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On the effect and monitoring of organic enrichment in marine sediments associated with Atlantic salmon farms: towards greater environmental sustainability

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A preliminary report of a Norwegian Case study

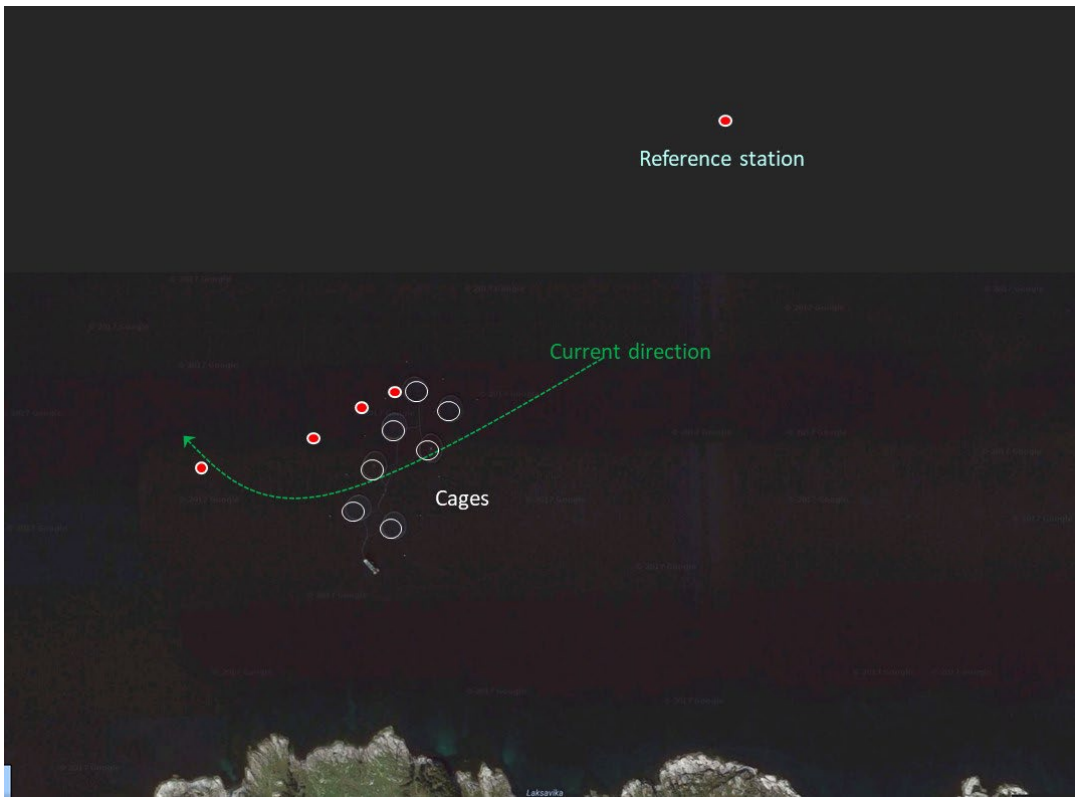

Figure 1. Location of aquaculture farm off Rennesoy, Rogaland, Norway. Location of sediment stations sampled are indicated with red dots and the cages with open white circles.

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Summary

This is a preliminary report of a case study around a Norwegian aquaculture farm designed to test novel suitable methods that could be applied across different locations within an EU project. The main goal was to validate methods to determine the effect and to monitor the carbon footprint of Atlantic salmon farms. Here we report on the highlights and these methods will be applied in more detail across different aquaculture farms.

Summary

The expansion of salmon aquaculture in fjords has raised concerns over the deposition of organic waste on the seafloor, affecting benthic communities and the overall health of these ecosystems. This calls for sensitive tools that can monitor and follow the effects of sediment organic enrichment. Here we report on highlights of a preliminary study testing new approaches. Combining measurements of carbon standing stock and carbon stable isotope signatures proved to be a sensitive tool to simultaneously determine contribution of aquaculture derived organic matter (AOM) to seafloor carbon stock and its spatial impact. Additionally, direct measure of organic carbon degradability in simples small bottle sediment-water slurry incubations was also a sensitive tool to follow spatial distribution of AOM. As commonly recorded, high organic enrichment close to under the cages led to severe drop in macrofauna biodiversity with strong dominance of the polychaete *Capitella*. Metagenomics of same sediment revealed that the corresponding genus among the bacteria is Sulfurovum, indicative of enhanced sulphur oxidation in accordance with high sulphide production at the cage and adjacent station (sulphide odor, strongest at the cage station).

Keywords: Aquaculture, sediment organic enrichment, biodiversity, organic carbon degradability, carbon stocks, stable isotopes

Introduction

This is a preliminary report of a case study around a Norwegian aquaculture farm designed to test novel suitable methods that could be applied across different locations within an EU project. The main goal was to validate methods to determine the effect and to monitor the carbon footprint of Atlantic salmon farms. Here we report on the highlights and these methods will be applied in more detail across different aquaculture farms.

Highlights of the Norwegian Case study

Cages are situated in deep water (100 m water depth) on a slope, with relatively strong bottom water currents resulting in coarse grained sediment under cages (median grain size 95 µm) and finer sediment down the slope, with very fine-grained material characterizing the sediment encountered at the reference, basin station at 218 m water depth (Fig. 2a).

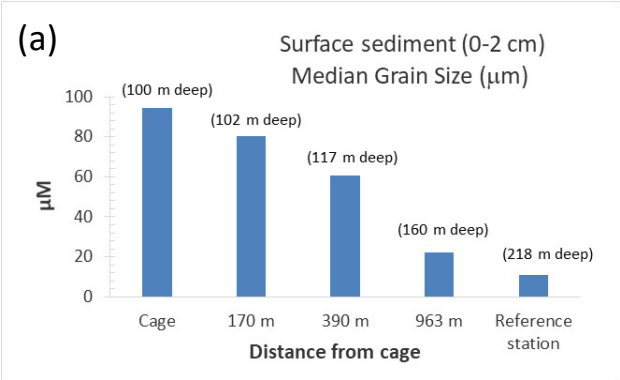


Figure 2. Median grain size and sediment % TOC

Direct estimates of organic carbon is commonly determined by quantifying total organic carbon (TOC) content as % of dry mass sediment (e.g. Fig. 2b). However, this may be complicated to interpret if there are major differences in sediment granulometry (i.e. strong differences in median grain size and silt content). Here, trends in TOC content as % DM (% dry mass) may indicate deposition of aquaculture derived organic matter (AOM) in the deep basin reference station (Fig. 2b). However, a better quantification is TOC content per surface area (Fig. 3a) and it is now evident that maximum sediment TOC is at the cage station, decreasing with distance from cage, but again elevated at the basin reference station (Fig. 3a). However, relative increase in the reference station may not be related to AOM input. A more sensitive method explored in this study is the lability (reactivity) of the organic matter (sediment oxygen consumption rates expressed per unit organic carbon, Fig. 3b).

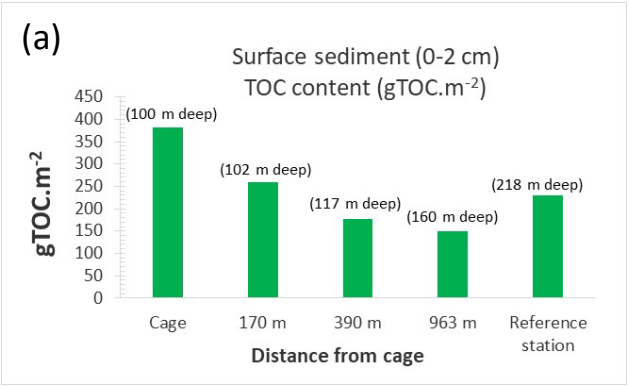


Figure 3 (a) Surface sediment TOC content as $gC.m^{-2}$ and **(b)** Surface sediment organic matter lability expressed as sediment oxygen consumption per gram organic carbon.

Clearly, the greatest impact of AOM is at the cage station (< 5 m from cage) and station closest to the cage (Fig. 3b). The strong reduction in reactivity with distance and the lowest in the refences, basin station suggest limited AOM input to these stations and that the origin of organic matter at the deepest, reference station is not AOM. Reactivity of organic carbon is evidently a very sensitive indicator of ecological footprint along the transect but also shows that the impact is restricted to close to the cage (fig. 3b). This is supported by sediment stable carbon isotope data (Fig. 4). The origin of the organic matter can be traced in the stable carbon isotope signature of the organic matter. The fish-feed used in Norway had a δ13C-TOC signature of -26.04 ± 0.20 ‰ which is very different from normal marine organic matter (-19 to -22 ‰); and most negative values of sediment stable carbon isotope signatures are found closest to the cages (Fig. 4a).

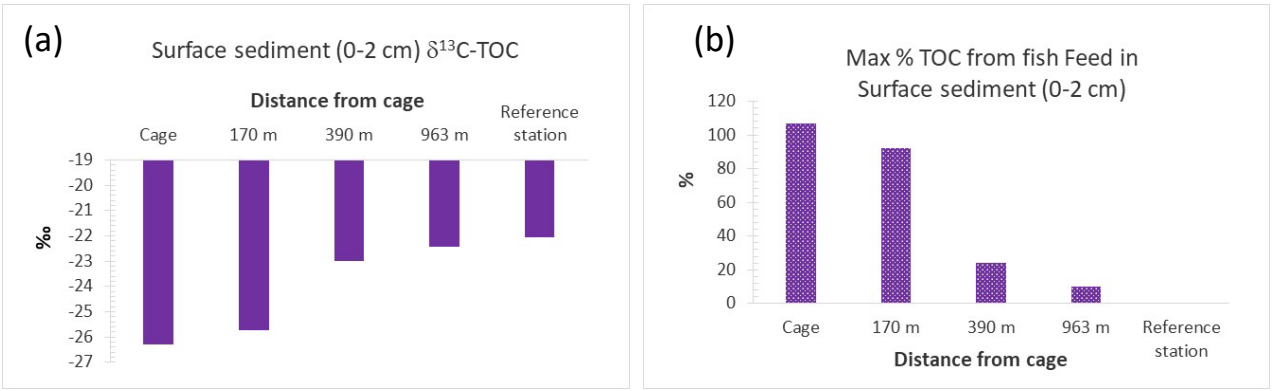


Figure 4 (a) Surface sediment stable isotope signatures and **(b)** maximum contribution of fish-feed to surface sediment organic matter content.

Surface sediment organic matter stable isotope signatures along the transect confirm that AOM input to the seafloor in primarily at the cage and adjacent station (Fig. 4a) with more typical marine organic values (-22 ‰) at the reference station. The reference station is evidently sink of fine material of more refractory marine organic matter. This is further support by estimation of maximum contribution of AOM to sediment C_{org} which was calculated using a two-end member linear mixing model. Isotope signature of AOM ($\delta^{13}C_{AOM}$) was estimated as average of fish-feed pellets (-26.04 ‰) Maximum % C_{org} AOM = $((\delta^{13}C_{sample}-\delta^{13}C_{REF})/(\delta^{13}C_{AOM}-\delta^{13}C_{REF}))$ x 100. $\delta^{13}C_{sample}$ is the isotope signature of the sediment sample and $\delta^{13}C_{ref}$ is the isotope signature of the sediment at the reference site (-22 ‰). Maximum contribution is at the cage station and undetectable at the distant, deep reference station (Fig 4b). Clearly, relatively high organic matter content in the deep reference station (Fig. 2 b & 3a) is an accumulation of refractory marine organic matter as reflected in low lability organic carbon (Fig. 3b).

The primary effect of AOM reaching the seafloor is evidently an input of highly reactive organic matter and the combination of organic matter lability and stable carbon isotope signatures is evidently a very sensitive tool to monitor the spatial impact of AOM.

Enhanced input of highly labile organic matter leads to strong increase in sediment oxygen consumption resulting in anoxic sediments followed by strong increase in anaerobic organic matter degradation rates and associated high sulphide production close to cages. This is often reflected in a drastic reduction in macrofauna biodiversity, resulting in the dominance of species indicative of high organics. However, sampling, processing, and taxonomic identification of macrofauna is very time consuming and alternative methods are required. Here we explored the utility of shotgun metagenomic analysis of bulk genomic DNA extracted from surface (0-2 cm) sediment along the transect studied (Fig. 2). Many time-consuming studies of the trends of macrofauna species response have repeatedly confirmed a significant reduction of species diversity with a strong dominance of polychaetes *Captilla* and Dorvilleidae characterizing the sediment most strongly impacted and disturbed by AOM input. In order to verify the utility of metagenomic analysis, a comparison was made with macrofauna trends. An impression of the impact on macrofauna was obtained by examining diversity and biomass of macrofauna (retained on a 500 µm mesh size sieve) in the upper 2 cm in half of replicate 0.1 m^{-2} van Veen grab sediment samples.

As commonly observed, a high input of AOM leads to significant reduction in macrofauna diversity (Table 1 & Fig. 5). Interestingly, maximum biomass is not found at the cage station with the highest supply of AOM and may reflect the inhibitory effects of accumulating toxic metabolites.

Table 1. Characteristics of macrofauna community analyzed in the upper 2 cm (average ± sd, N=2)

	# Species	# individuals.m^{-2}	Shannon (log2)	Margalef
Reference Station	20 (1)	226 (91)	2.82 (0.13)	4.06 (0.05)
963 m from cage	33 (2)	241 (61)	4.34 (0.15)	6.62 (0.80)
390 m from cage	26 (4)	368 (74)	3.45 (0.37)	4.79 (0.63)
170 m from cage	17 (1)	1180 (294)	1.63 (0)	2.52 (0.32)
Cage	4 (1)	1303 (847)	0.27 (0.21)	0.46 (0.17)

Comparison of the macrofauna species composition (ANOSIM and SIMPER analysis) revealed significant differences between stations but strongest AOM effect on cage and nearby sediment (Fig. 6) that clustered together and the other stations together constituting the second major cluster. The main difference between the cage and the reference station is the stronger dominance of polychaetes *Capitella sp*. and Dorvilleidae in cage sediment; in the reference sediment a stronger dominance of *Myriochele* sp., *Paramphinome jeffreysii*, Nemertea and Ophiuroidae.

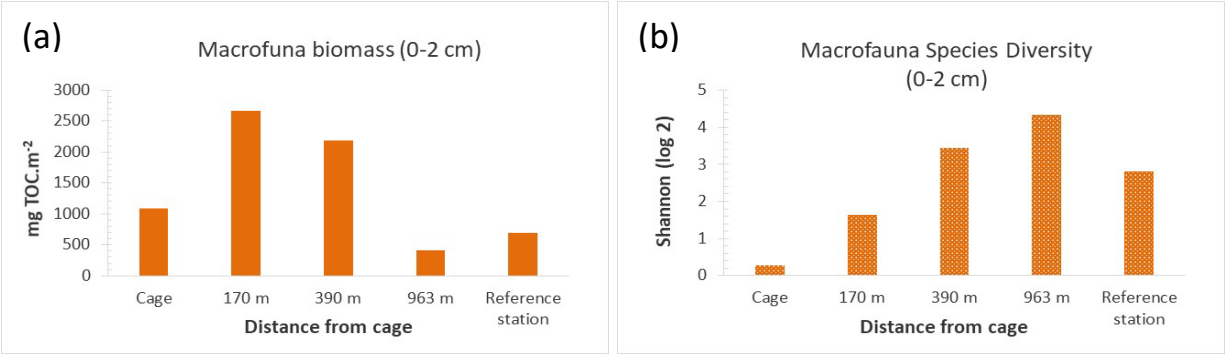


Figure 5 (a) Surface sediment macrofauna biomass and **(b)** macrofauna Shannon diversity index.

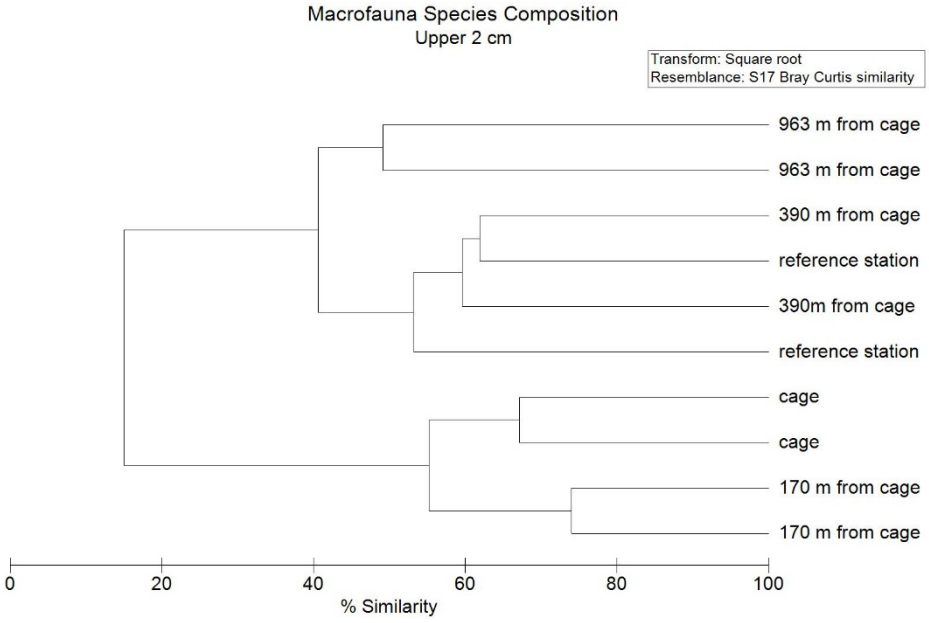


Figure 6. Cluster plot of macrofauna species in the upper 2 cm based on square root transformed data.

These trends found in macrofauna can be compared with trends observed in bacteria. In contrast to amplicon sequencing, shotgun metagenomic analysis offers additional information on Kingdom dominance alongside bacteria biodiversity and functioning. Here we report on Illumina HiSeq PE125 sequencing data which was processed using the open-source web application MG-RAST which is a server that provides automatic phylogenetic and functional analysis of metagenomes.

In MG-RAST, the highest taxonomic level at which trends can be extracted is at the Kingdom level to identify the dominant kingdom which can then be examined in more detail. Bacteria constitute the major group (Fig. 7) and with their quick responses to

changes in the environment, they are potential ideal tools to monitor AOM effect and fate in sediments.

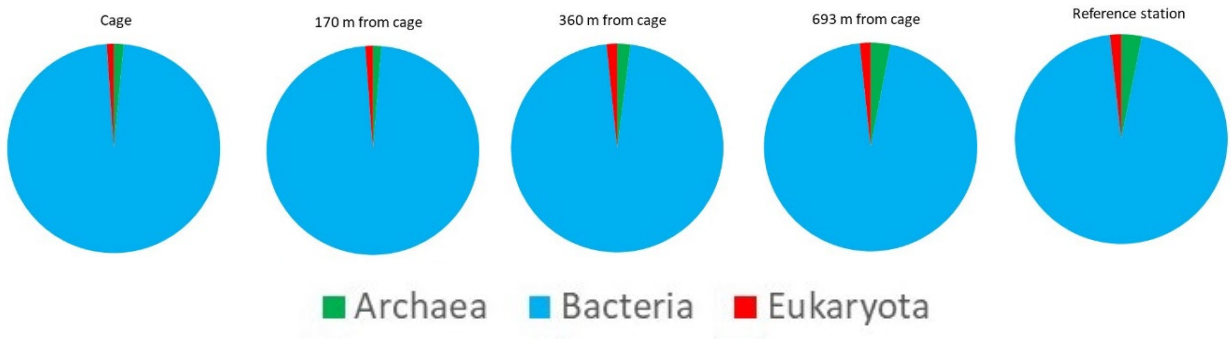


Figure 7. Taxonomic structure at the highest taxonomic level (Kingdom, Archaea, Bacteria and Eukaryota) close to under the cage and at different distances from the cage.

Bacteria were examined in more detail (Table 2, Fig. 8-10). Although not as extreme as in te case of macrofauna, lowest bacteria diversity is found at the cage station. This probably reflects the greater flexibility in bacteria. However, even with closer similarity between stations compared to that found in macrofauna (compare Fig. 6 & Fig 8); metagenomic bacteria analysis was sensitive enough to detect a similar clustering of stations as in the case of macrofauna (Fig. 8). Again, ANISOM and SIMPER analysis was used to identify key taxa responsible for differences across stations. There is a significant difference in the genus composition patterns between stations (Fig. 8) and the main genus accounting most for the differences with decreasing dominance away from the cage is Sulfurovum (Fig. 9), a genus indicative of enhanced sulphur oxidation in accordance of high sulphide production at the cage and adjacent station (sulphide odor, strongest at the cage station). This high sensitivity of metagenomic analysis of bacteria in revealing a biological effect of AOM comparable to time-consuming macrofauna analysis illustrates its advantageous application in monitoring ecological footprint of the AOM in the seafloor. Additionally, insight into biological functioning can also be extracted from the metagenomes (MG-RAST, Subsystem-Leve1 l Analysis, Fig. 10) which also revealed spatial impact patterns like that of macrofauna and bacteria diversity alteration as a function of AOM input. Analysis of surface sediment organic matter lability and stable carbon isotope signatures together with bacteria metagenomic analysis of structure and functioning constitute sensitive new tools to quantitatively and qualitatively monitor and study the seafloor ecological footprint of Atlantic salmon farms. This has the dual function of contributing to better management and providing insight into fundamental aspects of marine ecology such as the relationship between biota structure and ecological functioning.

Table 2. Characteristics of bacteria genera examined in the upper 2 cm (average ± sd, N=2)

	# Genus	# reads	Shannon (log2)	Margalef
Reference Station	301	5023132	7.69	19.45
963 m from cage	301	3696872	7.68	19.91
390 m from cage	301	3705975	7.68	19.84
170 m from cage	301	3452314	7.56	19.93
Cage	301	3491112	7.42	19.92

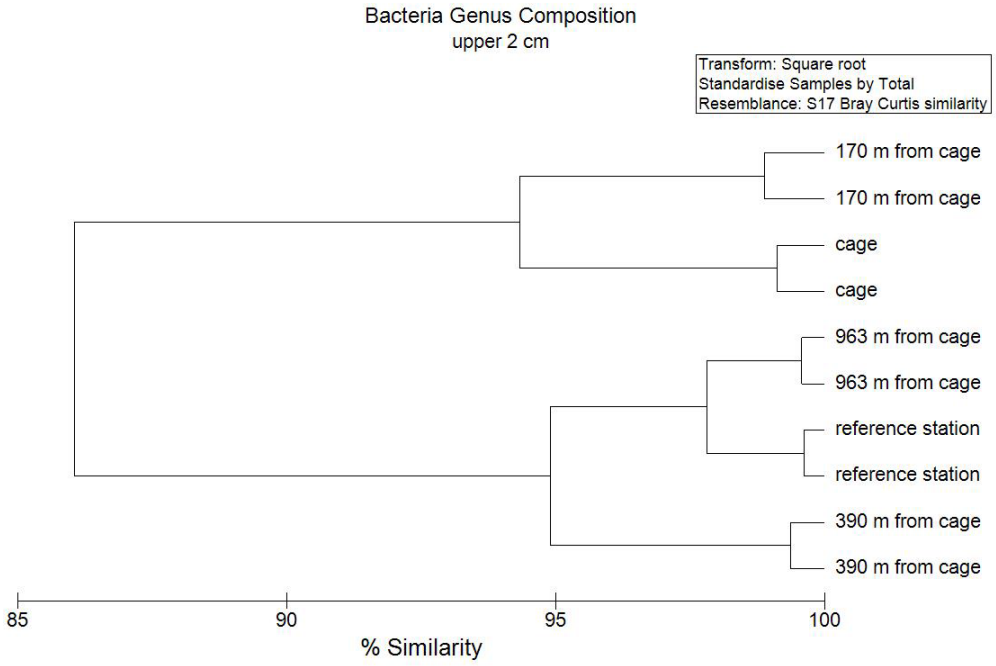
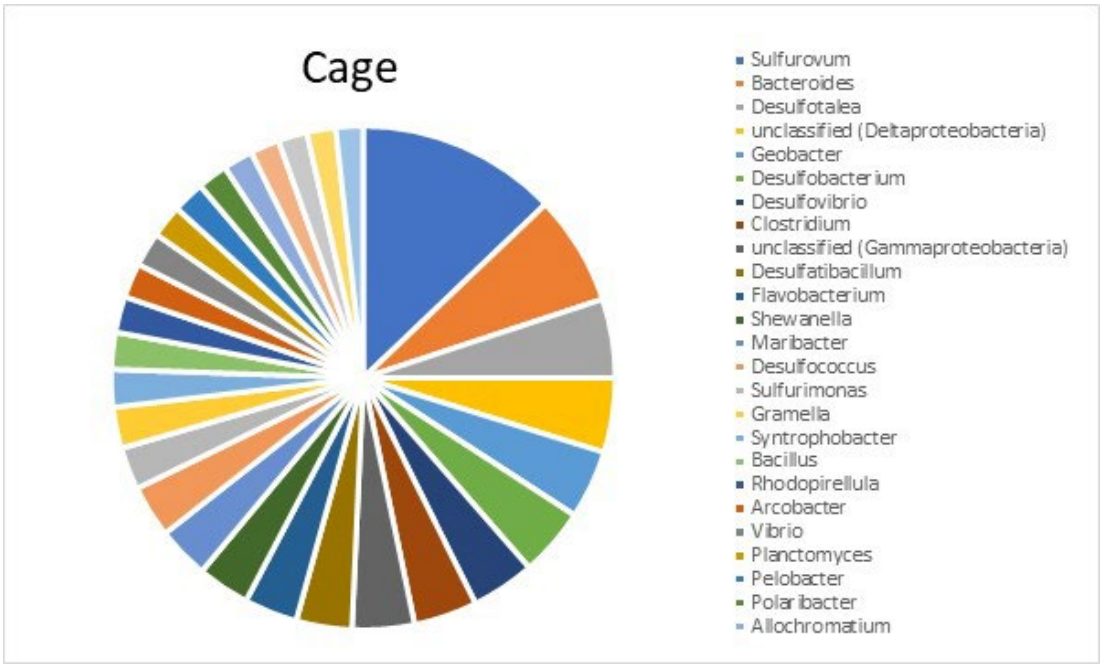
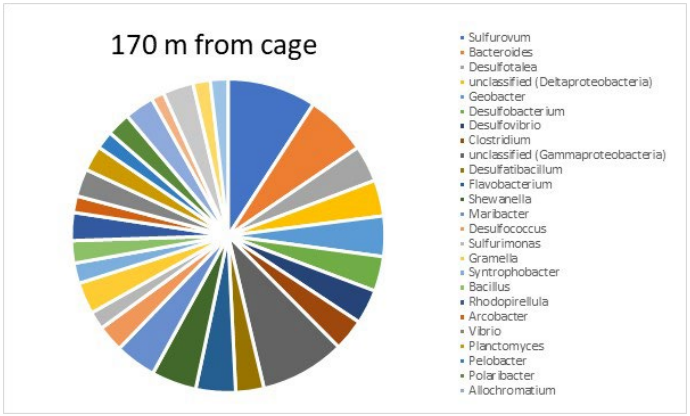
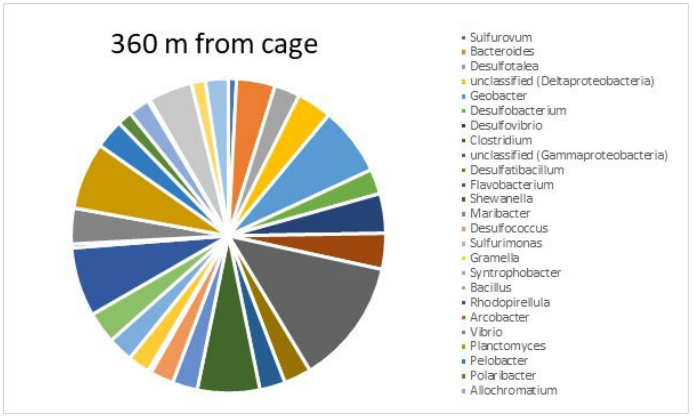
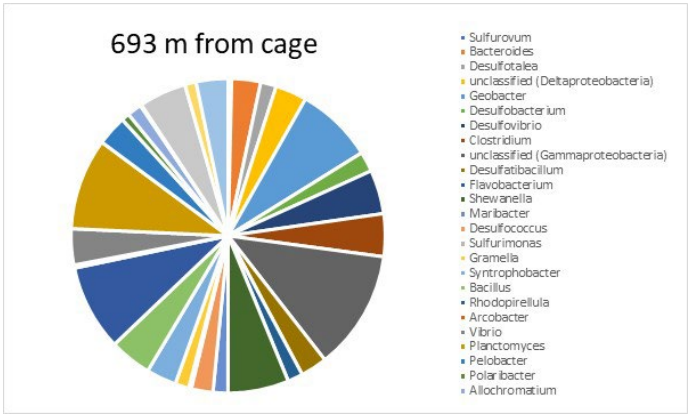


Figure 8. Cluster plot of bacteria genus composition in the upper 2 cm based on square root transformed standardized data (taxa having an occurrence ≥ 0.1 % in any single sample)









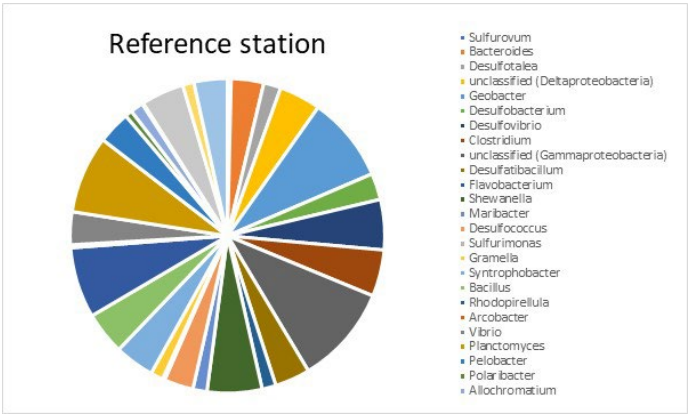


Figure 9. Bacteria genus composition in the upper 2 cm based on square root transformed standardized data (taxa having an occurrence ≥ 0.1 % in any single sample)

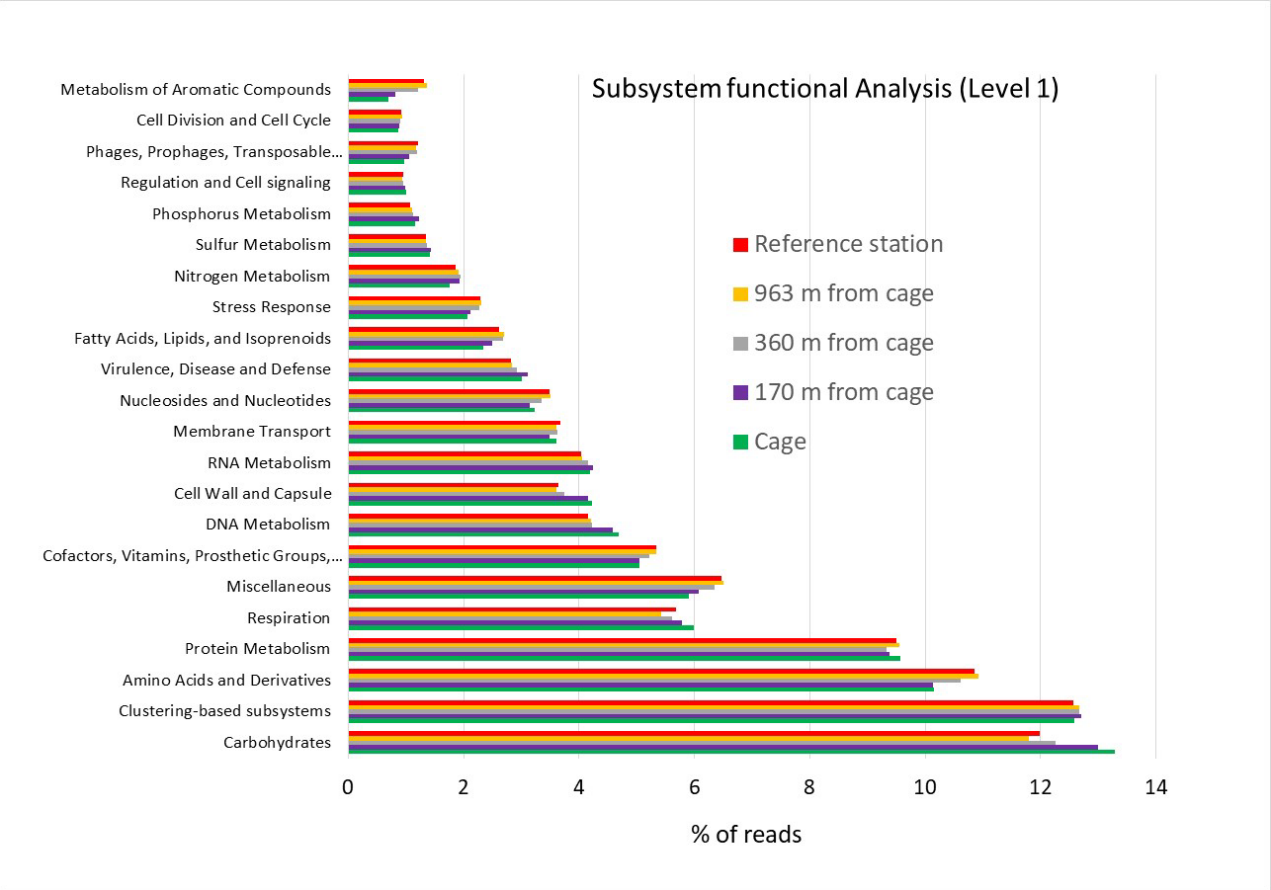


Figure 10. Average % occurrence of major subsystem functions (> 1 % in any sample) at the different stations.

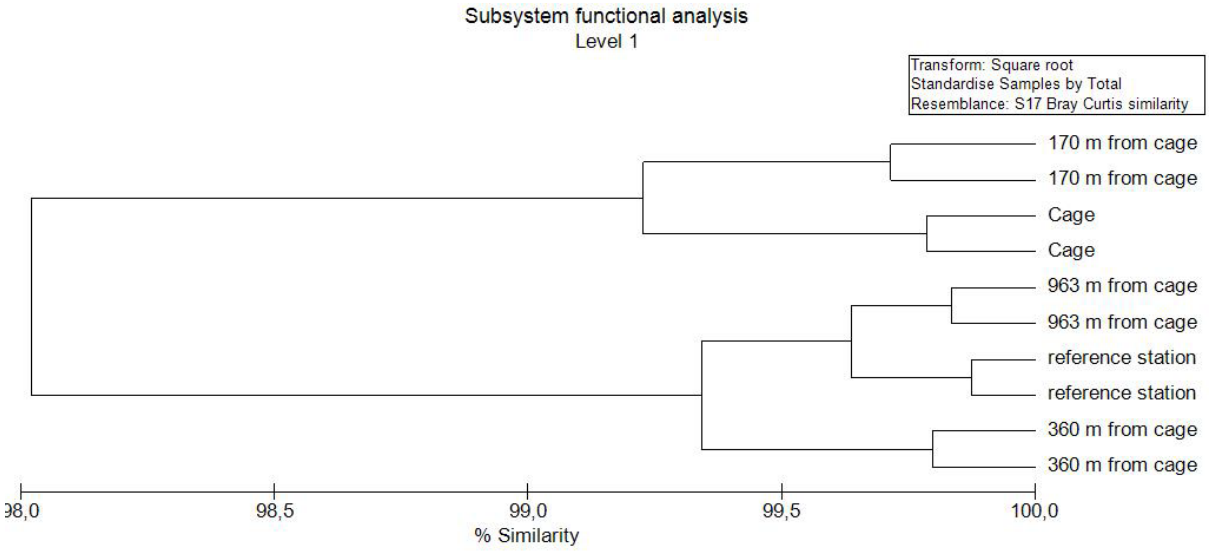


Figure 11. Cluster plot of major subsystem functions in the upper 2 cm based on square root transformed standardized data (1231248 – 2384306 reads of all 28 annotated processes were utilized for analysis).

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