AALBORG UNIVERSITET

Sustainable Biotechnology M.Sc.

Master Thesis

Analysing the Effects of Climate Induced Summer Warming has on Microorganism in Arctic Soil.

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1. Abstract

Climate Change has worldwide consequences, including warming the Artic, which is one of the fastest warming regions in the world. Increasing temperatures in the Arctic causes thawing of permafrost and overall warming of the soil. Permafrost in known to store large amounts of carbon and with warming soil, microbial activity is thought to increase. This can lead to potentially devastating increases of emissions of CO_2 and CH_4, causing a global feedback mechanism. Yet, much remains unknown about High Arctic microbial communities and how they could react to changing climate. This study analysed microbial communities in the High Arctic of northern Greenland, after seven years of induced summer warming by Open Top Chambers (OTC). From soil samples DNA and RNA was extracted. Sequencing of the 16S and ITS genes was used to analyse diversities and relative abundance. No statistically significant effects of the treatment were observed. However, depth dependent shift could be seen.

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2. List of Abbreviations

Abbreviation	**Explanation**
ANOVA	Analysis of Variance, statistical method
ASCP	Arctic soil carbon pool
ASV	Amplicon sequence variant
bp	Base pair
DNA	Deoxyribonucleic acid
cDNA	Complementary deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
GHG	Greenhouse gas
GMST	Global mean surface temperature
GSAT	Global surface air temperature
ha	hectare
IPPC	Intergovernmental Panel on Climate Change
ITEX	International Tundra Experiment
MAGT	mean annual ground temperature
OTC	Open-top chamber
OTU	Operational taxonomic unit
PERMANOVA	Permutational multivariate analysis of variance
PCoA	Principal Coordinate Analyses
PCR	Polymerase chain reaction
Pg	Petagram ($1Pg = 10^{15}$ g)
R	a programming language for statistical computing
RDP	Ribosomal Database Project
qPCR	quantitative polymerase chain reaction
SRES	Special Report Emissions Scenarios
%w/v	% Weight per volume

3. Introduction

3.1. Climate Change

Global climate is known to naturally change over time (Gulev et al., 2021). Yet there is a scientific consensus that humans have influenced climate in recent years, causing warming of the atmosphere, oceans, and land (Masson-Delmotte et al., 2021, Eyring et al., 2021). The evidence and consensus for this have been increasing for years (Eyring et al., 2021). Climate change is a complex topic, it is known to be caused by a variety of different factors. Impacts of climate change are also numerous and change throughout regions (Masson-Delmotte et al., 2021).

The Global Climate system depends on the energy that reaches the atmosphere and the Earth. This solar radiation can vary due to several circumstances. First, the radiation from the sun on earth is not constant. The orbits in the solar system change constantly, so does the emission from the sun (Masson-Delmotte et al., 2021). Secondly, the so-called albedo changes. Albedo refers to the reflection of solar radiation. This can vary based on vegetation, cloud coverage or particles in the atmosphere. Lastly, the outgoing longwave radiation can be hindered by Greenhouse gases (GHG) in the atmosphere (Le Treut et al., 2007).

3.1.1. Greenhouse Gases

Greenhouse gas emissions are at the centre of man-made climate change. By analysing ice cores from the Arctic or Antarctica, the gas composition of air can be determined for past centuries. It shows that by 2011 the ratio of carbon (390.5 ppm), methane (1803.2 ppb) and nitrous oxide (324.2 ppb) had increased and were higher than any values from the previous 800 thousand years. Human activity is responsible for increases of GHG in the atmosphere since about 1750 (Gulev et al., 2021). The overall increase in GHG emissions is due to a lack of equilibrium in sinks and sources of those gases, meaning, more gas is emitted than stored (Masson-Delmotte et al., 2021).

CO_2 increased by 2.0 ppm/yr. from 2000 to 2011, this ascended to 2.4 ppm/year until 2019. Methane has increased by 3.5% from 2011 to 2019. Nitrous oxide has increased by 2.4% in the same timeframe. Overall, the growth rate of GHG emissions is increasing (Gulev et al., 2021). The gases released into the atmosphere then affect climate all around the world. Since 1938 the impact that the CO_2 concentration in the atmosphere has on global climate has been known. The engineer Guy Stewart Callendar had shown a correlation by conducting several experiments. In addition, the increase of emissions from several industries were discussed (Khandekar et al., 2005).

3.1.2. Climate Change in the Arctic

Although climate change is a global phenomenon, the Arctic is of special interest, as near-surface warming is expected to increase twice as fast as the global average (Graham et al., 2017, Graversen et al., 2008). This is known as ‘Arctic amplification’ (Graversen et al., 2008). Dire impacts of warming are expected by 2050, much sooner than in other parts of the world.

Moreover, polar regions play a fundamental role in the global ecosystem, regulating global climate (Constable and Harper, 2021).

Many changes can be observed in the Arctic. Variations in temperature, precipitation, and an increase in extreme weather events have numerous and severe consequences. Ice is melting, permafrost is thawing, and Arctic wildfire will become more frequent (Constable and Harper, 2021, Solomon et al., 2007).

3.1.2.1 Temperature

When discussing global warming, it usually refers to the increase in surface temperature (Khandekar et al., 2005). Global surface temperature is rising due to man-made climate change. Comparing 1850-1990 to 2010-2019, the average temperature has increased by 1.07°C (Masson-Delmotte et al., 2021). A warming of the troposphere and cooling of the stratosphere has been observed since the 1950s. To measure, compare, and track temperatures globally several measurements are used, e.g., the Global mean surface temperature (GMST) and the Global surface air temperature (GSAT) (Gulev et al., 2021). Figure 1 shows the mean annual ground temperature (MAGT) of the Arctics (Obu et al., 2019).

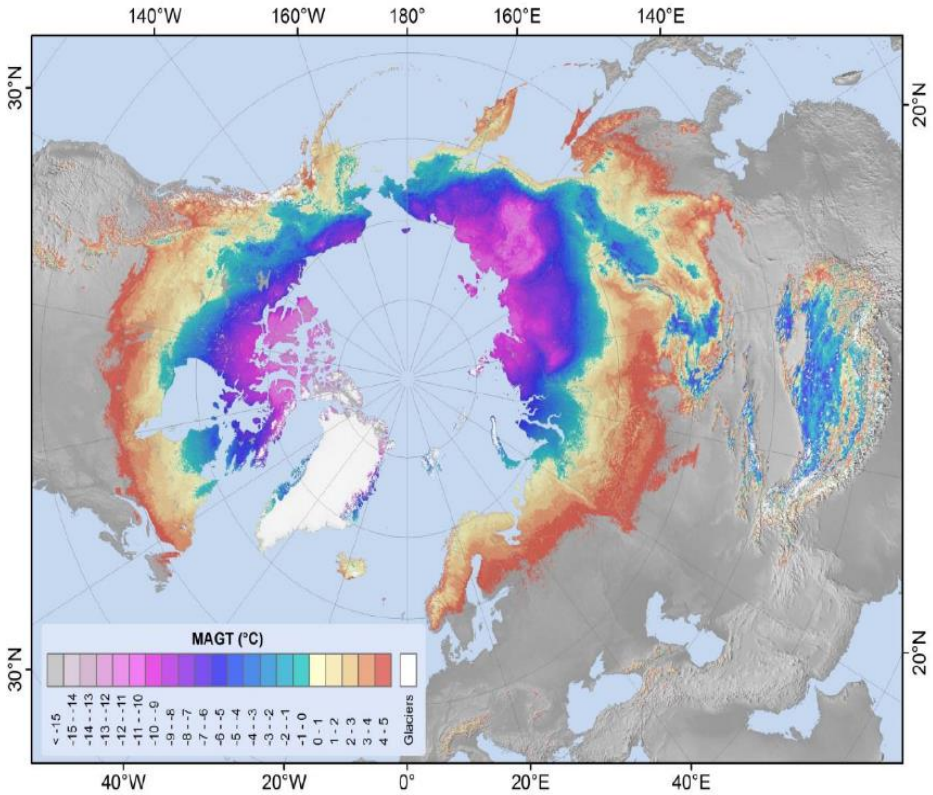

Figure 1: Average Mean Annual Ground Temperature for the northern Hemispheres.(Obu et al., 2019)

In the polar regions severe changes in temperature have been observed. In the Arctic and Iceland, since 1979 increases in extreme heat events, cold season warm days and night, as well as shifts in melt days, and increasing amounts in warm Arctic winter events were detected. In addition, the number of cold days and nights have significantly decreased (Ranasinghe, et al., 2021). In the last 400 years the Arctic has never been warmer than it is now (Chapin et al., 2005).

3.1.2.1.1 Summer Warming

Changes in albedo are believed to be the main cause for warming in the Arctic (Chapin et al., 2005). Warming rates in Alaska and Western Canada have increased from 0.15°C per decade (1961-1990) to 0.3-0.4°C per decade (1966-1995). Similar if not stronger increases have been observed in the European and Russian Arctic (Chapin et al., 2005).

The seasonal shift in temperature is often measured in days. E.g., the snowmelt has been moving forwards, from 1.3 to up to 9.1 days per decade. Thawing soil is also advancing, 2.0 to 3.3 days per decade (Chapin et al., 2005). In coming years, the decrease of ice-cover is expected to lead to a feedback mechanism. Ice-covered land reflects more solar radiation than water or land. With the ice-cover gone the land absorbs more solar-radiation, causing the ground to warm up further. This is expected to amplify summer warming in the future (Graversen et al., 2008).

3.1.2.1.2 Winter Warming

Strong winter warming has been observed in the Arctic (Chapin et al., 2005). Since 1950 when record keeping began in the arctic, the winter season 2015-2016 was the warmest on record (Graham et al., 2017). The mean winter temperature increases by about 1.27°C per decade. However, no strong increases are observed in the maximum winter temperature. This is most likely due to the fact that temperature is measured near-surface which will be affected by snow and ice (Graham et al., 2017). Winter warming events have become more frequent, warmer, and longer. Those events are related to winter storms, and warm and moist air which intrudes from southern regions (Doyle et al., 2011).

3.1.2.2. Precipitation

Within most scenarios of climate change, a further increase of precipitation is predicted in the Arctic (Walsh et al., 2011). However, larger interannual variations are also expected (Ranasinghe, et al., 2021). On land north of 55°N precipitation has increased by 5% since 1950. Since 2000 river discharge and precipitation has been exceeding previous measurements (Walsh et al., 2011). In addition, with the melting starting sooner due to increased temperatures, meltwater flooding in turn also occurs sooner in the year (Ranasinghe, et al., 2021). With increasing precipitation cloud cover has also been increasing (Walsh et al., 2011).

3.1.3. Treeline and Herbivores

The aforementioned changes in arctic climate have several consequences for the vegetation and as a result therefrom herbivores. Thanks to satellite imaging and indigenous observation an increase in shrubs covers can be observed (Vowles and Björk, 2019). For example, in the Alaskan North Slope tundra the shrubs cover has increased 1.2% per decade since 1950 (Chapin et al., 2005). Extension of shrub can happen mainly in three different ways. First, existing patches can fill in more. Secondly, through overall growth and an increase in density. And lastly if the existing shrub line advances north (Vowles and Björk, 2019).

Besides extension of Tundra shrubs, the arctic treeline is also advancing north. With permafrost thawing, tree seedlings and mature trees can grow in new areas. It is estimated that in the last 50 years 2.3% of the treeless area, or 11,600km^2, has been converted from tundra to forest (Chapin et al., 2005). In addition to the treeline itself extending, the existing forest is also known to grow denser. Tree species extending north include birch (*Betula* spp.), willow (*Salix* spp.), and alder white spruce (*Picea glauca*) (Vowles and Björk, 2019, Chapin et al., 2005).

Herbivores generally play an essential role in any ecosystem they occur in. In the arctic this is not different. Yet due to the harsh living conditions of polar climates the mammalian herbivores diversity is relatively low, with only roughly 50 species being found in the Arctic. Most of these are medium to small herbivores (Olofsson and Post, 2018). Most regions have a diversity lower than 10 (Barrio et al., 2016). When looking at large mammalian herbivores, 4 are unique to specific regions and not found elsewhere. This leaves two large herbivores that are distributed in the arctic, caribou or reindeer (*Rangifer tarandus)* and muskoxen (*Ovibos muschatus)* (Klein and Bay, 1991). In Greenland muskoxen are the only species of large mammalian herbivores (Olofsson and Post, 2018).

With herbivores feeding on plants, they have an influence on the expansion of shrubs and trees. Reindeers are known to slow down the advancing of treelines by grazing (Chapin et al., 2005). Reindeer exclusion experiments with fences have shown that tree and shrub expansion increased much more if especially large herbivores are unable to graze. Not many studies exist about the effects of muskoxen specifically, nevertheless, joint studies show that similar assumptions can be drawn (Olofsson and Post, 2018).

3.2. Arctic Soil

Large areas worldwide are considered to be Arctic habitat. Alone in the northern hemisphere, approximately 7.2×10^6 km^2 of landmass is north of the Arctic treeline. Of this 26% or 1.9×10^6 km^2 is covered by glaciers. 91% of those glaciers are located in Greenland (Margesin, 2009).

Although the climate in the Arctic can vary strongly throughout the year and across different areas, some characteristics are applicable for all. For the entire Arctic the summers are short and fairly cold, the winters however, are long, dark, and even colder than the summers. Only for short periods during the summer the mean temperature in a day can rise above 0°C. Temperatures in the Arctic range from -20°C to -40°C in winter and 3°C to 10°C in summer, with the coldest month being February and the warmest being July. The precipitation mostly occurs as snow and annually reaches 60-160 mm (Margesin, 2009). Those extreme conditions strongly influence and shape the Arctic ecosystem, including the soil (Bölter et al., 2006).

3.2.1. Soil Characteristics

Soil can form under various conditions which strongly influence the characteristics the soil will later have. The factors which have a strong impact on the soil include overall climate, time, local topography, biological elements, and parent material (Bölter et al., 2006, Tedrow and Cantlon, 1958). This leads to a large variety of different soils around the world. Starting in the 1950s, natural soil was categorised into 5 zones, tundra, podzol, chernozem, desert, and laterite

(Tedrow and Cantlon, 1958). This set the beginning of an organised and still ongoing systematic research effort into soil. In the Arctic tundra soils are most prevalent. Worldwide 5.5% of land surface is considered tundra and on the northern continents it is about 20% (Bölter et al., 2006). They are characterised by poor drainage, mostly due to the underlying permafrost. The upper layers often are high in mineral content (Tedrow and Cantlon, 1958).

With the Arctic covering large land surface, large heterogeneity can be observed for the soils overall. The soil can be sandy, of a fine granular structure, loamy, blocky, grained, or a combination of many of those. Many different variations of gravel and stones can be found in soil. The total organic carbon can also vary strongly and usually decreases with increasing depth. In the Artic the carbon to nitrogen ratio ranges from 10 to 20 in most regions. Due to the carbonates in the soil, most are alkaline (Bölter et al., 2006).

3.2.2 Arctic Classification

The Arctic can be divided into three major categories, High Arctic, Low Arctic and Subarctic. Figure 2 shows the distribution of them. The Subarctic stretches from the boreal forest to the treeline. Low Arctic is north of the treeline, and High Arctic is considered to be the northern most part (Blaud et al., 2015)

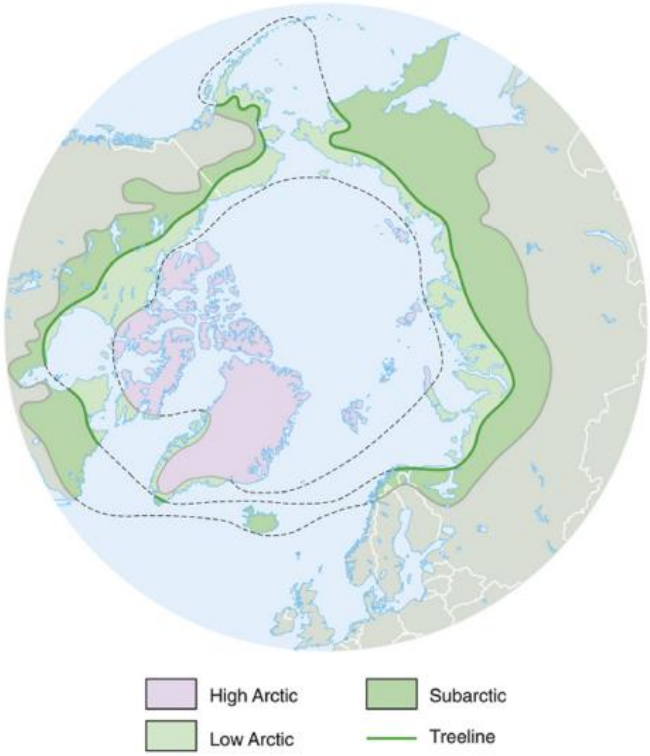

Figure 2: Map of the northern Hemisphere showing the three ecological zones of the Arctic. (Blaud et al., 2015)

Many factors change for the different kinds of Arctic. Most notably the growing season increases further south. In High Arctic the growing season lasts 1-2.5 months, in Low Arctic 3-4 months, and in Subarctic 3.5-12 months (Jones et al., 2010). The annual average temperature in the High Arctic is below -15°C, in the Low Arctic -15°C to -10°C. Precipitation also varies, High Arctic areas mostly have less than 250 mm per year, with Greenland being an exception

with over 1000 mm. In the Low Arctic ranges from 500 mm to below 250 mm. Subarctic has up to 750 mm annual precipitation. Yet some Subarctic areas also have less than 250 mm (AMAP, 2012, Blaud et al., 2015,).

Those climatic conditions strongly shape local ecosystems, ranging from polar desert to boreal forest and wetlands. In the Subarctic tundra transitions to boreal forest and wetlands. In the Low Arctic tundra and peatland increases and a shift towards semi-desert is seen. Lastly, in High Arctics polar deserts, polar semi-deserts and tundra are common. The strong variation can be seen in plant cover. The Subarctic is completely covert in plants. Low Arctics have a plant cover of 80-100%. This drops massively to 0-20% plant cover in the High Arctics (Jones et al., 2010).

3.2.3. Permafrost and Active Layer

A soil or sediment is considered permafrost if it is frozen for at least two consecutive years. Permafrost is present in most of the Arctic (Figure 3). Even though Arctic temperatures are typically low, warmer temperatures during the summer month can lead to thawing of soil. This is then referred to as the active layer of permafrost. The depth of the active layer can vary strongly (Bölter et al., 2006). In High Arctic conditions, the active layer can be only a few centimetres deep, approximately 2-8cm, whereas in Low Arctic areas the active layer can be up to 20m deep (Dobiński, 2020). However, most commonly the active layer is 20 to 100 cm deep (Margesin, 2009, Bölter et al., 2006). The permafrost itself is 100-500m deep. In Siberia it can reach thickness of more than 500m (Margesin, 2009).

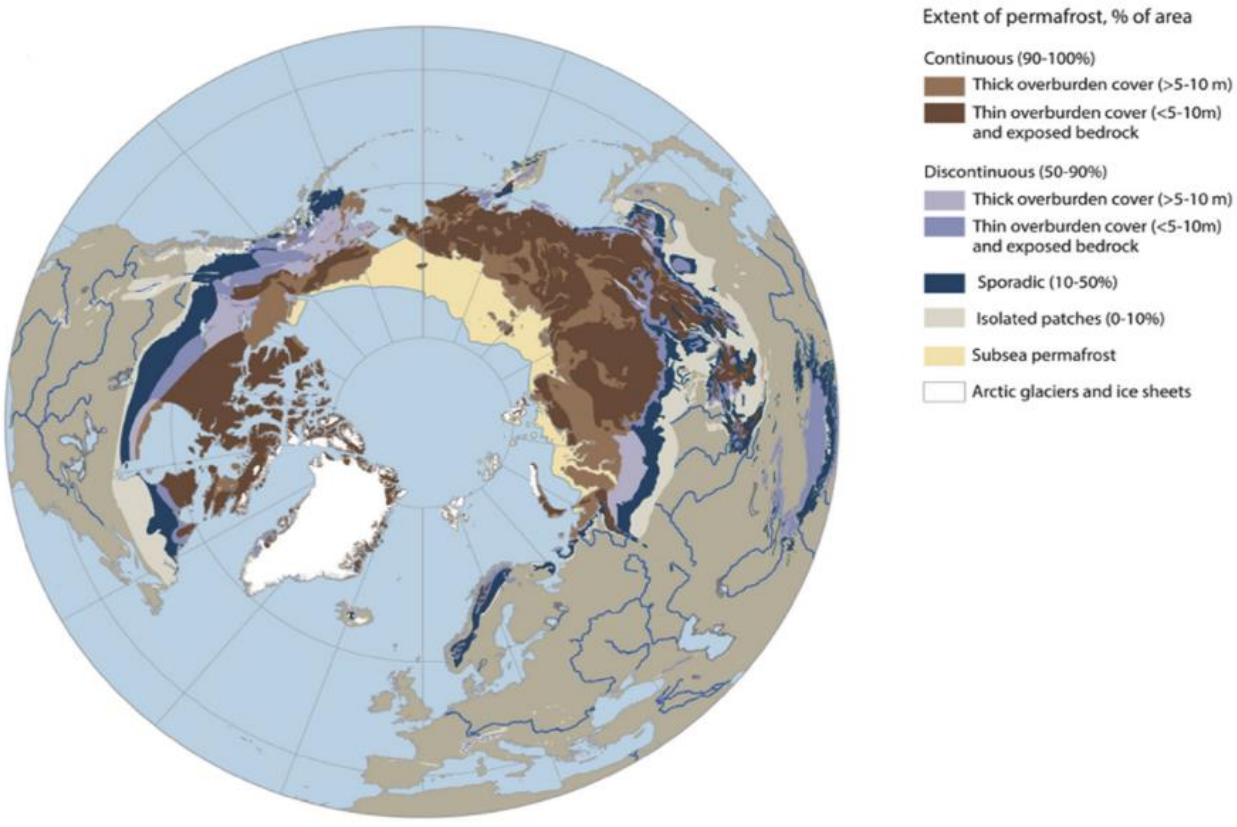

Figure 3: Map of the northern Hemisphere showing the distribution of permafrost (Blaud et al., 2015)

Ongoing cycles of freezing and thawing up, dry and wet seasons, and overall grim weather conditions, lead to short growing seasons. This causes a decoupling of biomass production and decay which can lead to an accumulation within the soil. Therefore, permafrost has generally a high residual carbon and organic matter content (Bölter et al., 2006).

3.2.4. Expected Changes in Arctic Soil

The effects of climate change are widely contested and discussed. Many different factors play a role and differ strongly through deficient systems. In the Arctic 4 major threats to the environment have been identified. All of those would have strong consequences for the microbial community and therefrom resulting for the Arctic soil overall (Blaud et al., 2015).

Firstly, temperature increases of 3°C to 6°C by 2080 will cause many changes. Besides thawing of permafrost, snow cover will significantly decrease, both overall and in the warmer summer month specifically. By 2050 snow cover is expected to drop by 10-20%. Thawing influences the availability of water in the area. Depending on the area, water content can either decrease due to improved drainage, or increase thanks to melting ice. Moreover, thawing permafrost will lead to an increase of biological activity. The treeline is expected to move further north, and plant roots grow deeper. Changes in microbiological communities are also expected. (AMAP, 2012)

Furthermore, changes in precipitation will be seen in the Arctic. Especially during autumn and winter months an increase is expected. Notably a shift from snow to rain is predicted. This shift will change snow cover to ice cover. Overall, depending on the specific area this will lead to both drier and wetter conditions (Blaud et al., 2015).

Effects of atmospheric pollution will also be seen in the Arctic. Nitrogen deposition of 1-5kg N per hectare per year are expected. Moreover, mercury residue will increase. This increase will show especially strong effects in otherwise nutrient deprived regions and change diversity of plants and microorganisms (Kühnel et al., 2012).

Lastly, human activity will drastically increase in the Arctic, mainly due to human presence overall, logistical efforts, including air and water traffic, and extraction of diverse resources. This inevitably causes land use change. Moreover, spillage, pollution and contamination from fuel, and extraction efforts are most likely unavoidable (Peters et al., 2011).

3.2.5. Arctic Soil Carbon Pool

In recent years the Arctic tundra environment has become a topic of increased research interest (Bölter et al., 2006, Margesin, 2009). As discussed previously man-made climate change will strongly affect temperatures and precipitation. Currently, the Arctic is thought to be an overall carbon sink, meaning storing more CO_2 than releasing into the atmosphere. However, with rising temperatures thawing of permafrost is expected which will have great impact on the stored carbon, potentially turning the Arctic into a carbon source (Bölter et al., 2006).

Compared to other ecosystems with more favourable growth conditions, for example temperate climate, Arctic regions produce substantially less biomass. Therefore, most of the carbon in the Arctic has been stored for thousands of years. The amount of carbon stored in the permafrost-affected ecosystems is quite significant in a global context, causing much concern (Margesin, 2009).

The question of how much carbon is stored in Arctic soil is strongly discussed and still much information and details are missing. In recent years the estimated size of the stored carbon pool has increased to more than twice of previous projections (van Huissteden and Dolman, 2012). Approximately 15% of the world soil carbon is believed to be stored currently in Arctic soil, in large parts as frozen, not fully composed plant material (Bölter et al., 2006, Yergeau et al., 2010).

In 1982 the Arctic soil carbon pool (ASCP) was thought to be 192 Pg (petagram), this increased to 268 Pg in 2003. By 2009 the estimated ASCP had increased to 1499 Pg, of which 88% is believed to be within soil that is permanently frozen. Recent studies estimate that circumpolar permafrost stores 1672 Pg which is almost double the amount of carbon currently in the atmosphere (van Huissteden and Dolman, 2012). Those estimations however are to be taken with a grain of salt as there are still many uncertainties, especially in deeper layers of soil. Moreover, the heterogeneity in soil composition, properties, and biological activity, cause difficulties in developing comprehensive models (van Huissteden and Dolman, 2012, Bölter et al., 2006).

3.2.6. Thawing Soil and Carbon Release

Mapping out ASCP proves to be challenging. As climate change is expected to affect the Arctic strongly, the effects on the ASCP are also widely discussed and researched. Analysing carbon isotopes has shown that “old carbon” had already been released into the atmosphere. Experts believe that by 2100 11-17% of the stored ASCP will be released (van Huissteden and Dolman, 2012) and up to 90% of near-surface Arctic permafrost is thawed (Yergeau et al., 2010). Those estimates are based on several different models which combine different climate models with estimates of the carbon pool (van Huissteden and Dolman, 2012).

A global vegetation-carbon model (MAGICC6) which works with a reduced complexity estimates a carbon release of 33-114 Pg by 2100. By 2300 it suggests that 50% of the carbon stored in the upper 3m could be released into the atmosphere. A more complex model called ORCHIDEE assumes a release of 62 ± 7 Pg of carbon and an emission of 41Tg up to 70Tg of methane per year by 2100. In this model microbial activity is included more which is predicted to increase emission of CO_2 by 37% and CH_4 by 14% in 2100 (Grosse et al., 2011).

Another model called ‘Terrestrial Ecosystem Model’ includes the emissions caused by wildfires which are expected to become a more recurring phenomenon even in the Arctic circle. The model assumes an increase in emission up to 473 Tg of carbon per year in 2100, most of which is attributed to fire emissions (Schaefer et al., 2011).

A model based on the IPCC SRES A1B (Special Report Emissions Scenario) takes into consideration the impact of vegetation. It comes to the conclusion that by 2200 up to 59% of permafrost is lost causing a carbon emission of 190±64 Pg. Furthermore, it assumes that by 2030 the Arctic will have switched from being an overall carbon sink to an overall carbon source (van Huissteden and Dolman, 2012).

Those vastly different models and therefrom resulting predictions show that still much is unknown. The impacts of climate change onto the Arctic ecosystem leave many open questions, but what all have in common is the large amounts of carbon expected to be released into the atmosphere.

3.2.7. Microorganism in Arctic Soil

As temperatures increase in the Arctic and soil thaws a shift in the ecosystem is expected, as it causes an overall longer growing season. Therefore, microbial activity is expected to increase, which leads to an increase in decomposition of organic material (van Huissteden and Dolman, 2012). What type of organisms, especially microorganisms become more dominant will have strong effects on the release of carbon into the atmosphere. As a consequence, this will determine if the Arctic environment becomes an overall sink or source for GHG (Yergeau et al., 2010).

3.2.7.1. Data Availability

In recent years increasing amounts of research effort has been dedicated to biodiversity worldwide. The same is true for soil biodiversity (Malard and Pearce, 2018). However, even though the Arctic is of huge importance to the global ecosystem and climate change, not much focus has been given to Arctic soils and its microbial biodiversity. This is notable as not many organisms of the Arctic tundra have been identified. Overall, almost 5% of the earth landmass is dominated by Arctic tundra, yet only 0.2% of 16S rRNA genes in common genebanks represent soil organisms from that area (Nemergut et al., 2005). However, an increased effort into identifying Arctic soil microbes can be observed (Malard and Pearce, 2018).

Previously research into microbial communities in Arctic soil have been focusing on summer periods when the regions are free of snow. This was the case because microbial activity was believed to be non or almost non existing in the winter months. However, studies show that snow covered soil have microbiological activity. As a matter of fact, snow can cause insulation of the soil and microbes continue to be active during those periods (Nemergut et al., 2005).

Strong disparity in sequenced microbes can be seen in different regions or countries, e.g., much more sequences of soil organisms are available from North America than from Icelandic or Russian Arctic soil (Malard and Pearce, 2018). In addition, most sequencing effort has been directed towards soil bacteria. Even though fungi play an integral part of Arctic nutrient cycles, most remain unidentified (Nemergut et al., 2005)

3.2.7.2. Overall Diversity

For a long time, microbial activity and diversity in Arctic soil was believed to be low, however recent studies into richness and relative abundance have shown that the diversity is similar to other soils in temperate or even tropical regions (Blaud et al., 2015). Although polar microorganisms have lower activity rates due to climatic conditions, their role in nutrient cycling is nevertheless essential (Malard and Pearce, 2018).

Recent studies have shown a significant decrease of detectable cells with increasing depths. Leading to the conclusion, that cells activity decreases with increasing depths of soil. It is believed that abundance and activity is higher in the active layer than in permafrost (Blaud et al., 2015). Yet the level of activity within permafrost is still barely understood (Nemergut et al., 2005).

3.2.7.3. Bacteria

Bacterial communities in Arctic soil are mostly dominated by Proteobacteria with 37% to 45% (Tveit et al., 2013). Of those nitrogen fixing Rhizobiales as well as Burkholderiales, Xanthomonadales, and Myxococcales are the most prominent Proteobacteria (Malard and Pearce, 2018). Myxococcales belong to Deltaproteobacteria which is the most common class of Proteobacteria. After Proteobacteria the phyla Actinobacteria and Planctomycetes, with 15% and 9%-14% respectively are most common. Verrucomicrobia, Acidobacteria, and Chloroflexi show an abundance of around 10% each (Tveit et al., 2013, Mackelprang et al., 2011).

A difference between active layer and permafrost can be observed. In permafrost mostly Bacteroidetes and Firmicutes can be found whereas in the active layer Actinobacteria, Proteobacteria and Chloroflexi dominate (Tveit et al., 2013). In future years an increase in relative abundance of Acidobacteria, Bacteroidetes, and Actinobacteria is expected (Malard and Pearce, 2018).

Nevertheless, the bacterial diversity changes greatly through regions. For example, are Bacteroidetes much more common in Greenland and Finland than Alaska, Canada or Svalvad. When looking at the diversity on class level, differences between sites become even more clear. This suggests strong biogeographical variation throughout Arctic soil (Malard and Pearce, 2018).

3.2.7.4. Fungi

Fungal diversity and communities in Arctic habitat are still largely unknown. Large variations across the region are expected, yet not fully understood. The most found phyla are Ascomycota, Basidiomycota, Chitriomycota, and Zygomycota. The latter two are significantly less common, with a combined relative abundance of less than 25%. In both Svalbard and Alaska Ascomycota are most prevalent. However, in Canada Basidiomycota are the dominating fungi phyla (Malard and Pearce, 2018).

3.2.7.5 Archaea and Cyanobacteria

Again, depending on region the composition can vary greatly. Amongst archaea 4 phylum are overall the most represented, Thaumarchaeota, Euryarchaeota and Crenarchaeota. However, in Svalbard only Thaumarchaeota and Crenarchaeota can be found. In Greenland and Alaska Euryarchaeota is by far the most prevalent. Within this phylum Methanomicrobia and Methanobacteria are the most abundant. Siberia and Canada show similar levels of relative abundance across all 4 phyla (Malard and Pearce, 2018).

CO_2 and N_2 uptake are mostly attributed to plants and their root bacteria. However, next to that, Cyanobacteria also contribute as they are capable of binding N_2 into the soil. For Cyanobacteria the most represented orders are Oscillatoriales, Synechococcales, and Nostocales (Zakhia et al., 2008, Malard and Pearce, 2018).

3.3. International Tundra Experiment

In the late 1980s the effects of warming climate were of increasing concern. A major point of interest were high latitude regions, as rising temperatures were thought to have more immediate and intense impacts there (Henry and Molau, 1997). Thus, at a meeting with Arctic tundra ecologists at the Kellogg Biological Station (Michigan State University) in December 1990 the International Tundra Experiment (ITEX) was established (Henry et al., 2013). Initially, the main goal of ITEX was to study tundra plant species and their response to warming climate and environmental shifts (Marion et al., 1997, Henry and Molau, 1997). The ITEX has grown ever since and is still an international network of researchers analysing tundra ecosystems in polar and alpine locations (Oberbauer et al., 2007). In the early 1990s several Arctic and Subarctic tundra sites had been established in Sweden, Greenland, Norway, Iceland, Finland, Canada, and the U.S. Furthermore, sites in Russia had been thought of, but lack of sufficient funding as well as political matters at the time have hindered the implementation of ITEX (Henry and Molau, 1997) (Henry et al., 2013). Now, more than 20 sites are established around the world to conduct years-long and standardized experiments about changes in the tundra biome (Oberbauer et al., 2007).

A second meeting regarding ITEX was held in February 1992 at the Danish Polar Centre in Copenhagen, Denmark. From this a manual for further ITEX field sites was developed. Due to many variations in biological factors as well as logistical and financial prospects, two levels of standard were established. This allows for adaptation to those variations and limitations while maintaining comparability (Molau, 1996).

To study warming climate a low-cost and uncomplicated approach was thought after. Therefore, a passive warming method in the form of open-top chambers (OTC) was chosen for ITEX (Henry and Molau, 1997) (Henry et al., 2013). OTCs consist of transparent walls of either plexiglass or fibreglass. This causes the air within to warm up. The chamber is not closed but rather has an open top, allowing for gas exchanges and precipitation to remain close to the conditions outside the chamber (Figure 4). Most OTCs are placed on location and unattended for several years. Sensors placed at the site provide information about temperatures and precipitations for the duration of the exposure experiment (Henry and Molau, 1997).

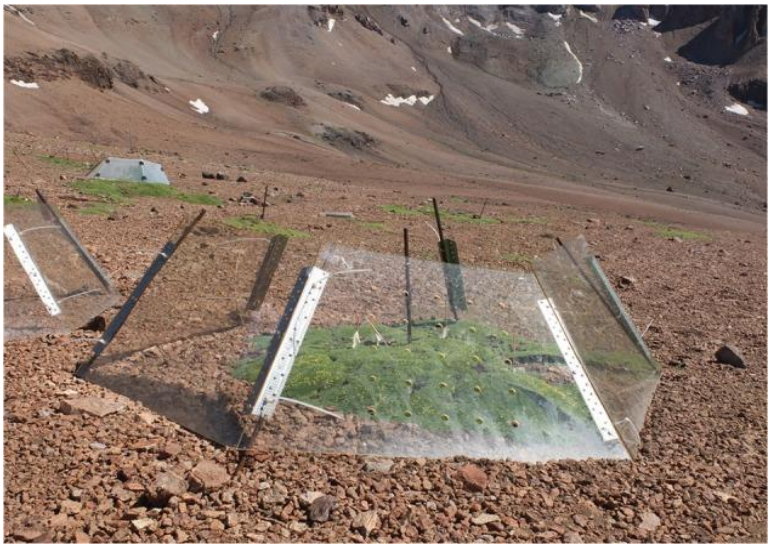

Figure 4: Photograph of an Open Top Chamber taken in central Chile (Anthelme 2014).

Research has shown that ITEX OTC have increased the temperature within the chambers in the growing season. On average an increase of 1°C to 3°C of near-surface temperature was observed (Henry and Molau, 1997). Depending on different properties of the site, the changes vary. Wet Arctic sites have shown lower temperature changes in the range of 1.2°C to 1.8°C. Dry polar deserts have shown higher temperature increases within the chambers, with 2.2°C to 5.2 °C (Marion et al., 1997). Those levels of temperature increase are in accordance with expected global warming but remain at the lower side of most models (Henry and Molau, 1997). Passive warming within ITEX has shown to increase growth rates as well as leaf area of plants. Overall, plants and soil biota are thought to have an increased respiration rate due to an increase in enzyme activity. Which is believed to be caused by warming. However, other components e.g., moisture content, can also influence and limit respiration rates (Oberbauer et al., 2007).

In combination with other global studies, ITEX aids in understanding and predicting future effects of climate change on Arctic and alpine ecosystems (Henry and Molau, 1997). Nevertheless, improvements to the ITEX are discussed to enable more efficient research and gain a more complete and complex insight into tundra ecosystems. Still a lack in ITEX setups in Russia is noticeable, including more Russian sites and scientists in the network would be crucial (Henry et al., 2013). Other factors than temperature, e.g., precipitation, as well as biological interactions e.g., with herbivores and pollinators are currently mostly disregarded. As they have significant impacts on the ecosystems including them in further studies is necessary (Post and Pedersen, 2008). Another aspect which is often overlooked is migration, which next to adaptation is an essential method used to cope with prompt environmental changes. Lastly, the implementation of modern techniques both on the ITEX sites and laboratories can lower costs and collect more data (Henry et al., 2013).

3.4. Analysing Soil Samples

To understand soil, its function, and microbial community, complex analysis is needed. The size and diversity of soil can be very large. In one gram of grassland soil the magnitude of prokaryotic cells found is 10^9 cells/g soil. For forest soils, it is about 10^7 cells/g soil (Daniel, 2005). This number only represents detected organisms. The true amount of cells is believed to be higher, in light of undetected and rare species (Torsvik and Øvreås, 2002). To analyse the microbiological diversity within soil, several approaches are possible. One can cultivate the organisms or use molecular methods (Daniel, 2005). It is estimated that only between 0.1% and 1.0% of soil bacteria can currently be cultivated (Torsvik and Øvreås, 2002, Amann et al., 1995).

Methods independent of cultivation can help examine soil diversity. Moreover, biases due to cultivation can be overcome (Daniel, 2005). Many techniques for DNA extraction of soil have been developed. Soil often has a complex matrix, with components changing depending on the soil. This can cause different challenges for extraction. Hence several different soil DNA extraction methods have been developed (Lloyd-Jones and Hunter, 2001). The heterogeneous nature of soil and some microorganisms attaching to soil particles, makes DNA extraction challenging (Martin-Laurent et al., 2001). Two main approaches are used. Firstly, co-extracting DNA with other soil components, by direct cell lysis and then purifying the DNA. Secondly, it is possible to first separate the cells and soil, then the cells can be lysed, and the DNA extracted (Daniel, 2005). Direct lysis has shown to extract more DNA compared to separating DNA and matrix first. Direct lysis is also a harsher method (Gabor et al., 2004). Extracted DNA can be used for sequencing or for constructing DNA libraries (Daniel, 2005).

For DNA libraries extracted sequences can be inserted into host organisms. Those sequences can vary in size, from 15 kb to over 40 kb (Daniel, 2005). Most commonly the host organism is *Escherichia coli*. Now other hosts are being established, including *Streptomyces* and *Pseudomonas* (Martinez 2004). By analysing such libraries, a functional analysis of soil is possible. This has led to understanding and discovering biomolecules and gives an insight into physiology, interactions within the ecosystem, and evolution (Gabor et al., 2004). However, this does not come without obstacles and limitations. A function-driven screening is dependent on expression working within the provided host organism. Moreover, often a large number of clones are needed, which is labour intensive (Daniel, 2005).

In addition to function-driven screenings, sequence-driven approaches are available. The main advantages of sequencing are that they are independent of any host organism, and that with similar methods a variety of targets can be reached (Daniel, 2005). However, sequences are compared to known genes in databases. This makes discovering the undiscovered challenging. Also, some biases cannot be avoided, e.g., due to different amplification efficiencies in PCRs. Moreover, it provides less information about functionality in the soil (Knight et al., 2018).

Overall, the different approaches provide different insights and come with unique challenges. Combining methods is always advisable, to minimise the effects of biases and to gather as much

information as possible. Further development and improvements will help better understand the large soil habitat (Daniel, 2005).

3.5. Aim of the Study

Climate change is expected to cause strong temperature increases in the Arctic, which then can cause changes in microbial communities. Increase microbial activity can cause the stored carbon to be emitted into the atmosphere. To better understand the changes in Arctic soil, this study looks at the effects of induced summer warming in northern Greenland. Specifically, the microbiological soil communities are analysed to see temperature driven changes.

3.6. Hypothesis

The ITEX set up, which simulates summer warming, is believed to affects both, diversity and abundance of microorganisms in high Arctic soil. The bacterial and fungal community in the top layer of the soil is expected to be affected most. The deeper soil is thought to show less change.

3.7. Flow-chart

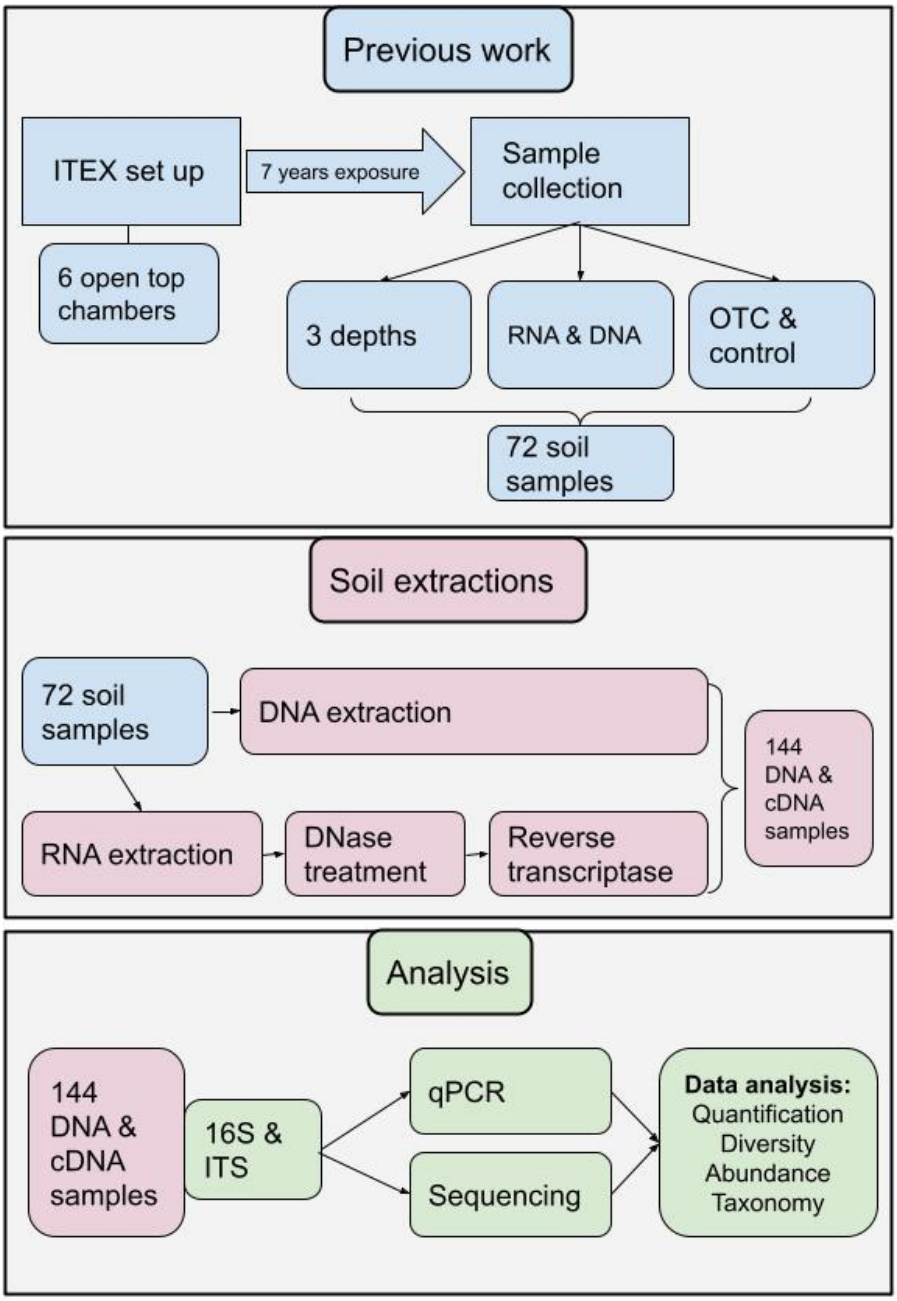

Figure 5: Overview of the experimental work

4. Material and Methods

This study is part of a years-long exposure experiment, for the purpose of this thesis called "The Brønlundhus Open Top Chamber Experiment". Therefore, some parts were executed previously by others.

4.1. Previous Work

The experiment started in July 2014 when six open-top polycarbonate chambers were set up by Bo Elberling from the Department of Geoscience and Resource Management, University of Copenhagen. Samples were taken 7 years later on July 26, 2021, by Bo Elberling and Elisabeth Biersma from the Natural History Museum, University of Copenhagen. The site was about 500 metres away from the research station Brønlundhus (Figure 6), Peary Land, North Greenland (82° 6' N, 32° 33' W). The site is an inland delta in which mainly grasses grow. The delta is surrounded by mostly barren soil.

Figure 6: Photograph of the Research Station Brønlundhus. Picture taken by Anders Priemé

The OTCs had a diameter of 150cm at the base and a height of 35cm. Sampling was done by 2.54-cm diameter steel corer at the depths of 0-5cm, 5-10 cm and 15-20cm (Table 1). Samples were taken from inside each OTC and outside, as control. For each spot three replicate soils were taken and homogenised, stones and woody parts were removed from the samples. The samples were placed into individual 2mL plastic tubes and 0.5mL LifeGuard Soil Preservation Solution (Qiagen, Vedbæk, Denmark) was added. The samples were stored and transported at about 5°C and placed into storage at University of Copenhagen at -80°C. A detailed overview of all samples can be found in Appendix 1. In the following, abbreviations for the samples will be used. The sample abbriviation consists of 4 digits, the first indicating if DNA or RNA extraction was used, with D and R respectively. The second digit refers to the chamber the sample was taken from. The third digit indicate to control with 'C', and OTC with 'O'. The last number represents the depths of the sample.

. *Table 1: Sample depth specified*

Name hereafter	Depth of sample
Depth 1	0-5 cm
Depth 2	5-10 cm
Depth 3	15-20 cm

4.2. Extraction

The samples collected in Greenland were stored at -80°C until the extraction process and thawed up before extraction began. To avoid degradation of RNA, all RNA extractions and steps to obtain cDNA from the RNA samples were carried out in a special dedicated laboratory. This laboratory uses appliances free of RNA degrading enzymes. Moreover, RNAZap (RNaseZap™, invitogen by Thermo Fisher Scientific Baltics, Vilnius, Lithuania) was used to remove these enzymes from objects brought into the laboratory, including but not limited to the sample tubes, gloves, and all surfaces before use.

4.2.1. DNA Extraction

Before extraction the previously added LifeGuard Soil Preservation Solution was removed, by centrifuging the samples and removing the supernatant. For extraction of the DNA the DNeasy® PowerSoil® Pro Kit (Qiagen, Vedbæk, Denmark) was used. The provided PowerBead Pro Tubes were centrifuged briefly in order to collect the beads on the bottom of the tubes. 250mg of each soil sample and 800µL of solution CD1 were added to the tubes. Those were then horizontally vortexed for 10 minutes. Afterward the PowerBead Pro Tubes were centrifuged for 1 minute at 15,000g. The supernatant was transferred to a new 2mL tube. Then 200µL of solution CD2 were added and shortly vortexed. The tube was then again centrifuged for 1 minute at 15,000g. 700µL of supernatant were transferred into fresh 2mL tubes and 600µL of solution CD3 were added and the mixture was shortly vortexed. 650µL of the mixture were transferred onto a provided MB Spin column and centrifuged for 1 minute at 15,000g. The flow-through was discarded. The remaining mixture was then added to the same spin column and again centrifuged. The flow-through was discarded again and the column was placed on new collection tubes. 500µL of solution EA were added to the column and again centrifuged for 1 minute at 15,000g. The flow-through was discarded. 500µL of solution C5 was added to the column and again centrifuged at the same conditions. The columns were then transferred into new 2mL tubes and centrifuged again for 2 minutes at 16,000g and then placed into new 1.5mL provided Elution Tubes. 75µL of solution C6 was added directly onto the membrane of the column. After a final centrifugation at 15,000g for 1 minute the column was discarded and the extracted DNA was stored at -20°C.

4.2.2. RNA Extraction

Similar to the DNA extraction, prior to the RNA extraction the sample tubes were briefly centrifuged, and the safeguard solution was removed. The RNeasy® PowerSoil® Total RNA Kit (Qiagen, Vedbæk, Denmark) was used for RNA extraction. Soil was added to the provided 15mL PowerBead Tube. A detailed list of the quantity of soil used for each sample can be found

in the Appendix 1. Then 2.5mL of PowerBead Solution, 0.25mL of solution SR1, 0.8mL of solution IRS, and 3.5mL of phenol/chloroform/isoamyl alcohol were added to the PowerBead tubes. The tubes were vortexed horizontally for 15 minutes at maximum speed and then centrifuged for 10 minutes at 2500g. The aqueous phase was then transferred into a new 15mL tube and 1.5mL of solution SR3 was added and vortex shortly. The mixture was incubated at 4°C for 10 minutes and centrifuged for 10 minutes at 2500g and room temperature. The supernatant was transferred into a new 15mL tube. 5 mL of SR4 was added. The mixture was vortex and incubated for 30 minutes at room temperature. Afterwards it was centrifuged for 30 minutes at 2500g. The supernatant was decanted, and the tube inverted on a paper towel for 5 minutes. Then 1mL of solution SR5 was added and resuspended to dissolve the pellet completely. The provided JetStar Mini Column were prepared by adding 2mL of Solution SR5 and letting it flow through completely. The column was not to dry out before loading the sample. The sample solution was added to the column and let gravity flow through the column into a tube. Afterwards 1mL of solution SR5 was added to the column and again let flow through. The columns were then transferred into new tubes and 1mL of solution SR6 was added and let flow through. 1mL of solution SR4 was added to the eluted RNA and inverted several times to mix. Then it was incubated at -20°C for 10 minutes and then centrifuged for 15 minutes at 13,000g. The supernatant was decanted, and the tubes were inverted on a paper towel for 10 minutes. The RNA was resuspended with 100µL of solution SR7. To prevent decay of the RNA samples a DNase treatment and reverse transcriptase were carried out on the sample immediately after the RNA extraction was finished.

To remove DNA that was extracted during the RNA extraction a DNase treatment was conducted. The DNA-*free*™ Kit from Ambion™ (ThermoFisher Scientific) was used. The 100µL extracted RNA from the previous step was used. 10µL DNase I Buffer and 1µL rDNaseI was added to the RNA. The mixture was incubated for 30 minutes at 37°C. The DNase Inactivation Reagent was resuspended before use and 10µL were added to the mixture. While occasionally mixing, the sample was incubated for 2 minutes at room temperature and then centrifuged for 1.5 minutes at 10,000g. The supernatant was then transferred into new tubes.

To transcribe the RNA to cDNA the kit SuperScript™ II Reverse Transcriptase from Invitrogen™ (Fisher Scientific) was used. 10µL of the RNA sample after DNase treatment were mixed with 1µL of random hexamers (5'-NNN NNN-Wobbles-3', MV: 1792, 100pmol/µL) and 1µL dNTP (10 mM each). The mixture was incubated for 5 minutes at 65°C and afterwards quickly cooled on ice. Then 4µL of 5X First-Strand Buffer and 2µL of 0.1 M DTT (Dithiothreitol) were added and the mixture was incubated for 2 minutes at 25°C. 1µL of SuperScript™ II RT was added and mixed gently. The mixture was incubated first for 10 minutes at 25°C, then for 50 minutes at 42°C and finally at 70°C for 15 minutes. Afterwards the samples were stored at -20°C until further steps were carried out.

4.3. Illumina Sequencing

The extracted DNA and cDNA were prepared for Illumina MiSeq sequencing. For that, sequencing libraries with dual-PCR setup were prepared. The primers for the first PCR were 341F and 801R for 16S. The targeted regions for 16S were V3 and V4 with an approximated

length of 460bp. The ITS primers were HS7 and HS4. Table 2 shows the primer sequences that were used. Table 3 and Table 4 show the PCR setup used for the first PCR. The PCR was set up using PCRBIO HiFi polymerase, modified to 25µL reactions (with 2µL template, Table 4) in accordance with the manufacturer's instructions.

Table 2: Primer sequences for the first PCR.

Primer	**Sequence**
Uni341F	5'-CCTAYGGGRBGCASCAG-3
Uni806R	5'-GGACTACNNGGGTATCTAAT-3
HS7	5'-GTGARTCATCGARTCTTTG-3
HS4	5'-TCCCTSCGCTTATTGTGC-3

Table 3: PCR thermo cycle setup for the first PCR

Temperature	**Time**	**cycles**
95°C	1 minute	1 cycle
95°C	15 sec	30 cycles
56°C	15 sec	
72°C	30 sec	
72°C	5 minutes	1 cycle
10°C	∞	

Table 4: First PCR component set-up for 25 µL reaction

Reagent	**Amount**
5x PCRBIO Reaction Buffer	5 µL
Forward primer (10 µM)	1 µL
Reverse primer (10 µM)	1 µL
Template DNA	2 µL
PCRBIO HiFi Polymerase (2u/µL)	0.25 µL
PCR grade dH_2O	15.75

After the first PCR a purification of the samples was conducted using the HighPrep™ PCR PCR Clean-Up System (MagBio Genomics Inc., USA) (0.65µL beads:1µL amplicon product). A second PCR was then performed on the purified product of the first PCR. This second PCR used primers with attached sequencing adaptors and barcode tags. For the 16S samples a plate with the tagged primer sets was used (Integrated DNA Technologies, Inc; '341f-806r - plate 1,

REPLICATE', IDT Plate#:13968476, 5µM in 50µL, Coralville, Iowa, USA). Again, the PCRBIO HiFi polymerase, modified to 25µL reactions (with 2µL template) was used.

For the second PCR of the ITS samples Nextera indexed primers (Nextera® XT Library Prep Kit, Illumina, Inc., San Diego CA, USA) were used. The adaptors for that were: N701, N702, N703, N704, N705, N706, N707, N710, N711, N712, N714, N715, S502, S503, S505, S506, S507, S507, S510, S511. For the ITS PCR mixture 5µL of both the Index S- and Index N-Primers, 5µL 5x PCRBIO Reaction Buffer, 2µL template, 7,75µL PCR grade dH_2O and 0,25µL PCRBIO HiFi Polymerase (2u/µL) were added together. The second PCR was run as depicted in Table 5.

Table 5: PCR thermo cycle setup for the second PCR

Temperature	**Time**	**cycles**
95°C	1 min	1
95°C	15 sec	15
56°C	15 sec	
72°C	30 sec	
72°C	5 min	1
10°C	∞	

The products of the second PRC were again purified using the HighPrep™ PCR-kit. Afterwards a normalisation step using the SequalPrep Normalization Plate (96) Kit (Invitrogen, Maryland, MD, USA) was performed. All samples, 16S and ITS, were then pooled together and concentrated using DNA Clean and Concentrator™-5 kit (Zymo Research, Irvine, CA, USA). The concentration was measured using a QuBit fluorometer (Quant-iT™ High-Sensitivity DNA Assay Kit (Life Technologies)) and then diluted to 1.34µg/mL.

The PCRs were run on 2720 Thermal Cycler (applied biosystems by life technologies). After both, first and second PCR, a gel electrophoresis was run to check if the PCR was successful. A 1%w/v agarose gel with Ethidium Bromide and Gene Ruler 100 bp (DNA Ladder, SM0241, Thermo scientific Baltics, Vilnius, Lithuania) was used. All gels were run at 110V for 30 minutes.

Then the samples were sequenced using the Illumina MiSeq Desktop Sequencer (Illumina Inc., CA, US) with a final concentration of 16 pM. All reagents used were taken from MiSeq Reagent Kits v2 (Illumina Inc., CA, US). 5.0% PhiX internal control was included for each run. For sequence demultiplexing the MiSeq Controller Software was used.

4.4. Bioinformatical Analysis

The data obtained by sequencing was analysed in several steps. First the tool BioDSL (https://github.com/maasha/BioDSL) was applied. Hereby the diversity spacers and sequencing adaptors were trimmed. It was also used for sequence mate-pairing, removal of short reads

under 100bp, dereplication of remaining sequences and singleton sequence removal. Operational taxonomic unit (OTU) were clustered at a sequence similarity of 97% using USEARCH v7.0.1090 (Edgar, 2010). The UCHIME algorithm (Edgar et al., 2011) was then used to check and remove chimera. Mothur v.1.25.0 (Schloss et al., 2009) was applied to designate sequences to OTUs at a threshold of 0.8 and the Ribosomal Database Project (RDP) trainset9 database (032012) (Cole et al., 2014) was used to then classify the sequences. The phylogenetic tree was constructed using FastTree (Price et al., 2009). From this sequencing tables were extracted at unique amplicon sequence variants (ASV) level. The taxonomy, if possible, was classified to the species level.

All statistical analyses were conducted using R and all plots were created using ggplot. The entire R code used can be found in Appendix 2.

A decontamination was conducted by applying the negative controls (decontam). To determine the effects of the treatment on the sample several statistical analysis tools were used. These tests were carried out on the entire sample set, as well as several subsets

The effect of the treatment, summer warming, on the samples was of interest. This was tested by using ANOVA (Analysis of Variance). Here both the Shapiro-test and the Bartlett-test were conducted. For calculating the alpha-diversity the OTU abundance matrices were normalised to obtain the same sequencing depth for all samples. Then ANOVA was again used to calculate the Shannon diversity and the observed richness. Moreover, a Principal Coordinate Analyses (PCoAs) was conducted. To analyse the community structures of different samples and subsets, a permutational multivariate analysis of variance (PERMANOVA) was carried out.

4.5. qPCR

On the unamplified DNA and cDNA, a qPCR (quantitative PCR) was performed in specialised plates (PCR® Microplate, white, PCR-96-LC480-W, nonpyrogenic & RNase-/DNase-free, corning Incorporated, Rexnosa, Mexico). For the thermo cycles the LightCycler® 96 System (Rocher Diagnostics, Mannheim, Germany) was used. The cycle details can be found in Table 6. For each sample 2µL template were mixed with 10µL qPCR master mix (2x qPCRBIO SyGreen Blue Mix Lo-ROX, PCR Biosystems Ltd., London, UK), 0.8µL of each of the two primers (as detailed previously) and 6.4µL PCR grade dH_2O.

Table 6: Thermo cycle set-up for the qPCR

Step	**Cycles**
Preincubation 95°C for 10 min	1
2 step amplications (95°C and 60°C)	45
Melting, 95°C for 180 sec	1

The sample concentrations per gram soil were calculated with the LightCycler® 96 System software 1.1 (Rocher Diagnostics, Mannheim, Germany). A standard curve was calculated based on a serial dilution for each plate.

5. Results and Discussion

5.1 Agarose Gel

Agarose gels were carried out to verify the first and second PCR of the Illumina preparations. When unsuccessful, the PCRs were repeated until the gel showed the desired bands. Thereby contaminated runs as well as non-functioning primer sets were identified and corrected before sequencing. Large gels were used, to be able to run all samples form one 96-plate at ones. The resulting images were cut to visualise them easier. Pictures of the entire gels are included in the Appendix 3 and 4. The gels were run only to verify if a band is visible or not. Therefore, the ladders are not fully separated.

5.1.1 16S

The agarose gel for the 16S plate showed all 4 ladders. The gel had 5 negative control samples, four from the first PCR and one from the second. The negative control of the second PCR showed no band, indicating no contamination for the second PCR. Of the negative controls of the first PCR three out of 4 showed no band. One shows a very faint band. As the band is very faint, and all others show no band, the run was accepted. Any sequences found in the negative control were later used to decontaminate the samples. Mock communities were used to verify the sequencing later. For all the three mock samples used bands were visible. For the purpose of analysing the gel, the mock communities can also be seen as positive controls.

Most out of the 72 samples showed a band. Overall, the bands on the left side seemed much fainter. However, this was in part due to gel imaging device. On one side the lamp was much weaker causing fainter bands. When the gel was flipped, this could be seen to the same extend on the other side of the gel.

Figure 7 shows a part of the gel, the entire gel is included in the Appendix 3. From all samples, R4C3 showed no band. Samples D6C1, R2O2, R1O3, R4O3, R5C1, R1C2, R3C3, and R4C2 showed very faint bands, compared to the other samples. This can be due to less DNA or RNA was extracted, or the specific Illumina primes for those chambers were not functioning properly. However overall, the PCR was verified by the gel and the samples were then prepared further for sequencing.

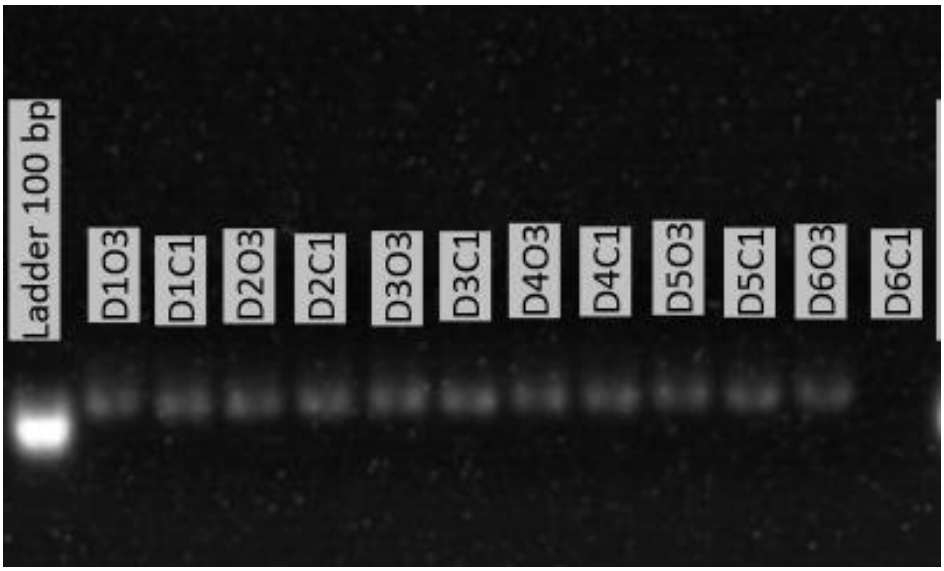

Figure 7: Section of the agarose gel from the second PCR for selected 16S samples

5.1.2. ITS

The gel for the ITS plate also showed all 4 ladders. This gel contains 7 samples from a lab college marked as 'Louis' and can be disregarded for this project. For the PCR 4 negative samples were used, two for the first PCR and two for the second. All four showed now bands, indicating that the negative controls were not contaminated.

Again, most of the samples showed a band, however, the bands especially for the RNA samples were less strong. Also, as in the previous gel, a decrease in intensity was be seen towards the left side of the gel. This was attributed to issues with the gel reader. Figure 8 shows an excerpt of the gel, the entire gel is attached in the Appendix 4.

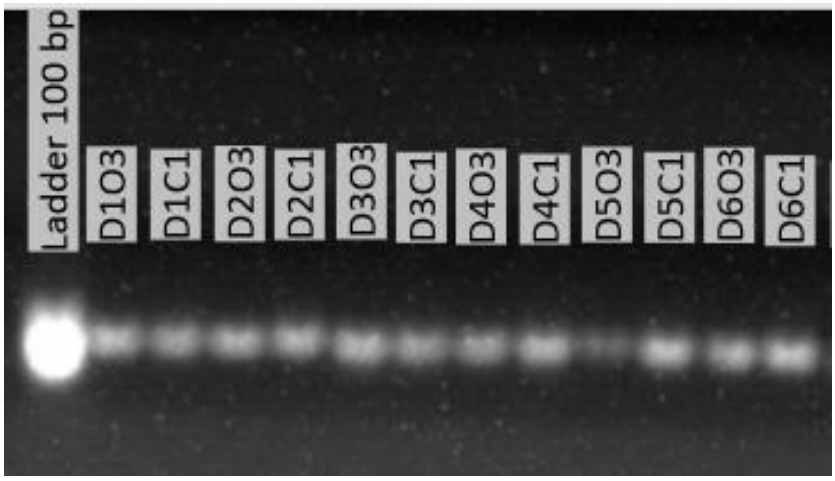

Figure 8: Section of the agarose gel from the second PCR for selected ITS samples

All DNA samples showed a band. Most had similar intensity. Sample D5O3 had the weakest band of the DNA samples. For the RNA sample the intensity varied much more. Samples R6O1, R6O3, R1C2, R5C3, and R6C3 showed no visible band.

The gels were performed to see whether or not the PCRs had worked. As most samples show bands and the negative controls show no contamination, the PCR products were then prepared for sequencing.

5.2 qPCR

5.2.1 Efficiency and R^2

The qPCR was carried out on four plates for the different sample groups. For each plate a standard curve was calculated. The corresponding R^2 scores for each group are listed in Table 7. The coefficients of correlation varied from 0.91 for both RNA sample sets, to 0.98 for the DNA sample with the 16S primer set. The DNA samples for ITS had a R^2 score of 0.96.

The efficiencies ranged from 166% to 301%. Sample groups with the same primer set show very similar efficiencies. The DNA samples with the 16S primers had an efficiency of 170% and the RNA samples with the same primers had an efficiency of 166%. The ITS samples showed much higher efficiencies, 301% for the DNA samples and 299% for the RNA samples.

Both, the R^2 scores and efficiency, were calculated based on the standard serial dilution for each plate. The standard curve was then used for the calculations of each sample. Unfortunately, the standard curves did not show satisfactory accuracy.

Table 7: R^2 score and Efficiencies calculated for for all 4 qPCRs

Sample plate	**R^2 score**	**Efficiency**
DNA samples with 16S primers	0.98	170 %
DNA samples with ITS primers	0.96	301 %
RNA samples with 16S primers	0.91	166 %
RNA samples with ITS primers	0.91	299 %

The R^2 value shows how well the measured standard curve fits the regression line. As the standard curve is used for further calculations, high standards were applied. Values of $R^2 > 0.98$ were expected for accurate calculations. With higher values being better as then the accuracy is higher (Mcdevittt et al., 2004, Helland, 1987). Of all four plates non showed R^2 values higher than 0.98. The R^2 score of the 16S DNA plate was 0.98, just right at the limit.

Another value to calculate the quality of the standard curve is the efficiency. It is a measurement for the rate at with reagents are converted to amplicons, by polymerase. In theory, this should be 100 %. However, in praxis value of 90% to 100% are desired. Efficiencies greater than 110% or lower than 90 % are indications for problems in the qPCR run (Svec et al., 2015, Čepin, 2017). As all four runs showed efficiencies significantly higher than the excepted threshold as well as low R^2 values, the qPCRs were most likely faulty. Several aspects can cause the efficiency to be higher than expected.

Most commonly primer-dimers or nonspecific amplicons cause increased efficiencies. In addition, impurities can cause increased and decreased efficiencies. The sample itself can be contaminated by e.g., heparin, haemoglobin, polysaccharides, or chlorophylls, depending on

the original sample. The extraction process can also lead to contamination with e.g., ethanol, phenol or SDS. This depends on the type of extraction that was used (Čepin, 2017). To prevent contamination, purification steps could be carried out. Further dilution could also decrease contaminations, as those would in turn also be diluted. Moreover, new sets of standards could be obtained.

Another aspect to consider, are outliers. It is possible, that only a very few amount of samples from the standard curve show strong discrepancies. If the incorrect concentration is off by a tenfold or hundredfold, one sample can cause significant effects when looking at the accuracy of the standard curve (Čepin, 2017). Hence, the concentrations of the measured standards were compared with the theoretical values of the serial dilution used. However, no specific outlier could be identified. Therefore, no correction was possible in this case.

Furthermore, a mistake in pipetting can be the reason for increased efficiencies. A wrongly calibrated pipet can be the problem. However, in this case this seems unlikely, as the pipets used were very new and recently calibrated. Moreover, no issued became apparent, when used in other experiments. Thus, human error appears to be more likely. This was the first time conducting this kind of experiment. Moreover, it was one of the first times using a multichannel pipet. This could have caused inaccuracies.

For both 16S plates and both ITS plates the same standard and serial dilution were used. If they were faulty, then the mistake would have carried out throughout all plates. The efficiencies for both plate sets were very similar. For the 16S plates the differed by 4% and for the ITS plates by 2 %. This strongly indicates that an error occurred during the setup of the standard curves. Therefore, it is strongly suggested to repeat the experiment. To ensure that the standard curves are within the desired range of accuracy, they could be tested separately until the problem is solved. Unfortunately, due to the time restrictions of this project, a repetition of the experiments was not possible. The qPCR was carried out at the end of the time in the laboratory.

With the low accuracy, small changes in concentrations, were disregarded. However, strong changes were observed when comparing the three depths.

5.2.2 DNA-16S samples

The DNA samples with 16S primers (Figure 9) showed the lowers differences between the groups. The highest concentration was measured for the control samples at depth-1, with ~$7x10^8$ copies per gram of soil. The lowest concentration was observed for the OTC samples at depth-3, with ~9×10^7. Moreover, an overall decline with increasing depth can be observed. In light of the aforementioned inaccuracies in the standard curve, this should be taken with a grain of salt. The differences between treatment and control were not very large.

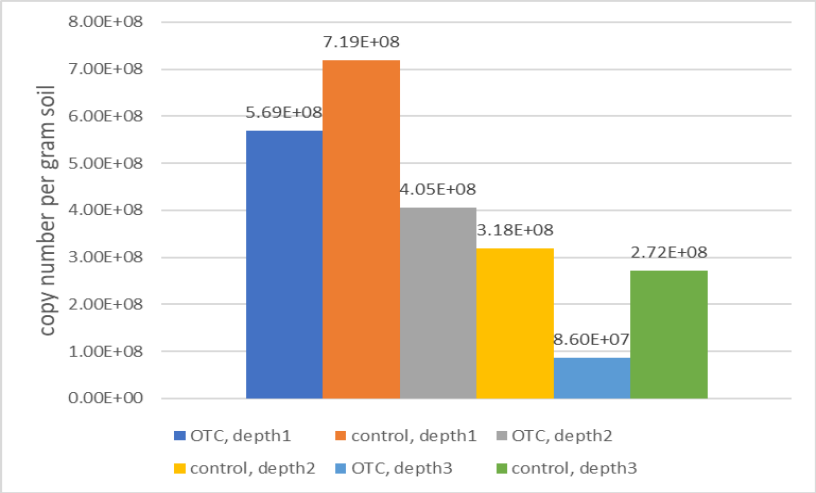

Figure 9: Concentration of copy numbers per gram of soil of the DNA-16S samples for OTC and control at all 3 depths. Based on qPCR measurements.

5.2.3 DNA-ITS samples

The DNA samples with the ITS primer showed a similar trend as the 16S samples. The highest concentrations were measured for depth-1, with both the OTC set and control in the range of 10^7 copies/g of soil. Then with each depth, the concentration decreased by a tenfold. The differences between control and OTC again were less apparent and due to the mentioned efficiency issues, the concentrations were not analysed further to avoid misleading conclusions.

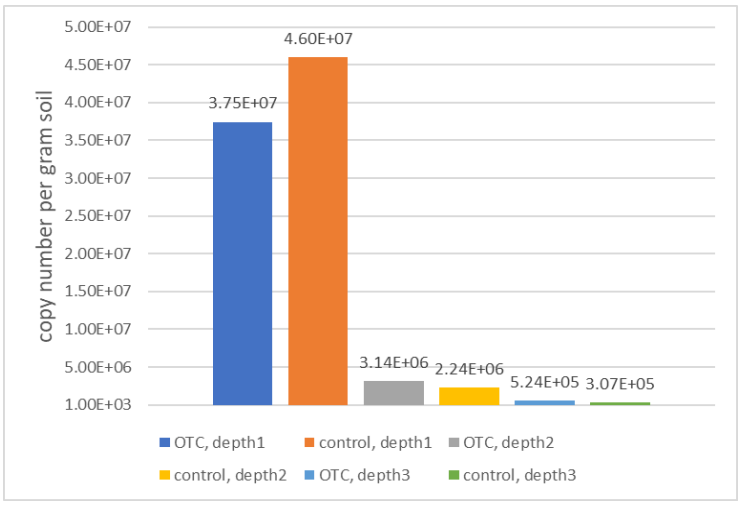

Figure 10: Concentration of copy numbers per gram of soil of the DNA-ITS samples for OTC and control at all 3 depths. Based on qPCR measurements

5.2.4 RNA-16S samples

When looking at the RNA samples with 16S primers, a similar pattern was observed (Figure 11). The highest concentration was measured for depth-1, for the OTC sample in the range of 10^6 copies/g of soil. The lowest concentration was observed for the depths-3, control sample at ~$2x10^{-1}$ copies/g of soil. Both samples at depth-2 had a concentration in the range of 10^2 copies/g of soil. The control sample at depth-1 had a concentration of ~$9x10^4$. Therefore, the

difference between OTC and control at depth-1 was over a tenfold. While keeping the previously discussed efficiencies in mind, this could indicate a difference in bacterial activity between the OTC and control.

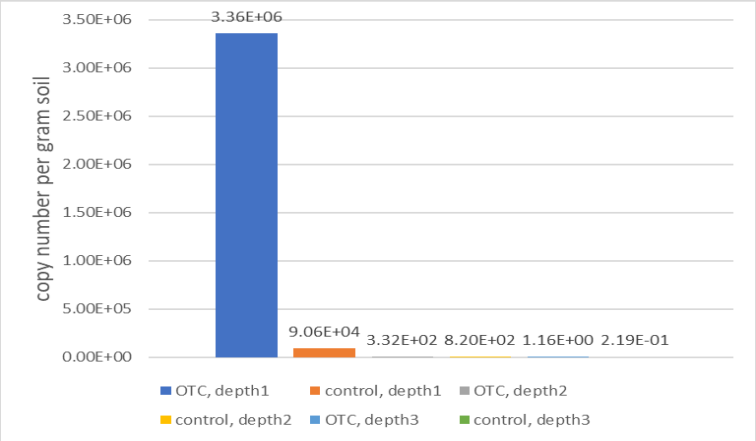

Figure 11: Concentration of copy numbers per gram of soil of the RNA-16S samples for OTC and control at all 3 depths. Based on qPCR measurements

5.2.5 RNA-ITS samples

The concentrations for the RNA samples with ITS primers were very similar to the RNA 16S samples (Figure 12). Again, the OTC sample at depth-1 had the highest concentration with ~$6x10^4$ copies/g of soil. The corresponding control had a concentration of ~$2x10^3$ copies/g of soil. Both measured concentrations a depth 2 were around zero. The concentrations at depth 3 were in the range of 10^{-3} copies/g of soil and 10^{-2} copies/g of soil, for OTC and control respectively.

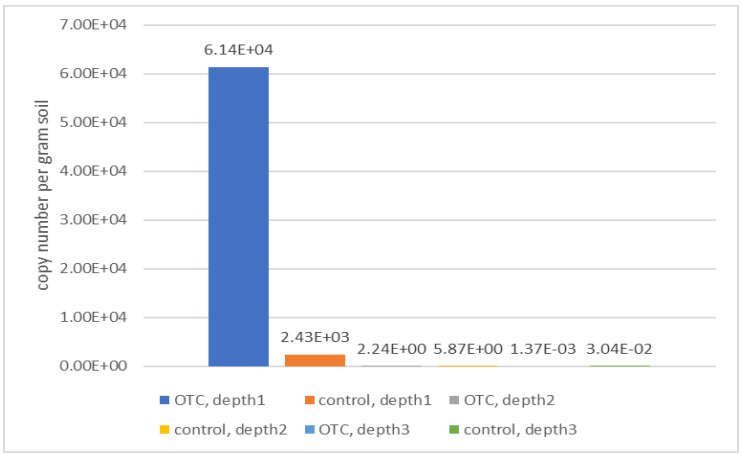

Figure 12: Concentration of copy numbers per gram of soil of the RNA-ITS samples for OTC and control at all 3 depths. Based on qPCR measurements.

The results of all four sets should be taken with a grain of salt, as previously discussed. However, in all measurements, significant decreases in concentrations with increasing depth were observed. Except for the DNA-16S samples, those observed decreases were by at least tenfold. Due to the high difference, it can be speculated that with increasing depth the amounts of cells decrease both for bacteria and fungi. Moreover, for both RNA samples, at least tenfold difference in concentration between OTC and control at depth-1 were observed. This could indicate that the biological activity is increased by the OTC. By repeating the qPCR these results

could be confirmed, and a stronger conclusion could be drawn. Also, more subtle differences between OTC and control, especially in deeper layers, could be analysed.

5.3 Sequencing Data

5.3.1 Sequencing Overview

To check the quality of the sequencing run, overall statistics on the amount of sequences and ASVs were calculated (Table 8). For the 16S samples 2,612,739 high quality sequences were obtained. The mean of all OTUs in the 16S samples was 32,659 ± 20,359 per sample. For the ITS samples a total of 13,094,908 high quality sequences were obtained, per sample 157,770 ± 86,234 OTUs. The 16S samples had overall 16,733 ASVs with a mean of 546 ± 357. The ITS samples had 307,005 ASVs, per sample about 94 ± 154.

One 16S sample showed an unusually low OTU of 42 and after further analysis was taken out of the samples. This was sample D6C1, which also showed no band in the second PCR. No visible problems with the sample were seen before extraction. Moreover, other samples that were extracted simulations with sample D6C1 showed no problem. The apparent lack of DNA in this sample could have several causes. The extraction could not have worked at all, meaning no DNA was ever extracted from the sample. More likely though, is that the extracted DNA was lost during the process, possibly while using the binding column. The sample could have either not bond to the column and thereby be washed away. Or the elution step was faulty, meaning sample was not released from the column.

Table 8: Overview from sequencing calculated for OTUs and ASV for the 16S and ITS sample groups

Measurement	**Values for 16S analysis**	**Values for ITS analysis**
Sum of OTUs	2,612,739	13,094,908
Mean of OTUs	32,659.24	157,770
Standard deviation of OTUs	20,359.3	86,234.35
Minimum OUT in sample (negative control included)	3	0
Minimum OUT in sample (negative control not included)	42	5,355
Maximum OUT in sample	84,590	307,005
Amount of ASVs	16,733	4,060
Mean of ASV	545.625	94.6988
Standard deviation of ASV	357.3422	154.4291
Minimum ASV (negative control included)	1	0
Minimum ASV (negative control included)	19	16
Maximum ASV in sample	1,311	723

5.3.2 Alpha Diversity

Alpha diversity is a measurement for the number of different species living in one habitat (Thukral, 2017). To analyse the alpha diversity, both the observed richness and Shannon diversity were calculated. The results can be seen in Figures 13 and 14.

Figure 13 shows the Shannon diversity of all samples. The 16S-DNA samples had a Shannon diversity index between 5 and 5.5, the highest can be seen for depth2 and the lowest for depth-1. The standard deviation was biggest for depth-1. The RNA-16S samples showed a lower Shannon diversity index overall, ranging from 2.5 to 4. Here the OTC sample at depth-1 showed the highest value. The lowest was observed for OTC at depth-3. Overall, for the 16S RNA sample a higher standard deviation was observed, being over 2.

The ITS-DNA samples were ranging from slightly under 1 to slightly over 2. The highest value was observed for the control sample at depth 2. However, the 4 values for, depth1 and 2 were all very similar, with a difference of less than 0.5. The overall lowest value was seen in the depth3 control sample. The standard deviations were highest at depth-3 with about 1. The RNA-ITS samples were highest at depth-3. The standard deviations for those samples are also large, around 3.

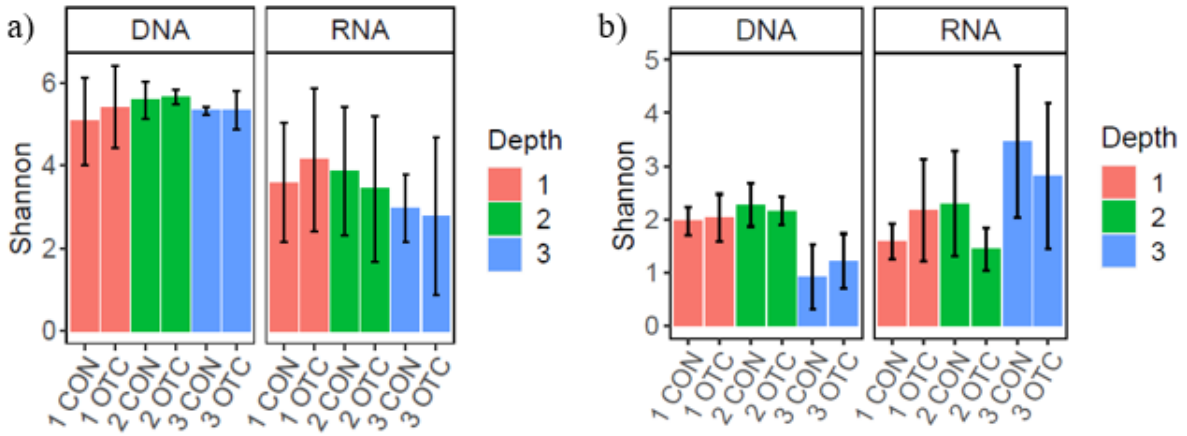

Figure 13: Effects of temperature treatment on diversity. Shannon Diversity (mean ± standard deviation, n=6). a) shows the 16S samples and b) the ITS samples

Another alpha diversity calculation was carried out, the observed richness (Figure 14). This value represents the amount of different species identified in the different sample. For the 16S-DNA sample, the highest Observed Richness was around 600 for the OTC sample at depth-1. The lowest was observed for the control sample at depth-1. Standard deviations of almost 200 were seen. The 16S-RNA samples showed an overall lower richness, but higher standard deviation. Again, the highest richness was observed for the OTC sample at depth-1 with about 500. The lowest was seen for the OTC sample at depth-2. Standard deviation of up to 400 were seen throughout the samples.

The ITS samples showed a much lower observed richness. For the DNA-ITS samples the values did not get over 50. The lowest values were observed for depth-3 with around 10. The RNA-ITS values were overall higher than the DNA samples. Most strongly, this was observed for

depth-3 where the value was 250. Yet the sample of depth-3 also show a very high variation of over 300. This could indicate that a few samples caused a significant shift in the overall richness.

To better determine whether the climate chambers had a significant effect on the soil microorganism's diversity, statistical calculations were carried out. For this the F-values and P-

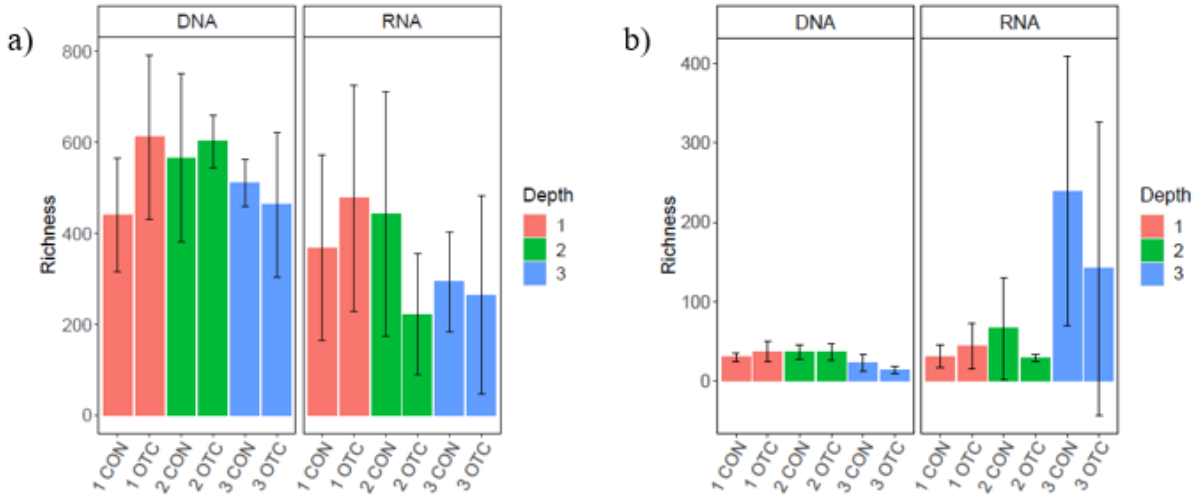

Figure 14: Effects of temperature treatment on diversity. Observed Richness (mean ± standard deviation, n = 6). a) shows the 16S samples and b) the ITS samples

values for the sample sets were calculated for the Shannon Diversity and the Observed Richness.

Table 9 shows the statistical analysis for the effects of treatment, meaning the difference between OTC and control samples. Overall, the results showed, that changes in diversity between the two treatment methods were not statistically significant. This was determined on both, the P-value and the F-value. The Null hypothesis of this test was that there would be a difference due to treatment. All four P-values are greater than 0.05, meaning the Null hypothesis was rejected. The F value was largest for the observed richness of the ITS samples. The higher F-value indicated a greater difference between the means within the group.

Overall, the statistical analysis of the treatment showed that no statistical significance was observed. This suggests that the number of different species did not vary significantly due to the treatment.

Table 9: Comparison of Observed Richness and Shannon Diversity for the effects of warming treatment.

Sample set	calculation	F value	P value	Degree of freedom
All 16S samples	Observed Richness	0.000	0.98	1
All 16S samples	Shannon Diversity	0.013	0,91	1
All ITS samples	Observed Richness	1.35	0.25	1
All ITS samples	Shannon Diversity	0.38	0.54	1

In addition to the effects of treatment on the samples, the effects of depths were calculated, to determine their significance (Table 10). The P-values for both 16S sample sets and the ITS

Shannon diversity were far above the threshold. Hence, the null hypothesis for them was also rejected. For the observed richness of the ITS samples, the P value was 0.004 and the F value was 6.03. Meaning for the ITS observed richness a statistically relevant difference was observed.

Table 100: Comparison of Observed Richness and Shannon Diversity for the effects of sampling depth.

Sample set	calculation	F value	P value	Degree of freedom
All 16S samples	Observed richness	1.64	0.20	2
All 16S samples	Shannon diversity	1.03	0.36	2
All ITS samples	Observed richness	6.03	0.004	2
All ITS samples	Shannon diversity	0.29	0.75	2

Overall, the alpha diversity did not show strong indications for changes caused by treatment. This, however, did not include a comparison of the different taxa, but rather the amount of them. Therefore, further analysis into the abundance and taxonomy of the samples were carried out.

5.3.3 PCoAs and PERMANOVA

A principal component analysis (PCoA) was conducted to further analyse the possible effect of the treatment on the soil and differences between the samples. The distance matrix visualises the phylogenetic differences in a two-dimensional space. Figure 15 shows the PCoA for all 16S samples, and Figure 16 for all ITS samples. They show the two treatment methods control, and OTC, the three depths, and the number of chambers.

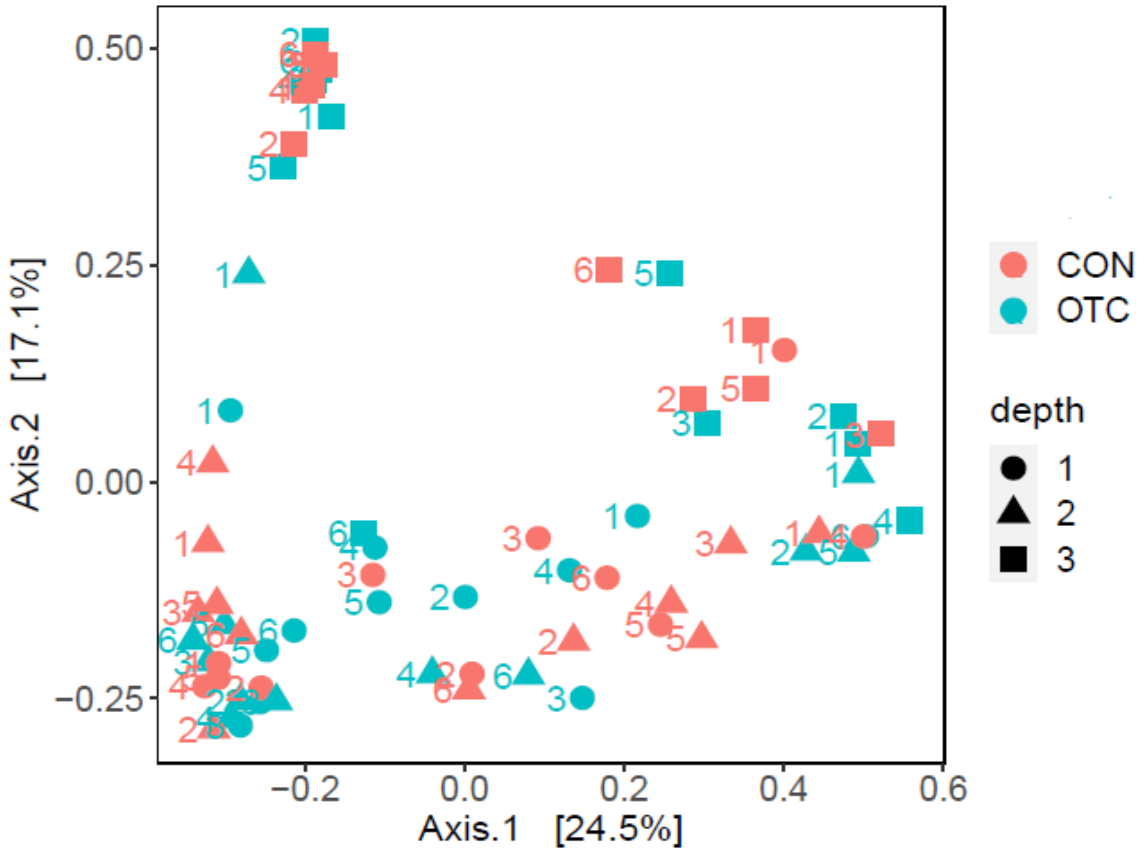

Figure 15: PCoA of community structure for all 16S samples, for the effect of warming treatment. The variation is explained by each axis in parentheses

For the treatment, no clear clustering was observed. In addition to the joint plots, additional PCoAs were calculated for each individual depth and separating DNA from RNA. However, for those plots, also no clear clustering was observed. A selection of those plots can be found in the Appendix 5-10.

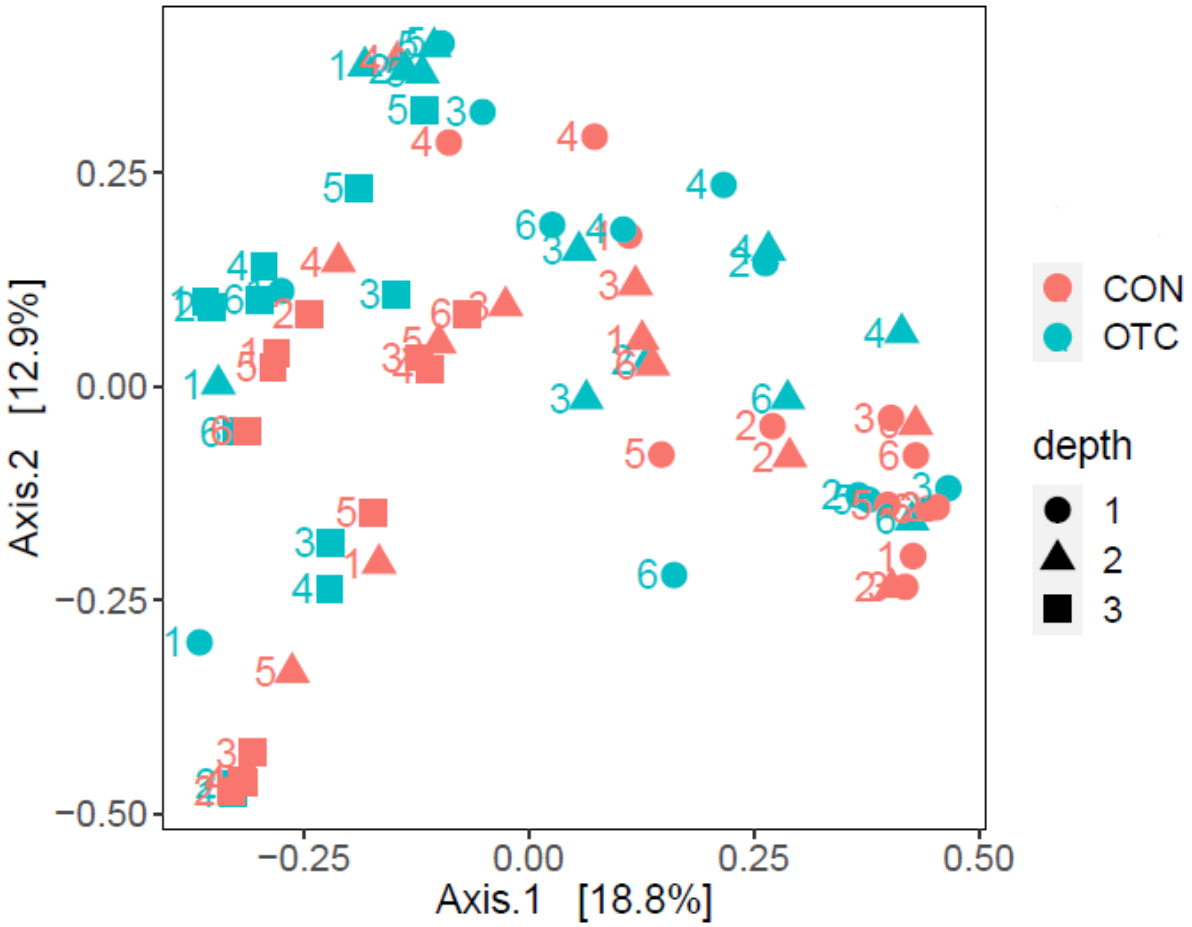

Figure 16: PCoA of community structure for all ITS samples, for the effect of warming treatment. The variation is explained by each axis in parentheses

As no clear clustering could be identified, PERMANOVA was used, to calculate whether or not a statistical relevance could be implied. This calculation was done for 16S and ITS samples of DNA and RNA at the different depths. Table 11 shows the results of those calculations. The P-values were between 0.91 for the ITS-DNA samples at depth-2 and 0.12 for the 16S-RNA samples at depth-2. Again, no p value was below the 0.05 mark. Therefore, the null hypothesis of treatment influencing the diversity was again rejected. This showed that the treatment had no statistically significant impact on the soil diversity, at any depth.

Table 11: PERMANOVA calculated for warming effects at different sampling depths.

Sample set	F-value	P-value	Degree of freedom
16S for DNA samples in depth 1	0.75	0.73	1
16S for DNA samples in depth 2	0.75	0.90	1
16S for DNA samples in depth 3	0.99	0.43	1
16S for RNA samples in depth 1	0.68	0.87	1
16S for RNA samples in depth 2	1.53	0.12	1
16S for RNA samples in depth 3	0.83	0.66	1
ITS for DNA samples in depth 1	0.75	0.74	1
ITS for DNA samples in depth 2	0.75	0.91	1
ITS for DNA samples in depth 3	0.99	0.43	1
ITS for RNA samples in depth 1	0.68	0.87	1
ITS for RNA samples in depth 2	1.53	0.12	1
ITS for RNA samples in depth 3	0.87	0.66	1

This calculation uses permutations to calculate statistical relevance and probability. The permutation groups here included all six chambers for the given subset, e.g., for the 16S-DNA set at depth-1, all 6 OTC samples were compared to all 6 control samples of that specification. By combining them, the heterogeneity of the samples could have affected the overall statistics. Hence, there is a possibility, that the variations between the chambers could overshadow the differences between control and OTC. All six OTCs were set up in the same valley, but soils are known to show great variations in small areas (Malard and Pearce, 2018).

5.3.3.1 Split-Plot Design

The six different chambers were believed to be heterogeneous, with differences in diversity and abundance. This could potentially cause limits in the statistical analysis. Therefore, an approach was thought, that compares each chamber individually with the corresponding control sample. PERMANOVA allows for such comparisons in so called 'split plot designs' (Anderson 2008). This could be used to compare directly chamber 1 with control 1, and then compare the changes between them, and chamber 2 and control 2 with each other, and so on for all 6 chambers. This could give insight into microbial changes due to the OTC while avoiding the problem of heterogeneity between the spots.

For this approach one of two conditions must apply. Either, each sample has replicates, meaning several samples from within the OTC and from the control. In this case, only one sample for each was taken. Or, several treatments apply, for example changes in temperature and changes in water availability were measured (Anderson 2008). This as well did not apply to the data and experimental setup. Without one of these two conditions, the permutation would not work, but rather pick the exact same set again and again.

5.3.4 Taxonomy and Relative Abundance

5.3.4.1 16S

The samples were further analysed to identify the taxa present in the soil and their relative abundance (Figure 17). For the 16S samples Proteobacteria showed the largest abundance

throughout the samples. Especially for the RNA measurements, Proteobacteria was the dominating phyla with over 50%. The second most abundant phyla were Actinobacteria and Firmicutes. Actinobacteria showed more relative abundance in the DNA samples as in the RNA samples. Moreover, a shift with depths was observed. For each set, Actinobacteria was least abundant in depth-3, followed by depth-1 and most abundant in depth-2. The opposite was observed for Firmicutes, which consistently showed the lowest abundance for depth-2. The highest abundance was seen for depth-3 at DNA and depth-1 for RNA. Moreover, in all samples Bacteroidetes were detected, most abundant in depth-3. With smaller relative abundance Patescibacteria, Acidobacteria, Crenarchaeota, Verrucomivrobia, and Euryarchaeota were found in some soils.

The abundance of organisms from the RNA samples gives an indication about activity. Higher abundance in RNA samples indicates that that phylum was more active. Proteobacteria by far showed the highest abundance for the RNA samples, followed by Actinobacteria and Firmicutes. By comparing controls with their corresponding OTC samples, no strong differences were observed. This indicated that the OTCs, had no significant effect on the bacterial relative abundance in the soil.

Other studies show similar results in Arctic community structure. Some studies show that Proteobacteria and Actinobacteria are the most prominent. Firmicutes and Bacteroidetes are also commonly found in northern Greenland soil, but less abundant (Ganzert et al., 2014, Yergeau et al., 2010, Tveit et al., 2013). Other studies also found Proteobacteria, Acidobacteria,

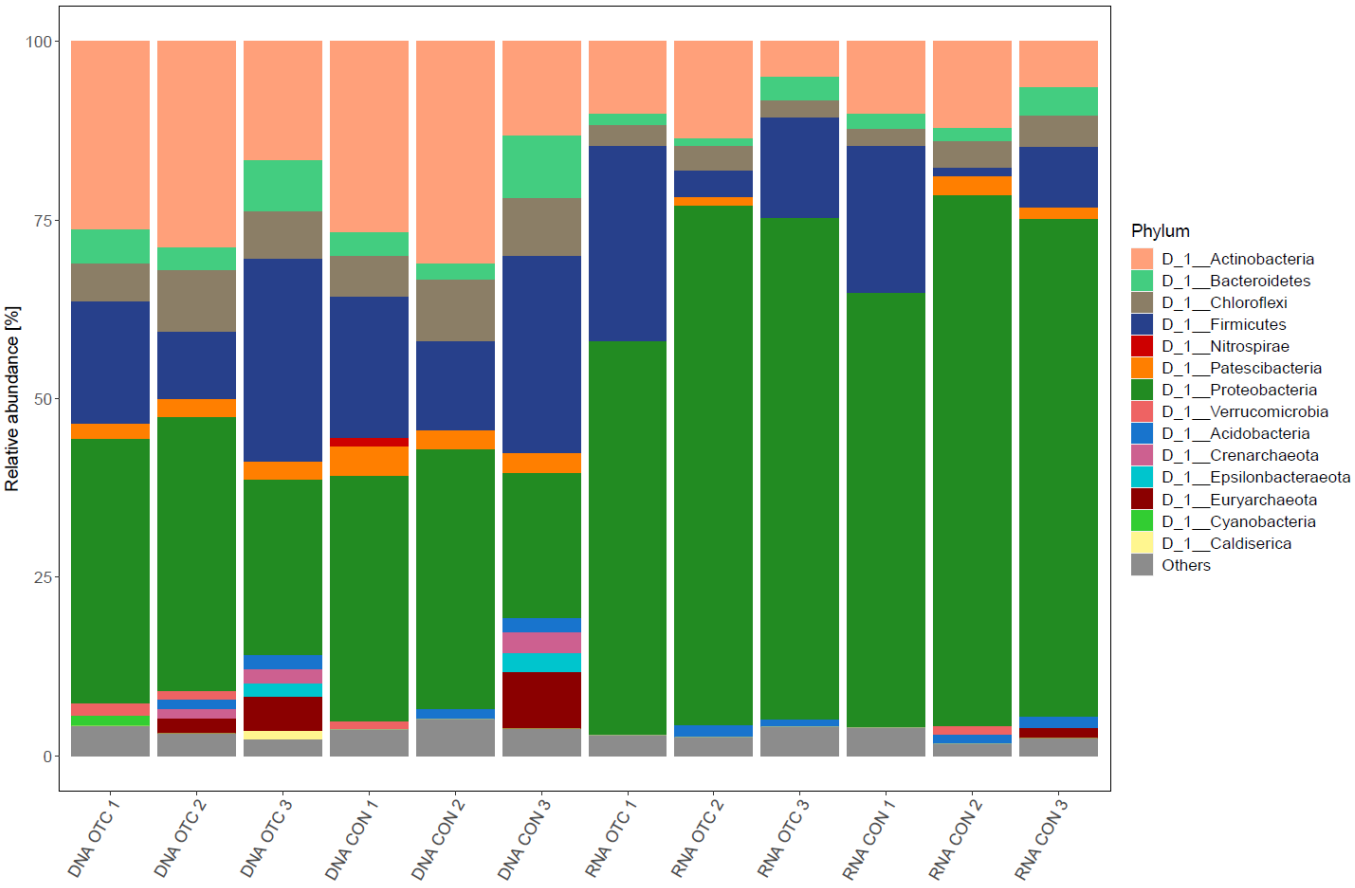

Figure 17: Relative abundance of sequences in 16S samples on phylum level.

Actinobacteria, Verrucomicrobia, Bacteroidetes, and Firmicutes to be most abundant, accounting for 84% of all analysed sequences (Gittel et al., 2014). Moreover, archaea, more specifically Euryarchaeota were fund, which is consistent with other Arctic soils (Malard and Pearce, 2018).

When analysing the relative abundance for the sample, a strong shift in Firmicutes was observed. The abundance was much higher for depth-1 and depth-3 than for depth-2. Especially for the RNA samples that was observed. Therefore, the Firmicutes phyla was looked at more in detail. Figure 18 shows the relative abundance of family within the Firmicutes phyla.

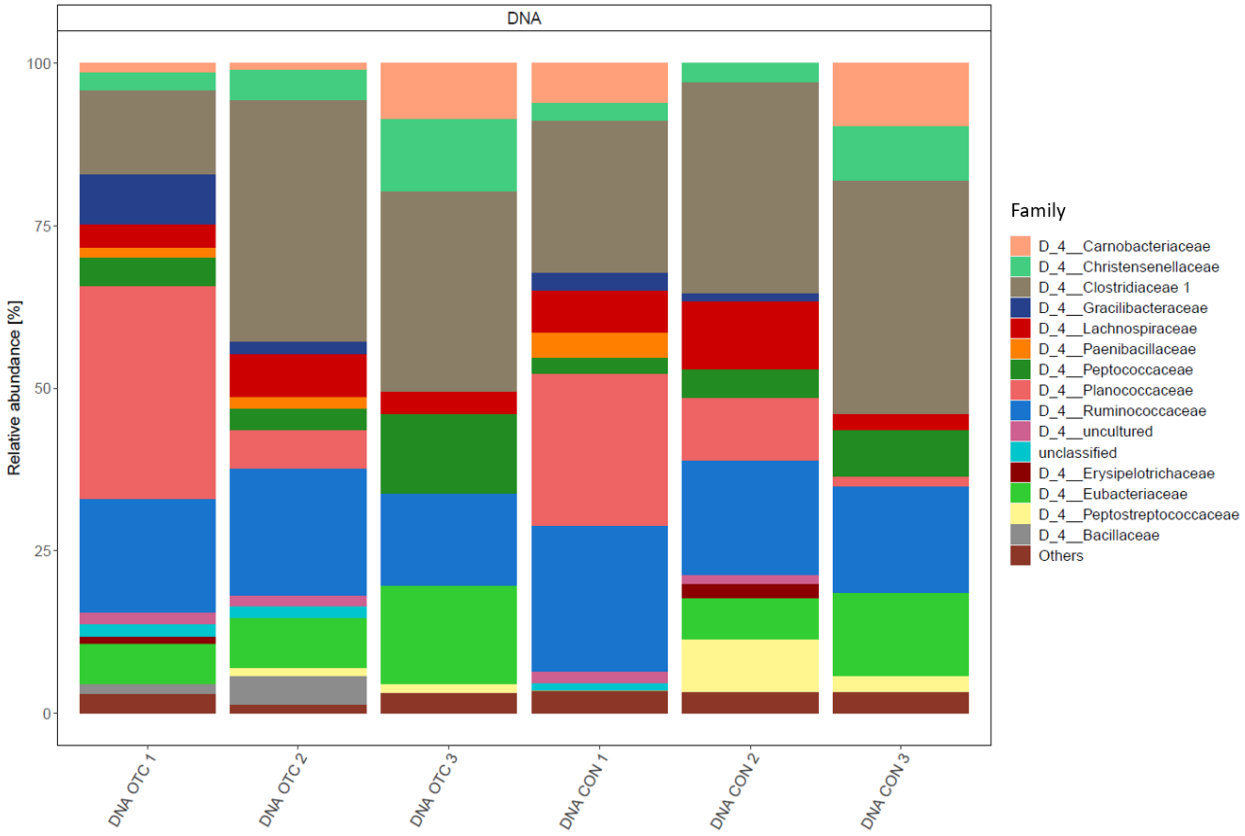

Figure 18: Relative abundance of the Firmicutes from sequences in 16S samples on family level.

When looking at the family level of Firmicutes, a shift within depth was be observed. Clostridiaceae 1 was most prominent in depth-3 and least in depth-1. Planococcaceae on the other hand was most prominent in depth-1 and very little present in depth-3. Also, depth dependent shifts were seen for Carnobacteriaceae, Christensenellaceae, Gracilibacteraceae, Lachnospiraceae, Peptococcaceae, and Eubacteriaceae. The shown abundance is only in relation to each other, not to the other phyla. Especially for depth2 this should be kept in mind, as there Firmicutes showed the lowest abundance on phyla level.

By looking at the family level, the changes for Firmicutes in the overall relative abundance (Figure 18) could be explained by an overall depth dependent shift in family within the Firmicutes phyla.

5.3.4.2 ITS

A taxonomic analysis was also carried out for the ITS samples (Figure 19). Ascomycota were by far the most abundant, reaching 75% to 95% of relative abundance. Besides that, Basidiomycota and Mortierellomycota were the identified phyla. This is consistent with other studies of fungi in the Arctics. Ascomycota and Basidiomycota are known to be the dominating phyla in the high Arctic soil, with Ascomycota being the more abundant in the soil (Tveit et al., 2013).

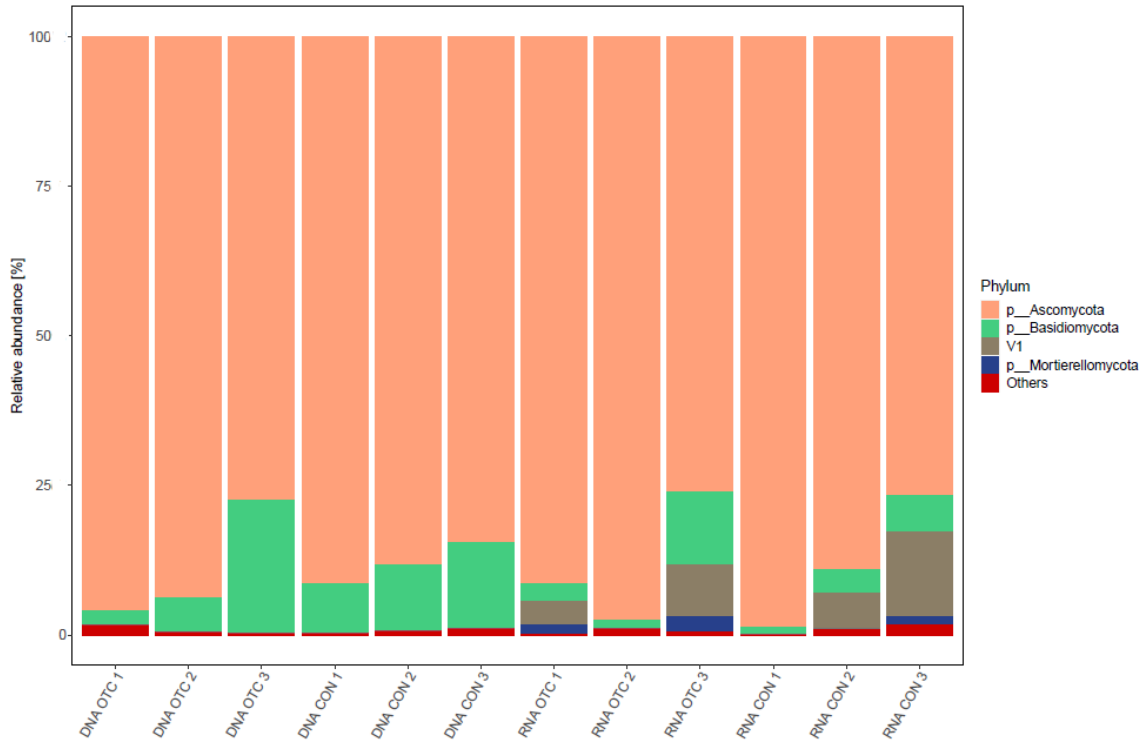

Figure 19: Relative abundance of sequences in ITS samples on phylum level.

Again, no shift between OTC and control was observed. Suggesting, that the OTCs had no significant impact on the fungal community.

On phylum level Ascomycota is dominating strongly. Therefore, the abundance on class level was also analysed (Figure 20). Three classes were the most abundant throughout, Dothideomycetes, Leotiomycetes, and Sordariomycetes. Moreover, Eurotiomycetes, Agaricomycetes, and Pezizomycetes were detected. Again, a shift dependent on depth was observed.

Not much is yet known about the fungal community in the High Arctic. However, Leotiomycetes, Dothideomycetes, Eurotiomycetes, and Sordariomycetes have been identified in other Artic soils as well as in Tibet and Antarctica (Zhang et al., 2020).

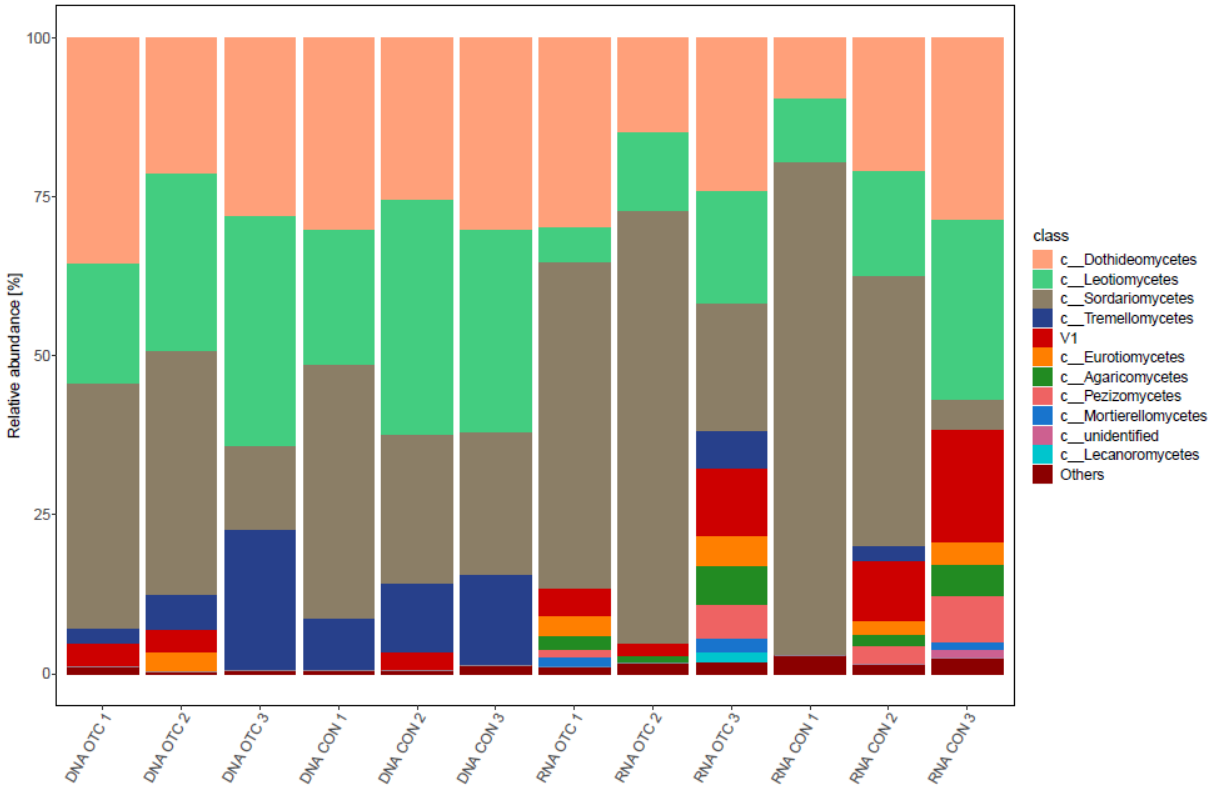

Figure 20: Relative abundance of sequences in ITS samples on class level.

5.4. General Discussion

During sampling, visual changes in the plant community were observed. Inside the chambers, the plants had grown 20-30 cm high, whereas the plants outside were only a few centimetres high (Personal communication with Anders Priemé). Detailed plant sample were taken and will be analysed in further studies. The visible change in plant size led to the presumption, that some changes had occurred.

The increase in plant growth due to OTC treatment is in accordance with the overall greening of the Arctic that is observed (Vowles and Björk, 2019). However, the chambers are also known to have an herbivore exclusion effect. So much so, that in some studies, plants are damaged throughout the experiment to simulate grazing from large herbivores (Rinnan et al., 2009). Around the research station Brønlundhus two larger herbivores have been observed, muskox and gees (Klein and Bay, 1991). The exclusion of those could have led to the visible difference between the plants inside the OTC and outside.

Consistently shifts in the community based on depth were observed. This goes along with other studies that have shown that in the top layers microbiological activity is higher. A strong difference can also be observed between permafrost and active layer (Blaud et al., 2015). However, the results did not indicate a shift in the microbial community based on treatment method. Other studies have shown that OTCs can influence the plant and microbial community. ITEX setups have led to increased plant cover and density (Welker et al., 1997). Moreover, shifts in the microbial community were observed. However, some of those changes were only

observed after 10 to 13 years (Rinnan et al., 2009). Further studies have shown that changes in microbial communities due to warming treatment are slow (Lim et al., 2018). With warming soil increases in relative abundance of Acidobacteria, Bacteroidetes, and Actinobacteria in Arctic soils are speculated (Malard and Pearce, 2018).

On the other hand, a few studies have also found, little to no shifts due to OTC treatment. A study from Svalbard, Norway showed no significant effect on microbial community, after about one year of OTC treatment. In that study the samples were collected in late July (Lim et al., 2018), similar to sampling in this study. Moreover, a study into *Dryas octopetala*, an Arctic and alpine plant, showed, that OTC treatment shows significant changes in only 3 out of 4 sites (Welker et al., 1997).

Several aspects could have caused no changes to be observed. One possibility is, that the OTCs did not have a strong warming effect on the soil. ITEX chambers on average increase the near-surface soil temperature by 1-3°C (Henry and Molau, 1997). Wet soil is known to warm up less, 1.2°C to 1.8°C, and dry polar deserts have shown temperature increases of 2.2°C to 5.2°C (Marion et al., 1997). The soil in this study was wet, Figure 6 shows the ground where the samples were taken, and water is clearly visible. The water was believed to be melted water, from nearby, snow covert mountains (personal communication with Anders Priemé). Moreover, the site had a slight slope, so the water was not still but instead was flowing through. This could have caused the OTC to have a smaller warming effect than expected.

This ITEX setup, was the most norther, yet. Figure 21 shows the ITEX sites around the world. The difference in longitude and thereby sunlight exposure could have several consequences. In northern Greenland, the snowmelt is between June and August (Pedersen et al., 2015) and the annual growing season in the High Arctic lasts 1-2.5 months (Blaud et al., 2015). Other regions in the Arctic, observed snow melt as early as late April (Pedersen et al., 2015). For the OTC to have a warming effect on soil the snow above must have melted. The timeframe in which the indirect warming can occur might be significantly shorter in northern Greenland. In addition, the samples were taken at the end of July, therefore, the soil might just have been exposed to the annual warming effect from the OTC for a few weeks. Climate data from the site could help analyse this further.

Figure 21: Map showing the sites of International Tundra Experiments worldwide. The yellow start represents the site of this project. The red circles represent sites with experimental warming, the blue diamonds, sites without experimental warming. (Sites & People - International Tundra Experiment (ITEX))

Other than the warming not having been sufficient, statistical limitations could have been hindering. Soil in known to be very heterogenic, artic soil is not exempt from that. Studies have shown that Arctic soil have high levels of heterogeneity (Makhalanyane et al., 2016). The differences between the chambers could have outweighted possible slight shifts between OTC and control samples.

6. Future Perspective

This study was part of a larger project, the Brønlundhus Open Top Chamber Experiment. Therefore, in the near future, several other analyses will be conducted to gather more insight and form a more conclusive picture about this site.

As mentioned previously, the qPCR results showed some inaccuracies and could be repeated to confirm the effect of depth and make a conclusive inference about the effect of the treatment. Moreover, the taxonomic data could be further analysed to gain knowledge about species and their functions in the soil. Sequencing in this study only targeted the 16S and ITS regions, leading to an experimental bias. This could be minimalized by including other sequencing methods. Other aspects of the site could also be further analysed. Several plant samples were taken, so the previously discussed visual difference in plant size will be investigated. Moreover, soil samples could be analysed regarding composition, water availability, and physical characteristics.

Still much is unknown about the about the microbial community, diversity, and functionality of Arctic soil (Malard and Pearce, 2018). This makes predicting the impacts of climate change on Arctic soil difficult. Further field studies and research help gaining a more accurate picture of the current state and potential future developments. The Arctic carbon soil pool could potentially lead to a global feedback mechanism, strongly accelerating GHG emissions and global warming (van Huissteden and Dolman, 2012). By conducting more research into warming effects of the Arctics, carbon and methane fluxes could be better understood (Gulev et al., 2021). Moreover, by understanding the impact climate change has on the Arctic and the world entirely, more adaptation and mitigation strategies could be developed.

7. Conclusion

This study did not obtain the results that were initially hypothesised. After seven years of induced summer warming, no significant changes in the microbial community were observed. The soil samples from the ITEX set-up were analysed for composition, diversity, and relative abundance. Although no changes in community were observed due to treatment, depth dependent changes were seen. The unique position of the experimental site was thought to have caused the warming within the chamber to be minimal. Moreover, heterogeneity between the OTCs could have outweighed the treatment dependent changes in the statistical analysis.

Nonetheless, the experimental set up and insight into Artic soil warming will be essential to understand climate change in the Artic and how it will affect the global system.

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Johanna Ettingshausen

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Appendix

Appendix 1: Overview of all samples. Sample name refers to the name used in this study. The chambers were numbered from 1 to 6. The sample weight refers to the amount used for DNA or and RNA extraction.

Sample name	chamber	Treatment	DNA or RNA	Depths	sample weight [g]
D1O1	W1	OTC	DNA	0-5 cm	0.25
D2O1	W2	OTC	DNA	0-5 cm	0.25
D3O1	W3	OTC	DNA	0-5 cm	0.25
D4O1	W4	OTC	DNA	0-5 cm	0.25
D5O1	W5	OTC	DNA	0-5 cm	0.25
D6O1	W6	OTC	DNA	0-5 cm	0.25
D1O2	W1	OTC	DNA	5-10 cm	0.25
D2O2	W2	OTC	DNA	5-10 cm	0.25
D3O2	W3	OTC	DNA	5-10 cm	0.25
D4O2	W4	OTC	DNA	5-10 cm	0.25
D5O2	W5	OTC	DNA	5-10 cm	0.25
D6O2	W6	OTC	DNA	5-10 cm	0.25
D1O3	W1	OTC	DNA	15-20 cm	0.25
D2O3	W2	OTC	DNA	15-20 cm	0.25
D3O3	W3	OTC	DNA	15-20 cm	0.25
D4O3	W4	OTC	DNA	15-20 cm	0.25
D5O3	W5	OTC	DNA	15-20 cm	0.25
D6O3	W6	OTC	DNA	15-20 cm	0.25
D1C1	W1	control	DNA	0-5 cm	0.25
D2C1	W2	control	DNA	0-5 cm	0.25
D3C1	W3	control	DNA	0-5 cm	0.25
D4C1	W4	control	DNA	0-5 cm	0.25
D5C1	W5	control	DNA	0-5 cm	0.25
D6C1	W6	control	DNA	0-5 cm	0.25
D1C2	W1	control	DNA	5-10 cm	0.25
D2C2	W2	control	DNA	5-10 cm	0.25
D3C2	W3	control	DNA	5-10 cm	0.25
D4C2	W4	control	DNA	5-10 cm	0.25
D5C2	W5	control	DNA	5-10 cm	0.25
D6C2	W6	control	DNA	5-10 cm	0.25
D1C3	W1	control	DNA	15-20 cm	0.25
D2C3	W2	control	DNA	15-20 cm	0.25
D3C3	W3	control	DNA	15-20 cm	0.25
D4C3	W4	control	DNA	15-20 cm	0.25
D5C3	W5	control	DNA	15-20 cm	0.25
D6C3	W6	control	DNA	15-20 cm	0.25
R1O1	W1	OTC	RNA	0-5 cm	1.3037
R2O1	W2	OTC	RNA	0-5 cm	1.7079

R3O1	W3	OTC	RNA	0-5 cm	2.5282
R4O1	W4	OTC	RNA	0-5 cm	2.9867
R5O1	W5	OTC	RNA	0-5 cm	2.083
R6O1	W6	OTC	RNA	0-5 cm	2.0225
R1O2	W1	OTC	RNA	5-10 cm	1.4491
R2O2	W2	OTC	RNA	5-10 cm	1.8973
R3O2	W3	OTC	RNA	5-10 cm	1.8445
R4O2	W4	OTC	RNA	5-10 cm	2.1739
R5O2	W5	OTC	RNA	5-10 cm	3.0944
R6O2	W6	OTC	RNA	5-10 cm	1.9385
R1O3	W1	OTC	RNA	15-20 cm	1.7231
R2O3	W2	OTC	RNA	15-20 cm	1.983
R3O3	W3	OTC	RNA	15-20 cm	2.5733
R4O3	W4	OTC	RNA	15-20 cm	2.3116
R5O3	W5	OTC	RNA	15-20 cm	2.7099
R6O3	W6	OTC	RNA	15-20 cm	2.3618
R1C1	W1	control	RNA	0-5 cm	2.9891
R2C1	W2	control	RNA	0-5 cm	1.6883
R3C1	W3	control	RNA	0-5 cm	2.3681
R4C1	W4	control	RNA	0-5 cm	1.7687
R5C1	W5	control	RNA	0-5 cm	2.1465
R6C1	W6	control	RNA	0-5 cm	2.3801
R1C2	W1	control	RNA	5-10 cm	2.3607
R2C2	W2	control	RNA	5-10 cm	2.4573
R3C2	W3	control	RNA	5-10 cm	2.356
R4C2	W4	control	RNA	5-10 cm	2.2206
R5C2	W5	control	RNA	5-10 cm	2.6295
R6C2	W6	control	RNA	5-10 cm	2.2127
R1C3	W1	control	RNA	15-20 cm	2.3057
R2C3	W2	control	RNA	15-20 cm	2.1825
R3C3	W3	control	RNA	15-20 cm	1.903
R4C3	W4	control	RNA	15-20 cm	2.1791
R5C3	W5	control	RNA	15-20 cm	3.0449
R6C3	W6	control	RNA	15-20 cm	2.3618

Appendix 2: R code used for calculations of diversity and creation of plots. This code represents, the calculation for the 16S samples. The same calculations were used to calculate ITS data. To avoid repetition, the ITS was not added as the same were used.

```
library(phyloseq)
library(vegan)
library(grid)
library(reshape2)
library(egg)
library(RColorBrewer)
library(decontam)
#Template for plots
plot.theme1 <- theme(panel.grid.major = element_blank(),
                     panel.grid.minor = element_blank(),
                     panel.background = element_rect(fill = "white",
                                                     colour        =
"black",
                                                     size    =    0.5,
linetype = "solid"),
                     panel.border=  element_rect(fill=NA,size  =  0.5,
linetype = 'solid',colour = "black"),
                     axis.text.x  =  element_text(size=13),axis.text.y
= element_text(size=13),legend.text = element_text(size=13),
                     axis.title = element_text(size=14),
                     legend.title  =  element_text(color  =  "black",
size = 14),
                     strip.text.x = element_text(size=14),
                     strip.background  =  element_rect(colour="black",
fill="white")
)
# function to create colors
ColCreate    <-    colorRampPalette(brewer.pal(9,    "Set1"))#function
interpolating palette to desired number of colors; sample can be used
to randomize the vector

### check working directory ###
getwd()
#######Import Biom files (containing count table and taxonomy)
biom             <-             import_biom(BIOMfilename             =
"C:/Users/Johanna/Documents/Copenhagen/10.
Semester/R/input_files/feature-table_taxonomy-16S.biom")
#### Read Samples sheet
sample     <-     read.csv("C:/Users/Johanna/Documents/Copenhagen/10.
Semester/R/input_files/sample_names.csv", encoding = "UTF-16", sep =
",", check.names = F)
sample[,2] <- paste0("Run20220408", sample[,2])
rownames(sample) <- sample[,2]
# create phyloseq object for samples
phySample <- sample_data(sample)
#### Merge phyloseq (Create common phyloseq object containing count
table, taxonomy and sample information)
phy <- merge_phyloseq(biom,phySample)
colnames(tax_table(phy)) <- c("kingdom", "phylum", "class", "order",
"family",  "genus", "species", "Rank8", "Rank9", "Rank10", "Rank11",
"Rank12", "Rank13", "Rank14", "Rank15")
# ***********************************************
```

```
## Overall statistics on number of Seqs and ASVs ######
# ************************************************
#Numbers of seqs (mean +- sd, min, max)
#total seqs in dataset
sum(phy@otu_table@.Data)
#value for each sample
colSums(phy@otu_table@.Data) #or rowSums if samples are rows
#mean
mean(colSums(phy@otu_table@.Data))
#sd
sd(colSums(phy@otu_table@.Data))
#min
min(colSums(phy@otu_table@.Data))
#max
max(colSums(phy@otu_table@.Data))
#Numbers of ASVs unfiltered (mean +- sd, min, max)
#total number of  ASVs
nrow(phy@otu_table@.Data)
#value for each sample
apply(phy@otu_table@.Data>0,2,function(x)length(which(x)))          #
apply(object,1,function) if samples are rows
#mean
mean(apply(phy@otu_table@.Data>0,2,function(x)length(which(x))))
#sd
sd(apply(phy@otu_table@.Data>0,2,function(x)length(which(x))))
#min
min(apply(phy@otu_table@.Data>0,2,function(x)length(which(x))))
#max
max(apply(phy@otu_table@.Data>0,2,function(x)length(which(x))))
###   Function   for   preparing   tables   for   abundance   barplots
**********************************************************************
*********** ####
#countab: counttable only numeric part,taxa are rows
#taxo=taxonomy table
#col2matchcount vector of rownames or column in counttab to which
order of taxonomy table should be matched
#col2matchtax:vector of rownames or column in taxo to match
#Taxlevel: taxonomic level on which should be aggregated
#Samp: sample data
#fac: factor in Sample of which the mean should be made
#Summarize: should rare taxa be summarized and represented as others
#sumlevel: abundance threshold below which taxa are summarkued as
others
#!function does not work using summary as others if there is only one
category left that is not part of others => error in extraction of
list
#!after melt: when reordering levels of variable: it has to be
unique(as.character(...));         originally         it         was
as.character(unique(...))

#! update of function: for soil: as.vector has to be used in addition
to unlist to extract taxa
#after summarizing as others from list <=> otherwise results as matrix;
some names are used twice and values are counted more than once
AbuBarTable=function(countab,taxo,col2matchcount,col2matchtax,Taxlev
el,Samp,fac,Summarize=F,sumlevel){
```

```
  #replace NA with unclassified
  taxo=as.data.frame(apply(taxo,2,function(x){
    sapply(x,function(y){ifelse(is.na(y),"unclassified",y)})
  }))
  tax=taxo[match(col2matchcount,col2matchtax),]
  ##Abundance barplots at kingdom level
  taxabu=aggregate(countab,list(tax[,Taxlevel]),sum)
  rownames(taxabu)=taxabu[,1]
  taxabu=taxabu[,-1]
  taxabu.rel=apply(taxabu,2,function(x)x/sum(x))
  taxabu.rel=as.data.frame(t(taxabu.rel))
  taxabu.rel=taxabu.rel[rownames(Samp),]
  for(i in 1:ncol(Samp)){
    Samp[,i]=as.character(Samp[,i])
  }

  taxabu.rel.mean=aggregate(taxabu.rel,list(Samp[,fac]),mean)
  colnames(taxabu.rel.mean)[1]=fac
  rownames(taxabu.rel.mean)=taxabu.rel.mean[,1]
  a=c()
  for (i in colnames(taxabu.rel.mean)){
    a[i]=is.numeric(taxabu.rel.mean[,i])
  }
  taxabu.rel.mean=taxabu.rel.mean[,a]
  if(Summarize==T){
    num=taxabu.rel.mean
    num.l=as.list(as.data.frame(t(num)))
    num.l=lapply(num.l,function(x){
      names(x)=colnames(num)
      Others=sum(x[which(x<sumlevel)])
      x=x[-which(x<sumlevel)]
      names(Others)="Others"
      x=c(x,Others)
    })
    taxabu.rel.mean   <-as.data.frame(do.call(rbind,   lapply(num.l,
"[",unique(as.vector(unlist(sapply(num.l,names)))))))
    #lapply(x,"[",element) => extracts elements from each element of
the list => if name does not exist: NA
    colnames(taxabu.rel.mean)                                        <-
unique(as.vector(unlist(sapply(num.l,names))))
    taxabu.rel.mean=apply(taxabu.rel.mean,2,function(x){
      sapply(x,function(y){ifelse(is.na(y),0,y)})
    })
  }
  a=as.data.frame(Samp[!duplicated(Samp[,fac]),])
  if(all(rownames(taxabu.rel.mean)==a[,fac])){
    taxabu.rel.mean=cbind(taxabu.rel.mean,a)
  }else{

taxabu.rel.mean=taxabu.rel.mean[match(a[,fac],rownames(taxabu.rel.me
an)),]
    taxabu.rel.mean=cbind(taxabu.rel.mean,a)
  }
  require(reshape2)
  taxabu.rel.mean.long=melt(taxabu.rel.mean)
  a=unique(as.character(taxabu.rel.mean.long$variable))
```

```
  if("Others"%in%taxabu.rel.mean.long$variable){

taxabu.rel.mean.long$variable=factor(taxabu.rel.mean.long$variable,l
evels=c(a[-(which(a=="Others"))],"Others"))
  }

  taxabu.rel.mean.long[,fac]=factor(
taxabu.rel.mean.long[,fac],levels=unique(taxabu.rel.mean.long[,fac])
)
  return(taxabu.rel.mean.long)
}
#make subsets with only negative controls
phyNeg <- subset_samples(phy,DNARNA %in% c("neg"))
phyNeg <- prune_taxa(taxa_sums(phyNeg)>0,phyNeg) #remove ASVs which
do not occur any more after subsetting
negtab <- as.data.frame(as.matrix(otu_table(phyNeg)))
negtax <- as.data.frame(as.matrix(tax_table(phyNeg)))
sam <- (as.data.frame(as.matrix(sample_data(phyNeg))))
negabu    <-    AbuBarTable(negtab,    negtax,    rownames(negtab),
rownames(negtax), "genus", sam, "seq_name")
#bar plot of neg control
pdf("C:/Users/Johanna/Documents/Copenhagen/10.
Semester/R/results/16S/negControlComposition_16S.pdf",  width  =  15,
height = 10)
ggplot(negabu,aes(x=my_name,y=value,fill=variable))+geom_bar(stat="i
dentity")+
  ylab("Read counts")+xlab("") + labs(fill="Genus")+
  theme(axis.text.x = element_text(angle = 60, hjust = 1))+
  #hier kann man eigenen farb vecotr dann einsetzen
  #scale_fill_manual(values=col2)+
  plot.theme1
dev.off()
#subsets with only mock community
phyMock <- subset_samples(phy,DNARNA %in% c("Mock"))
phyMock  <-  prune_taxa(taxa_sums(phyMock)>0,phyMock)  #remove  ASVs
which do not occur any more after subsetting
mocktab <- as.data.frame(as.matrix(otu_table(phyMock)))
mocktax <- as.data.frame(as.matrix(tax_table(phyMock)))
sam <- (as.data.frame(as.matrix(sample_data(phyMock))))
mockabu                                                          <-
AbuBarTable(mocktab,mocktax,rownames(mocktab),rownames(mocktax),"gen
us",sam,"seq_name")
#bar plot of mock communities at genus level
pdf("C:/Users/Johanna/Documents/Copenhagen/10.
Semester/R/results/16S/mockCommunityComposition_16S.pdf", width = 15,
height = 10)
ggplot(mockabu,aes(x=my_name,y=value,fill=variable))+geom_bar(stat="
identity")+
  ylab("Relative abundance")+xlab("") + labs(fill="Genus")+
  theme(axis.text.x = element_text(angle = 60, hjust = 1))+
  #hier kann man eigenen farb vecotr dann einsetzen
  #scale_fill_manual(values=col2)+
  plot.theme1
dev.off()
rm(phyMock,mocktab,mocktax,sam,mockabu,Negtab,Negtax,phyNeg,negabu,n
egtab,negtax)
```

```
#### Remove contaminant sequences
# remove mock community from phyloseq
phyData <- subset_samples(phy,!(DNARNA %in% c("Mock")))
phyData <- prune_taxa(taxa_sums(phyData)>0,phyData) #remove ASVs
which do not occur any more after subsetting
#run decontimation tool
contaminations <- isContaminant(phyData,neg = "neg", method =
"prevalence")
# Filter contaminant from phyloseq
phyDataNC <- prune_taxa(!contaminations$contaminant, phyData)
# remove negative controls
phyDataNC <- subset_samples(phyData,!(DNARNA %in% c("neg")))
phyDataNC <- prune_taxa(taxa_sums(phyDataNC)>0,phyDataNC) #remove
ASVs which do not occur any more after subsetting
## subset samples ***********************************
# if an analysis should be done with only a subset of samples
#phy_seq has only experimental seq, neg and mock excluded
#2 samples were taken out manually with "check" due to lab
irregularities.
#phy_seq consists of samples were contamination was taken out and the
2 samples are out.
phy_seq <- subset_samples(phyDataNC, DNARNA %in% c("DNA", "RNA")) #
e.g. subset to samples for which the ID is among the ones in the vector
phy_seq <- prune_taxa(taxa_sums(phy_seq)>0,phy_seq) #remove ASVs
which do not occur any more after subsetting
#subset for only DNA
phy_seq_DNA <- subset_samples(phyDataNC, DNARNA %in% c("DNA"))
phy_seq_DNA                                                    <-
prune_taxa(taxa_sums(phy_seq_DNA)>0,phy_seq_DNA)#remove ASVs which do
not occur any more after subsetting
#subset for Firmicutes
phy_seq_DNA_Firmicutes                                         <-
prune_taxa(as.data.frame(tax_table(phy_seq))[,"phylum"]=="D_1__Firmi
cutes",phy_seq)
#subset for DNA depth 1
phy_seq_DNA_d1 <- subset_samples(phy_seq_DNA, depth %in% c("1"))
phy_seq_DNA_d1       <-       prune_taxa(taxa_sums(phy_seq_DNA_d1)>0,
phy_seq_DNA_d1)
###define colors###
col1=c("blue2","red2","grey20","cyan3","blue3","forestgreen","orange
red","darkgoldenrod3",      "firebrick3","coral3",      "dodgerblue4",
"greenyellow", "deeppink3", "yellow3", "darkviolet", "saddlebrown",
"navyblue",   "darkolivegreen4",   "lightskyblue2",   "chartreuse3",
"orchid3","mediumpurple3")
col2=c("lightsalmon1","seagreen3","wheat4","royalblue4","red3","dark
orange1","forestgreen","indianred2","dodgerblue3","hotpink3","turquo
ise3","darkred","limegreen","khaki1","gray55","tomato4","lightgolden
rod4","springgreen4","bisque4","lawngreen")
names(col2)      <-      c("D_1__Actinobacteria","D_1__Bacteroidetes",
"D_1__Chloroflexi","D_1__Firmicutes","D_1__Nitrospirae","D_1__Patesc
ibacteria","D_1__Proteobacteria","D_1__Verrucomicrobia","D_1__Acidob
acteria","D_1__Crenarchaeota","D_1__Epsilonbacteraeota","D_1__Euryar
chaeota","D_1__Cynobacteria","D_1__Caldiserica","Others")
# ********************************************
#  Alphadiversity ***************************** #####
# ***************************
```

```
## Calculate indices  ####
## rarefy
phyRar=rarefy_even_depth(phy_seq,rngseed=T)
#estimate richness (calculate alphadiversity indices)
Alphadiv=estimate_richness(phyRar, measures = c("Observed","Shannon",
"Chao1"))
rm(phyRar)
#combine with sample data
all(rownames(Alphadiv)==rownames(sample_data(phy_seq)))#check  order,
cbind overwrites rownames
Alphadiv=cbind(sample_data(phy_seq),Alphadiv)
#### plot
#create directory for results
#dir.create("./results/",recursive = T)
#combined factor depth / treatment
Alphadiv$DxT <- paste(Alphadiv$depth,Alphadiv$control)
Alphadiv$depth <- as.factor(Alphadiv$depth)

#observed Richness
#with stat summary mean and sd are calculated for all values grouped
through combination of x and facet_wrap
p=ggplot(data            =            Alphadiv,            aes(x=DxT,
y=Observed,fill=depth))+stat_summary(fun.y="mean",
geom="bar",position=position_dodge())+
  stat_summary(fun.y = mean,
               fun.ymin = function(x) mean(x) - sd(x),
               fun.ymax = function(x) mean(x) + sd(x),
               geom = "errorbar",width=0.2) +
  ylab("Richness")+xlab("") + labs(fill="Depth")+
  facet_wrap(~DNARNA)+
  theme(axis.text.x = element_text(angle = 60, hjust = 1))   + #for
rotated x axes label
  plot.theme1
  #scale_fill_manual(values=col1)
p=set_panel_size(p,margin = unit(0, "mm"), width = unit(2, "inch"),
height = unit(4, "inch"))
pdf("C:/Users/Johanna/Documents/Copenhagen/10.
Semester/R/results/16S/ObservedDNAvsRNA_16.pdf", width = 10, height =
10)
grid.arrange(p)
dev.off()
#Shannon diversity
#with stat summary mean and sd are calculated for all values grouped
through combination of x and facet_wrap
p=ggplot(data            =            Alphadiv,            aes(x=DxT,
y=Shannon,fill=depth))+stat_summary(fun.y="mean",
geom="bar",position=position_dodge())+
  stat_summary(fun.y = mean,
               fun.ymin = function(x) mean(x) - sd(x),
               fun.ymax = function(x) mean(x) + sd(x),
               geom = "errorbar",width=0.2) +
  ylab("Shannon")+xlab("") + labs(fill="Depth")+
  facet_wrap(~DNARNA)+
  theme(axis.text.x = element_text(angle = 60, hjust = 1))   + #for
rotated x axes label
  plot.theme1
```

```
#scale_fill_manual(values=col1)
p=set_panel_size(p,margin = unit(0, "mm"), width = unit(1.3, "inch"),
height = unit(2, "inch"))
pdf("./results/16S/ShannonDNAvsRNA_16S.pdf", width = 10, height = 10)
grid.arrange(p)
dev.off()
# ****************************************************
# Beta diversity ********************************** ####
# ****************************************************
## PCoA ************************************************ ####
#calculate relative abundance
phyRel <- transform_sample_counts(phy_seq,function(x)x/sum(x))
#### PCOA
ord1 <- ordinate(phyRel,distance = "bray",method="PCoA")
#extract sample data
sam <- as.data.frame(as.matrix(sample_data(phyRel)))
#convert depth to factor
phyRel@sam_data$depth <- as.factor(phyRel@sam_data$depth)
#convert chamber to factor
phyRel@sam_data$chamber <- as.factor(phyRel@sam_data$chamber)
#plot
p <- plot_ordination(phyRel,ord1,color= "control",shape = "depth")+
  geom_point(size=4)+
  geom_text(aes(label = chamber), size = 5, hjust = 1.7) +
  #scale_color_manual(values=col1)+
  labs(color="Factor1")+
  plot.theme1
p <- set_panel_size(p,margin = unit(0, "mm"), width = unit(4, "inch"),
height = unit(4, "inch"))
#save plot
pdf("./results/16S/pcoaTreatment_16S.pdf", width = 7, height = 7)
grid.arrange(p)
dev.off()
#plot
p <- plot_ordination(phyRel,ord1,color="DNARNA",shape = "depth")+
  geom_point(size=4)+
  #scale_color_manual(values=col1)+
  labs(color="Factor1")+
  plot.theme1
#fix panel size
p <- set_panel_size(p,margin = unit(0, "mm"), width = unit(4, "inch"),
height = unit(4, "inch"))
#save plot
pdf("./results/16S/pcoaDNAvsRNA_16S.pdf", width = 7, height = 7)
grid.arrange(p)
dev.off()
##                 changing                  pcoa                  Plots
************************************************* ####
### new pcoa with DNA samples comparing OTC to Con
#calculate relative abundance
phyRel<- transform_sample_counts(phy_seq_RNA_d1,function(x)x/sum(x))
#### PCOA
ord1 <- ordinate(phyRel,distance = "bray",method="PCoA")
#create color gradient from light to dark for sites within each biome:
Start gradient from white and don't use first color (white)
#extract sample data
```

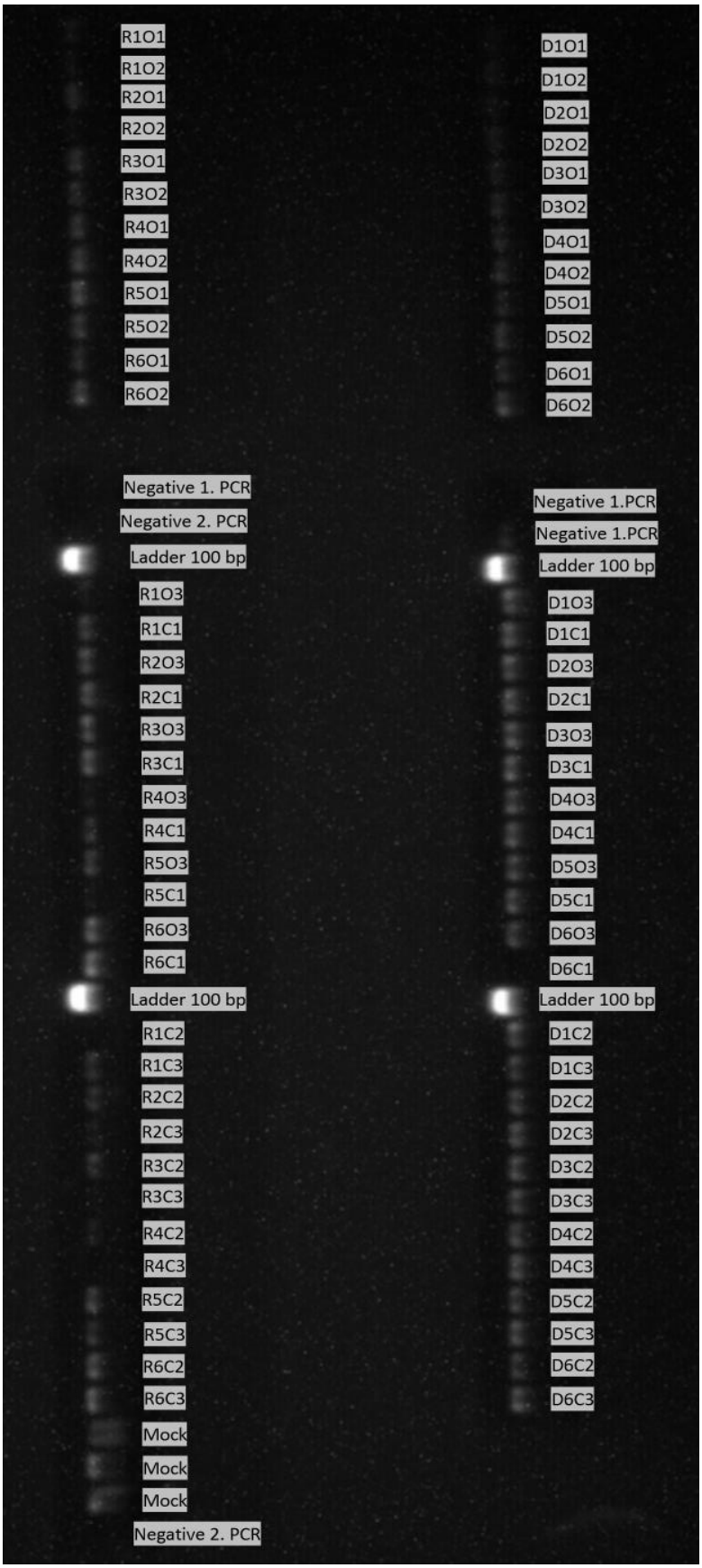
```
sam <- as.data.frame(as.matrix(sample_data(phyRel)))
#convert depth to factor
phyRel@sam_data$depth <- as.factor(phyRel@sam_data$depth)
#convert chamber to factor
phyRel@sam_data$chamber <- as.factor(phyRel@sam_data$chamber)
#plot
p <- plot_ordination(phyRel,ord1,color="control",shape = "depth")+
  geom_point(size=4)+
  geom_text(aes(label = chamber), size = 5, hjust = 1.7) +
  scale_color_manual(values=col1)+
  labs(color="Factor1")+
  plot.theme1
#fix panel size
p <- set_panel_size(p,margin = unit(0, "mm"), width = unit(4, "inch"),
height = unit(4, "inch"))
#save plot
pdf("./results/16S/PCOA_RNA_depth1_16S.pdf", width = 7, height = 7)
grid.arrange(p)
dev.off()
## Plots ************************************************** ####
### Function for preparing tables for abundance barplots **** ####
#countab: counttable only numeric part,taxa are rows
#taxo=taxonomy table
#col2matchcount vector of rownames or column in counttab to which
order of taxonomy table should be matched
#col2matchtax:vector of rownames or column in taxo to match
#Taxlevel: taxonomic level on which should be aggregated
#Samp: sample data
#fac: factor in Sample of which the mean should be made
#Summarize: should rare taxa be summarized and represented as others
#sumlevel: abundance threshold below which taxa are summarkued as
others
#!function does not work using summary as others if there is only one
category left that is not part of others => error in extraction of
list
#!after melt: when reordering levels of variable: it has to be
unique(as.character(...)); originally it was
as.character(unique(...))
#! update of function: for soil: as.vector has to be used in addition
to unlist to extract taxa
#after summarizing as others from list <=> otherwise results as matrix;
some names are used twice and values are counted more than once
AbuBarTable=function(countab,taxo,col2matchcount,col2matchtax,Taxlev
el,Samp,fac,Summarize=F,sumlevel){
  #replace NA with unclassified
  taxo=as.data.frame(apply(taxo,2,function(x){
    sapply(x,function(y){ifelse(is.na(y),"unclassified",y)})
  }))
  tax=taxo[match(col2matchcount,col2matchtax),]
  ##Abundance barplots at kingdom level
  taxabu=aggregate(countab,list(tax[,Taxlevel]),sum)
  rownames(taxabu)=taxabu[,1]
  taxabu=taxabu[,-1]
  taxabu.rel=apply(taxabu,2,function(x)x/sum(x))
  taxabu.rel=as.data.frame(t(taxabu.rel))
  taxabu.rel=taxabu.rel[rownames(Samp),]
```

```
  for(i in 1:ncol(Samp)){
    Samp[,i]=as.character(Samp[,i])
  }
  taxabu.rel.mean=aggregate(taxabu.rel,list(Samp[,fac]),mean)
  colnames(taxabu.rel.mean)[1]=fac
  rownames(taxabu.rel.mean)=taxabu.rel.mean[,1]
  a=c()
  for (i in colnames(taxabu.rel.mean)){
    a[i]=is.numeric(taxabu.rel.mean[,i])
  }
  taxabu.rel.mean=taxabu.rel.mean[,a]
  if(Summarize==T){
    num=taxabu.rel.mean
    num.l=as.list(as.data.frame(t(num)))
    num.l=lapply(num.l,function(x){
      names(x)=colnames(num)
      Others=sum(x[which(x<sumlevel)])
      x=x[-which(x<sumlevel)]
      names(Others)="Others"
      x=c(x,Others)
    })
    taxabu.rel.mean   <-as.data.frame(do.call(rbind,   lapply(num.l,
"[",unique(as.vector(unlist(sapply(num.l,names)))))))
    #lapply(x,"[",element) => extracts elements from each element of
the list => if name does not exist: NA
    colnames(taxabu.rel.mean)                                        <-
unique(as.vector(unlist(sapply(num.l,names))))
    taxabu.rel.mean=apply(taxabu.rel.mean,2,function(x){
      sapply(x,function(y){ifelse(is.na(y),0,y)})
    })
  }
  a=as.data.frame(Samp[!duplicated(Samp[,fac]),])
  if(all(rownames(taxabu.rel.mean)==a[,fac])){
    taxabu.rel.mean=cbind(taxabu.rel.mean,a)
  }else{

taxabu.rel.mean=taxabu.rel.mean[match(a[,fac],rownames(taxabu.rel.me
an)),]
    taxabu.rel.mean=cbind(taxabu.rel.mean,a)
  }
  require(reshape2)
  taxabu.rel.mean.long=melt(taxabu.rel.mean)
  a=unique(as.character(taxabu.rel.mean.long$variable))
  if("Others"%in%taxabu.rel.mean.long$variable){

taxabu.rel.mean.long$variable=factor(taxabu.rel.mean.long$variable,l
evels=c(a[-(which(a=="Others"))],"Others"))
  }
  taxabu.rel.mean.long[,fac]=factor(
taxabu.rel.mean.long[,fac],levels=unique(taxabu.rel.mean.long[,fac])
)
  return(taxabu.rel.mean.long)
}
#make new subsets
phy_seq_DNA_d1_ind  <-  subset_samples(phy_seq_RNA_d3,  chamber  %in%
c("6"))
```

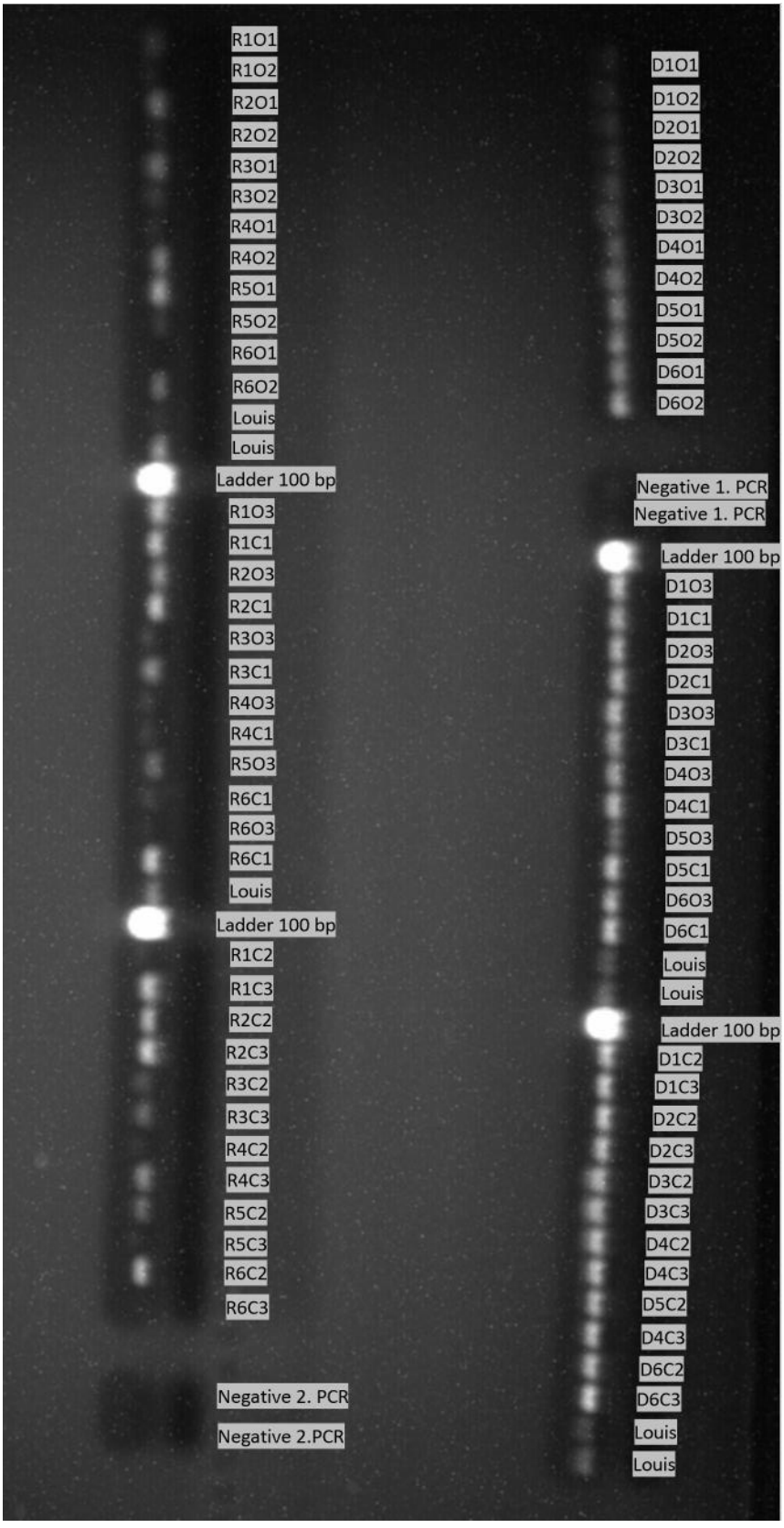
```
phy_seq_DNA_d1_ind   <-   prune_taxa(taxa_sums(phy_seq_DNA_d1_ind)>0,
phy_seq_DNA_d1_ind)
#### Input files
#calculate relative abundance
phyRel<-
transform_sample_counts(phy_seq_DNA_Firmicutes,function(x)x/sum(x))
#### PCOA
ord1 <- ordinate(phyRel,distance = "bray",method="PCoA")
#create color gradient from light to dark for sites within each biome:
Start gradient from white and don't use first color (white)
#extract sample data
sam <- as.data.frame(as.matrix(sample_data(phyRel)))
#convert depth to factor
phyRel@sam_data$depth <- as.factor(phyRel@sam_data$depth)
#convert chamber to factor
phyRel@sam_data$chamber <- as.factor(phyRel@sam_data$chamber)
#otutable
Otu=as.matrix(phyRel@otu_table@.Data)
#extract Taxonomy
Tax <- as.matrix(phyRel@tax_table@.Data)
#sample data (subset NABO)
sam=as.data.frame(as.matrix(phyRel@sam_data))
#Add column for combination of site and extraction to sam
sam$RDxtreatmentxdepth <- paste(sam$DNARNA,sam$control, sam$depth)
#### Stacked barplots
#Make table
AbuPhyl                                                                <-
AbuBarTable(Otu,Tax,rownames(Otu),rownames(Tax),"family",sam,"RDxtre
atmentxdepth", Summarize = TRUE, sumlevel = 0.01)
#convert abundance to %
AbuPhyl$value=AbuPhyl$value*100
#plot with facets by site => in each facet comparison soil extracted
vs elutriated
# if facets are created by biome differences between extraction methods
seem to be compensated
pdf("C:/Users/Johanna/Documents/Copenhagen/10.
Semester/R/results/16S/Individual_bar_plots/firmicutes_all.pdf",
width = 15, height = 10)
ggplot(AbuPhyl,aes(x=RDxtreatmentxdepth,y=value,fill=variable))+geom
_bar(stat="identity")+
  ylab("Relative abundance [%]")+xlab("") + labs(fill="phylum")+
  theme(axis.text.x = element_text(angle = 60, hjust = 1))+
  #hier kann man eigenen farb vecotr dann einsetzen
  scale_fill_manual(values=col2)+
  plot.theme1+
  facet_wrap(~DNARNA)
dev.off()
# ************************************************
# Statistics Alphadiversity ********************** ####
# ************************************************
######## with all samples (including those with very few seqs)
#do in subset for soil and elutriated (for effect of site and biome)
as well as for full dataset (for effect of extraction method)
#for statistical analyses:  in subsets use subsets rarefied separately

## Effect site by ANOVA ************************ ####
```

```
Alphadiv.stats=list()
for (i in c("Observed","Shannon")){
  Alphadiv.stats[[i]]=list()

Alphadiv.stats[[i]][["aov"]]=aov(data=Alphadiv,get(i)~control*depth*
DNARNA)

Alphadiv.stats[[i]][["shapiro"]]=shapiro.test(Alphadiv.stats[[i]][["
aov"]]$residuals)
Alphadiv.stats[[i]][["bartlett"]]=bartlett.test(data=Alphadiv,get(i)
~interaction(control,depth,DNARNA))
}
#summarize in dataframe results of shapiro (normality) and bartlett
test (homoskedasticity)
stats.valid=data.frame()
for(i in c("Observed","Shannon")){

stats.valid[i,"p_shapiro"]=Alphadiv.stats[[i]][["shapiro"]]$p.value

stats.valid[i,"p_bartlett"]=Alphadiv.stats[[i]][["bartlett"]]$p.valu
e
}
#  normatlity and homoskedasticity fulfilled
print(stats.valid)
# Effects Observed
summary(Alphadiv.stats$Observed$aov)
# Effects Shannon
summary(Alphadiv.stats$Shannon$aov)
##                                                          Permanova
********************************************************** ####
#extract table of rel. abundances from physeq
Rel <- as.data.frame(as.matrix(phy_seq_RNA_d2@otu_table@.Data))
Rel <- as.data.frame(t(Rel)) #sample have to be rows
#extract sample table from physeq
sam=as.data.frame(as.matrix(phy_seq_RNA_d1@sam_data))
all(rownames(Rel)==rownames(sam))
#### For one Factor
#permanova
adonis2(Rel~control,data=sam,method="bray",permutations = 9999)
#homogeneity of variance
betad <- betadisper(vegdist(Rel,method="bray"),sam)
permutest(betad,permutations = 9999)
#### For two Factors
#permanova
#adonis2(Rel~,data=sam,method="bray",permutations = 9999)
#betadisper does not work with more than one factor
sam$chamber <- as.character(sam$chamber)
adonis2(Rel~chamber+control,data=sam,method="bray",permutations    =
9999)
```

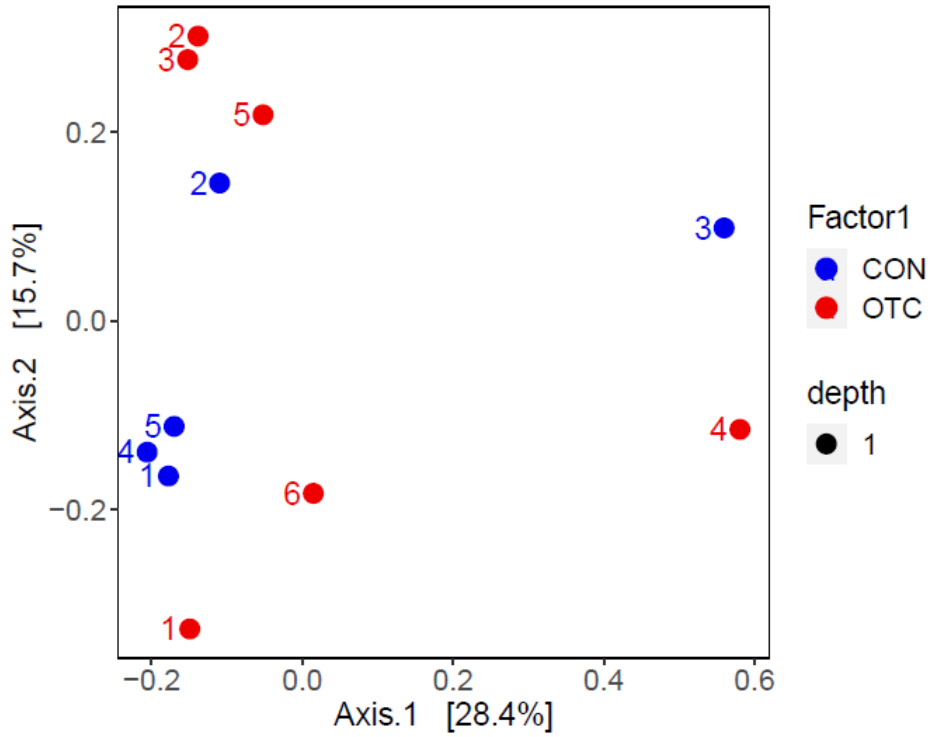
Appendix 3: Agarose gel of all 16S samples after the second PCR for sequencing preparation.



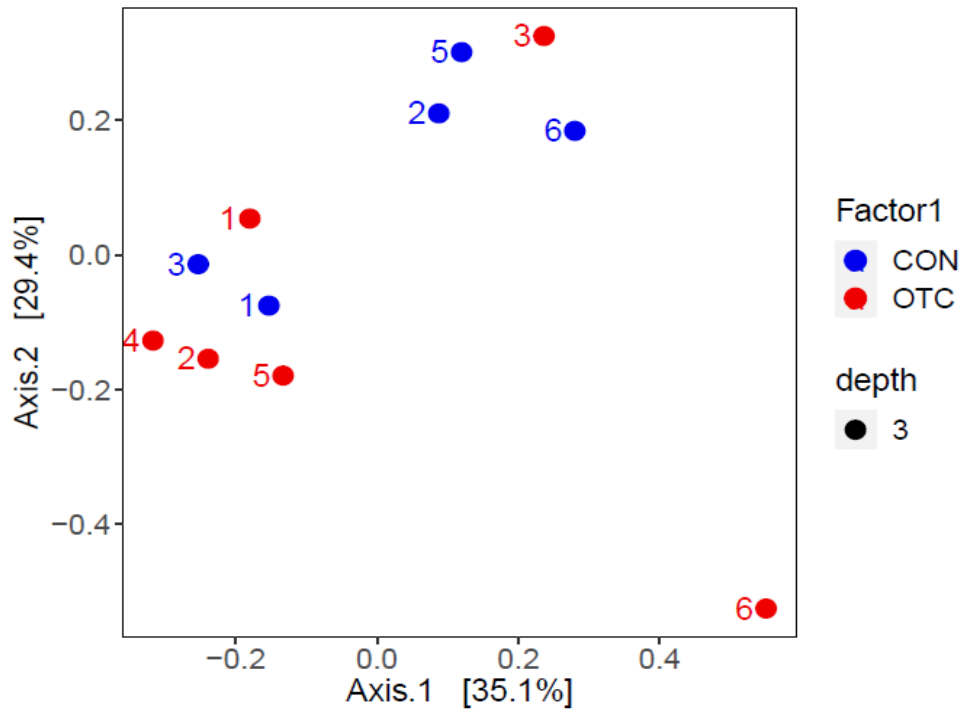
Appendix 4: Agarose gel of all ITS samples after the second PCR for sequencing preparation. Samples called 'Louis' can be disregarded as they were part of another project.



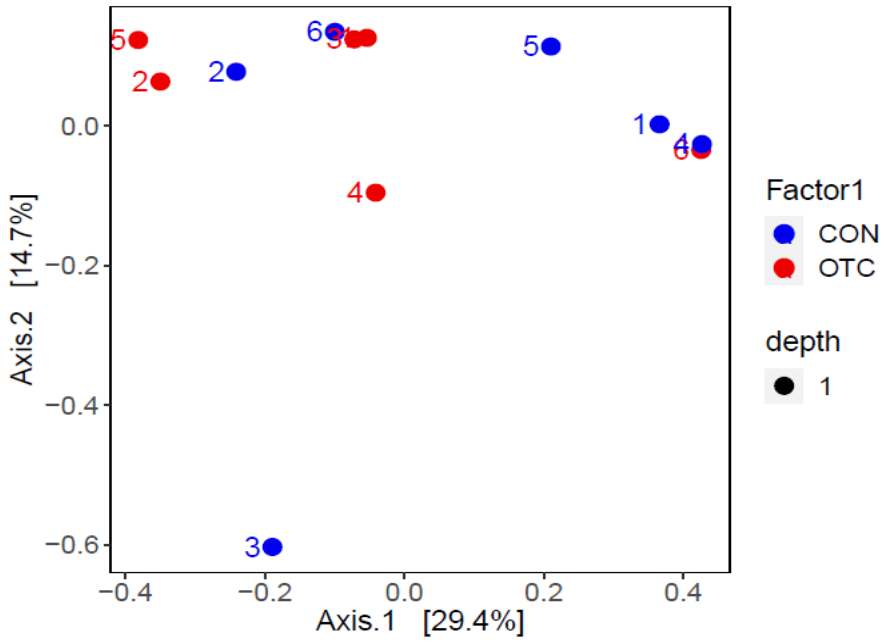
Appendix 5: PCoA of community structure for the DNA-16S samples at depth 1, for the effect of warming treatment. The variation is explained by each axis in parentheses.



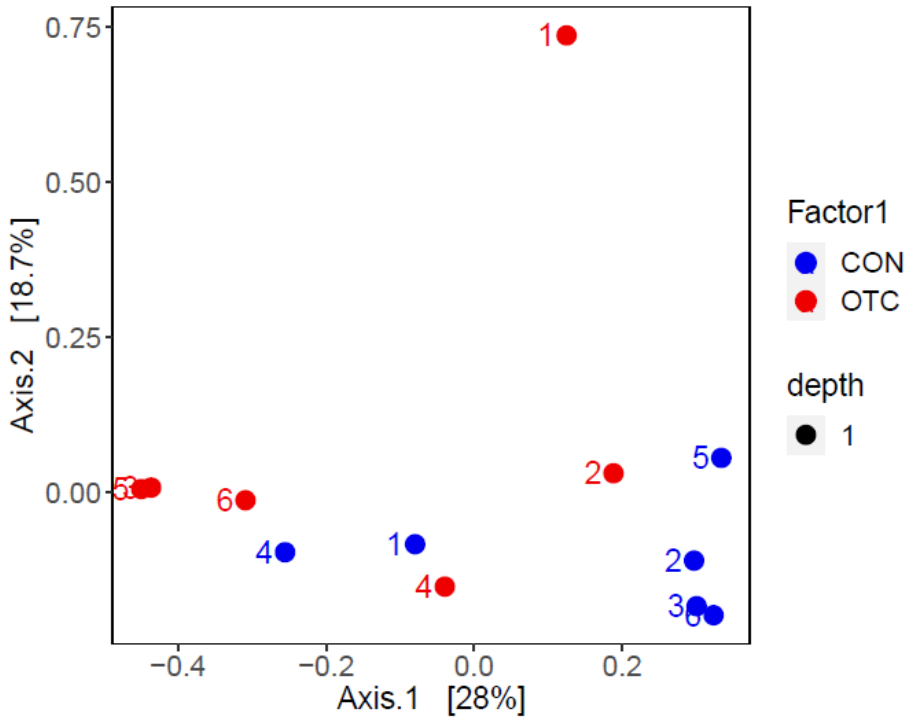
Appendix 6: PCoA of community structure for the RNA-16S samples at depth 3, for the effect of warming treatment. The variation is explained by each axis in parentheses.



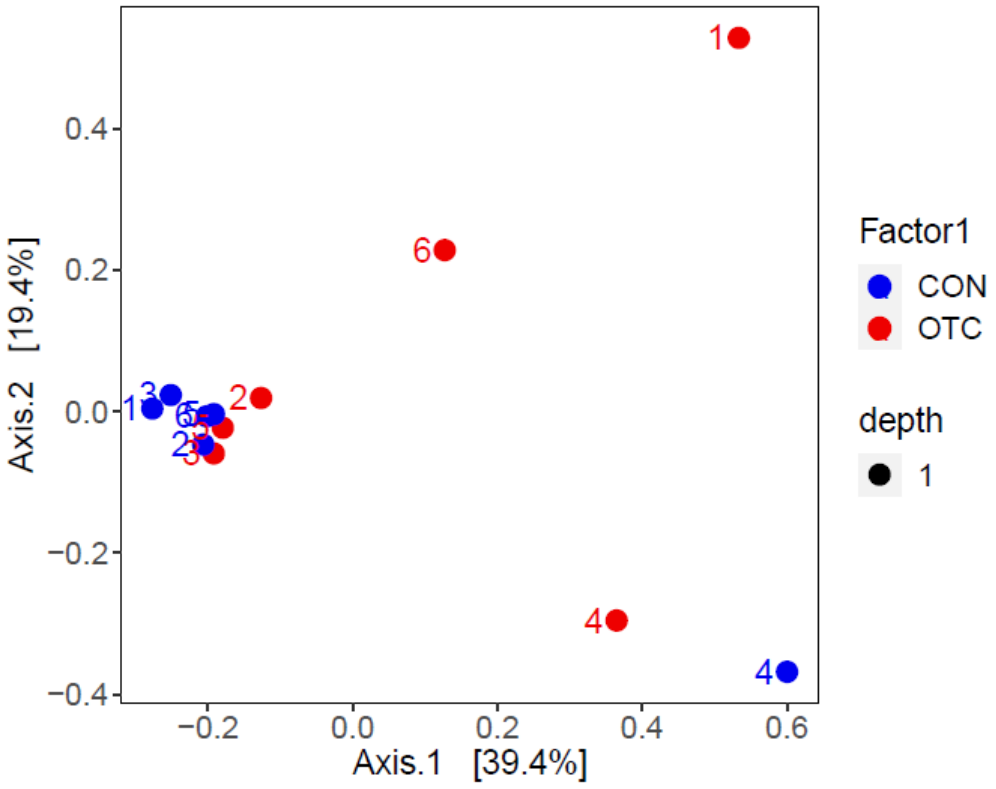
Appendix 5: PCoA of community structure for the RNA-16S samples at depth 1, for the effect of warming treatment. The variation is explained by each axis in parentheses.



Appendix 6: PCoA of community structure for the DNA-ITS samples at depth 1, for the effect of warming treatment. The variation is explained by each axis in parentheses.



Appendix 7: PCoA of community structure for the RNA-ITS samples at depth 1, for the effect of warming treatment. The variation is explained by each axis in parentheses.



Appendix 8: PCoA of community structure for the RNA-ITS samples at depth 3, for the effect of warming treatment. The variation is explained by each axis in parentheses.