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Quantification of specific microbial functional guilds in Arctic soil

Bachelor’s thesis

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Annotation: The quantity of specific bacterial and archaeal groups were determined in permafrost soils. The selected groups were quantified by quantitative polymerase chain reaction (qPCR) of specific genes: total archaea (*16SrRNA gene*), methanogenic archaea (*mcrA gene*), nitrogen fixators (*nifH gene*) and denitrifiers (*nosZ gene*). The quantities of these groups were compared between different soil horizons and furthermore correlations between the quantity of gene copies and basic soil parameters were computed.

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Abstract

Arctic permafrost soils are most affected by global warming. Thawing of permafrost soil may stimulate the activity of microorganisms and accelerate the degradation of the largest organic carbon pool. This study quantified microbial guilds in Arctic soil horizons; such as the nitrogen fixing bacteria, the denitrifying bacteria, methanogens and the total archaea.

The samples have been collected on Disko Island in Greenland. The specific genes, such as *nifH*, *nosZ*, *mcrA* and *16SrRNA*, have been quantified from purified DNA samples with qPCR. The data was then analysed with the statistical programme Rstudio to detect significant differences of gene copies in soil horizons with the ANOVA followed by TukeyHSD test. Regressions and correlations were computed between the gene copies and abiotic soil parameters and the depth.

The number of gene copies per gram of soil were in all cases highest in the organic top soil and equally high in the permafrost soil. The second highest was the abundancy in the topsoil and buried organic material pockets. These are the result of a continuous thaw and freeze process, which mixes soil layers, known as cryoturbation. Whereas the lowest number of copies was found in the mineral subsoil. A significant regression between the genes and parameters, such as the percentage of nitrogen and carbon in the soil, the dissolved nitrogen and the dissolved organic carbon, the water content and the depth were found.

The high abundance of genes in permafrost soil show a potential for fast degradation of buried carbon and nitrogen when the global warming is continuing. Further investigations must clarify how the number of gene copies are affected by increasing temperatures.

CONTENT

1. Introduction ... 1
  1.1. Permafrost-process of cryoturbation ... 2
  1.2. Soil horizon properties ... 3
  1.3. Microorganisms in Arctic soil ... 4
  1.4. Methane cycle ... 4
  1.5. Nitrogen cycle ... 5
  1.6. Data from other studies in Arctic regions on the population of microorganisms ... 7
  1.7. Aim of the work ... 7
2. Material and methods ... 8
3. Results ... 11
4. Discussion ... 19
  4.1 Comparison to other studies ... 19
  4.1.1. Abundancies of gene copies in the soil horizons ... 19
  4.1.2. Correlations of selected prokaryotic groups with environmental parameters ... 20
  4.2. Limitations of the study and future perspectives ... 21
5. Conclusion ... 21
Index of abbreviations ... 26
Index of figures ... 27
Index of tables ... 29
Supplementary material ... 30

1. INTRODUCTION

The aim of this research has been, to quantify microbial guilds in Arctic permafrost soils; such as the nitrogen fixing bacteria, the denitrifying bacteria, methanogens and archaea. These microorganisms are involved in global nitrogen and carbon cycling. Both cycles must be considered when investigating the global warming and positive carbon-cycle feedback. The quantification of the microbial guilds was determined with the aid of qPCR of the genes *nifH*, *nosZ*, *mcrA* and *16SrRNA* and the software R was used to evaluate the statistics (RStudio 2016).

Some previous studies focused on the soil and its microbial community in Siberia, Svalbard, Canada and other Arctic regions. Little is known of the quantitative numbers of microorganism in Disko island in Greenland. The quantification of microbial groups involved in global C and N cycle in the Arctic soil are recently of major interest. This information may be used to attest previous observations and further establish new predictions. It is commonly known that temperatures on earth are continuously rising. Looking at some statistics from NASA we see an uprising trend in the last 100 years (NASA). In high latitude regions the temperature has risen twice as fast as anywhere else on earth. It increased by 0.6°C per decade in the last 30 years (Schuur and Grosse 2015). It was predicted that this trend will continue or even accelerate when taking carbon-cycle feedbacks into account (Cox et al. 2000).

Despite such kind of studies, it is difficult to predict, what effects global warming will have on the ecosystems of our planet. All kind of factors are interconnected and must be taken into consideration when predicting our future in terms of the climate. The climate influences the population and metabolic activities of the organisms on earth and together with the organisms, the cycling of nutrients is shifted. These changes affect the composition of the atmosphere and influence therefore also the climate. The temperatures are rising because greenhouse gasses in the atmosphere enhance the radioactive forcing. The rising temperatures affect the thawing of ice and permafrost, the desiccation of peatlands and other changes in ecosystems. It was previously studied that rising temperatures increase soil respiration and decomposition of soil organic matter (SOM). The study of Hicks Pries et al. showed an increase of 34-37% in the annual soil respiration on a temperature increase of 4°C (Hicks Pries et al. 2017). In another 122 days incubation experiment of permafrost soil in -2°C and 8°C the microbial community and the abundancy of functional genes changed only significantly in 8°C incubations. In these incubated samples a degradation of the soil

organic carbon (SOC) of the organic-rich Arctic soil was observed. Thereby also the abundancy of functional genes decreased (Yang et al. 2017).

An important factor of positive feedback to global warming may be given when peatlands dry and are exposed to oxic conditions. The degradation of stored organic matter to greenhouse gases is accelerated in aerobic metabolism. The other considerable influence of warming may be the thawing of permafrost (Davidson and Janssens 2006). The northern permafrost region is very rich in organic carbon and it is estimated that approximately 1672Pg of organic carbon are stored in that soil. This amount of organic carbon accounts half of the estimated belowground organic carbon pool on earth (Tamocai et al. 2009). The Arctic was a sink of carbon in the past because the cold temperatures decreased the rate of decomposition. However, upon an increase of the global temperature, the Arctic may serve as a carbon source.

1.1. Permafrost-process of cryoturbation

Figure 1 Typical profile of Cryosols (permafrost soils affected by cryoturbations): Uppermost layer is O- (organic layer), followed by A- (organic topsoil), jj- (cryoturbated topsoil), BC- (mineral subsoil) and PF-horizon (permafrost)

The Permafrost is a remainder from the last ice age and increasing temperatures causes its thawing. This results in unstable ground, earth slides and increased erosion. A lake may disappear suddenly, because the grounds ability to retain water is lost when permafrost melts. A sudden release of gases can make the earth collapse (Wagner et al. 2018). Permafrost affected soils cover approximately 18 million km^2, or about 16% of the earths land surface (Tamocai et al. 2009). Permafrost affected soil are typical by cryoturbation, which are driven by repeating freezing/thawing events. The cryoturbation mixes higher soil layers, rich in organic carbon, with the deeper layers. This leads to the high carbon content in deeper soil layers, due to mixing of organic topsoil and mineral subsoil and an accumulation of organic matter on the permafrost table (Mergelov and Targulian 2011).

1.2. Soil horizon properties

The soil is composed of varies layers. The uppermost layer is high of organic matter and called the O-horizon. The organic topsoil is mainly composed by slightly to highly decomposed organic matter. The mineral topsoil is the A-horizon, it is mineral soil mixed with humus and of very dark greyish brown colour. It is moderately to well decomposed litter, with a lot of nutrients and rich in microorganisms. Some fine roots are abundant in the A-horizon, and it is tilled for agriculture. The mineral subsoil is called B-horizon and is composed of very little organic matter and smaller abundancy of microorganisms. The leached minerals from the soil surface accumulate here. The BC-horizon is the transition between B-and C-horizon but has more similar properties to the upper B-layer. The C-horizon is the soil base, which develops directly from the underlying bedrock and has a low microbial activity (Figure 2). The major difference of Arctic soil to the soil in warmer regions, is the existence of buried topsoil. These cryoturbated pockets with high organic content were labelled with jj and can occur in different depths. The Permafrost layer PF is characterized by its permanent sub-zero temperatures and reduced availability of water in a liquid state (Altshuler, Goordial, and Whyte 2017). Permafrost is not always reached after the same depth. Some regions in the Arctic have a continues permafrost plate, but in lower altitudes the permafrost is underlying the soil in discontinues patches. In two of our sampling sites the permafrost could not been reached. Disko island is between the border of the continues and discontinues permafrost layer (Christiansen 1999).

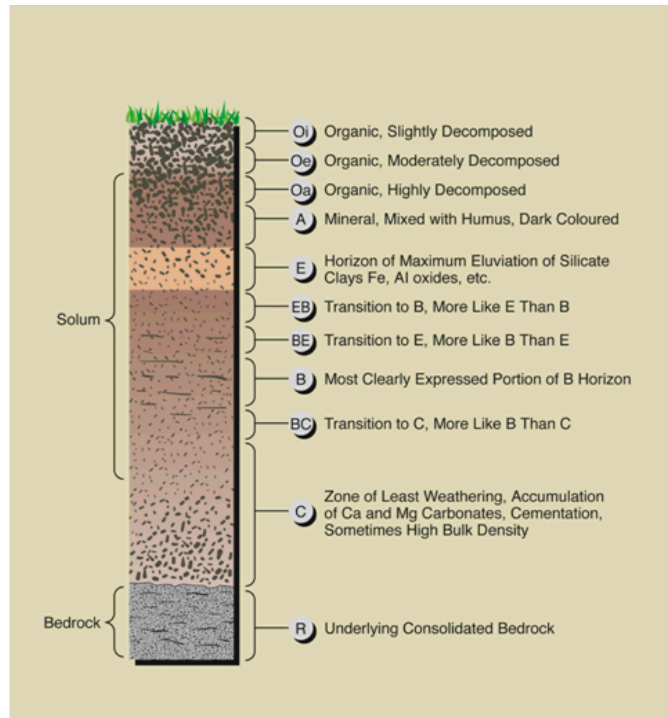

Figure 2 Soil horizon classification and properties

1.3. Microorganisms in Arctic soil

Microorganisms can survive and adapt to cold temperatures. Under these conditions, they are not only able to run catabolic and anabolic processes (Drotz et al. 2010). Microorganisms which have their optimum temperature below 20°C are called psychrophiles. They can survive sub-zero temperatures, in some extreme cases even down to -40°C. But even at soil temperatures down to -20°C it is possible to find fluid parts in the soil. These fluids make it possible for microorganisms to survive and be metabolically active even in permafrost soil. Because majority of microorganisms are osmotrophs, it is important for them to be in fluids, to efficiently exchange substances and get nutrients from the environment. To survive in sub-zero temperatures, they must maintain a fluid cell membrane. The cell membranes tend to have a higher content of unsaturated fatty acids and shorter-chain fatty acids. These fatty acids remain semifluid even in cold temperatures. The lipids even contain polyunsaturated fatty acids, which is very uncommon in prokaryotes. Cold-active enzymes have more alpha-helixes instead of beta-sheets because alpha-helixes are less rigid than beta-sheets. These enzymes also tend to be composed of more polar and less hydrophobic amino acids. Furthermore, they have a low number of weak bonds and less interactions between regions (Altshuler, Goordial, and Whyte 2017; MADIGAN et al. 2009).

1.4. Methane cycle

We were looking closer to methanogens, which is especially interesting for investigating in the release of the greenhouse gas methane from the Arctic soil. Methane is the third most common greenhouse gas on earth, after CO_2 and H_2O. However methane has a 28% higher global warming potential (GWP) than CO_2 without considering climate-carbon feedbacks (www.ipcc.ch n.d.). Even though under oxic conditions more permafrost carbon is degraded to CO_2, we cannot forget about anoxic conditions. In these conditions less carbon is degraded, but the methane has a higher GWP than CO_2 (Knoblauch et al. 2018). In the Arctic, methane is released form methanogenesis and from methane, which was trapped in the permafrost for several years. Upon global warming the permafrost melts and releases the trapped methane. The carbon decomposition potential across the regions is comparable. Major variabilities are dependent on the soil composition and mainly the carbon to nitrogen ratio. It was discovered that release of methane changes over the years, as carbon pools are exhausted or new ones are exposed. The Arctic region is not only dominated by cold temperatures. The underlying permafrost determines the surface appearance. This region is rich in lakes and waterlogged soils because the permafrost retains the water. In wetlands and waterlogged soils anaerobic conditions prevail, which are ideal conditions for methanogens.

Not all the methane is released into the atmosphere, some amount is oxidised to CO_2 before it leaves the soil (Schuur and Grosse 2015).

Methanogens produce methane as a product of anaerobic respiration of simple carbon molecules. They use H_2 as reducing agent and CO_2 or simple carbon molecules as carbon source. Methanogens belong to archaea and most of them are obligate anaerobes. Until now it was thought that microbial methanogenesis can only occur in anoxic environments. Despite that concept methanogens in well oxygenated soils exists. One of the identified organisms is *Candidatus Methanothrix paradoxum*. In a study of 2017 even a ten times greater methane production was observed in oxygenated soil, in comparison to anoxic soil in a freshwater wetland (Angle, 2017). Researchers found a correlation between nitrogen fixation and methanogens. The release of methane decreased when the soil was rich in Ammonium (NH_4^+) and increased in nitrogen-poor soils. It was predicted, that some methanogens can fix nitrogen. This energetically expensive reaction is sustained by gaining Energy from the synthesis of methane (Bae, Morrison, and Chanton 2018).

1.5. Nitrogen cycle

Nitrogen is a very important chemical compound for the biosynthesis of organic matter. Very often nitrogen is the limiting factor for growth of plants and must be supplemented artificially to the soil for sustainable agriculture. Even though 78% of the atmosphere is nitrogen gas, this form of nitrogen is an inert gas and very stable and therefore it cannot be

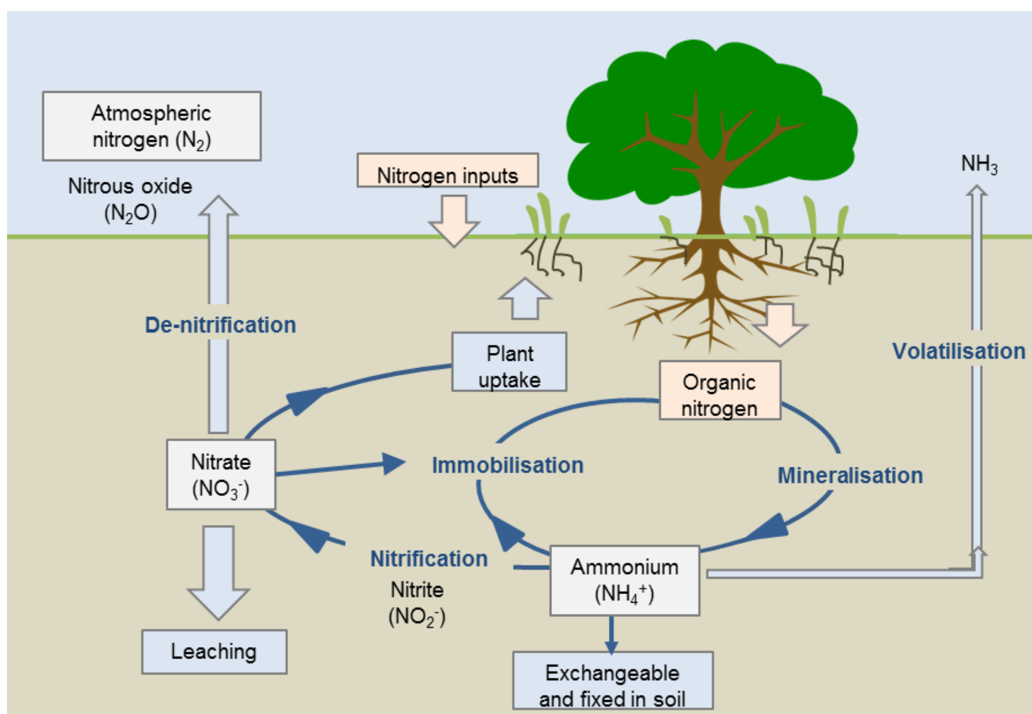

Figure 3 Cartoon of the nitrogen cycle

used by plants and animals. Atmospheric nitrogen must be fixed to reactive nitrogen species such as NO_3^-, NO_2, NH_3. The Arctic is not used for agriculture and is solely dependent on natural ways of nitrogen fixation or decomposition of organic nitrogen. Natural nitrogen fixation is occurring either by lightnings, which is very rare in polar regions, or by nitrogen fixing bacteria. Nitrogen fixing bacteria convert N_2 to ammonium ions, which are accessible by plants and other microorganisms. Ammonia is converted to nitrite and nitrate in the nitrification steps. Nitrate is either used by plants or by denitrifying bacteria and released after the final reduction step to the atmosphere in the form of N_2, NO or N_2O gasses. The process of denitrification includes all steps from nitrate to the reduced gaseous nitrogen forms. The three enzymes nitrate reductase, nitrite reductases and nitric oxide reductase catalyse these chemical transformations (Bothe, Ferguson, and Newton 2006) (Fig. 3). In the Arctic soil, nitrate (NO_3^-) is present at very low levels, in various cases even under the detection limit. Therefore, it was often excluded when considering the available reactive nitrogen sources for plants. Even though the amount is very low, it is absorbed by the plants at similar rates as in soils with high nitrate concentrations (Liu et al. 2018). In the polar regions the permafrost must be considered. Due to these characteristic features, Nitrogen is stored over years and melting of permafrost can be a sudden nitrogen source upon global warming (Liu et al. 2018). Nitrogen regulates topsoil carbon dynamics by altering microbial metabolic efficiencies. The carbon and nitrogen cycle are closely related (Chen et al. 2018). It was previously shown that nitrogen transformation rates were significantly slower in cryoturbated pockets than in the organic horizon. This may be, because of a different community composition, for example less fungi are found in the cryoturbated pockets (Wild et al. 2013). In the Siberian Arctic in buried topsoil degradation rates of soil organic matter (SOM) were only 13% as fast as in the topsoil. When substituting the cryoturbated pockets with amino acids and proteins, the SOM decomposition rate doubled. This suggests that nitrogen was the main limiting factor. In the topsoil only a weak change in degradation rates was achieved when adding organic compounds (Wild et al. 2014).

Dissolved nitrogen (DN) originates mainly from biological matter. It is the remainder of plants or nitrogenous waste from animals. Dissolved organic nitrogen (DON) includes compounds such as proteins, free amino acids, nucleic acids, amino sugars and urea. DON are the nitrogen containing molecules from the dissolved organic carbon pool (DOC). Furthermore, dissolved inorganic nitrogen (DIN) can be found in soil, which includes ammonium, nitrate and nitrite. DN serves as a valuable nitrogen and carbon source for microorganisms (Jørgensen 2009).

1.6. Data from other studies in Arctic regions on the population of microorganisms

In an experiment in the Canadian high Arctic researcher compared microorganism communities and abundancies of functional genes between active soil and permafrost soil. They found very similar community compositions in permafrost and active soil and most functional genes have been found in both layers. They also discovered that the *nifH* was quiet abundant in their sampling site, but had a low diversity, which makes it more vulnerable to changes (Yergeau et al. 2010). Another study in the Canadian Arctic was correlating the abundance of functional genes to the depth and C/N in four different locations. They studied the genes *nifH*, *mcrA*, *pmoA* and *amoA*. For the *mcrA gene* they did not find a consistent trend in their sampling sites. The most abundant gene was *nifH*, reaching numbers of 10^8 copies per gram wet soil. The *nifH gene* had also a negative correlation to depth in two of the four sampling sites. They found a high diversity of bacteria and archaea, which decreased by depth (Frank-Fahle et al. 2014). In Greenland, bacterial, fungal and archaeal gene copies have been obtained with qPCR for the different soil layers. For bacteria highest numbers of gene copies have been found in the topsoil and the buried organic pockets and the lowest number was present in the permafrost affected soil. A similar distribution of gene copies along the soil horizons was found for archaea (Gittel et al. 2014). The abundance of bacterial SSU decreased with depth in a sampling site in northeast Siberia. Although the genes in buried subsoils were significantly higher than in its surrounding subsoil samples. Bacterial SSU gene copies were lowest in the permafrost samples (Gittel et al. 2013).

1.7. Aim of the work

The main goal of the thesis was to determine the quantity of specific bacterial and archaeal groups in Arctic permafrost soils. The quantification of the selected groups was conducted with quantitative polymerase chain reaction (qPCR) of specific genes, such as: total archaea (*16SrRNA gene*), methanogens (*mcrA gene*), nitrogen fixators (*nifH gene*) and denitrifiers (*nosZ gene*). The quantities of gene copies were compared between the different soil horizons and correlated to basic soil parameters.

2. MATERIAL AND METHODS

The samples were collected in August 2016 during the vegetation season in west Greenland on the Disco Island, Qeqertarsuaq -CENPERM Arctic Station. Three sites with different plant domination were selected to take samples of the soil horizons. In the end we got 140 samples, of which 31 samples were from the A-horizon, 44 of the B-horizon, 40 of the jj-horizon, 16 of the O-horizon and 9 of the PF-horizon. 39 samples were from the sampling site a (pit A-D), 61 from the sampling site b (pit E-H) and 40 from the sampling site c (pit I-L). Permafrost samples could only be taken from sampling site b in the other sampling sites the permafrost was not reached. The sampling site b was close to a lake and more humid.

The DNA of these soil samples was purified, and the abundance of some functional genes involved in C- and N-cycling were analysed with quantitative real time PCR (qPCR). Total quantity of bacterial, archaeal and fungal communities was conducted by targeting *16SrRNA gene* and *18SrRNA gene* respectively. The data for total bacteria was provided by the supervisor. Furthermore, extracted DNA samples were tested for the functional gene abundance of methanogens in the soil horizons by quantifying *methyl coenzyme M* reductase genes (*mcrA gene*). The functional gene abundance of denitrifying bacteria was determined by the quantification of *nitrous oxide reductase* genes (*nosZ gene*) and the functional gene abundancy of nitrogen fixing bacteria was determined with the amplification of the *nitrogen reductase* coding genes (*nifH gene*).

The qPCR assays were conducted with StepOne Real-Time PCR in 48-well reaction plates. With one plate 18 different samples could be analysed with two analytical replicates. The standard was always diluted by a factor of ten. The amplification curves of these wells were then used as a reference to calculate the standard curve and therefore determine the concentration of our DNA samples. In the last well we used an inner standard (VS), which is used to calibrate all plates of the same gene assays to a similar C_T value for the inner standard. The Master Mix consisted of water, Fast SYBR™ Green Master Mix, the respective primers, BSA and DMSO. 17 µl of the Master Mix were mixed with 3µl of DNA template. The specific primers and other details to the qPCR procedure are found in the table below (Table 1).

Table 1 Data of how the qPCR was conducted for the different genes

	16SrRNA gene	***mcrA gene***	***nifH gene***	***nosZ gene***
Primer forward (5′-3′)	16SrRNA787F ATTAGATACCCSBGTAGTCC	ME1 GCMATGCARATHGGWATGTC	IGK3 GCIWTHTAYGGIAARGGIGGIATHGGIAA	nosZ-F-1181 CGCTGTTCITCGACAGYCAG
Primer reverse (5′-3′)	16SrRNA1059R GCCATGCACCWCCTCT	MCR1R CADATYTGRTCRTA	DVV ATIGCRAAICCICCRCAIACIACRTC	nosZ-R-1880 ATGTGCAKIGCRTGGCAGAA
Quantity [nmol]	0,5	0,3	1	1
Annealing temp.	60°C	50°C	58°C	56°C
No. of cycles	45	40	40	35
Standard	*Pyrococus furiosus*	*Methanosarcina sp.*	*Methylocystis heyeri*	*Pseudomonas aeruginosa*
Dilutions	10^{-3}-10^{-7}	10^{-3}-10^{-7}	10^{-4}-10^{-8}	10^{-3}-10^{-7}
Start quantity (copies per ml)	$2,01*10^{11}$	$9,87*10^{9}$	$3,39*10^{11}$	$2,42*10^{10}$
Reference	(Yu et al. 2005)	(Hales et al. 1996)	(Gaby and Buckley 2012)	(Rich et al. 2003)

The samples which had a high standard deviation between the replicates were repeated. Some samples showed multiple peaks in the melting curve and had to be checked for possible different gene amplifications with gel electrophoresis.

The threshold was adjusted so that the C_T values of the VS was consistent for all plates. The C_T value is the cycle number at which the increase of the fluorescence signal is first detectable, and it depends on the initial amount of template after how many cycles this is reached. When we set the C_T values for the VS we ensure that all the time the same number of amplification cycles were used to get significant amplifications.

The qPCR data was used for statistical analysis with the software R Studio (RStudio 2016). After checking the distribution of the data with a histogram, the data was normalized by log10 transformation. For the comparison of the soil horizons we used ANOVA test, followed by Tukey-HSD test and visualized the data with graphs. A comparison of the three

different sites was also shown with graphs with the function ggplot. For the graphs we arranged the horizons according to this order: O, A, B, jj, PF. For the computation of correlation, the Pearson correlation test was used. The regression test calculated the explained variation (R^2), which provides a measure how close the data are to the fitted regression line. Furthermore, gene copies were correlated to the depth, the water content in the soil and the percentage of carbon and nitrogen in the soil (C% and N%) and the dissolved organic carbon and the dissolved Nitrogen (DOC and DN).

3. RESULTS

Looking at the boxplots of all genes we saw a clear trend of decreasing gene copies per gram dry weight of soil till the B-horizon, in the centre of the boxplots and increased values at both sides. On the right side were placed the O-horizon and A-horizon and on the left side of the B-horizon were the jj-pockets and the PF-layer. We got significant results (p-value < 0.05) for all studied genes.

The bacteria had highest numbers of genes in the O- horizon and significantly lower numbers in the jj- and PF-horizon and the lowest number in the A- and B-horizon (Figure 4). The qPCR data of bacteria was obtained from MSc. Milan Varsadiya.

Total bacteria

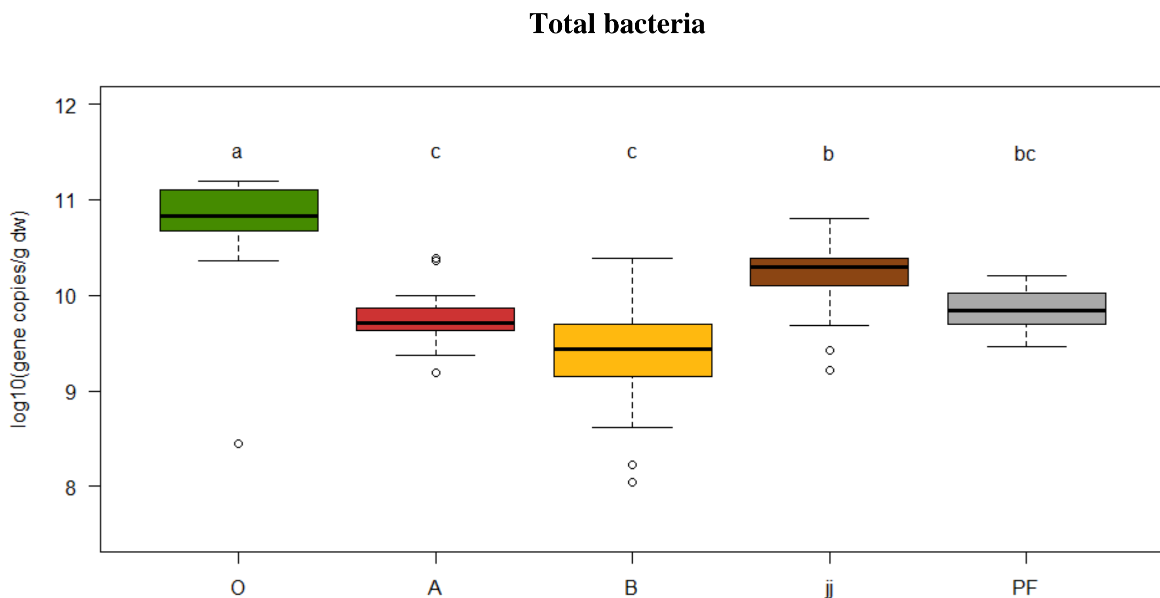

Figure 4 Boxplot of bacteria number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons.

Total archaea *16SrRNA gene* was significantly the lowest in the B-horizon (5.66 x 10^6 genes per gram dry weight). However, no significant difference was found between all other horizons, with the median in the other soil horizons of 1.89 x 10^7 genes per gram dry weight, which is two orders of magnitude higher (Figure 5).

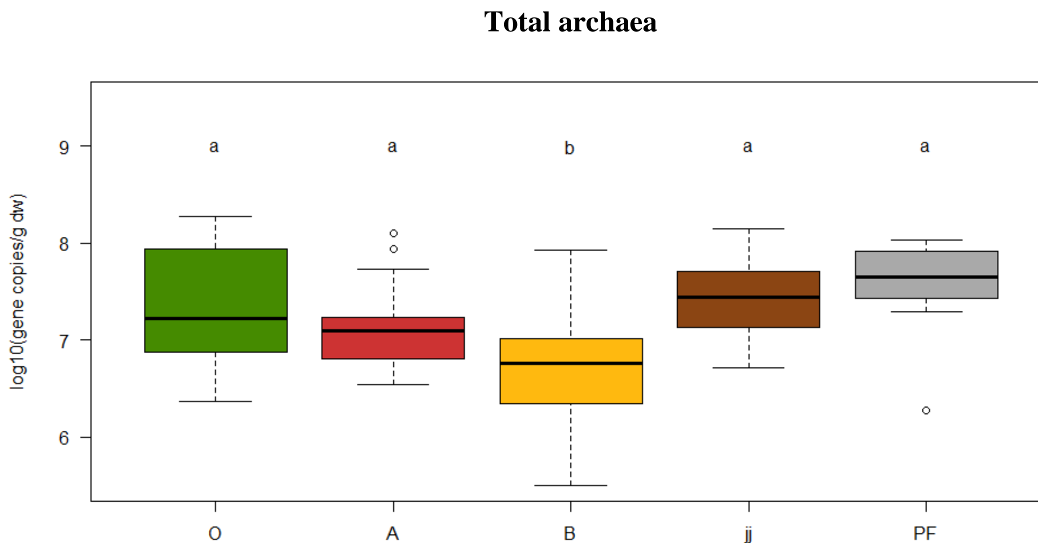

Figure 5 Boxplot of *16SrRNA* number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons.

The *mcrA gene* had a statistically significant highest gene abundance in the PF- and O-horizon (median = 6.89 x 10^5 genes per gram dry weight). Second highest the gene number was in the jj-horizon (1.20 x 10^5 genes per gram dry weight) and lowest by a factor of ten in the A- and B-horizons (1.76 x 10^4 genes per gram dry weight) (Figure 6).

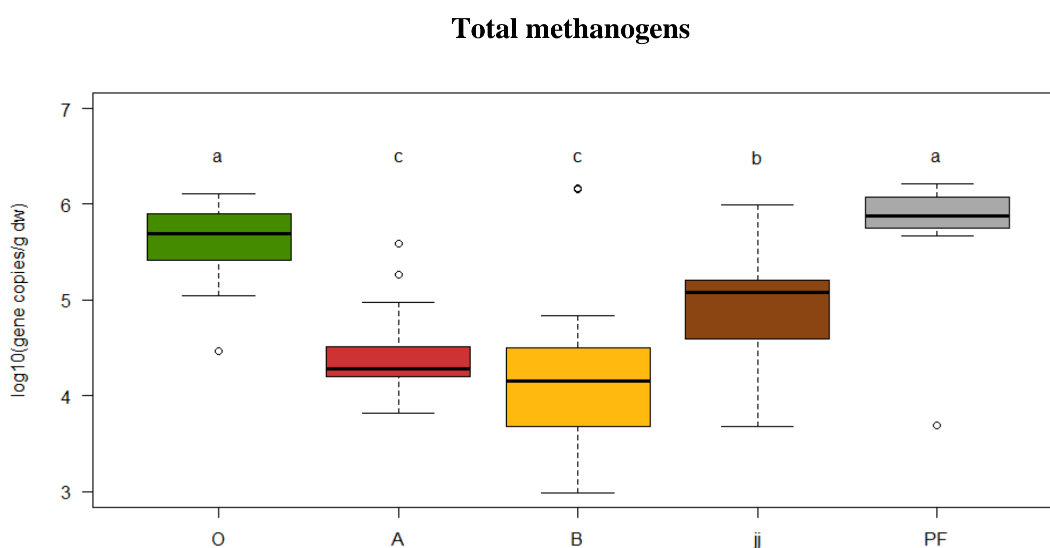

Figure 6 Boxplot of *mcrA* number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons.

The *nifH gene* had also the highest numbers of genes in the PF- and O-horizons (1.01×10^6 genes per gram dry weight). In the jj-horizon it was not significantly less abundant but can be grouped also with the significantly lower A- and B-horizon (1.21×10^5 genes per gram dry weight, Figure 7).

Nitrogen fixators

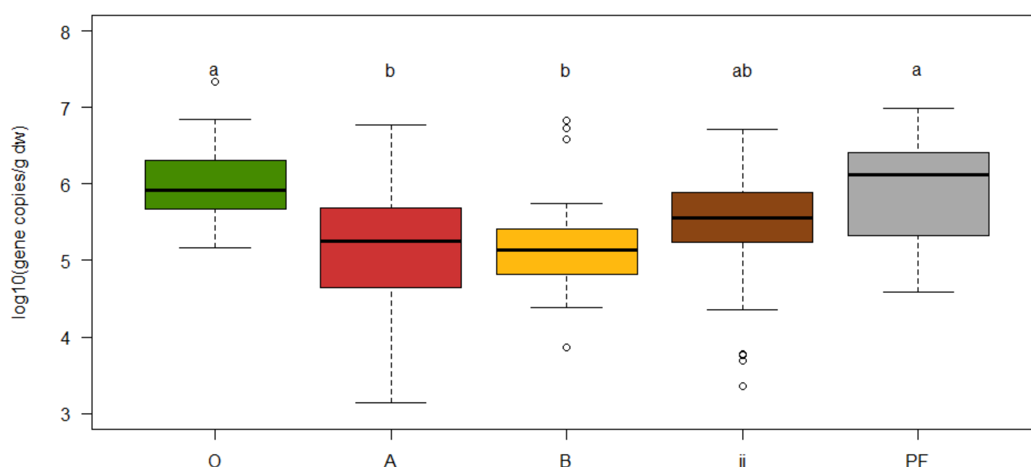

Figure 7 Boxplot of *nifH* number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons.

The *nosZ gene* is highest in the O-horizon (1.31×10^9 genes per gram dry weight), but not significantly lower in PF, which groups also with A and jj. Gene copies in the jj-horizon were significantly lower than in the O-horizon and significantly higher than in the A-horizon. The B-horizon was again the horizon with the lowest *nosZ gene* abundance (2.95×10^7 per gram dry weight, Figure 8).

Denitrifiers

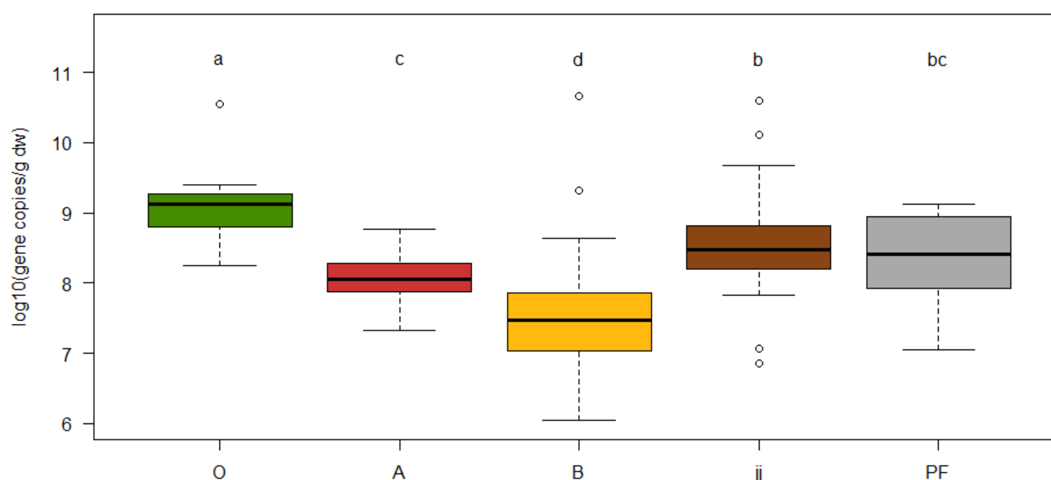

Figure 8 Boxplot of *nosZ* number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons.

Furthermore, we computed correlations of the gene numbers with each other and abiotic soil parameters. The correlation of the genes *nifH*, *nosZ*, *mcrA* and *16SrRNA* with each other was for all computations significant with p-value < 0.001. We were able to show significant positive correlation (cor) between the percentage of nitrogen (N%) in the soil and all amplified genes per gram of dry weight (p-value < 0.001). The strongest correlation exists between N% and *mcrA* (cor=0.74, R^2=55%). Furthermore, the correlation was significant for all genes and the dissolved nitrogen (DN) (p-value < 0.001) (Table 2). When doing the same correlations with the gene copy numbers per ng of DNA the results were not as significant. This means that the percentage of the *nosZ* per total microbial biomass in the soil is not dependent on N% nor on DN. Whereas, the absolute number of gene copies per gram dry soil was dependent on the nitrogen parameters.

Table 2 Regression of gene copies per gram dry weight (/g dw) of *16SrRNA*, *mcrA*, *nifH* and *nosZ* to the percentage of nitrogen in the soil (N%) and the dissolved nitrogen in the soil (DN)

N%	**p-value**	**cor**	**R^2 in [%]**
Total archaea (16SrRNA gene)	<0.001	0.58	33
Methanogens (mcrA gene)	<0.001	0.74	55
Nitrogen fixators (nifH gene)	<0.001	0.35	12
Denitrifiers (nosZ gene)	<0.001	0.64	41
DN	**p-value**	**cor**	**R^2 in [%]**
Total archaea (16SrRNA gene)	<0.001	0.38	14
Methanogens (mcrA gene)	<0.001	0.61	37
Nitrogen fixators (nifH gene)	<0.001	0.37	13
Denitrifiers (nosZ gene)	<0.001	0.43	18

Moreover, we computed the correlation between the gene numbers and the carbon in the soil. For the carbon we had values of the total percentage of carbon in the soil (C%) and the dissolved organic carbon in the soil (DOC). Both carbon values, C% and DOC had a positive correlation to the quantified number of genes per gram dry weight. The correlation was not as significant when the quantified gene numbers per ng of DNA were used for computation of the correlations. Details can be seen in the table 2.

Table 3 The correlation between the gene copies per gram dry weight (/g dw) and the carbon percentage (C%). The lower half represents the correlations of the genes with the dissolved organic carbon (DOC).

C%	**p-value**	**cor**	**R^2 in [%]**
Total archaea (16SrRNA gene)	<0.001	0.57	32
Methanogens (mcrA gene)	<0.001	0.77	59
Nitrogen fixators (nifH gene)	<0.001	0.39	15
Denitrifiers (nosZ gene)	<0.001	0.66	43
DOC	**p-value**	**cor**	**R^2 in [%]**
Total archaea (16SrRNA gene)	<0.001	0.46	21
Methanogens (mcrA gene)	<0.001	0.76	58
Nitrogen fixators (nifH gene)	<0.001	0.48	23
Denitrifiers (nosZ gene)	<0.001	0.57	33

Moreover, one of the soil parameters which we used for regression, was the water content in the soil. A significant correlation and regression were found for all studied genes. One example graph is seen in Figure 9, other graphs are in the supplement and an overview can be found in table 4.

Table 4 Regression of gene copies per gram dry weight (/g dw) and the water content of the soil.

Water content	**p-value**	**cor**	**R^2 in [%]**
Total archaea (16SrRNA gene)	<0.001	0.56	33
Methanogens (mcrA gene)	<0.001	0.79	67
Nitrogen fixators (nifH gene)	<0.001	0.46	25
Denitrifiers (nosZ gene)	<0.001	0.67	47

Another parameter which we used to correlate to our gene numbers and for computing the regression was the depth of the taken sample. For the depth not all the time a significant correlation was found. We found a significant regression for *mcrA* and *nosZ* when taking the gene copies per gram dry weight. In both cases the gene number per gram dry soil decreased by depth with a similar slope (Table 5, Figure 10, Supplement 8).

Table 5 Regression of gene copies to the depth, with gene copies per gram dry weight (/g dw) ns= not significant.

Depth	**p-value**	**cor**	**R^2 in [%]**
Total archaea (16SrRNA gene)	ns		
Methanogens (mcrA gene)	<0.001	-0.37	13
Nitrogen fixators (nifH gene)	ns		
Denitrifiers (nosZ gene)	<0.001	-0.34	12

Moreover, the ratio of *mcrA* to *16SrRNA* was calculated and shown in a boxplot. The ratio of *mcrA* and *16SrRNA* was higher in O and PF than in A, B, jj (Fig. 11). Therefore, we can conclude that in the horizons A, B and jj other archaea than methanogens were abundant in higher quantities. Additionally, we did not find any correlation between archaea and the C/N ratio.

When comparing abundancies of the genes in the three different sampling sites, some significant differences were observed. In sampling site c the functional gene abundance of archaea was generally higher. In sampling site a, the functional genes *mcrA*, *nifH* and *nosZ* were all present in significantly smaller quantities than in sampling site b and c (Supplement 1, 2, 3, 4, 5).

After we found similar trends of some genes to abiotic soil properties, we tried to correlate the number of gene copies with each other. All observed genes had correlations with each other. The strongest correlation was between *mcrA* and *nosZ* per gram dry weight. All the details are seen in Table 6.

Table 6 Correlation of gene copies per gram dry weight (/g dw) of the selected functional groups with each other. (p = p-value)

Gene copies /g dw	*Nitrogen fixators (nifH gene)*			*Denitrifiers (nosZ gene)*			*Methanogens (mcrA gene)*		
	p	**cor**	**R^2**	**p**	**cor**	**R^2**	**p**	**cor**	**R^2**
Denitrifiers (nosZ gene)	<0.001	0.49	25						
Methanogens (mcrA gene)	<0.001	0.58	34	<0.001	0.66	43			
Total archaea (16SrRNA gene)	<0.001	0.35	12	<0.001	0.62	38	<0.001	0.60	36

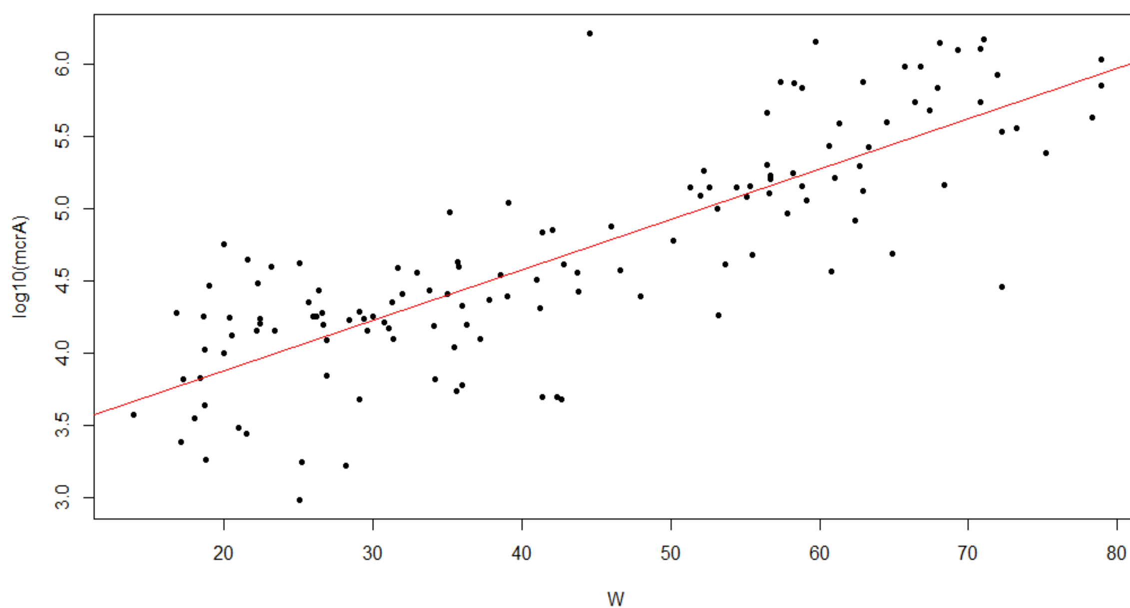

Figure 9 Regression between the water content (W) of the soil and the gene abundance of *mcrA* per gram dry weight with log transformation.

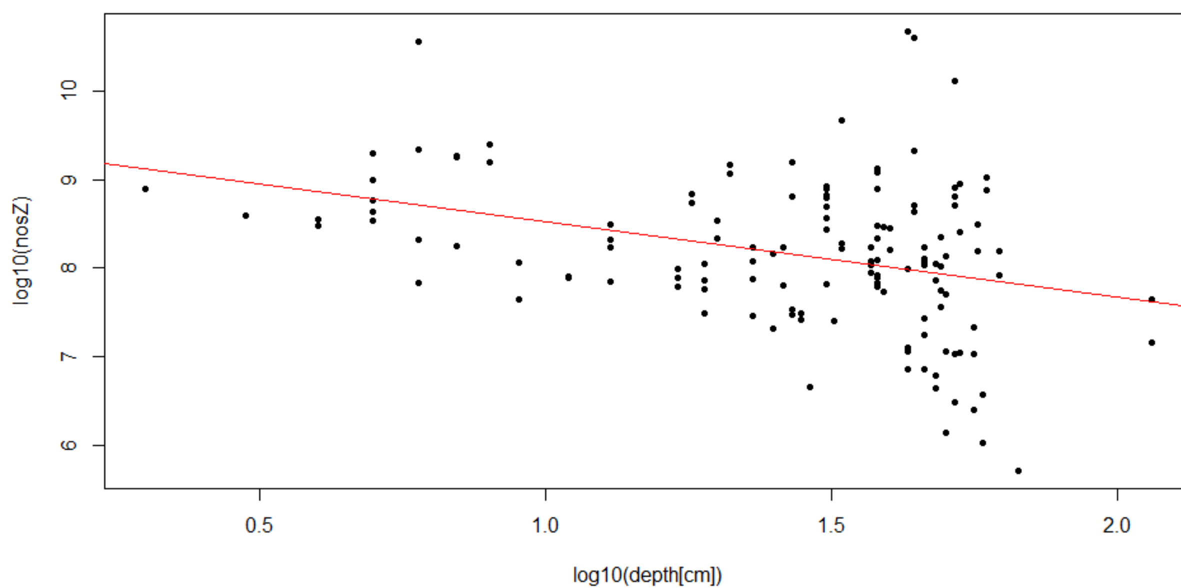

Figure 10 Regression between the depth and the gene abundance *nosZ* per gram dry weight with log transformation.

Ratio of methanogens out of total archaea

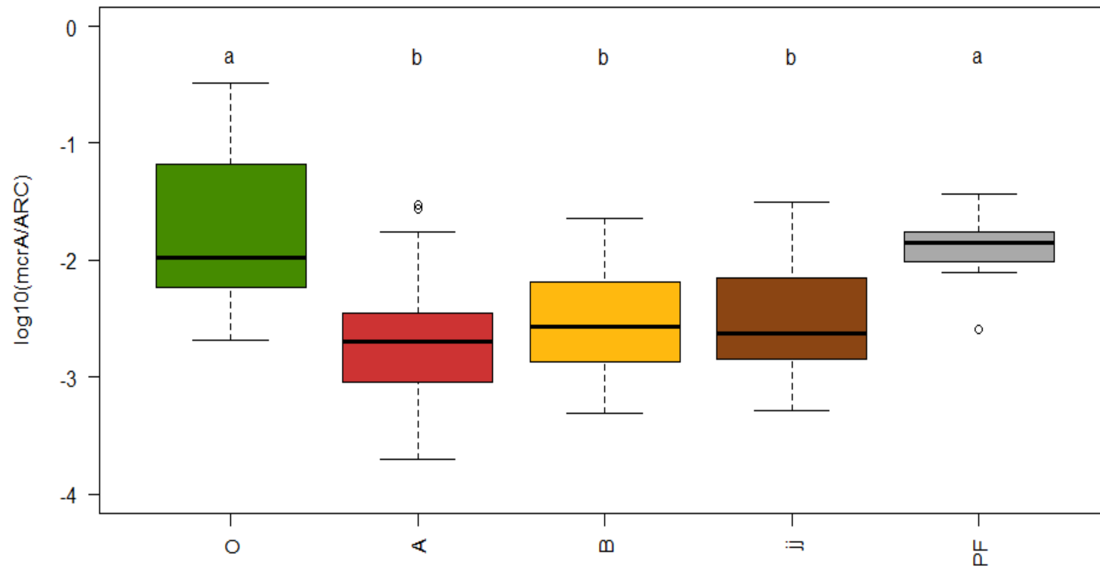

Figure 11 Ratio of the gene abundance of *mcrA* over *16SrRNA* in comparison of the different soil horizons

4. DISCUSSION

The soil composition is not only dependent on abiotic factors, moreover the number and divergence of microorganisms is an important factor to determine the rate of decomposition and nutrient cycling. It is confirmed that increased temperatures accelerate the decomposition rate of organic matter (Hicks Pries et al. 2017; Yang et al. 2017). As an increase in the global temperature is affecting mostly the poles, it is of major interest to observe the changes in the Arctic (Schuur and Grosse 2015).

The current study focused on the enumeration of the number of the classifying genes *nifH*, *nosZ*, *mcrA* and *16SrRNA* with qPCR. The DNA samples were extractions from soil samples of different soil horizons in the sampling site on Disko island in Greenland. These gene numbers were correlated with different soil properties and the gene abundancies in the various soil horizons were compared with each other. The gene numbers are used to estimate an approximate abundance of these specific microorganisms in the soil. The knowledge about the distribution of these microorganisms is important to determine the soil composition and rates of decomposition, which may accelerate upon global warming. Up to now most attention was payed to the topsoil only, but we sampled all the horizons down to the permafrost table, as the deeper layers should not be neglected. We found a high number of those genes in buried topsoil and permafrost layers. The number was comparably high to the gene number in organic topsoil samples. Moreover, this confirms the importance to consider and study the whole soil profile. It is estimated that in the northern permafrost region is about half of the global carbon stored. The comparably high number of microorganisms in that buried organic soil represents a high potential for decomposition. Until now the decomposition of buried organic material was slowed down my low temperatures, and restricted availability of nutrients (Yang et al. 2017; Wild et al. 2013). However, the temperatures are currently rising and will make buried carbon sources more easily accessible, which may result in a positive carbon feedback.

4.1 Comparison to other studies

4.1.1. Abundancies of gene copies in the soil horizons

In the results it can be seen that there is a higher abundancy of every gene and microorganisms in the O-horizon. The jj-horizon had comparable or slightly less genes than the O-horizon. This was also seen in the northeast Siberian tundra and northeast Greenland, where for Bacteria the highest numbers of genes were found in the organic topsoil and the cryoturbated organic pockets. Although this trend was also mostly consistent for our samples, in these previous studies the lowest number of genes was often found in the

permafrost samples. (Gittel et al. 2014, 2013; Yergeau et al. 2010) Surprisingly in our sampling site the number of gene copies for the genes *mcrA*, *16SrRNA* and *nifH* was as high in the permafrost layer as in the organic topsoil. The Permafrost samples belonged only to the sampling site b, therefore we cannot generalize this outcome for the whole region. Most probably permafrost affected soil can have varies different properties depending on the region. Some permafrost affected soil is rich in organic matter, coming from cryoturbated pockets, but at the same time permafrost samples can include frozen mineral soil. It may be, that the abiotic properties of the soil have a stronger impact on the number of gene copies, than the frozen properties. The frozen conditions only slow down degradation and conserve the soil for a long period.

4.1.2. Correlations of selected prokaryotic groups with environmental parameters

The correlation between *nifH gene* per gram dry soil with the depth was not significant, even though in a previous studies in the Canadian Arctic a negative correlation was found in two of the four samples of polygonal soil (Frank-Fahle et al. 2014). Furthermore, the correlation between *mcrA gene* per gram wet weight and the depth was studied in the research project of Frank-Fahle et al., but no consistent trend was found. Whereas, we could find a negative correlation of *mcrA* and *nosZ genes* per gram dry soil to the depth. *NosZ* and *mcrA genes* have a very similar correlation to the depth. On the same time a negative correlation to the depth for archaea and bacteria was found by a previous research project, which was not represented by our data (Frank-Fahle et al. 2014).

Furthermore, we detected a strong correlation between the water content in the soil and the abundance of the studied genes *nifH*, *nosZ*, *mcrA* and *16SrRNA*. For all these genes it is typical trend because they require microaerophilic or anoxic conditions.

Additionally, there was a correlation of the gene numbers per gram dry weight and the soil parameters C%, N% DOC and DN. The values for C% and DOC in the soil sample were interconnected with the gene number of all the studied genes. It is difficult to define whether these bacteria and archaea prefer and prosper in soils high in carbon, or if the high amount of C% and DOC is caused by the microorganisms. The N% and DN in the soil correlated also significantly with all genes. Not only the nitrogen fixing and the denitrifying bacteria, which are both involved in the nitrogen cycle. The gene *mcrA* has the strongest correlation with the nitrogen parameters. It was suggested by Bae et al. that some methanogens are able to fix nitrogen and are therefore also actively involved in the nitrogen cycle (Bae, Morrison, and Chanton 2018).

4.2. Limitations of the study and future perspectives

There are several limitations to the current study. One limitation of the study is, that only permafrost samples from one sampling sites were taken. Despite these limitations, this study is the first to analyse the soil and its properties along the soil horizons on Disko island. However, the results warrant further validation in other Arctic regions around the poles. Further work is necessary to understand the observed correlation between the gene abundancies and the soil properties. Furthermore, future studies may shed more light on the issue, how much the numbers of genes in a soil sample are correlated to the degradation and how much it is affected by global warming.

5. CONCLUSION

We found highest numbers of genes in the soil horizons O and PF and lowest in the B-horizon. Additionally, significant positive correlations were found between the four studied genes per gram dry weight and the depth and the water content. We also observed correlations to the total carbon content in the soil and to the dissolved organic carbon. Further correlations were found between the genes to the total nitrogen content and to the dissolved nitrogen in the soil.

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INDEX OF ABBREVIATIONS

A	organic topsoil
B and BC	mineral subsoil
C%	percentage of carbon in the soil
DIN	dissolved inorganic nitrogen
DN	dissolved nitrogen
DOC	dissolved organic carbon
DON	dissolved organic nitrogen
GWP	global warming potential
jj	cryoturbated topsoil
mcrA gene	methyl coenzyme M reductase gene
nifH gene	nitrogen reductase coding gene
N%	percentage of nitrogen in the soil
nosZ gene	nitrous oxide reductase gene
SOC	soil organic carbon
SOM	soil organic matter
O	organic soil
PF	permafrost soil
qPCR	quantitative polymerase chain reaction

INDEX OF FIGURES

FIGURE 1 TYPICAL PROFILE OF CRYOSOLS (PERMAFROST SOILS AFFECTED BY CRYOTURBATIONS): UPPERMOST LAYER IS O- (ORGANIC LAYER), FOLLOWED BY A- (ORGANIC TOPSOIL), JJ- (CRYOTURBATED TOPSOIL), BC- (MINERAL SUBSOIL) AND PF-HORIZON (PERMAFROST) 2
FIGURE 2 SOIL HORIZON CLASSIFICATION AND PROPERTIES 3
FIGURE 3 CARTOON OF THE NITROGEN CYCLE 5
FIGURE 4 BOXPLOT OF BACTERIA NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. 11
FIGURE 5 BOXPLOT OF *16SRRNA* NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. 12
FIGURE 6 BOXPLOT OF *MCRA* NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. 12
FIGURE 7 BOXPLOT OF *NIFH* NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. 13
FIGURE 8 BOXPLOT OF *NOSZ* NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. 13
FIGURE 9 REGRESSION BETWEEN THE WATER CONTENT (W) OF THE SOIL AND THE GENE ABUNDANCE OF *MCRA* PER GRAM DRY WEIGHT WITH LOG TRANSFORMATION. 17
FIGURE 10 REGRESSION BETWEEN THE DEPTH AND THE GENE ABUNDANCE *NOSZ* PER GRAM DRY WEIGHT WITH LOG TRANSFORMATION. 17
FIGURE 11 RATIO OF THE GENE ABUNDANCE OF *MCRA* OVER *16SRRNA* IN COMPARISON OF THE DIFFERENT SOIL HORIZONS 18
FIGURE 12 BOXPLOT OF BACTERIA NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. ADDITIONALLY, THE COMPARISON OF THE THREE SAMPLING SITES IN THE LOWER BOXPLOTS. 30
FIGURE 13 BOXPLOT OF ARCHAEA (ARC, *16SRRNA*) NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. ADDITIONALLY, THE COMPARISON OF THE THREE SAMPLING SITES IN THE LOWER BOXPLOTS. 31
FIGURE 14 BOXPLOT OF *MCRA* NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. ADDITIONALLY, THE COMPARISON OF THE THREE SAMPLING SITES IN THE LOWER BOXPLOTS. 32
FIGURE 15 BOXPLOT OF *NIFH* NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. ADDITIONALLY, THE COMPARISON OF THE THREE SAMPLING SITES IN THE LOWER BOXPLOTS. 33
FIGURE 16 BOXPLOT OF *NOSZ* NUMBER OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) WITH LOG TRANSFORMATION TO COMPARE THE DIFFERENT SOIL HORIZONS. ADDITIONALLY, THE COMPARISON OF THE THREE SAMPLING SITES IN THE LOWER BOXPLOTS. 34
FIGURE 17 THE RATIO OF DENITRIFIERS (*NOSZ GENE*) PER TOTAL BACTERIA IN THE SOIL. 35
FIGURE 18 THE RATIO OF NITROGEN FIXATORS (*NIFH GENE*) PER TOTAL BACTERIA IN THE SOIL. 35
FIGURE 19 REGRESSION BETWEEN THE WATER CONTENT (W) OF THE SOIL AND THE GENE ABUNDANCE OF *NOSZ* PER GRAM DRY WEIGHT 36
FIGURE 20 REGRESSION BETWEEN THE WATER CONTENT (W) OF THE SOIL AND THE GENE ABUNDANCE OF *NIFH* PER GRAM DRY WEIGHT 36
FIGURE 21 REGRESSION BETWEEN THE WATER CONTENT (W) OF THE SOIL AND THE GENE ABUNDANCE OF *16SRRNA* PER GRAM DRY WEIGHT 36
FIGURE 22 REGRESSION BETWEEN THE WATER CONTENT (W) OF THE SOIL AND THE GENE ABUNDANCE OF *MCRA* PER GRAM DRY WEIGHT WITHOUT THE LOG TRANSFORMATION. 37
FIGURE 23 REGRESSION BETWEEN THE DEPTH AND THE GENE ABUNDANCE *NOSZ* PER GRAM DRY WEIGHT. 37
FIGURE 24 THE CORRELATION OF *NOSZ* GENE COPY NUMBERS PER GRAM DRY WEIGHT AND DISSOLVED ORGANIC CARBON (DOC) WITH LOG TRANSFORMATION. 38
FIGURE 25 THE CORRELATION OF *NOSZ* GENE COPY NUMBERS PER GRAM DRY WEIGHT AND PERCENTAGE OF CARBON IN THE SOIL (C%) WITH LOG TRANSFORMATION. 38
FIGURE 26 THE CORRELATION OF TOTAL ARCHAEA (*16SRRNA* GENE COPY NUMBERS PER GRAM DRY WEIGHT) AND DISSOLVED ORGANIC CARBON (DOC) WITH LOG TRANSFORMATION. 38
FIGURE 27 THE CORRELATION OF TOTAL ARCHAEA (*16SRRNA* GENE COPY NUMBERS PER GRAM DRY WEIGHT) AND PERCENTAGE OF CARBON IN THE SOIL (C%) WITH LOG TRANSFORMATION. 39

FIGURE 28 THE CORRELATION OF *MCRA* GENE COPY NUMBERS PER GRAM DRY WEIGHT AND DISSOLVED ORGANIC CARBON (DOC) WITH LOG TRANSFORMATION. 39
FIGURE 29 THE CORRELATION OF *MCRA* GENE COPY NUMBERS PER GRAM DRY WEIGHT AND PERCENTAGE OF CARBON IN THE SOIL (C%) WITH LOG TRANSFORMATION. 39
FIGURE 30 THE CORRELATION OF *NIFH* GENE COPY NUMBERS PER GRAM DRY WEIGHT AND DISSOLVED ORGANIC CARBON (DOC) WITH LOG TRANSFORMATION. 40
FIGURE 31 THE CORRELATION OF *NIFH* GENE COPY NUMBERS PER GRAM DRY WEIGHT AND PERCENTAGE OF CARBON IN THE SOIL (C%) WITH LOG TRANSFORMATION. 40

INDEX OF TABLES

TABLE 1 DATA OF HOW THE QPCR WAS CONDUCTED FOR THE DIFFERENT GENES 9
TABLE 2 REGRESSION OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) OF *16SRRNA*, *MCRA*, *NIFH* AND *NOSZ* TO THE PERCENTAGE OF NITROGEN IN THE SOIL (N%) AND THE DISSOLVED NITROGEN IN THE SOIL (DN).... 14
TABLE 3 THE CORRELATION BETWEEN THE GENE COPIES PER GRAM DRY WEIGHT (/G DW) AND THE CARBON PERCENTAGE (C%). THE LOWER HALF REPRESENTS THE CORRELATIONS OF THE GENES WITH THE DISSOLVED ORGANIC CARBON (DOC). 15
TABLE 4 REGRESSION OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) AND THE WATER CONTENT OF THE SOIL. 15
TABLE 5 REGRESSION OF GENE COPIES TO THE DEPTH, WITH GENE COPIES PER GRAM DRY WEIGHT (/G DW) NS= NOT SIGNIFICANT. 16
TABLE 6 CORRELATION OF GENE COPIES PER GRAM DRY WEIGHT (/G DW) OF THE SELECTED FUNCTIONAL GROUPS WITH EACH OTHER. (P = P-VALUE) 16

SUPPLEMENTARY MATERIAL

Supplement 1

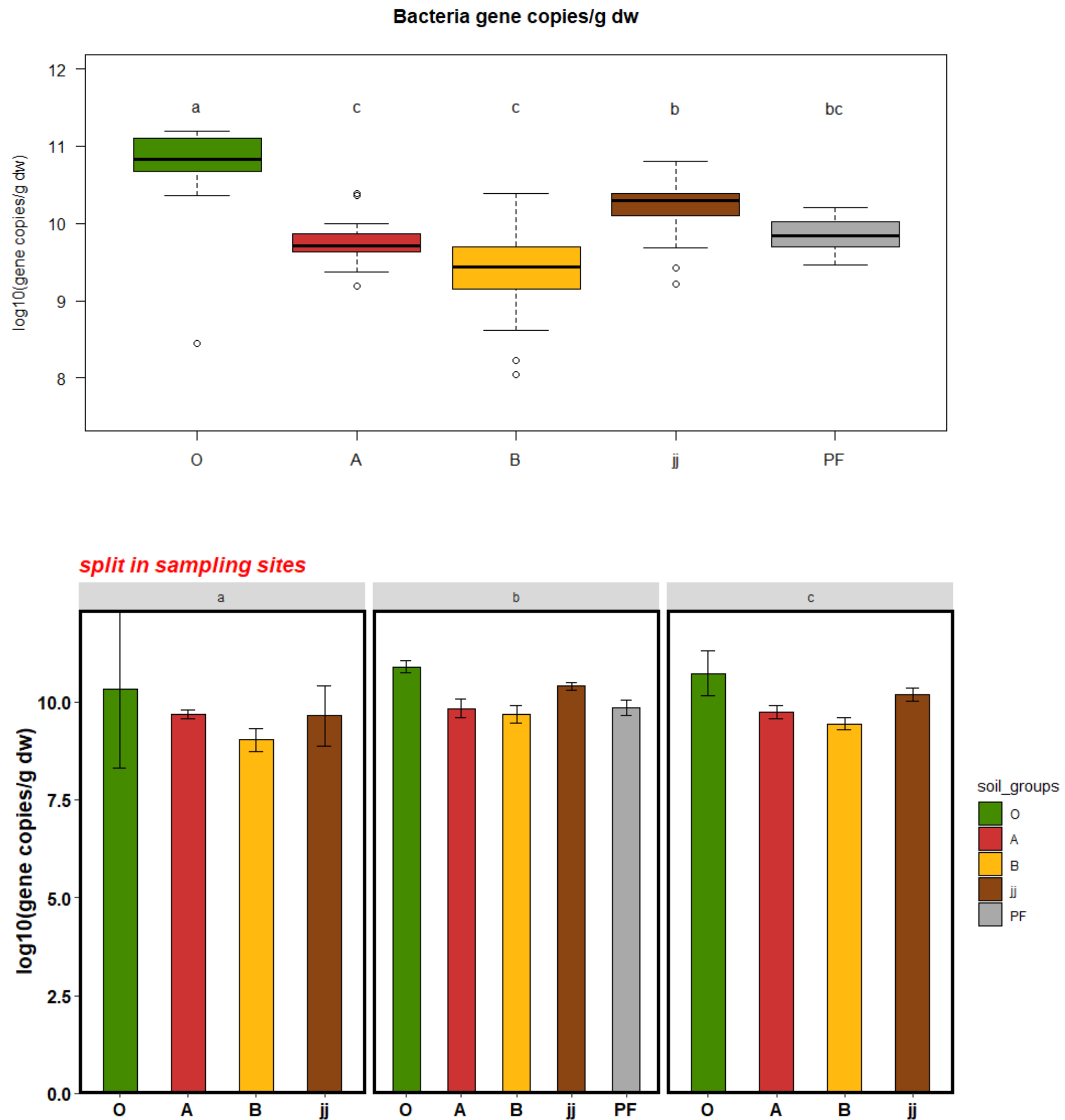

Figure 12 Boxplot of bacteria number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons. Additionally, the comparison of the three sampling sites in the lower boxplots.

Supplement 2

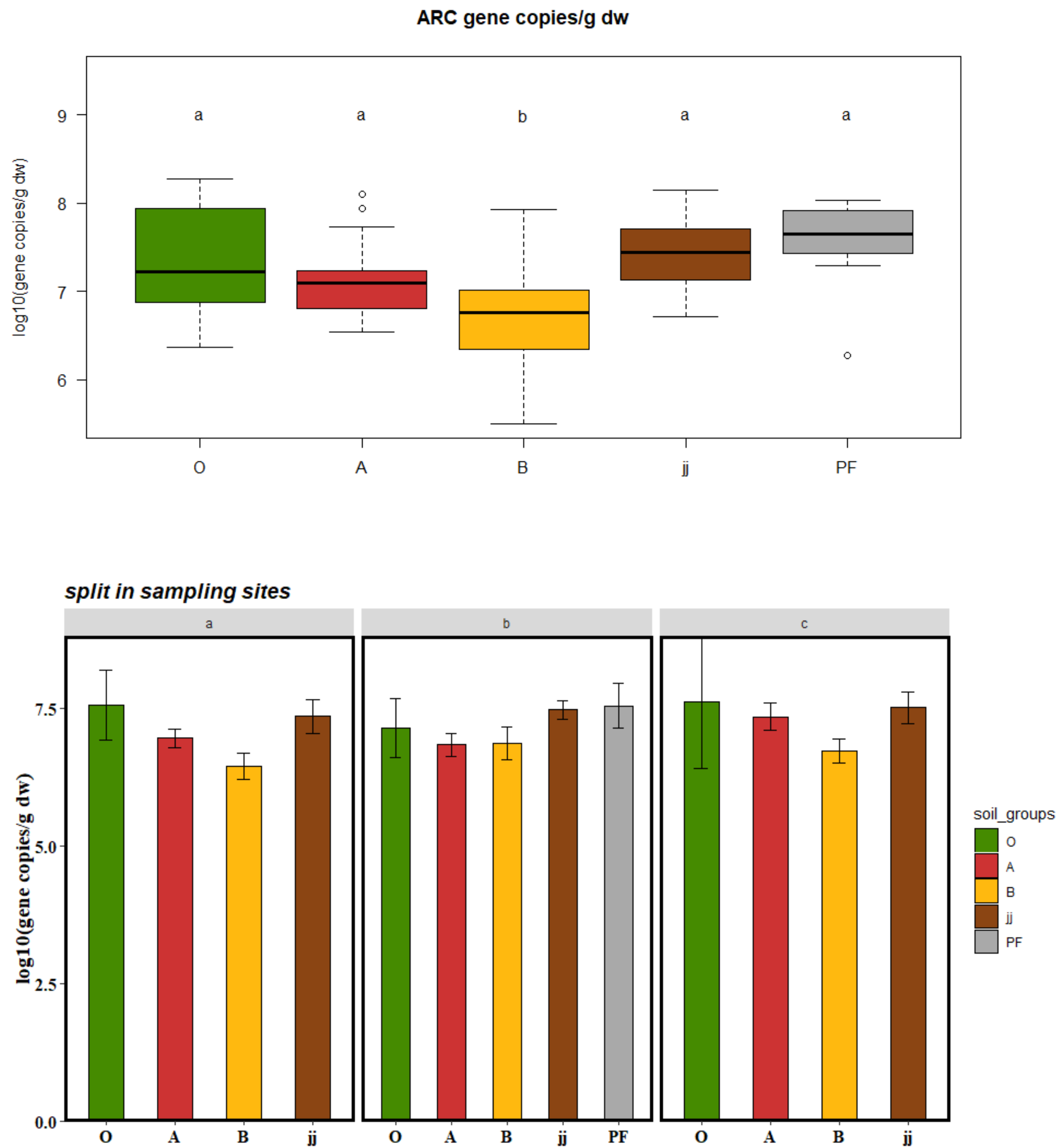

Figure 13 Boxplot of archaea (ARC, *16SrRNA*) number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons. Additionally, the comparison of the three sampling sites in the lower boxplots.

Supplement 3

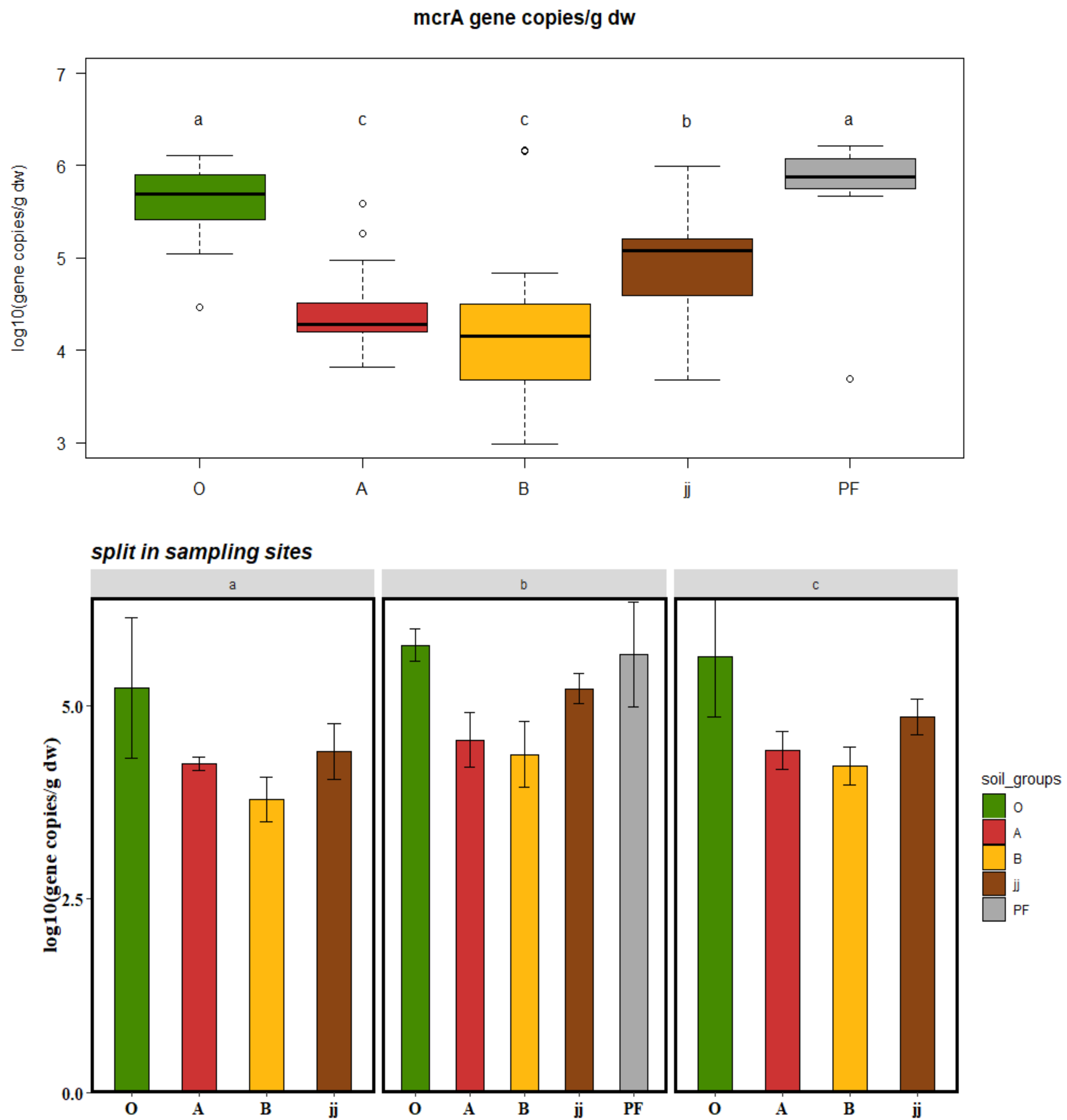

Figure 14 Boxplot of *mcrA* number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons. Additionally, the comparison of the three sampling sites in the lower boxplots.

Supplement 4

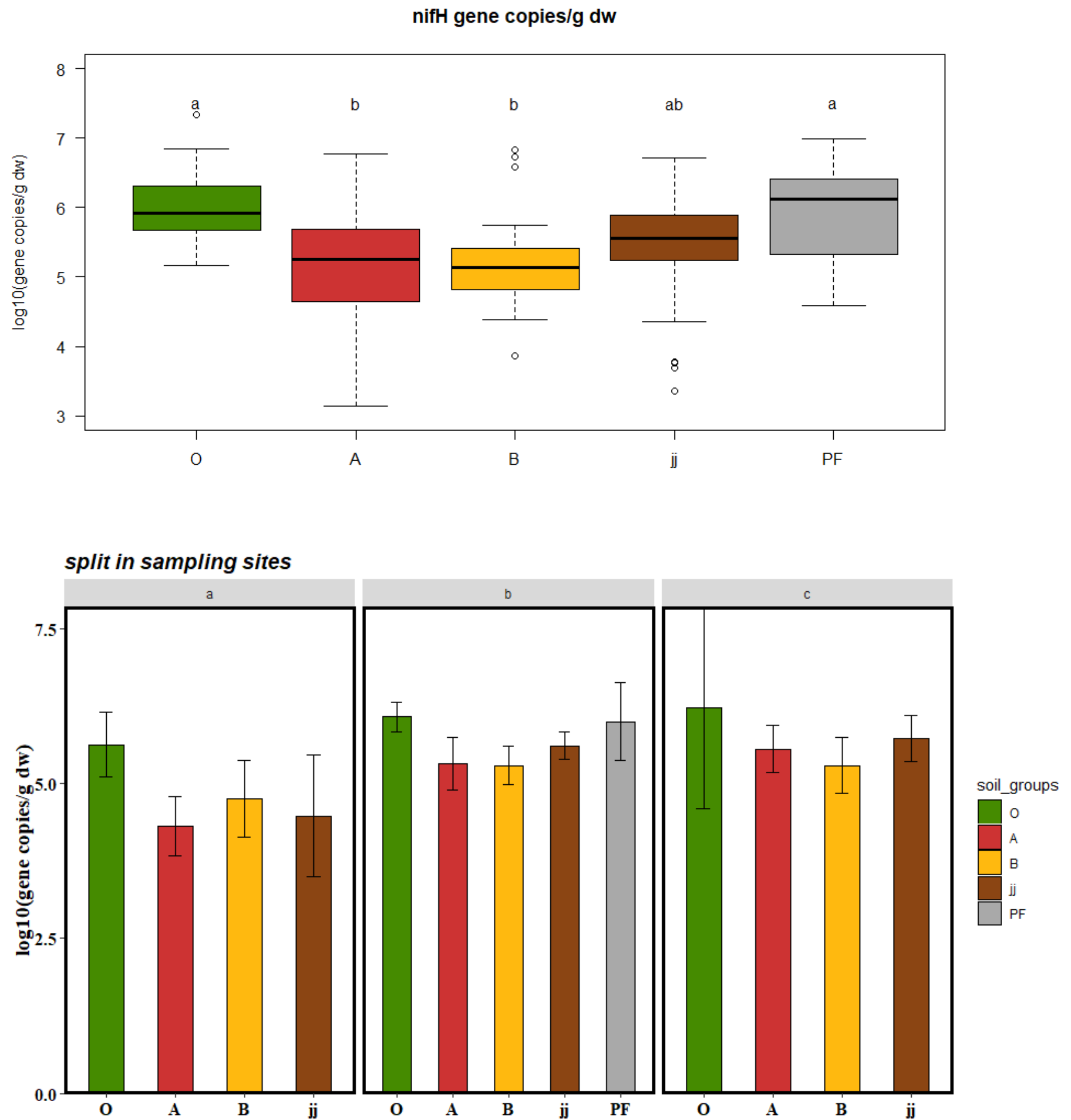

Figure 15 Boxplot of *nifH* number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons. Additionally, the comparison of the three sampling sites in the lower boxplots.

Supplement 5

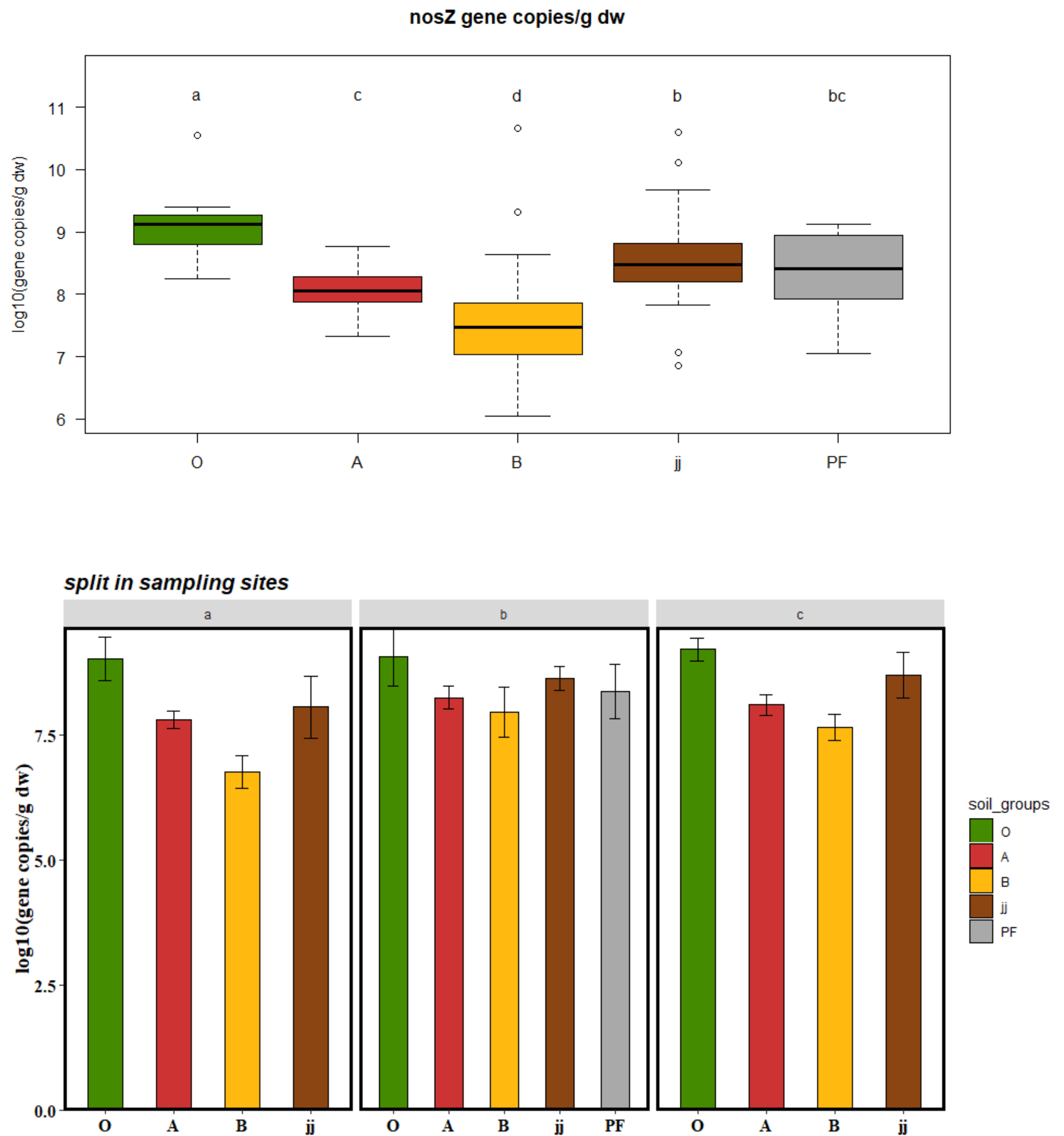

Figure 16 Boxplot of *nosZ* number of gene copies per gram dry weight (/g dw) with log transformation to compare the different soil horizons. Additionally, the comparison of the three sampling sites in the lower boxplots.

Supplement 6

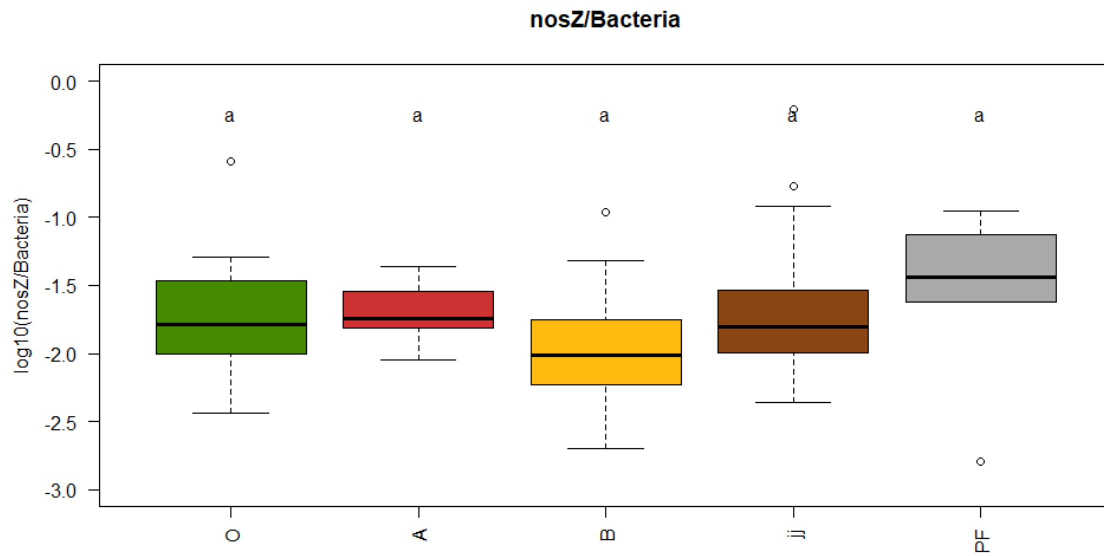

Figure 17 The ratio of denitrifiers (*nosZ gene*) per total bacteria in the soil.

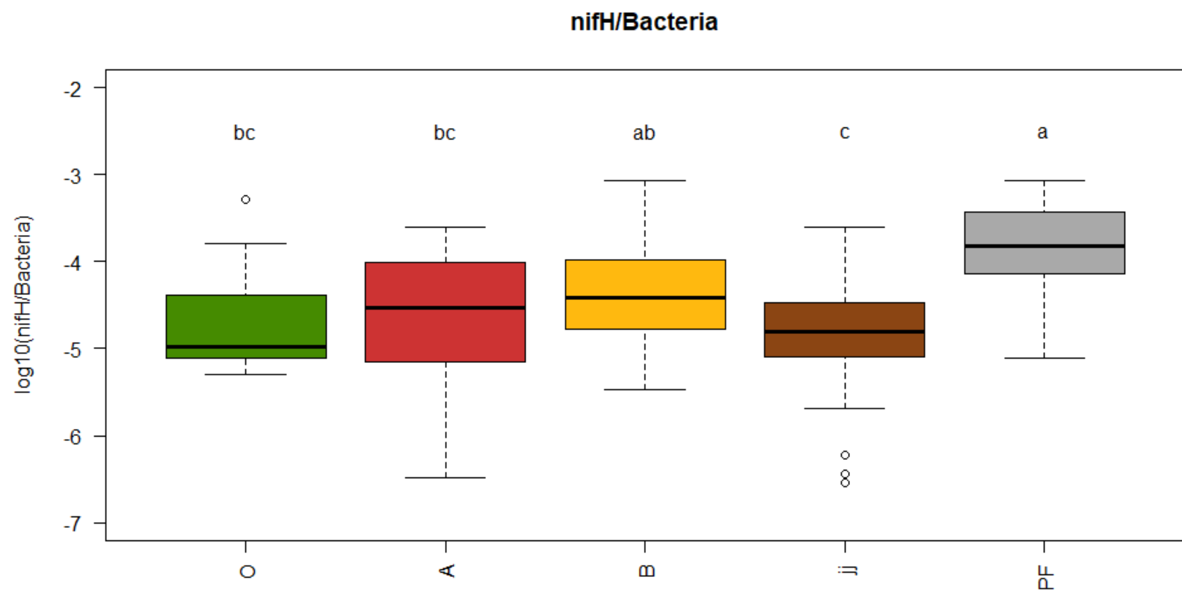

Figure 18 The ratio of nitrogen fixators (*nifH gene*) per total bacteria in the soil.

Supplement 7

Regression of denitrifiers to the water content

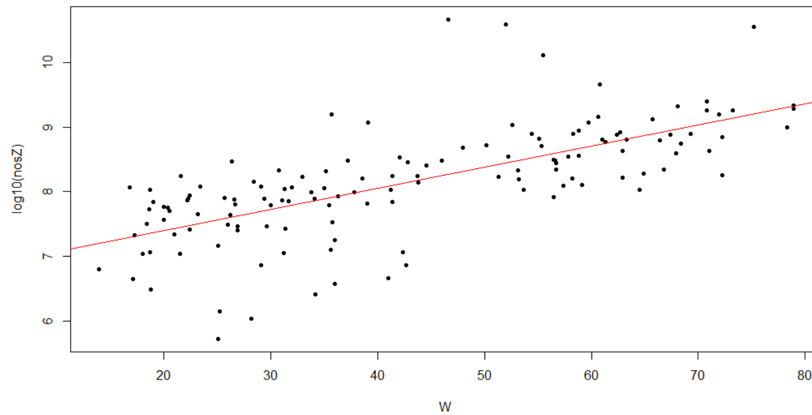

Figure 19 Regression between the water content (W) of the soil and the gene abundance of *nosZ* per gram dry weight

Regression of nitrogen fixators to the water content

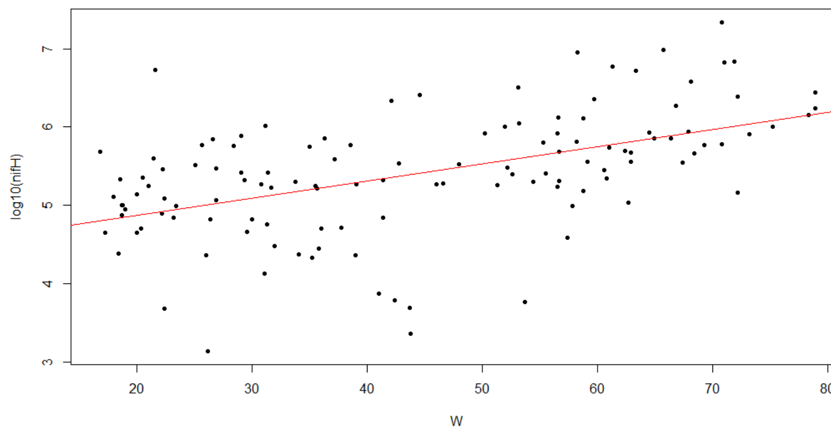

Figure 20 Regression between the water content (W) of the soil and the gene abundance of *nifH* per gram dry weight

Regression of total archaea to the water content

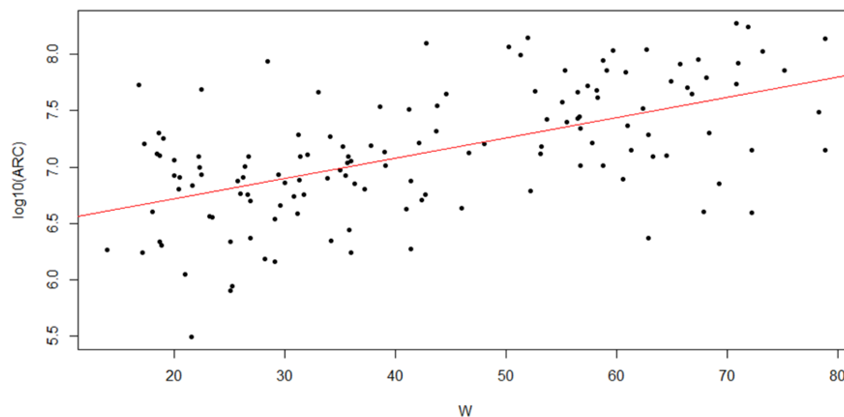

Figure 21 Regression between the water content (W) of the soil and the gene abundance of *16SrRNA* per gram dry weight

Regression of methanogens without log to water content

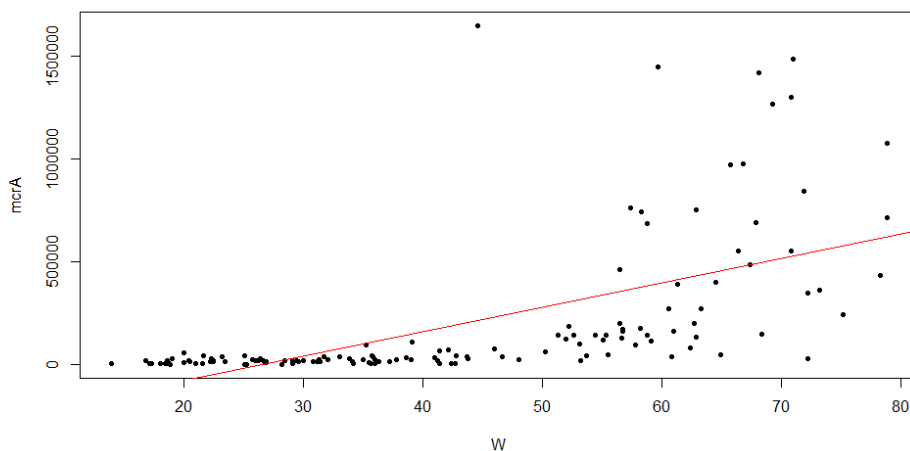

Figure 22 Regression between the water content (W) of the soil and the gene abundance of *mcrA* per gram dry weight without the log transformation.

Supplement 8

Regression of methanogens to the depth

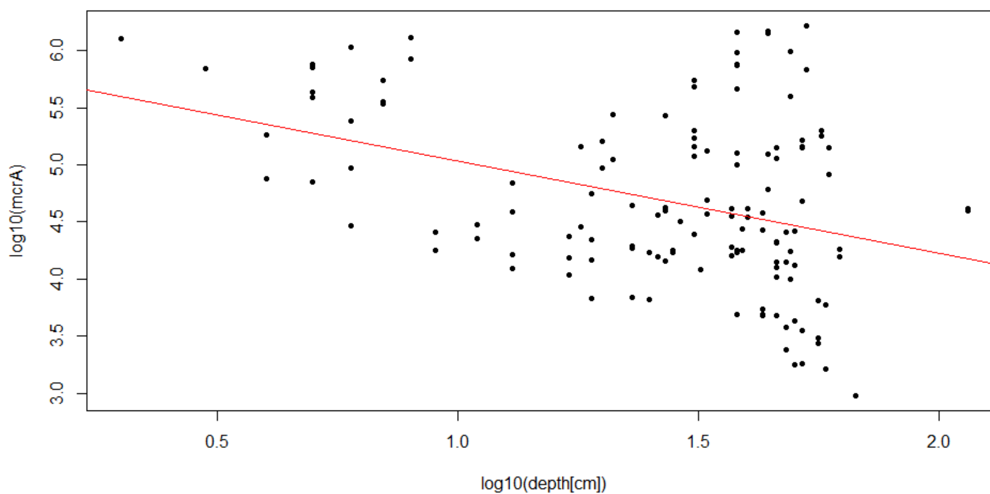

Figure 23 Regression between the depth and the gene abundance *nosZ* per gram dry weight.

Supplement 9

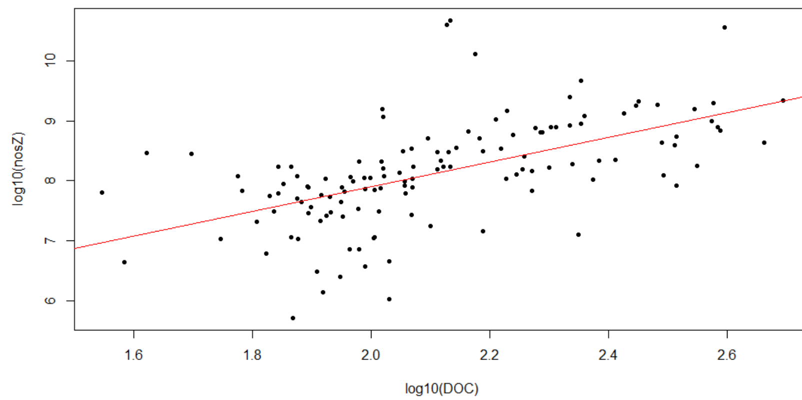

Figure 24 The correlation of *nosZ* gene copy numbers per gram dry weight and dissolved organic carbon (DOC) with log transformation.

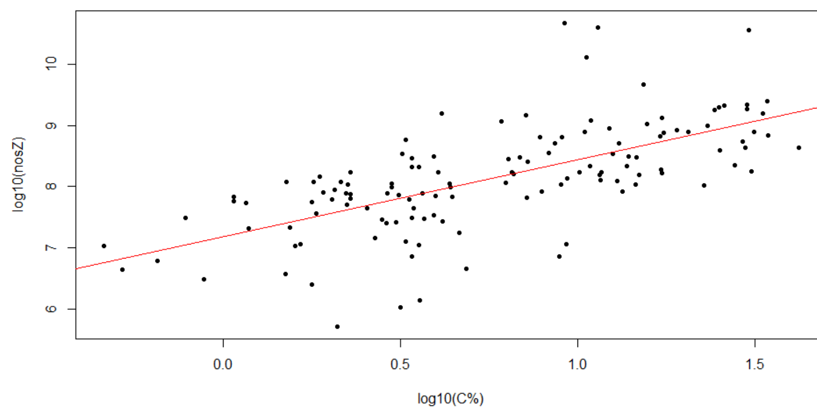

Figure 25 The correlation of *nosZ* gene copy numbers per gram dry weight and percentage of carbon in the soil (C%) with log transformation.

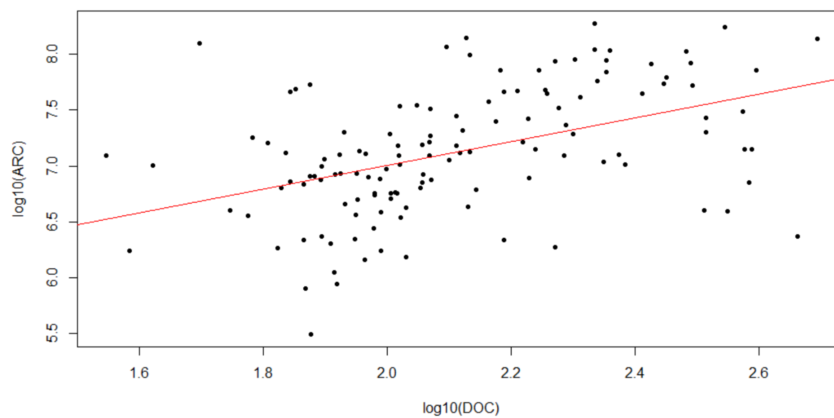

Figure 26 The correlation of total archaea (*16SrRNA* gene copy numbers per gram dry weight) and dissolved organic carbon (DOC) with log transformation.

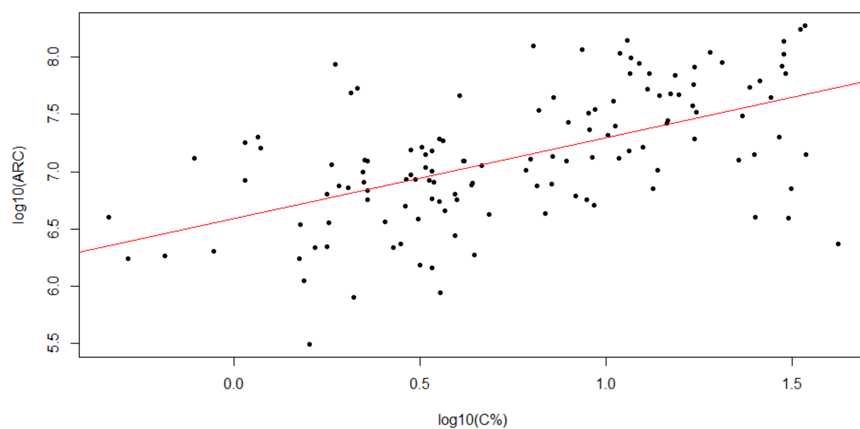

Figure 27 The correlation of total archaea (*16SrRNA* gene copy numbers per gram dry weight) and percentage of carbon in the soil (C%) with log transformation.

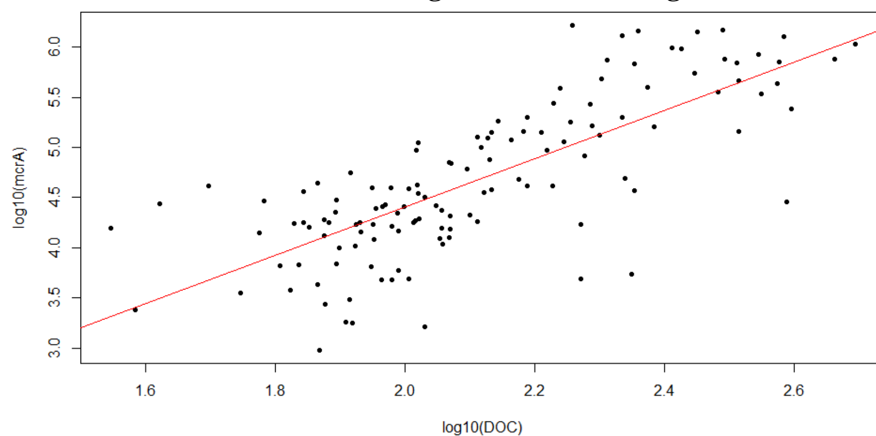

Figure 28 The correlation of *mcrA* gene copy numbers per gram dry weight and dissolved organic carbon (DOC) with log transformation.

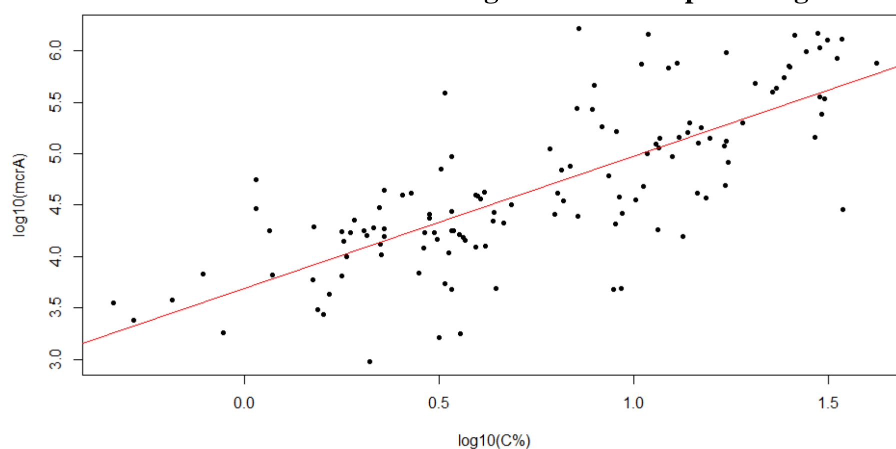

Figure 29 The correlation of *mcrA* gene copy numbers per gram dry weight and percentage of carbon in the soil (C%) with log transformation.

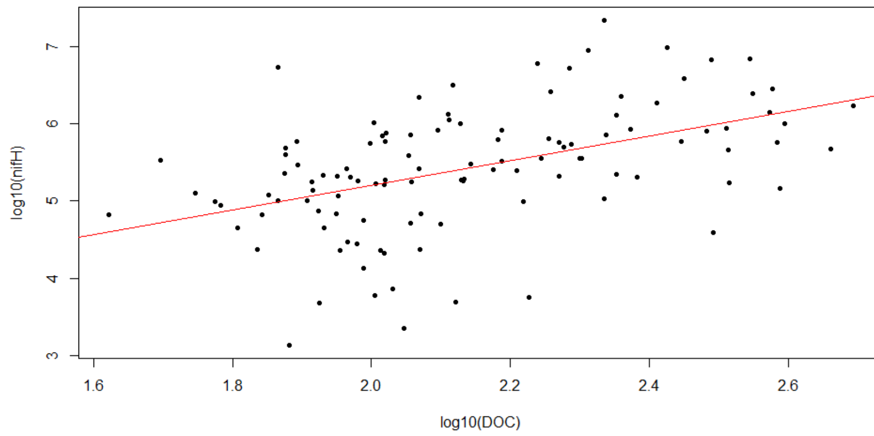

Figure 30 The correlation of *nifH* gene copy numbers per gram dry weight and dissolved organic carbon (DOC) with log transformation.

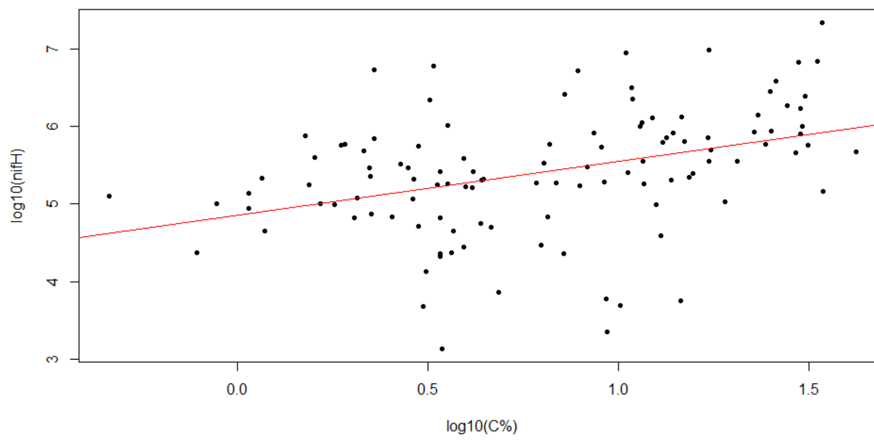

Figure 31 The correlation of *nifH* gene copy numbers per gram dry weight and percentage of carbon in the soil (C%) with log transformation.