emissions from tidal marshes. Wetlands 31, 831–842.

Poffenbarger, H. J., Needelman, B. A., and Megonigal, J. P. (2011). Salinity influence on methane emissions from tidal marshes. Wetlands 31, 831–842.

Postgate, J. R. (1982). The fundamentals of nitrogen fixation. Cambridge University Press.

Qadir, M., Ghafoor, A., and Murtaza, G. (2000). Amelioration strategies for saline soils: a review. L. Degrad. Dev. 11, 501–521.

Qi, Q., Zhao, M., Wang, S., Ma, X., Wang, Y., Gao, Y., et al. (2017). The Biogeographic Pattern of Microbial Functional Genes along an Altitudinal Gradient of the Tibetan Pasture. Front. Microbiol. 8. doi: 10.3389/fmicb.2017.00976.

Quesada, E., Ventosa, A., Rodriguez-Valera, F., and Ramos-Cormenzana, A. (1982). Types and properties of some bacteria isolated from hypersaline soils. J. Appl. Bacteriol. 53, 155–161.

Quince, C., Curtis, T. P., and Sloan, W. T. (2008). The rational exploration of microbial diversity. ISME J. 2, 997–1006.

Quintero, J. M., Fournier, J. M., and Benlloch, M. (2007). Na+ accumulation in shoot is related to water transport in K+-starved sunflower plants but not in plants with a normal K+ status. J. Plant Physiol. 164, 60–67.

Rabotnova, I. L. (1963). The importance of physical-chemical factors (pH and rH2) for the life activity of microorganisms. ARMY BIOLOGICAL LABS FREDERICK MD.

Rahman, M. M., and Yamamoto, A. (2020). "Methane cycling in paddy field: a global warming issue," in Agrometeorology (IntechOpen), 21.

Ramel, F., Brasseur, G., Pieulle, L., Valette, O., Hirschler-Réa, A., Fardeau, M. L., et al. (2015). Growth of the obligate anaerobe Desulfovibrio vulgaris Hildenborough under continuous low oxygen concentration sparging: impact of the membrane-bound oxygen reductases. PLoS One 10, e0123455.

Rath, K. M., Maheshwari, A., and Rousk, J. (2017). The impact of salinity on the microbial response to drying and rewetting in soil. Soil Biol. Biochem. 108, 17–26. doi: 10.1016/j.soilbio.2017.01.018.

Rath, K. M., Maheshwari, A., Bengtson, P., and Rousk, J. (2016). Comparative toxicities of salts on microbial processes in soil. Appl. Environ. Microbiol. 82, 2012–2020.

Rath, K. M., Murphy, D. N., and Rousk, J. (2019). The microbial community size, structure, and process rates along natural gradients of soil salinity. Soil Biol. Biochem. 138, 107607.

Ravi, R. K., Walton, K., and Khosroheidari, M. (2018). MiSeq: a next generation sequencing platform for genomic analysis. Dis. gene Identif. methods Protoc., 223–232.

Reddy, K. R., and DeLaune, R. D. (2008). Biogeochemistry of wetlands: science and applications. CRC press.

Reed, S. C., Cleveland, C. C., and Townsend, A. R. (2008). Tree species control rates of free-living nitrogen fixation in a tropical rain forest. Ecology 89, 2924–2934.

Rejmánková, E., and Sirová, D. (2007). Wetland macrophyte decomposition under different nutrient conditions: relationships between decomposition rate, enzyme activities and microbial biomass. Soil Biol. Biochem. 39, 526–538.

Rideout, J. R., Chase, J. H., Bolyen, E., Ackermann, G., González, A., Knight, R., et al. (2016). Keemei: cloud-based validation of tabular bioinformatics file formats in Google Sheets. Gigascience 5, s13742-016.

Rietz, D. N., and Haynes, R. J. (2003). Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biol. Biochem. 35, 845–854.

Robeson, M. S., O'Rourke, D. R., Kaehler, B. D., Ziemski, M., Dillon, M. R., Foster, J. T., et al. (2021). RESCRIPt: Reproducible sequence taxonomy reference database management. PLoS Comput. Biol. 17, e1009581.

Rodrigues, J. L., Wertz, J. T., Schmidt, T. M., and Breznak, J. A. (2004). A genomic DNA library of Verrucomicrobium isolated from termite guts reveals nitrogen fixation genes. in 10th International Symposium on Microbial Ecology ISME-10 Microbial Planet: Sub-surface to Space, Cancun, Mexico August, 22–27.

Roemmich, D., Belbéoch, M., Freeland, H., Garzoli, S., Gould, W. J., Grant, F., et al. (2009). Argo: The Challenge of Continuing 10 Years of Progress. Oceanography 22, 46–55. doi: 10.5670/oceanog.2009.65.

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584.

Roh, S. W., Abell, G. C. J., Kim, K.-H., Nam, Y.-D., and Bae, J.-W. (2010). Comparing microarrays and next-generation sequencing technologies for microbial ecology research. Trends Biotechnol. 28, 291–299.

Romaní, A. M., Fischer, H., Mille-Lindblom, C., and Tranvik, L. J. (2006). Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87, 2559–2569.

Romesser, J. A., Wolfe, R. S., Mayer, F., Spiess, E., and Walther-Mauruschat, A. (1979). Methanogenium, a new genus of marine methanogenic bacteria, and characterization of Methanogenium cariaci sp. nov. and Methanogenium marisnigri sp. nov. Arch. Microbiol. 121, 147–153.

Rondon, M. R., August, P. R., Bettermann, A. D., Brady, S. F., Grossman, T. H., Liles, M. R., et al. (2000). Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. Appl. Environ. Microbiol. 66, 2541–2547.

Rousk, J., Elyaagubi, F. K., Jones, D. L., and Godbold, D. L. (2011). Bacterial salt tolerance is unrelated to soil salinity across an arid agroecosystem salinity gradient. Soil Biol. Biochem. 43, 1881–1887. doi: 10.1016/j.soilbio.2011.05.007.

Rozainee, M., Ngo, S. P., Salema, A. A., Tan, K. G., Ariffin, M., and Zainura, Z. N. (2008). Effect of fluidising velocity on the combustion of rice husk in a bench-scale fluidised bed combustor for the production of amorphous rice husk ash. Bioresour. Technol. 99, 703–713.

Sagot, B., Gaysinski, M., Mehiri, M., Guigonis, J.-M., Le Rudulier, D., and Alloing, G. (2010). Osmotically induced synthesis of the dipeptide N-acetylglutaminylglutamine amide is mediated by a new pathway conserved among bacteria. Proc. Natl. Acad. Sci. 107, 12652–12657.

Santos, H., and Da Costa, M. S. (2002). Compatible solutes of organisms that live in hot saline environments. Environ. Microbiol. 4, 501–509.

Saqib, Z. A., Akhtar, J., Haq, M. A., and Ahmad, I. (2012). Salt induced changes in leaf phenology of wheat plants are regulated by accumulation and distribution pattern of Na ion. Pak. J. Agric. Sci 49, 141–148.

Sardinha, M., Müller, T., Schmeisky, H., and Joergensen, R. G. (2003). Microbial performance in soils along a salinity gradient under acidic conditions. Appl. Soil Ecol. 23, 237–244.

Sarig, S., and Steinberger, Y. (1994). Microbial biomass response to seasonal fluctuation in soil salinity under the canopy of desert halophytes. Soil Biol. Biochem. 26, 1405–1408.

Sarig, S., Fliessbach, A., and Steinberger, Y. (1996). Microbial biomass reflects a nitrogen and phosphorous economy of halophytes grown in salty desert soil. Biol. Fertil. Soils 21, 128–130.

Satlewal, A., Agrawal, R., Bhagia, S., Das, P., and Ragauskas, A. J. (2018). Rice straw as a feedstock for biofuels: availability, recalcitrance, and chemical properties. Biofuels, Bioprod. Biorefining 12, 83–107.

Saunois, M., Stavert, A. R., Poulter, B., Bousquet, P., Canadell, J. G., Jackson, R. B., et al. (2020). The Global Methane Budget 2000–2017. Earth Syst. Sci. Data 12, 1561–1623. doi: 10.5194/essd-12-1561-2020.

Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science (80-.). 270, 467–470.

Schimel, J., Balser, T. C., and Wallenstein, M. (2007). Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.

Schlesinger, W. H., and Bernhardt, E. S. (2013). Biogeochemistry: an analysis of global change. Academic press.

Schofield, R. V., and Kirkby, M. J. (2003). Application of salinization indicators and initial development of potential global soil salinization scenario under climatic change. Global Biogeochem. Cycles 17, n/a-n/a. doi: 10.1029/2002GB001935.

Segers, R. (1998). Methane production and methane consumption: a review of processes underlying wetland methane fluxes. Biogeochemistry 41, 23–51.

Seo, D. C., and DeLaune, R. D. (2010). Effect of redox conditions on bacterial and fungal biomass and carbon dioxide production in Louisiana coastal swamp forest sediment. Sci. Total Environ. 408, 3623–3631.

Serrano-Silva, N., Valenzuela-Encinas, C., Marsch, R., Dendooven, L., and Alcántara-Hernández, R. J. (2014). Changes in methane oxidation activity and methanotrophic community composition in saline alkaline soils. Extremophiles 18, 561–571.

Servais, S., Kominoski, J. S., Charles, S. P., Gaiser, E. E., Mazzei, V., Troxler, T. G., et al. (2019). Saltwater intrusion and soil carbon loss: Testing effects of salinity and phosphorus loading on microbial functions in experimental freshwater wetlands. Geoderma 337, 1291–1300.

Servais, S., Kominoski, J. S., Fernandez, M., and Morales, K. (2021). Saltwater and phosphorus drive unique soil biogeochemical processes in freshwater and brackish wetland mesocosms. Ecosphere 12, e03704.

Setia, R., Marschner, P., Baldock, J., and Chittleborough, D. (2010). Is CO 2 evolution in saline soils affected by an osmotic effect and calcium carbonate? Biol. Fertil. Soils 46, 781–792.

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., and Verma, V. (2011). Relationships between carbon dioxide emission and soil properties in salt-affected landscapes. Soil Biol. Biochem. 43, 667–674. doi: 10.1016/j.soilbio.2010.12.004.

Shahariar, S., Farrell, R., Soolanayakanahally, R., and Bedard-Haughn, A. (2021). Elevated salinity and water table drawdown significantly affect greenhouse gas emissions in soils from contrasting land-use practices in the prairie pothole region. Biogeochemistry 155, 127–146.

Shibagaki-Shimizu, T., Nakayama, N., Nakajima, Y., Matsuya, K., Kimura, M., and Asakawa, S. (2006). Phylogenetic study on a bacterial community in the floodwater of a Japanese paddy field estimated by sequencing 16S rDNA fragments after denaturing gradient gel electrophoresis. Biol. Fertil. soils 42, 362–365.

Sinsabaugh, R. L., Carreiro, M. M., and Repert, D. A. (2002). Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1–24.

Siringam, K., Juntawong, N., Cha-um, S., and Kirdmanee, C. (2011). Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (Oryza sativa L. spp. indica) roots under isoosmotic conditions. African J. Biotechnol. 10, 1340–1346.

Six, J., Frey, S. D., Thiet, R. K., and Batten, K. M. (2006). Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci. Soc. Am. J. 70, 555–569.

Smith, P., Davies, C. A., Ogle, S., Zanchi, G., Bellarby, J., Bird, N., et al. (2012). Towards an integrated global framework to assess the impacts of land use and management change on soil carbon: current capability and future vision. Glob. Chang. Biol. 18, 2089–2101.

Snakin, V. V, and AG, D. (1980). USE OF THE MAGNITUDE OF THE OXIDATION POTENTIAL OF SOILS FOR A THERMODYNAMIC DESCRIPTION OF BIOGEOCENOTIC PROCESSES.

Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., et al. (2006). Microbial diversity in the deep sea and the underexplored "rare biosphere." Proc. Natl. Acad. Sci. 103, 12115–12120.

Sørensen, K. B., Canfield, D. E., and Oren, A. (2004). Salinity responses of benthic microbial communities in a solar saltern (Eilat, Israel). Appl. Environ. Microbiol. 70, 1608–1616.

Speece, R. E. (1996). Anaerobic Biotechnology for Industrial Wastewater Treatment, 1st edn Archae Press.

Spiers, G. A., and McGill, W. B. (1979). Effects of phosphorus addition and energy supply on acid phosphatase production and activity in soils. Soil Biol. Biochem. 11, 3–8.

Strickland, M. S., and Rousk, J. (2010). Considering fungal: bacterial dominance in soils–methods, controls, and ecosystem implications. Soil Biol. Biochem. 42, 1385–1395.

Su, G., Wang, S., Yuan, Z., and Peng, Y. (2016). Enhanced volatile fatty acids production of waste activated sludge under salinity conditions: Performance and mechanisms. J. Biosci. Bioeng. 121, 293–298.

Suenaga, H. (2012). Targeted metagenomics: a high-resolution metagenomics approach for specific gene clusters in complex microbial communities. Environ. Microbiol. 14, 13–22.

Sugano, A., Tsuchimoto, H., Tun, C. C., Asakawa, S., and Kimura, M. (2005). Succession and phylogenetic profile of eubacterial communities in rice straw incorporated into a rice field: estimation by PCR-DGGE analysis. Soil Sci. Plant Nutr. 51, 51–60.

Sundareshwar, P. V, Morris, J. T., Koepfler, E. K., and Fornwalt, B. (2003). Phosphorus limitation of coastal ecosystem processes. Science (80-.). 299, 563–565.

Sutton-Grier, A. E., and Megonigal, J. P. (2011). Plant species traits regulate methane production in freshwater wetland soils. Soil Biol. Biochem. 43, 413–420.

Szent-Gyorgi, A. (1957). Bioenergetics Academic Press. New York [Back Transl. from a Russ. Ed. B. GIZ Fiz-Mat. Lit. Moscow, 1960, pp 54–56].

Taketani, R. G., Franco, N. O., Rosado, A. S., and van Elsas, J. D. (2010). Microbial community response to a simulated hydrocarbon spill in mangrove sediments. J. Microbiol. 48, 7–15.

Tanahashi, T., Murase, J., Matsuya, K., Hayashi, M., Kimura, M., and Asakawa, S. (2005). Bacterial communities responsible for the decomposition of rice straw compost in a Japanese rice paddy field estimated by DGGE analysis of amplified 16S rDNA and 16S rRNA fragments. Soil Sci. Plant Nutr. 51, 351–360.

Tchobanoglous, G., Burton, F. L., and Stensel, H. D. (2003). Wastewater engineering: treatment and reuse, Metcalf & Eddy Inc. McGraw-Hill, Inc., New York. doi 10, 70418780.

Tester, M., and Davenport, R. (2003). Na+ tolerance and Na+ transport in higher plants. Ann. Bot. 91, 503–527.

Theint, E. E., Bellingrath-Kimura, S. D., Oo, A. Z., and Motobayashi, T. (2016). Influence of gypsum amendment on methane emission from paddy soil affected by saline irrigation water. Front. Environ. Sci. 3, 79.

Tong, C., Cadillo-Quiroz, H., Zeng, Z. H., She, C. X., Yang, P., and Huang, J. F. (2017). Changes of community structure and abundance of methanogens in soils along a freshwater–brackish water gradient in subtropical estuarine marshes. Geoderma 299, 101–110.

Topno, S. E. (2015). Environmental performance and energy recoverable from stored rice straw bales in humid climate.

Townsend, T. G. (2018). Landfill bioreactor design and operation. Routledge.

Tringe, S. G., Von Mering, C., Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., et al. (2005). Comparative metagenomics of microbial communities. Science (80-.). 308, 554–557.

Tully, K., Gedan, K., Epanchin-Niell, R., Strong, A., Bernhardt, E. S., BenDor, T., et al. (2019). The invisible flood: The chemistry, ecology, and social implications of coastal saltwater intrusion. Bioscience 69, 368–378.

Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., et al. (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428, 37–43.

Updegraff, K., Bridgham, S. D., Pastor, J., and Weishampel, P. (1998). Hysteresis in the temperature response of carbon dioxide and methane production in peat soils. Biogeochemistry 43, 253–272.

Vaishnav, A., Varma, A., Tuteja, N., and Choudhary, D. K. (2016). PGPR-mediated amelioration of crops under salt stress. Plant-microbe Interact. an approach to Sustain. Agric., 205–226.

Van Nguyen, H., Nguyen, C. D., Van Tran, T., Hau, H. D., Nguyen, N. T., and Gummert, M. (2016). Energy efficiency, greenhouse gas emissions, and cost of rice straw collection in the Mekong River Delta of Vietnam. F. Crop. Res. 198, 16–22.

Van, D. T., Anh, T. T., and Van, L. V. (2022). Decomposition of rice straw residues and the emission of CO2, CH4 under paddy rice and crop rotation in the Vietnamese Mekong Delta region–A microcosm study. Plant, Soil Environ. 68, 29–35.

Vance, C. P. (1997). Enhanced agricultural sustainability through biological nitrogen fixation. in Biological fixation of nitrogen for ecology and sustainable agriculture (Springer), 179–186.

Vasconcelos, C., and McKenzie, J. A. (2000). Sulfate Reducers--Dominant Players in a Low-Oxygen World? Science (80-.). 290, 1711–1712.

Venkataramanan, M. (2011). Causes and effects of global warming. Indian J. Sci. Technol. 4, 226–229.

Vieites, J. M., Guazzaroni, M.-E., Beloqui, A., Golyshin, P. N., and Ferrer, M. (2008). Metagenomics approaches in systems microbiology. FEMS Microbiol. Rev. 33, 236–255.

Walter, A., Probst, M., Hinterberger, S., Müller, H., and Insam, H. (2016). Biotic and abiotic dynamics of a high solid-state anaerobic digestion box-type container system. Waste Manag. 49, 26–35.

Walters, W., Hyde, E. R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., et al. (2016). Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. Msystems 1, e00009-15.

Wan, W., Liu, S., Li, X., Xing, Y., Chen, W., and Huang, Q. (2021). Bridging rare and abundant bacteria with ecosystem multifunctionality in salinized agricultural soils: from community diversity to environmental adaptation. Msystems 6, e01221-20.

Wang, H., Guo, C. L., Yang, C. F., Lu, G. N., Chen, M. Q., and Dang, Z. (2016). Distribution and diversity of bacterial communities and sulphate-reducing bacteria in a paddy soil irrigated with acid mine drainage. J. Appl. Microbiol. 121, 196–206.

Wartiainen, I., Eriksson, T., Zheng, W., and Rasmussen, U. (2008). Variation in the active diazotrophic community in rice paddy—nifH PCR-DGGE analysis of rhizosphere and bulk soil. Appl. Soil Ecol. 39, 65–75.

Watkins, A. J., Roussel, E. G., Parkes, R. J., and Sass, H. (2014). Glycine betaine as a direct substrate for methanogens (Methanococcoides spp.). Appl. Environ. Microbiol. 80, 289–293.

Weinstock, G. M. (2012). Genomic approaches to studying the human microbiota. Nature 489, 250–256.

Weston, N. B., Vile, M. A., Neubauer, S. C., and Velinsky, D. J. (2011). Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils. Biogeochemistry 102, 135–151.

Whitmire, S. L., and Hamilton, S. K. (2005). Rapid removal of nitrate and sulfate in freshwater wetland sediments. J. Environ. Qual. 34, 2062–2071.

Wichern, F., Islam, M. R., Hemkemeyer, M., Watson, C., and Joergensen, R. G. (2020). Organic amendments alleviate salinity effects on soil microorganisms and mineralisation processes in aerobic and anaerobic paddy rice soils. Front. Sustain. Food Syst. 4, 30.

Wichern, J., Wichern, F., and Joergensen, R. G. (2006). Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100–108. doi: 10.1016/j.geoderma.2006.08.001.

Widdel, F. (1988). Microbiology and ecology of sulfate-and sulfur-reducing bacteria. Biol. Anaerob. Microorg., 469–585.

Widdel, F., Rouviere, P. E., and Wolfe, R. S. (1988). Classification of secondary alcohol-utilizing methanogens including a new thermophilic isolate. Arch. Microbiol. 150, 477–481.

Wiessner, A., Kappelmeyer, U., Kuschk, P., and Kästner, M. (2005). Sulphate reduction and the removal of carbon and ammonia in a laboratory-scale constructed wetland. Water Res. 39, 4643–4650.

Wolfe, R. S. (2011). "Techniques for cultivating methanogens," in Methods in enzymology (Elsevier), 1–22.

Wong, V. N. L., Dalal, R. C., and Greene, R. S. B. (2009). Carbon dynamics of sodic and saline soils following gypsum and organic material additions: a laboratory incubation. Appl. Soil Ecol. 41, 29–40.

Wright, A. L., and Reddy, K. R. (2001). Phosphorus loading effects on extracellular enzyme activity in Everglades wetland soils. Soil Sci. Soc. Am. J. 65, 588–595.

Xiong, H., Li, J., Liu, P., Duan, J., Zhao, Y., Guo, X., et al. (2014). Overexpression of OsMYB48-1, a novel MYB-related transcription factor, enhances drought and salinity tolerance in rice. PLoS One 9, e92913.

Xu, H. L., Wang, J. Y., Zhang, H., and Tay, J. H. (2003). A comparative study of anaerobic digestion of food waste in a single pass, a leachate recycle and coupled solid/liquid reactors. Water Sci. Technol. 47, 319–324.

Yan, N., Marschner, P., Cao, W., Zuo, C., and Qin, W. (2015). Influence of salinity and water content on soil microorganisms. Int. Soil Water Conserv. Res. 3, 316–323. doi: 10.1016/j.iswcr.2015.11.003.

Yang, C., Lv, D., Jiang, S., Lin, H., Sun, J., Li, K., et al. (2021). Soil salinity regulation of soil microbial carbon metabolic function in the Yellow River Delta, China. Sci. Total Environ. 790, 148258.

Yang, C., Wang, X., Miao, F., Li, Z., Tang, W., and Sun, J. (2020). Assessing the effect of soil salinization on soil microbial respiration and diversities under incubation conditions. Appl. Soil Ecol. 155, 103671.

YOKOHAMA, S., and MATSUMURA, Y. (2017). The Asian Biomass Handbook: A Guide for Biomass Production and Utilization. The Japan Institute of Energy.

Young, J. P. W. (1992). Phylogenetic classification of nitrogen-fixing organisms. Biol. nitrogen Fixat. 1544, 43–86.

Yu, I. K., and Hungate, R. E. (1983). Isolation and characterization of an obligately halophilic methanogenic bacterium. in Annual Meeting of the American Society for Microbiology, Abstract I, 139.

Yu, Y., Zhao, C., Zheng, N., Jia, H., and Yao, H. (2019). Interactive effects of soil texture and salinity on nitrous oxide emissions following crop residue amendment. Geoderma 337, 1146–1154. doi: 10.1016/j.geoderma.2018.11.012.

Yuan, B.-C., Li, Z.-Z., Liu, H., Gao, M., and Zhang, Y.-Y. (2007). Microbial biomass and activity in salt affected soils under arid conditions. Appl. Soil Ecol. 35, 319–328.

Yuan, J., Liu, D., Ji, Y., Xiang, J., Lin, Y., Wu, M., et al. (2019). Spartina alterniflora invasion drastically increases methane production potential by shifting methanogenesis from hydrogenotrophic to methylotrophic pathway in a coastal marsh. J. Ecol. 107, 2436–2450.

Zahran, H. H. (1997). Diversity, adaptation and activity of the bacterial flora in saline environments. Biol. Fertil. Soils 25, 211–223.

Zahran, H. H., Moharram, A. M., and Mohammad, H. A. (1992). Some ecological and physiological studies on bacteria isolated from salt-affected soils of Egypt. J. Basic Microbiol. 32, 405–413.

Zeng, L., and Shannon, M. C. (2000). Salinity Effects on Seedling Growth and Yield Components of Rice. Crop Sci. 40, 996–1003. doi: 10.2135/cropsci2000.404996x.

Zewdu, S., Suryabhagavan, K. V, and Balakrishnan, M. (2017). Geo-spatial approach for soil salinity mapping in Sego Irrigation Farm, South Ethiopia. J. Saudi Soc. Agric. Sci. 16, 16–24.

Zhang, C.-J., Chen, Y.-L., Pan, J., Wang, Y.-M., and Li, M. (2020). Spatial and seasonal variation of methanogenic community in a river-bay system in South China. Appl. Microbiol. Biotechnol. 104, 4593–4603.

Zhang, J., Zhang, R., He, Q., Ji, B., Wang, H., and Yang, K. (2020). Adaptation to salinity: Response of biogas production and microbial communities in anaerobic digestion of kitchen waste to salinity stress. J. Biosci. Bioeng. 130, 173–178.

Zhang, J., Zhang, R., He, Q., Ji, B., Wang, H., and Yang, K. (2020). Adaptation to salinity: Response of biogas production and microbial communities in anaerobic digestion of kitchen waste to salinity stress. J. Biosci. Bioeng. 130, 173–178.

Zhang, K., Shi, Y., Cui, X., Yue, P., Li, K., Liu, X., et al. (2019). Salinity Is a Key Determinant for Soil Microbial Communities in a Desert Ecosystem. mSystems 4. doi: 10.1128/mSystems.00225-18.

Zhang, K., Song, L., and Dong, X. (2010). Proteiniclasticum ruminis gen. nov., sp. nov., a strictly anaerobic proteolytic bacterium isolated from yak rumen. Int. J. Syst. Evol. Microbiol. 60, 2221–2225.

Zhang, L., Liu, W., Liu, S., Zhang, P., Ye, C., and Liang, H. (2020). Revegetation of a barren rare earth mine using native plant species in reciprocal plantation: effect of phytoremediation on soil microbiological communities. Environ. Sci. Pollut. Res. 27, 2107–2119.

Zhang, L., Zhu, K., and Li, A. (2016). Differentiated effects of osmoprotectants on anaerobic syntrophic microbial populations at saline conditions and its engineering aspects. Chem. Eng. J. 288, 116–125.

Zhang, W., Chong, W., Rui, X. U. E., and Wang, L. (2019). Effects of salinity on the soil microbial community and soil fertility. J. Integr. Agric. 18, 1360–1368.

Zhang, Y., Alam, M. A., Kong, X., Wang, Z., Li, L., Sun, Y., et al. (2017). Effect of salinity on the microbial community and performance on anaerobic digestion of marine macroalgae. J. Chem. Technol. Biotechnol. 92, 2392–2399.

Zhang, Y., Ghaly, A. E., and Li, B. (2012). Physical properties of rice residues as affected by variety and climatic and cultivation conditions in three continents. Am. J. Appl. Sci. 9, 1757–1768.

Zhang, Z., and Furman, A. (2021). Soil redox dynamics under dynamic hydrologic regimes-A review. Sci. Total Environ. 763, 143026.

Zhao, M., Han, G., Li, J., Song, W., Qu, W., Eller, F., et al. (2020). Responses of soil CO2 and CH4 emissions to changing water table level in a coastal wetland. J. Clean. Prod. 269, 122316.

Zheng, W., Xue, D., Li, X., Deng, Y., Rui, J., Feng, K., et al. (2017). The responses and adaptations of microbial communities to salinity in farmland soils: A molecular ecological network analysis. Appl. Soil Ecol. 120, 239–246. doi: 10.1016/j.apsoil.2017.08.019.

Zheng, Y., Bu, N.-S., Long, X.-E., Sun, J., He, C.-Q., Liu, X.-Y., et al. (2017). Sulfate reducer and sulfur oxidizer respond differentially to the invasion of Spartina alterniflora in estuarine salt marsh of China. Ecol. Eng. 99, 182–190.

Zhou, J., Bruns, M. A., and Tiedje, J. M. (1996). DNA recovery from soils of diverse composition. Appl. Environ. Microbiol. 62, 316–322.

Zhou, J., Deng, Y., Luo, F., He, Z., Tu, Q., and Zhi, X. (2010a). Functional molecular ecological networks. MBio 1, e00169-10.

Zhou, J., He, Q., Hemme, C. L., Mukhopadhyay, A., Hillesland, K., Zhou, A., et al. (2011). How sulphate-reducing microorganisms cope with stress: lessons from systems biology. Nat. Rev. Microbiol. 9, 452–466.

Zhou, J., He, Z., Van Nostrand, J. D., Wu, L., and Deng, Y. (2010b). Applying GeoChip analysis to disparate microbial communities. Microbe 5, 60–65.

Zhou, M.-X., Renard, M.-E., Quinet, M., and Lutts, S. (2019). Effect of NaCl on proline and glycinebetaine metabolism in Kosteletzkya pentacarpos exposed to Cd and Zn toxicities. Plant Soil 441, 525–542. doi: 10.1007/s11104-019-04143-5.

Zhou, Z., Chen, J., Cao, H., Han, P., and Gu, J.-D. (2015). Analysis of methane-producing and metabolizing archaeal and bacterial communities in sediments of the northern South China Sea and coastal Mai Po Nature Reserve revealed by PCR amplification of mcrA and pmoA genes. Front. Microbiol. 5, 789.

Zinder, S. H. (1993). Physiological ecology of methanogens. Methanogenes. Ecol. Physiol. Biochem. Genet., 128–206.

8-Abbreviations

GHGs: greenhouse gases
Tg: Teragrams
SWI: Seawater intrusion or saltwater intrusion
SRB: Sulfate reducing bacteria
VFAs: Volatile Fatty Acids
DNB: Denitrifying bacteria
nifH: a subunit of nitrogenase gene
dsrB: dissimilatory sulfite reductase subunit beta
mcrA: Methyl-coenzyme M reductase subunit alpha
MCR: Methyl-coenzyme M reductase
mRNA: Messenger RNA
bp: base pair
NaCl: sodium chloride
ppm: Parts per million
ppb: Parts per billion
cDNA: Complementary DNA
NCBI: National center for Biotechnology Information
NGS: Next Generation sequencing
pMMO: Particulate methane monooxygenase
pmoA: Particulate methane monooxygenase alpha
PCR: Polymerase Chain Reaction
qPCR: Quantitative PCR
RT-qPCR: Reverse Transcription Quantitative PCR
QIIME: Quantitative Insights Into Microbial Ecology
OUT: Operational taxonomic unit
ASV: amplicon sequence variant
PCoA: Principle coordinate analysis
rpoB: RNA polymerase subunit beta
SSU rRNA: Small subunit rRNA (SSU rRNA)
SOM: Soil organic matter
DGGE: Denaturing gradient gel electrophoresis
T-RFLP: Terminal restriction fragment length polymorphism
16S rRNA: 16S ribosomal ribonucleic acid
ANOVA: Analysis of Variance
MANOVA: Multivariate Analysis of Variance
FISH: Fluorescence in situ hybridization
GC: Gas Chromatography
HPLC: High Performance Liquid Chromatography

9-Acknowledgements

First and foremost, I would like to express my deepest gratitude to PD Dr. Werner Liesack for his invaluable guidance and mentorship throughout my PhD studies. His unwavering support and encouragement have been instrumental in shaping my research and academic journey.

I am also immensely grateful to my family for their unwavering financial support and constant kindness throughout my PhD. Their firm support has been the cornerstone of my academic success.

Furthermore, I would like to extend my sincere appreciation to Mr. Prof. Dr. Robert Junker and Mr. Prof. Dr. Lars Voll for their valuable contributions, guidance, and acceptance of my research work. Their insights and expertise have been invaluable in shaping my thesis writng and helping me navigate through academic challenges.

Lastly, I would like to express my gratitude to Mr. Prof. Dr. Tobias Erb for his financial support during a few critical months of my PhD. His support enabled me to overcome financial challenges and focus on my research work.

10-Pledge

I hereby declare that the thesis entitled:

"Impact of saltwater intrusion on paddy soil microbial communities"

was composed solely by myself, without any external assistance. Moreover, I confirm that the thesis has never been previously submitted to any other academic institutions or universities, and has not been utilized in any prior examinations.

Marburg, March 2023

Mehrdad Rabiei Nematabad