

# Mesocosm study with thermally treated (TCC) and water-based drill cuttings (WBM)



# Norwegian Institute for Water Research

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# REPORT

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## Abstract

Mesocosm and bottle slurry experiments were conducted to assess and compare the effects of thermally treated cuttings (TCC) versus cuttings with water-based mud (WBM) on benthic communities. While WBM has been discharged for a long time, discharges of TCC have not yet been practised routinely. In a mesocosm experiment, cuttings were added in a layer thickness of 6.3 mm in box-core samples from the Oslofjord, and effects measured on benthic community structure, microprofiles of O<sub>2</sub> and biogeochemical fluxes. In addition a bottle incubation experiment was performed on the same mud materials. Results from both experimental approaches showed significantly increased biodegradation measured as consumption of O<sub>2</sub> and nitrate+nitrite in WBM and TCC treatments compared to controls. The biodegradation product ΣCO<sub>2</sub> was released from WBM, but surprisingly consumed in TCC. This was presumably caused by precipitation of CaCO<sub>3</sub>(s) triggered by the mud ingredient Ca(OH)<sub>2</sub>(s) present in TCC. There was a significantly different impact on the benthic communities with mass mortality and reduction in macrofaunal biomass in TCC treatments, but unaltered faunal response in WBM exposure. The documented adverse effect of TCC cuttings was possibly due to intolerable alkaline conditions induced by the calcium oxide.

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Mesocosm study with thermally treated (TCC) and water-based drill cuttings (WBM)

## Preface

Total E&P Norge (TEPN) received a permit from the Environment agency for discharges of non- aqueous base mud cuttings after treatment through a thermo mechanical cuttings cleaner (TCC) system. This permit was associated with requirements for environmental monitoring of the water column and sediment.

The present study represents the first mesocosm experiment conducted with thermally treated oil-based drill cuttings (TCC). Also water-based drill cuttings (WBM) were used in order to provide complementary information to studies previously performed with WBM. This also provides a thorough comparison of benthic responses of TCC and WBM. Also a slurry incubation experiment was conducted on the same materials.

Total E&P Norge (TEPN) was the client for the main experiment with TCC, while Norog (Norwegian Oil and Gas Association) financed the WBM-part of the study. Both Total E&P Norge and Norog are acknowledged for initiating this project and for constructive inputs and discussions.

Hilde C. Trannum has been project leader, and has also been involved in fieldwork and experimental work at Solberstrand, together with Morten T. Schaanning. Joachim T. Johansen was responsible for the daily follow-up at Solberstrand. Kuria Ndungu at NIVA assisted in microprofile measurements. The crew on the ship “Trygve Braarud” is acknowledged for their assistance in the field work. Per I. Johannessen from NIVA also assisted in the field work.

Sieving of macrofauna was conducted by Siri Moy, Tage Bratrud, Anne Winge, Vibeke Hoff and Hilde C. Trannum. Sorting and weighing of soft bottom fauna were conducted by Siri Moy and Tage Bratrud and identification by Gunhild Borgersen, Marijana S. Brkljacic and Jesper Hansen (Akvaplan-niva AS). Database-work and calculation of biodiversity indices were performed Gunhild Borgersen. Multivariate statistics were performed by Hilde C. Trannum.

Leon Moodley designed and conducted the slurry incubations, facilitated by Stig Westerlund. Thierry Baussant provided quality assurance and discussed the preliminary framework with NIVA.  $\Sigma$ -CO<sub>2</sub> concentration and  $\delta^{13}\text{C}$ - $\Sigma\text{CO}_2$  signatures were measure at the Royal Netherlands Institute of Sea Research (NIOZ, Yerseke, The Netherlands). This research was also financially supported by IRIS, Marine Environment. Special thanks to Dr CJ Beets (Earth Science Department, Free University, Amsterdam, The Netherlands) for valuable discussions on isotope geochemistry.

Grimstad, 10<sup>th</sup> June 2016

*Hilde C. Trannum*

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## Summary

The present study represents the first mesocosm experiment conducted with thermally treated oil-based drill cuttings (TCC). Also water-based drill cuttings (WBM) were used in order to provide complementary information to studies previously performed with WBM. This also allowed a thorough comparison of benthic responses of TCC and WBM.

In a box-core setup, cuttings were added in a layer thickness of 6.3 mm, a threshold value currently used in environmental risk assessment models, and where WBM effects were observed in previous experiments. Effects were measured on benthic community structure, microprofiles and biogeochemical fluxes. After addition of test materials, the experiment was run for three months.

Although the organic carbon (OC) content in cuttings (both WBM and TCC) was lower than in control sediments, the OC degradability was significantly higher in the cuttings, and maximum in WBM (4 x higher than in control sediments). Consequently, cuttings resulted in increased sediment community oxygen and nitrate consumption. At the same time, these values were within the range typical of organic-rich coastal sediments.

Furthermore, while WBM cuttings did not alter macrofaunal community structure, sediments exposed to TCC showed high mortality and strong decrease in biomass. The TCC-boxes in average had less than one fourth of the number of individuals than the controls and half of the number of species. The most sensitive species showed almost 100% mortality. Surface deposit feeding and tube-building species seemed to be most severely affected by the cuttings. The multivariate test PERMANOVA confirmed the significant effect of TCC, but not of WBM, on the composition of the faunal communities. In this test the TCC-treatment was significantly different from all other treatments, while none of the other treatments differed between each other.

There was no evidence of enhanced toxicity (heavy metals and hydrocarbons) or severe oxygen depletion to account for the adverse impact of TCC cuttings.  $\Sigma$ -CO<sub>2</sub> evolution patterns (with regard to both concentration and stable carbon isotope signatures) in bottle sediment-water slurry incubations revealed that while CO<sub>2</sub> was produced in WBM slurries due to organic carbon degradation, CO<sub>2</sub> was actually consumed in TCC slurries. This reaction was triggered by the presence of calcium hydroxide in TCC cuttings and resulted in an increase of alkalinity. One and half day old slurries containing 100 % TCC cuttings had a pH > 9.4 compared to 8.1 in seawater. Elevated pH may have been toxic for the majority of the macrofauna and caused the significant inhibition of bacterial respiration of <sup>13</sup>C-labelled diatom carbon observed in the presence of TCC cuttings.

If the negative impact of TCC results from pH changes due to calcium hydroxides, adverse effects may not necessarily prevail *in situ* where more open systems would presumably allow recolonisation as soon as the pH is normalised. In the mesocosm, recolonisation by migration from adjacent areas is excluded and larvae settling from the water are restricted to those being able to pass by the water inlet and pumps. The rate of cuttings input to the sediment is likely to be crucial with regard to the actual change of *in situ* pH experienced by the fauna at the offshore location.

## Sammendrag

Denne studien er det første mesocosm eksperimentet som er utført med termisk behandlet oljebasert borekaks (TCC). Også vannbasert borekaks (WBM) ble anvendt for å komplettere tidligere studier med WBM. Dette tillot også en grundig sammenligning av bentiske responser på TCC og WBM.

I et box-core oppsett ble borekaks tilsatt i tykkelser på 6,3 mm, en terskelverdi som benyttes i miljørisikovurderingsmodeller, og hvor effekter av WBM er observert i tidligere forsøk. Effekter ble målt på bløtbunnsfauna, mikroprofiler og biogeokjemiske flukser. Etter tilsetning av testmaterialer ble forsøket kjørt i tre måneder.

Selv om innholdet av organisk karbon (OC) i borekaks (både WBM og TCC) var lavere enn i kontrollsedimentene, var nedbrytbarheten av OC signifikant høyere i kaksen, og viste maksimum i WBM (4 x høyere enn i kontrollsedimenter). Borekaks førte altså til økt oksygen- og nitratforbruk i sedimentene. Samtidig er disse verdiene innenfor det som er normalt for organisk rike, kystnære sedimenter.

Mens WBM ikke endret makrofaunaens struktur, viste sedimentene behandlet med TCC høy dødelighet og kraftig nedgang i biomasse. TCC-boksene hadde i gjennomsnitt mindre enn en fjerdedel av antall individ enn kontrollboksene og halvparten av artene. De mest følsomme artene viste opptil 100 % dødelighet. Overflatespisende og rørbyggende arter syntes å være mest sensitive. Den multivariate testen PERMANOVA bekreftet en signifikant effekt av TCC, men ikke av WBM, på sammensetningen av bunnfaunaen. I denne testen var TCC-behandlingen signifikant forskjellig fra alle andre behandlinger, mens ingen av de andre behandlingene skilte seg mellom hverandre.

Verken toksisitet (tungmetaller og hydrokarboner) eller kraftig oksygenmangel anses å kunne forklare den negative effekten av TCC. Utvikling av  $\Sigma\text{-CO}_2$  (både mht. konsentrasjon og stabile karbonisotoper) i inkuberingsforsøk viste at mens  $\text{CO}_2$  ble produsert i WBM-behandlinger som følge av nedbrytning av organisk karbon, ble  $\text{CO}_2$  derimot forbrukt i TCC-behandlingene. Denne reaksjonen ble utløst av kalsiumhydroksyd i TCC-kaksen, og resulterte i en økning i alkaliteten. Behandlinger med 100 % TCC kjørt i 1,5 dag hadde pH > 9,4 sammenliknet med 8,1 i sjøvann. Forhøyet pH kan ha vært skadelig for makrofaunaen, og kan også ha medført en vesentlig inhibering av bakteriell respirasjon av  $^{13}\text{C}$ -merket diatomer-karbon som ble observert for TCC-kaksen.

Hvis den negative effekten av TCC skyldes pH-endringer som følge av kalsium hydroksid, vil ikke de negative effektene nødvendigvis vedvare *in situ* ettersom mer åpne systemer formodentlig vil være gjenstand for rekolonisering så snart pH er normalisert. I mesocosm er rekolonisering fra tilstøtende områder og larvedadslag fra sjøvannet begrenset til de artene som er i stand til å passere vanninntaket og pumpene. I en feltsituasjon antas frekvensen til sedimenteringen av kaks å være avgjørende for hvilke endringer i pH faunaen utsettes for.

# 1. Background

Total E&P Norge (TEPN) received a permit from the Norwegian Environment Agency for discharges of non-aqueous base mud cuttings after treatment through a thermo mechanical cuttings cleaner system at the Martin Linge field in the North Sea. Such discharges have been allowed since 1993 in the British Sector. TEPN's permit was associated with the requirements from the Norwegian Environmental Agency for effect monitoring of the water column and sediment. The main concern associated with discharges of this type of cuttings is related to the presence of oil residues on particles and the associated effect in the water column and sediments. The TCC technology applies no external heat, but friction heat provides a temperature between 250 and 300 °C. Base oil is recycled, and the water is rinsed in a condensation process. The base oil is a low aromatic oil (C<sub>16</sub>-C<sub>22</sub>), which is of low toxicity, readily biodegradable, but which has a high log P<sub>ow</sub> value. In the TCC-drilling mud at Martin Linge only yellow and green chemicals will be used.

Drill cuttings eventually settle to the seafloor and this sedimentation may alter sediment community structure and functioning, and their related services. A common approach to examine the impact of drill cutting sedimentation is to sample sediment macrofauna over time in the field and follow possible change in biodiversity only. However, in order to separate the effects of possible contaminants (e.g. hydrocarbons) from the effects that result from enhanced sedimentation of particles, controlled experiments are required. For this purpose, mesocosm exposure studies on intact sediment communities have proven very reliable, and have been important to investigate effects of oil-, synthetic and water-based drill cuttings since the 1980ties (e.g. Schaanning et al., 2008; Trannum et al. 2010; 2011a).

The present study represents the first mesocosm experiment conducted with cuttings treated with the thermo mechanical cuttings cleaner system (TCC). Also water-based drill cuttings (WBM) were used in the present experiment in order to allow comparison to studies previously performed with WBM.

In addition to the mesocosm study, early diagenesis of the organic matter in control sediment versus cuttings was further examined in bottle slurry incubations (e.g. Moodley et al., 2005; Moodley et al., 2011). In separate incubations, sediment oxygen consumption and  $\Sigma$ -CO<sub>2</sub> evolution (sum oxic and anoxic processes through changes in  $\Sigma$ -CO<sub>2</sub> concentration and  $\delta^{13}\text{C}-\Sigma\text{CO}_2$ ) were measured. This provided a complementary insight into the chemical functioning characteristics of the different cuttings and sediments (e.g. relative Organic Carbon reactivity of the different substrates and impact of cutting concentrations on sediment functioning).



## 2. Experimental setup and methods

### 2.1 Collection of test communities

On 26<sup>th</sup> October 2015, 16 box core samples were collected at 120 m depth (59,643 N/ 10,629 E) with a KC box-corer with transparent inner liner (0.09 m<sup>2</sup>) in the outer Oslofjord, S.E. Norway (Figure 1). On 27<sup>th</sup> October four grab samples were collected with a 0.1 m<sup>2</sup> van Veen grab and sieved and preserved according to standard procedures (NS-EN ISO 16665:2013) in the field. These samples are a control of the experimental core communities. The vessel FF Trygve Braarud, belonging to University of Oslo, was used for the field work.

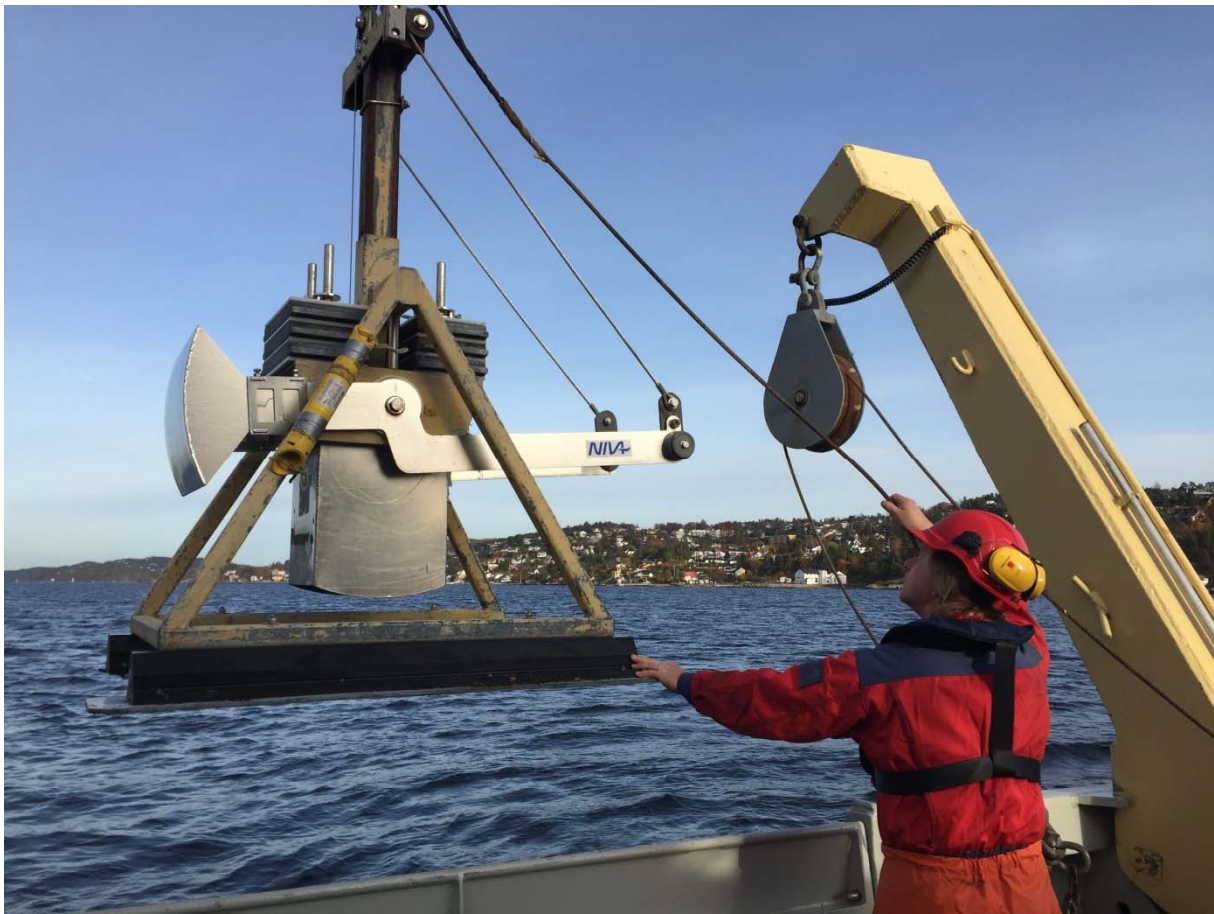


Figure 1. Collection of box cores for the mesocosm experiment, Oslofjord 2015.

### 2.2 Experimental facility

Within eight hours of sampling, the boxes were installed in the mesocosm facility at Solbergstrand (Berge et al. 1986, Trannum et al. 2010). The mesocosm resembles the conditions at the fjord sampling locations as the mesocosm is kept relatively cool, almost dark and is supplied with unfiltered seawater from 60 m depth. Benthic communities can be maintained under these conditions for several months (Schaanning et al. 2008). A lid with an aquaria pump covered the boxes. Each box received 10-15 ml min<sup>-1</sup> of water from a common header tank, and the flow rates were calibrated regularly.

## 2.3 Treatments and addition of test materials

The experiment included four treatments, TCC (thermally treated cuttings), WBM (water-based cuttings), SED (sediment) and CON (control), with four replicates each. TCC-treated drill cuttings were delivered from IRIS, and correspond with the cuttings used in previous experiment at IRIS (8 ½ inch). Drill cuttings with WBM were delivered from Det Norske, and had been used during the Trolla drilling (17 ½ inch). TCC was delivered as dry matter, while WBM as a mud. Sediment (SED) collected at the same location as the experimental communities was used as a control of the burial component. The layer thickness of TCC, WBM and SED was approximately 6.3 mm, as this represents the thickness where effects of WBM started to occur in previous experiments, and further as this represents the NOEC-value (no observed effect concentration) for burial (Smit et al., 2008). Lastly, such layer thickness is considered representative for sediments surrounding wells drilled with water-based drill cuttings, as a drill cuttings layer in the scale of mm to cm is fairly characteristic for the area between 100 m and 250 m from the discharge point (Statens Strålevern et al. 2008; Rye & Furuholt 2010). The treatment CON (controls) represents the boxes without any manipulation.

Prior to the additions, the WBM was washed three times in order to remove the water-soluble glycol. This was performed by mixing the mud with seawater and then shaking the slurry roughly, to let the particles settle in the bottle. The day after the supernatant was decanted, and this procedure was repeated three times. The TCC as well as SED was mixed with seawater prior to additions and settled for one day. The slurry of all three materials was then mixed with seawater again before performing the actual additions, in order to make aliquots suitable for pouring into the boxes, enabling a smooth surface (Figure 2). The estimation of the weight of material corresponding to a 6.3 mm layer thickness was based on density of the material, adjusted for the volume after mixing it with seawater. The weight of the material added into each box was 780 g for WBM, 591 g for TCC and 693 g for SED. It should be mentioned that the main principle in the mesocosm-tests is to perform test with materials "as discharged", i.e. without any particular pre-treatment except of getting the materials suitable for making aliquots for the addition procedure. However, due to concern of water-soluble glycol in the WBM, which is claimed to be washed out in the water column after discharge and before settling on the seabed, the WBM was subject to washing prior to addition. There was no information available on TCC indicating the need of a similar pre-treatment for this material.

The additions were performed 9<sup>th</sup> December 2015, i.e. after an acclimatization period of 6 weeks. The various treatments were randomly assigned to the boxes (by the random-function in excel). The boxes were left for particle settling until the next day. At that time the majority of the particles had settled. Fast sedimentation accords well with sedimentation experiments presented by Aquateam-COWI (2014). However, when the TCC was mixed with seawater, it developed a light "mousse", which still was evident in the boxes two days after addition (Figure 2). This mousse has also been recorded in other TCC-studies (Aquateam-COWI, 2014) including the Martin Linge TCC (Aquateam-COWI, 2015.).



Preparation of slurries; left SED, middle WBM, right TCC.



Addition of SED.



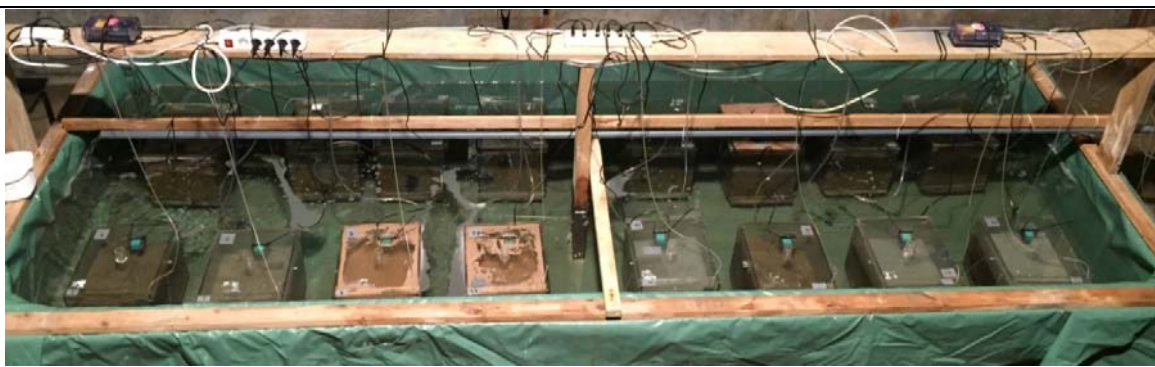
Addition of WBM.



Addition of TCC.



Appearance the same day as the additions was performed (09.12.2015). Left CON, middle SED, right TCC.



Two days after additions (one day after water supply had started up) (11.12.2015).

Figure 2. Addition of experimental treatments in the mesocosm facility, December 2015.

## 2.4 Measurements during the experiment

Concentrations of oxygen, ammonium, nitrate/nitrite, phosphate and silicate were measured in all boxes and in the header tank prior to addition of the test materials. Additionally, oxygen was measured before the water supply started up again after addition of test materials, as well as the day after (i.e. two days after additions). After addition of the test substances, oxygen measurements were performed regularly. Also the flow and temperature in the water baths were regularly monitored. Nutrient fluxes were measured five days after addition and then just before termination (unfortunately not ammonium in the last measurement).

O<sub>2</sub>-profiles were measured directly in the boxes with a Unisense™ Clark-type microelectrode with an internal reference and a guard cathode (Figure 3). Readings were taken at 0.1-0.5 mm depth intervals from a few mm above the sediment-water interface down to zero O<sub>2</sub>. The exact position of the sediment-water interface was adjusted according to the measured profiles, as this not always could be judged by the visual observations. On 1<sup>st</sup> December 2015 (prior to addition of test materials) microelectrode measurements were done in a few arbitrarily selected boxes (box 1, 3, 4 and 22). Then all boxes were measured on the 4<sup>th</sup> January (26 days after additions) and again on the 16<sup>th</sup> March, i.e. just before termination of the experiment. Due to electrode breakage one box (box 8) was not measured during the last series. Three measurements were performed pr. box, and sometimes 4 in the pre-addition measurement. The pre-addition measurements were replicated by lowering the electrode several times at the same location, while in the post-additions measurements the electrode was moved horizontally a small distance (2-5 cm) along the sediment surface before repeating the profile.



Figure 3. Microelectrode measurement.

## 2.5 Termination of experiment

When ending the experiment, the sediment from the boxes was subject to standard sieving (0.5 mm mesh sieve) and fixation with 10% buffered formaldehyde stained with Rose Bengal. Extra borax was added to the samples in order to neutralize the formaldehyde. Notes were taken pr. box with regard to sediment characteristics (incl. colour according to Munsell ® soil color chart), visible animals and eventual odour.

Subsamples were taken with a hand corer for analyses of grain size (0-5 cm) and total organic carbon (0-1 cm) according to specification in NS-EN ISO 16665:2013. Also samples for contaminants (0-5 cm) were taken pr. box. Some photos from the termination are shown in Figure 4.

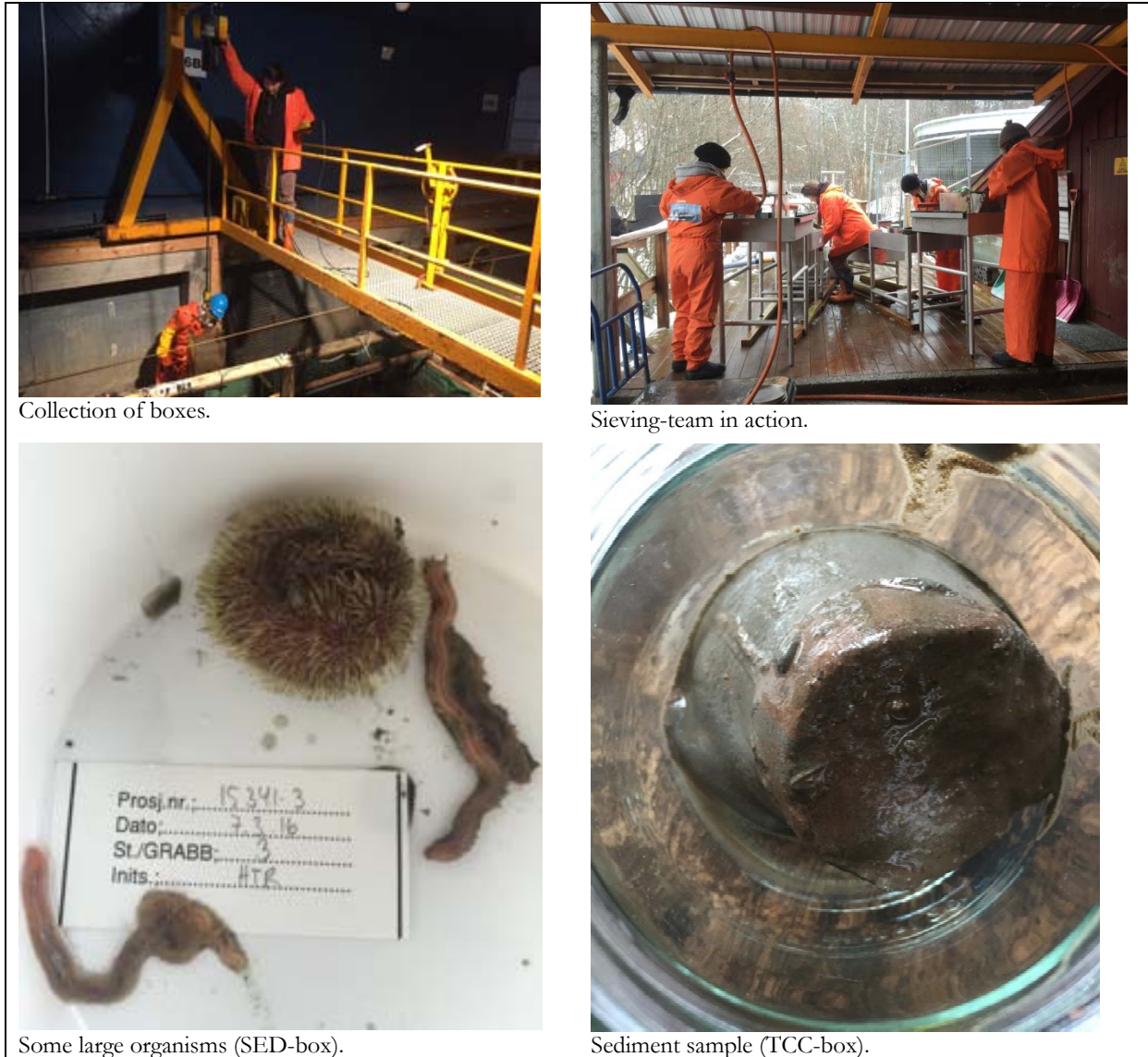


Figure 4. Termination of experiment.

## 2.6 Analyses of sediment and water

The vials for nutrients were stored in the dark at 4 °C, until performing chemical analyses using automated spectrophotometric methods modified after (Grasshoff et al., 1983).

To analyse total organic carbon (TOC), sediment samples were dried at 70 °C and treated with 10% HCl at least three times to remove calcium carbonate. The remaining carbon was determined by combustion at 480 °C and analyses of gaseous CO<sub>2</sub> using a Leco IR 212 carbon analyser.

Grain size analysis was done by ALS Laboratory Group Norway AS using wet sieve analysis with laser diffraction (fraction from 2 µm to 63 mm). Fractions > 63 mm, 31.5-63 mm, 16-31.5 mm, 8-16 mm, 4-8

mm, 2-4 mm, 1-2 mm, 0.5-1 mm, 0.25-0.50 mm, 0.125-0.25 mm and 0.063-0.125 mm were determined by the wet sieving method, other fractions were determined from the fraction "<0.063 mm" by laser particle size analyser using liquid dispersion mode.

## 2.7 Calculation of fluxes

Fluxes of oxygen and nutrients were calculated based on the concentration difference between the inlet water and the core water, with the following equation:

$$F = \frac{(C_i - C_o) \times Q}{A}$$

where  $C_i$  = concentration in header-tank,  $C_o$  = concentration in core-water,  $Q$  = flow of water through the box, and  $A$  = sediment area of box.  $Q$  was measured gravimetrically.

## 2.8 Faunal analyses

The organisms were sorted, preserved in 80% ethanol and then identified at NIVA's laboratory according to standard methods (NS-EN ISO 16665:2013). Bivalves were identified by Akvaplan-niva AS. Biomass measurement was performed for the main taxonomic groups after sorting. The species list was transferred to NIVA's database, which ensures updated taxonomic names. During the processing, sample treatments were blind to the analyst. .

## 2.9 Calculations and statistics

The number of species and total abundance were calculated pr. sample. Indices were calculated according to the indices required for offshore monitoring (Miljødirektoratet, 2015), which again accords with the classification system in relation to the Water Framework Directive specified in the guidelines (Veileder 02:2013; Direktoratgruppen, 2013). The equations can also be found here.

- $H'$  (Shannon–Wiener diversity index)
- $ES_{50}$  (Hurlbert's diversity index)
- NQI1 (Norwegian Quality Index)
- $ISI_{2012}$  (Indicator Species Index, version 2012)
- NSI (Norwegian Sensitivity Index)

$H'$  and  $ES_{50}$  are indices for biodiversity.  $H'$  was calculated with log<sub>2</sub> as a base.  $ES_{50}$  is number of species found for 50 randomly picked individuals in a sample, and had to be used instead of the commonly used  $ES_{100}$  due to <100 individuals in some samples. NSI and ISI are sensitivity indices that describe to what extent the macrobenthic community mainly consists of tolerant or sensitive species. NQI1 is an index which provides a combined measure of species diversity and sensitivity. It is based on AMBI index for disturbance, and the number of individuals and species in a sample.

ANOVA was performed on univariate faunal parameters as well as oxygen and nutrient data. Dunnett's test was used as a post-hoc test to look for significant differences between controls and the other treatments. Univariate statistical analyses were performed with the software package JMP version 6.

To analyse for similarities in the community structure, cluster-analysis and MDS-ordination were performed, based on Bray-Curtis similarity measure (Shannon & Weaver, 1963). Similarity-calculation was based on square-root transformed data. To test for significant differences in faunal composition,

PERMANOVA (permutational multivariate analysis of variance) was performed. PERMANOVA is a distance-based nonparametric multivariate analysis of variance that provides a pseudo- $F$  statistics and an associated p-value derived from permutation tests (Anderson et al., 2008). The multivariate analyses were performed with the PRIMER software package version 6 and PERMANOVA+ version 1.0.3.

## 2.10 Bottle slurry incubation

Bottle sediment-water slurries are a useful method to directly examine the degradability of organic carbon (OC) in the different cuttings as well as in control sediments. Additionally, mixing cuttings with sediment in different ratios can be incubated to imitate different thicknesses in layers deposited in the field. WBM mud has been proposed to negatively impact seafloor ecological functioning through high content of reactive OC leading to severe oxygen depletion for the benthos (e.g. Schaanning et al. 2008, Trannum et al. 2010). Here, in separate, independent incubations we examined oxygen consumption (sum of biological and chemical demand) and  $\Sigma$ -CO<sub>2</sub> evolution of different substrates as well as mixtures of cuttings and sediment (imitating sedimentation event and mixing in the seafloor). In order to prevent oxygen limitation, oxygen consumption incubations were run for 1.5 days at the end of which slurry water was still oxic.  $\Sigma$ -CO<sub>2</sub> evolution incubations were run for 3.5 days and reflect sum of oxic and anoxic processes. All preparations, processing and incubations were conducted at IRIS, experimental facility at Mekjarvik, in a climate room maintained at 10°C. Whatman GF/F filtered natural seawater was used for preparation of substrates and slurries (see below). While removing particulate organic matter, bacteria pass through the filter so that a separate bacteria inoculation was not required for incubation of cuttings.

Four substrates were utilized (Figure 5) and pre-treated in similar way as in the mesocosm experiment (sec 2.3):

- 1) Control sediment (CS): this was obtained from control mesocosms processed at the end of the 3-month incubation (see mesocosm experiment above). The sediment excluding large fauna (fraction passing through a sieve with 1 mm mesh size) was collected and transported cool to the laboratory. The sediment was then gently homogenized and allowed to settle, after which the overlying water was carefully removed. This constituted the basic CS substrate.
- 2) Defaunated sediment (DS): Time zero CS maintained at -20 °C to kill fauna. This substrate was prepared by thawing and gently homogenizing (693 grams) in filtered seawater. After allowing settlement for 1 day, the overlying water was carefully removed.
- 3) Water-based mud cuttings (WBM): 780 grams were washed three times in order to remove the water-soluble glycol. This was performed by mixing the mud with filtered seawater and particles allowed to settle. The day after the supernatant was poured off, and this procedure was repeated three times. After 3 rinses, the overlying water was carefully removed.
- 4) Thermally treated oil-based mud cuttings (TCC). 591 grams was mixed with seawater and after 1 day the overlying water was carefully removed.

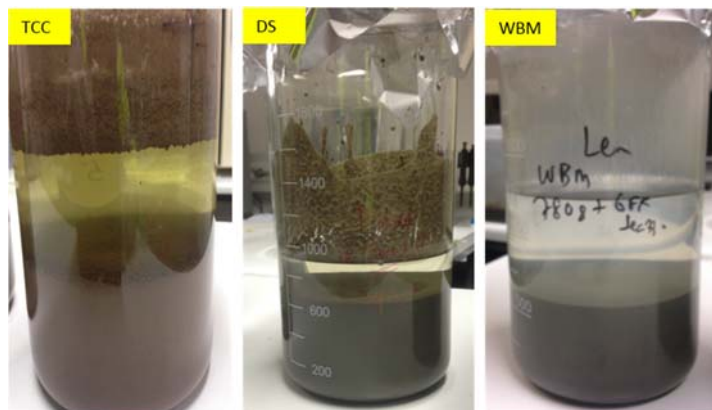


Figure 5. Preparation of the different substrates.

Slurry preparation:

Different volumes of wet substrate (sampled by filling a cut-off syringe) were transferred into pre-weighed 117 ml glass incubation bottles (Chrompack). Bottles were then filled to brim with GF/F filtered seawater (reweighed), sealed with screw caps fitted with rubber septa and then vigorously shaken by hand to thoroughly mix the slurry. Slurries were shaken daily and incubated in the absence of light (e.g. Moodley et al., 2011) In total 3 sets of incubations were conducted (see Figure 6. for depiction of bottle slurries):

- 1) Sediment oxygen consumption: Dissolved oxygen content was measured with oxygen micro-optodes (Presens and Pyroscience, Germany), at start and at the end when slurry caps were temporarily replaced by caps fitted with the mini-optodes through the septum. The amount of oxygen consumed was calculated as: (end concentration-start concentration)/volume of water. In order to prevent oxygen limitation, incubations were run for 1.5 days.

Three sub-experiments were conducted:

- 1a) Oxygen consumption was measured in slurries consisting of 10 ml of each substrate (CS, DS, WBM and TCC) in order to determine the presence of degradable OC and its relative reactivity ( $\mu\text{molO}_2\cdot\text{gC}^{-1}\cdot\text{hr}^{-1}$ ):
  - 1b) In order to examine the impact of a very thin layer of deposit mixed into surface sediment: oxygen consumption was measured in control sediment mixed with 40 % DS, WBM or TCC.
  - 1c) In order to examine the impact of a thicker layer of deposit mixed into surface sediment: oxygen consumption was measured of a thicker layer of sediment (14 ml) mixed with 57 % WBM or TCC.
- 2) Evolution of  $\Sigma\text{-CO}_2$ : Because anaerobe process may be equally or more important, we measured the start and end concentration of  $\Sigma\text{-CO}_2$  after 3.5 days incubation. Due to lack of sufficient amounts of Control Sediment, defaunated sediment (DS) was used to make mixtures with the drill cuttings in concentrations of 100 %, 60 % and 40 % cuttings. 10 ml of different mixtures were mixed with filtered seawater as described above for oxygen consumption measurements. A separate set of bottles were prepared for start  $\text{CO}_2$  samples: after vigorously mixing, 20 ml water was taken with a syringe and transferred to an Exetainer® tube with screw cap vials fitted with rubber septa (sample poisoned with  $\text{HgCl}_2$ ). The rest of the bottles were shaken once a day and after 3.5 days, water samples were collected. In the Netherlands, a headspace was made with helium and water acidified with 99% phosphoric acid to convert all species of  $\text{CO}_2$  to  $\text{CO}_2$  gas.  $\Sigma\text{-CO}_2$  concentration and  $\delta^{13}\text{C}\text{-}\Sigma\text{CO}_2$  were measured using a Carlo Erba 1106 Elemental Analyser coupled online with a Finnigan Delta S isotope ratio mass spectrometer (Van Nugteren et al., 2009, Moodley et al., 2011).
  - 3) Respiration of fresh highly degradable diatom carbon: respiration of  $^{13}\text{C}$ -labelled diatom carbon (25 %  $^{13}\text{C}$ ) in different substrates and substrate mixtures was compared to respiration in seawater without substrate. Slurries prepared and sampled as described in 2) and now with addition of 1 ml of tracer diatom solution (equivalent to an addition of 620  $\mu\text{g}$  carbon). Respiration of tracer diatoms documented through excess  $^{13}\text{C}$  in  $\Sigma\text{-CO}_2$  produced during 3.5 days incubations measured and calculated as described in Moodley et al. (2000).

Slurry incubations were initially not included in the project, and this part of the study was conducted partly based on IRIS's own financing. Partly due to this, there were two numbers of replicates in this part of the study, vs. four in the macrofaunal part. But most importantly, large replication for macrofauna is needed because of patchy distribution of some of the benthic species, while slurries are made with homogenized sediment and are mainly a microbial study, therefore number of replicates is less critical.





Figure 6. Slurry after water samples taken for  $\Sigma\text{-CO}_2$ . From left to right: DS, WBM, TCC,  $\text{H}_2\text{O}$ .

### 3. Results and discussion

#### 3.1 Sediment characteristics

The fraction of fine sediments ( $<63 \mu\text{m}$ ) in the 16 cores sampled at the end of the experiment (Figure 7) ranged 47-68% and had a mean value of 56 % which was the same as in the added sediments (as expected). Compared to the source sediment, TCC had less fines (36%) and WBM had more fines (80%), but because of the doses added, this had no clear effect on the fine contents in the cores.

The grain size distribution (Figure 8) showed that the coarser TCC material was dominated by particles  $>63 \mu\text{m}$  with a maximum in the 125-250  $\mu\text{m}$  fraction. However, particles were present in all size fractions down to  $<2 \mu\text{m}$ . The more fine-grained WBM was dominated by particles  $<125 \mu\text{m}$  with a maximum in the 8-16  $\mu\text{m}$  fraction. The sediment added (SED) was dominated by particles  $<500 \mu\text{m}$  and two maxima occurred at 8-16  $\mu\text{m}$  and 125-150  $\mu\text{m}$ , respectively. It may be important to note that significant amounts of particles were present in all of the smaller size ranges. Small particles may fill in the space between the larger particles and thus increase the diffusion resistance. Therefore, the small variation in size distribution between the added materials does not necessarily imply that the diffusion resistance varies between the different treatments.

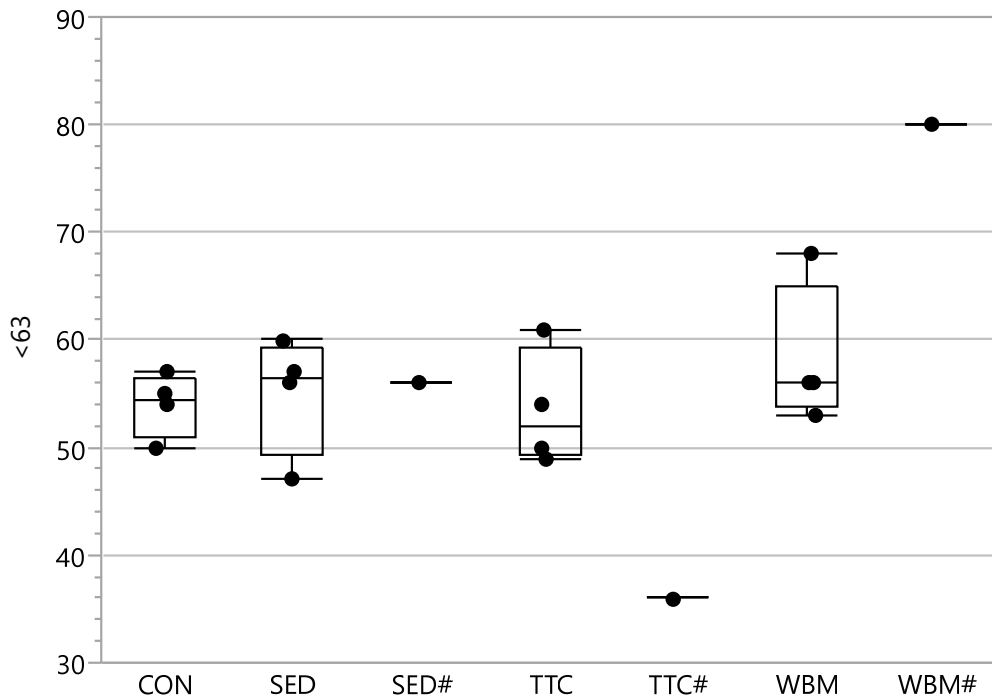


Figure 7. Analyses of grain size ( $<63 \mu\text{m}$ ) in added materials (SED#, TCC#, WBM#) and surface sediments (0-2 cm) sampled at the end of the experiment.

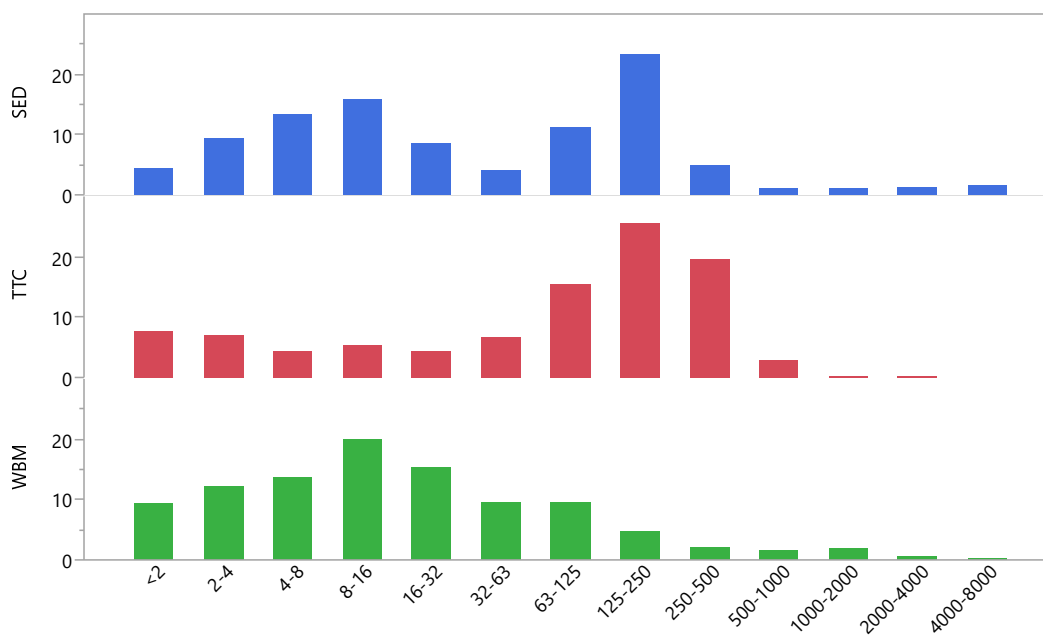


Figure 8. Size distribution of added materials (% within  $\mu\text{m}$  range).

Chemical analyses of the added materials are presented in Table 1. Similar analyses of the sediment in all mesocosm boxes are given in Appendix, Table 8. The concentrations are classified according to Bakke et al. 2007. All metals were classified in class I, except of a slight exceedance of lead in SED (class I < 30 mg/kg), which is actually due to some background pollution in the Oslofjord. There has been some concern of elevated heavy metals and in particular copper and zinc in TTC (Aquateam-COWI, 2014). Slightly elevated, but still low concentrations of these metals have also been recorded in the offshore monitoring of Martin Linge (DNV GL, 2015). However, as the concentrations of these metals in the cuttings are within class I/background level, such discharges cannot be considered a risk. In the present experiment, the Zn-content in the TTC was in fact the lowest of the three materials. It can also be mentioned that the Cu-concentration in the present cuttings corresponds very well with the Martin Linge TTC analysed by Aquateam- COWI (2015), while the Zn-concentration was lower in the present material.

Regarding hydrocarbons, the concentrations of the various fractions accorded fairly well with the cuttings analysed by Aquateam-COWI (2015), but the fraction >C12-C16 was considerable higher in the present study, while the opposite was true for the fraction >C10-C12. However the results from analyses of the same TTC materials used in the Martin Linge lab study (IRIS, 2015) also showed slightly different partition of the oil fractions. The differences we see here reflect most likely bias due to analytical artefacts due to the fact that the detection limits does not allow us to study the results in this detail.

Table 1. Chemical analyses (mg/kg) of the added materials (blue= class I/background concentration, green=class II/good condition).

ELEMENT	SED	TCC	WBM
Tørrstoff	66.3	99.7	76.2
TOC (Total organic carbon)	15.7	8.7	4.4
%<63 µm	56	36	80
As (Arsenic)	6.49	3.41	5.11
Ba (Barium)	63.2	8270	2700
Be (Beryllium)	0.706	0.2	0.565
Cd (Cadmium)	<0.10	0.15	0.17
Co (Cobalt)	10.3	4.87	10.4
Cr (Cromium)	24.2	26.3	32.3
Cu (Copper)	19.4	27.5	13.1
Fe (Iron)	21700	12600	23400
Hg (Mercury)*	<0.20	<0.20	<0.20
Mn (Manganese)	411	1160	287
Mo (Molybdenum)	0.6	7.2	0.55
Ni (Nickel)	21	23	23.2
P (Phosphor)	799	94	594
Pb (Lead)	31.3	9.1	11.2
Sr (Strontium)	67.4	739	150
V (Vanadium)	44.5	10.9	37
Zn (Zinc)	83.2	14.9	54.2
Li (Lithium)	38	7.4	42.6
Ti (Titanium)	477	32.2	749
Fraction >C10-C12	<2.0	6.7	<2.0
Fraction >C12-C16	<3.0	34.1	16.3
Fraction >C16-C35	53	41	18
Sum >C12-C35	53	75.1	34.3
Fraction >C35-C40	11.9	11	<5.0
Fraction >C10-C40	66	93	39

\* Detection level exceeds class I (<0.15 mg/kg)

## 3.2 Oxygen consumption and oxygen sediment penetration

### 3.2.1 O<sub>2</sub> microelectrode profiles

Typical examples of profiles measured in each core are shown in

. All profiles are shown in Appendix 6.2.1. O<sub>2</sub> is supplied from the overlying water and decreases below the sediment-water interface due to biological and chemical consumption. The depth of the O<sub>2</sub> penetration is determined by the rate of consumption and diffusion resistance in the sediment. As shown above, the differences in grain size distribution was small and fine fractions were present in all materials. Therefore, there is no reason to assume any systematic differences in diffusion resistance between the treatments. A frequently observed difference between profiles was that some profiles decrease almost linearly (constant rate) all the way to zero saturation, whereas other profiles showed that the decrease slowed down at low saturation levels, i.e. below ca 10% saturation. This decrease is observed as a rounded

tail in CON-18 which is not seen in WBM-16 and TCC-23. The tails could be the expected result of reduced metabolism of heterotrophic ( $O_2$  consuming) bacteria when  $O_2$  becomes scarce. It therefore appears likely that the absence of the tail in TCC and WBM, in particular during the first electrode survey, is a result of a different degradation process in the sediments with cuttings added.

The depth at which zero  $O_2$  saturation was reached was determined from each profile and the variations were assumed to result from the different treatments (TCC, WBM, SED or CON) and possibly also from different times (17<sup>th</sup> Dec. or 16<sup>th</sup> March) and different cores. This three factor model was run in the statistical program JMP® 11.0 and the results showed clear effects of treatment ( $p < 0.0001$ ) and time ( $p < 0.0001$ ), but no effects of core ( $p = 0.0677$ ) (Table 2 and appendix 6.2.2).

The time effect resulted from a general increase from 6.4 mm (grand mean) in December to 12.1 mm in March which is likely to occur from reduced reservoirs of labile organic carbon and reduced biodegradation towards the end of the experiment.

The effects of treatment was further analysed by Dunnett's comparison of least square means (these differ marginally from mean values) with control (appendix 6.2.2). This test showed that compared to a mean depth of 12 mm in CON, the depth was significantly lowered to 10 mm in SED, and 7 mm in WBM and TCC. The factor explaining this is most likely increased biodegradation due to added labile organic carbon.

In addition a significant interaction ( $p = 0.03$ ) was found between time and treatment. This means that the zero depth level did not change with time in the same way in all treatments. This interaction was further investigated by Tukey's comparison of all least square means (appendix 6.2.2). This test showed that on Dec. 17<sup>th</sup>, all treatments with added layers had significantly reduced  $O_2$  penetration, whereas on March 1<sup>st</sup> only TCC and WBM treatments were different from controls. This interaction is also evident from Figure 10 which shows that the increase of the penetration depth was larger in SED (6 to 13.5 mm) than in WBM (5 to 10 mm) and TCC (4 to 10 mm), whereas the major change within CON (12 to 13 mm) was reduced variability.

The small change in control suggests that the change in the other treatments resulted from changes in the added materials and not from systematic errors or external factors such as water quality. E.g. decreased water temperature would reduce  $O_2$  consumption in all treatments, CON included, and increased concentration of  $O_2$  in the source water would increase the downward diffusion in all treatments. Therefore, reduced  $O_2$  consumption in the added materials due to burnout of organic carbon appears to be the most likely explanation to the increase of the penetration depth between December and March.

Table 2. Results of statistical model on effects of treatment, time and core number on O<sub>2</sub> penetration and O<sub>2</sub> and nutrient fluxes. Lower part of the table shows results of a Dunnett's comparison (one way-ANOVA) of each treatment with control. More details on analyses are given in appendix 6.2. Red values are significant at the p=0.05 threshold.

	O <sub>2</sub> penetration	O <sub>2</sub> flux	NH <sub>4</sub> flux	NO <sub>3</sub> +NO <sub>2</sub> flux	PO <sub>4</sub> flux	Si(OH) <sub>4</sub> flux
<b>Whole model</b>						
Observations (n)	93	93	16	32	32	32
R2	0.72	0.89	0.71	0.81	0.89	0.91
p	<0.0001*	<0.0001*	0.081	0.0022*	<0.0001*	<0.0001*
<b>Effect test (p-values)</b>						
Treatment	<0.0001*	0.0008*	0.084	0.0001*	0.0156*	0.0013*
Time	<0.0001*	<0.0001*	-	0.0266*	<0.001*	<0.0001*
Core	0.067	0.065	0.951	0.764	0.972	0.119
Treatment × time	0.034*	0.0014*	-	0.051	0.0392*	0.342
Treatment × core	0.814	0.175	0.140	0.742	0.0117*	0.436
Time × core	0.339	0.698	-	0.478	0.081	0.0137*
Treatment × time × core	0.155	0.196	-	0.416	0.386	0.048
<b>Dunnett's comparison of treatment (p-values)</b>						
SED-CON	0.020*	0.961	0.789	0.274	0.636	0.0049*
TTC-CON	<0.001*	0.0049*	0.053	<0.0001*	0.0216*	0.0007*
WBM-CON	<0.001*	0.0032*	0.995	0.0030*	0.0182*	0.0238*

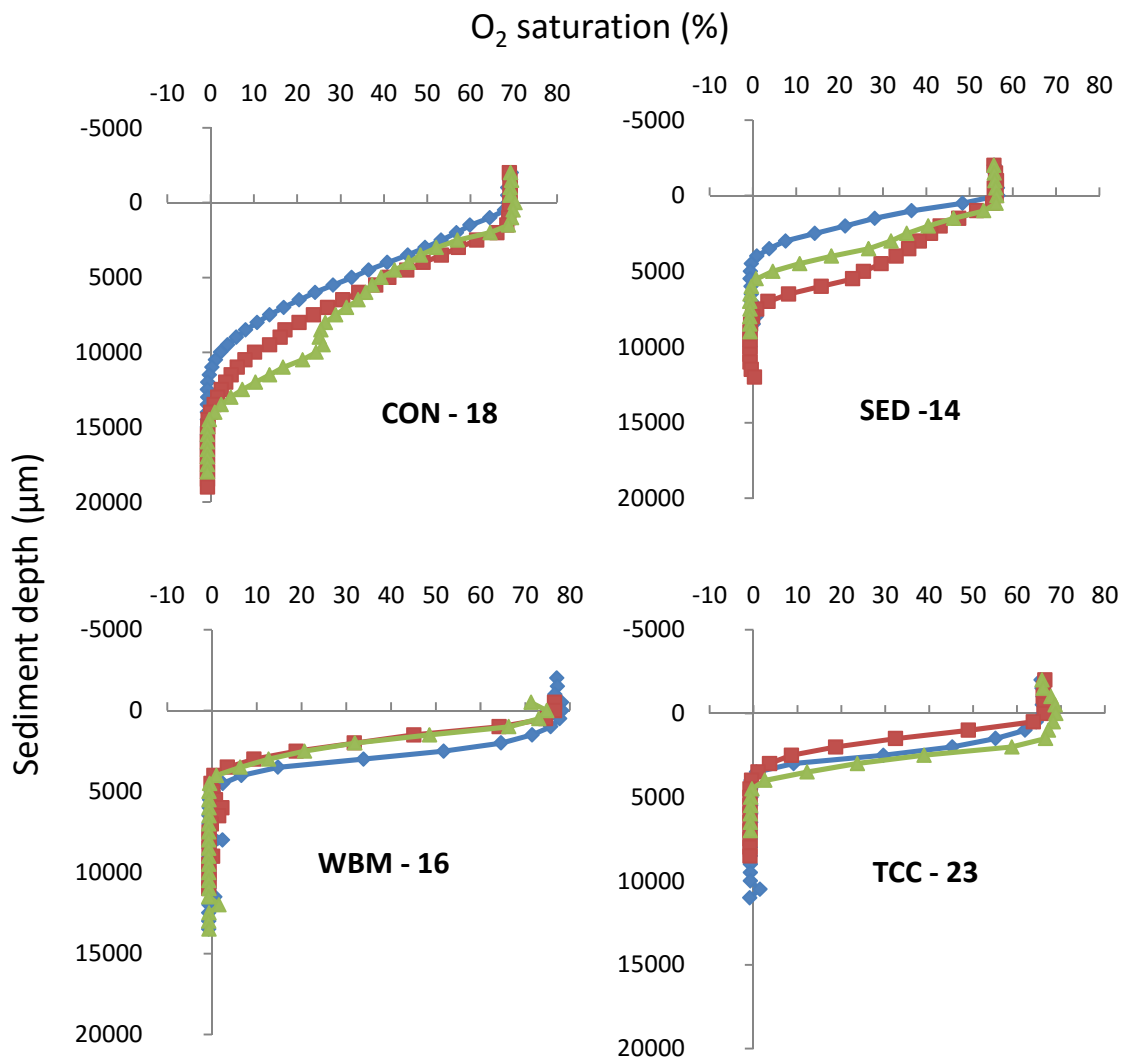


Figure 9. Typical microelectrode profiles determined 17<sup>th</sup> Dec. 2015.

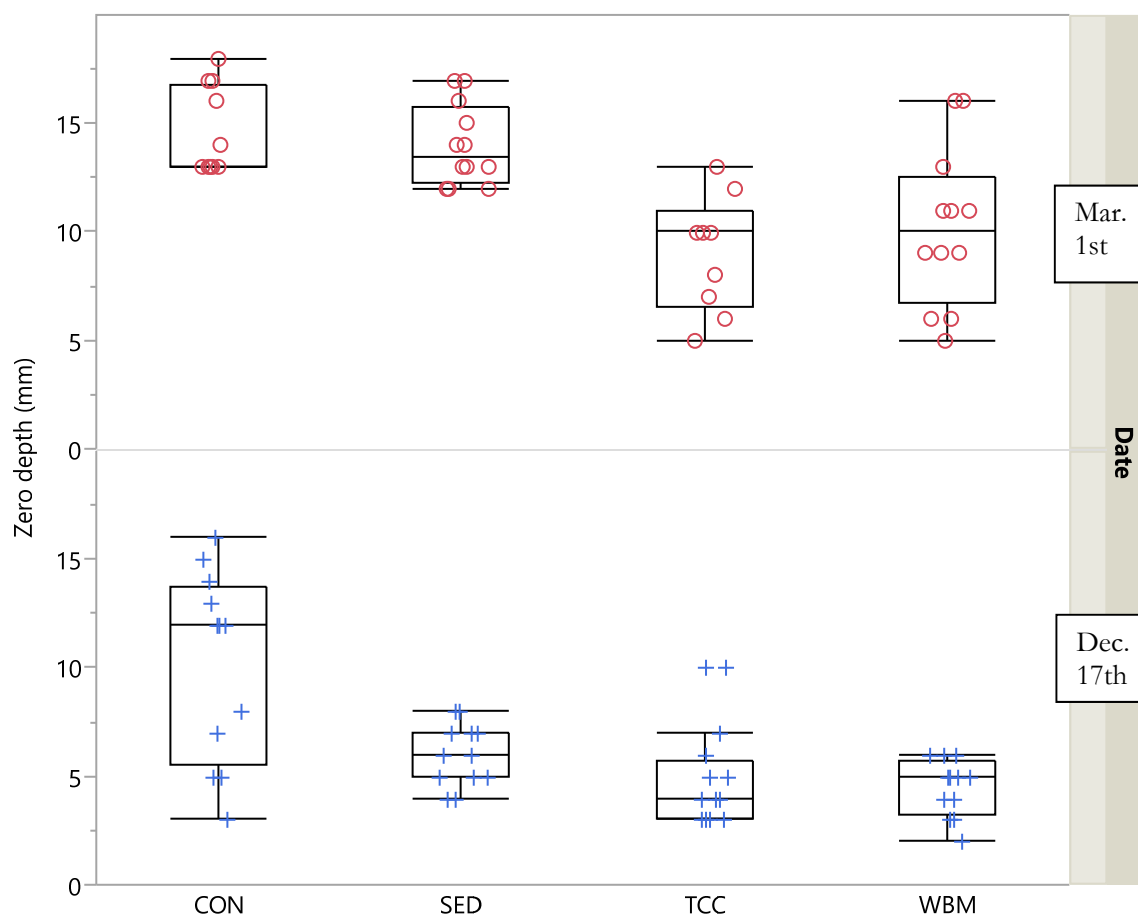


Figure 10. Box plots of oxygen zero depths determined from the microelectrode profiles in each treatment. Zero depth level of TCC and WBM in December was significantly lower ( $p < 0.001$ ) than control at both dates and all treatments in March (ref. appendix 6.2.2).

### 3.2.2 Sediment oxygen consumption (SOC)

Sediment oxygen consumption is measured as the difference in concentration between water going into and out of each boxcore. The change of the SOC-rates is shown in Figure 11 and the cumulative consumption after addition is shown in Figure 12. Before addition, the SOC rates ranged 419-541  $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ . SOC peaked in all treatments on day 8. Before day 8 the bacteria population is most likely the limiting factor for SOC, after day 8 labile organic carbon in the substrate may be limiting. The highest rates on day 8 occurred in TCC (954  $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) and the lowest occurred in control (588  $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ). At the end of the experiment, the range of 249-329  $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$  was somewhat reduced compared to the range before addition. The general decrease of the rates of SOC after day 8 was consistent with the increase of the penetration depth observed with the microelectrodes between Dec 17<sup>th</sup> and March 16<sup>st</sup> (Figure 10).

For the entire experimental period, the mean cumulative oxygen consumption was very similar in TCC and WBM and almost identical in SED and CON (Figure 12). The difference might suggest the presence of degradable substances in both types of cuttings. However, the higher mortality in the TCC treatments may provide an additional source of labile organic carbon to be degraded in these sediments. As discussed in Chapter 3.5, some kind of chemical oxygen consumption may also occur shortly after TCC addition.



The same statistical model as for the oxygen penetration depth was also used for the SOC rates. The result (ref. appendix 6.2.3) was very similar with significant effects of treatment ( $p=0.008$ ), time ( $p<0.0001$ ) and treatment  $\times$  time interactions ( $p=0.0014$ ). Dunnett's test showed that compared with control, the SOC was higher in TCC ( $p=0.0049$ ) and WBM ( $p=0.0032$ ) but not in SED ( $p=0.96$ ). This was consistent with the penetration depth measured at the end of the experiment. Tukey's comparison of SOC at each time of observations showed a significant increase from day 2 to day 8, and a significant decrease from day 8 to day 37. Day 51 and 65 were not different from day 37, but on day 95 SOC was significantly lower than all the previous days except day 65. The general decrease with time after the peak SOC on day 8, was expected from the burning out of the labile fractions of carbon present both in mud chemicals and natural organic matter in the sediment transferred from field. Peak SOC is often seen after about one week of incubation and results from exponential growth of bacteria populations reaching very high numbers if excess organic carbon is present. In TCC this excess carbon source may have been macrofauna suffering acute toxicity due to  $\text{Ca}(\text{OH})_2$  added in mud recipe (ref. Chapter 3.6).

The time  $\times$  treatment interaction ( $p=0.0146$ ) was probably a result of the degradation of the organic phase providing a different variation with time in WBM and TCC than in the two treatments without cuttings added. So far, the analyses of the factors controlling SOC was very similar to the results obtained for the microelectrode measurements.

Significant effects of sediment core were not found for any of the  $\text{O}_2$  parameters. Such effects might have resulted from various experimental bias (e.g. circulation pumps may differ slightly with regard to power and jet direction, temperatures may be systematically higher in one end of the basin than in the other etc.), or maybe more likely from inherent differences in the benthic communities in each box. Several observations noted during the experiment showed that such differences are present. Thus, in one of the SED-boxes (box 1) there was one large and one small sea anemone. These organisms apparently lead to a very large oxygen consumption ( $1036 \mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) before addition and  $870 (\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1})$  on day 2 after addition. The two anemones were removed after day 2 and on day 8 the SOC was down to  $205 \mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$  before stabilizing at a normal level of  $309\text{-}433 \mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$  throughout the remaining experimental period. Other large individuals, such as sea urchins, were present in some boxes but not in others. These are major bioturbators bull-doing through the sediment just below the surface and may significantly enhance exchange of oxygen and nutrient species between the pore water and the overlying water. We did not observe any clear impacts on SOC of such organisms, but in one box measured before addition, such organisms appeared responsible for an anomalous deep penetration of  $\text{O}_2$ . Fortunately, experimental bias was not sufficiently large or persistent to provide any significant core effects on oxygen penetration or oxygen consumption.

Increased oxygen consumption of sediments treated with WBM agrees well with previous field- and mesocosm-experiments (Trannum et al., 2010; Trannum et al 2011a, b). As this experiment is the first mesocosm-experiment with TCC, there are no comparable data. However, ecotox-tests have documented increased oxygen consumption of TCC (Aquateam, 2014), which fits well with the present work.

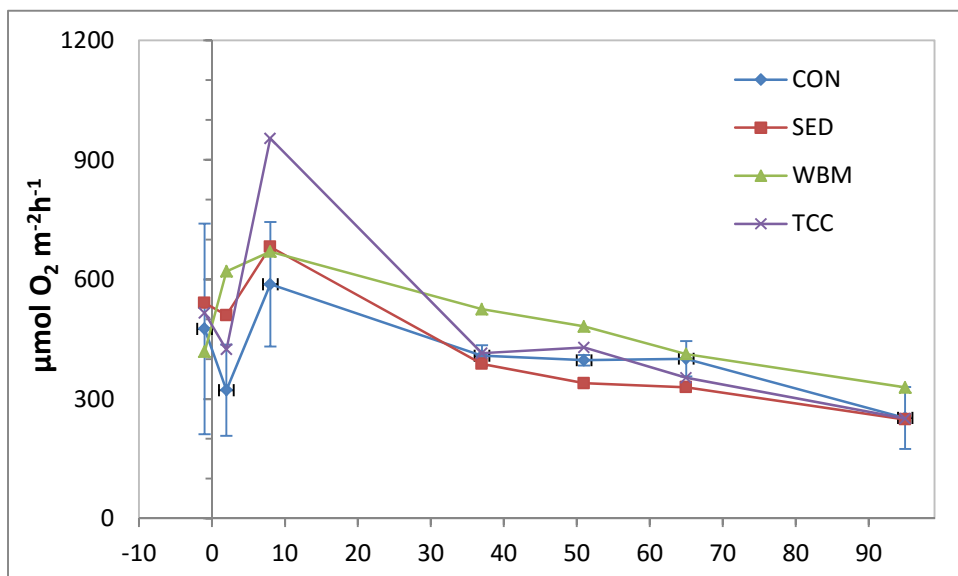


Figure 11. Sediment oxygen consumption in each treatment plotted against time (days). Vertical bars represent  $\pm$  SD (Standard Deviation) of mean SOC in control boxes.

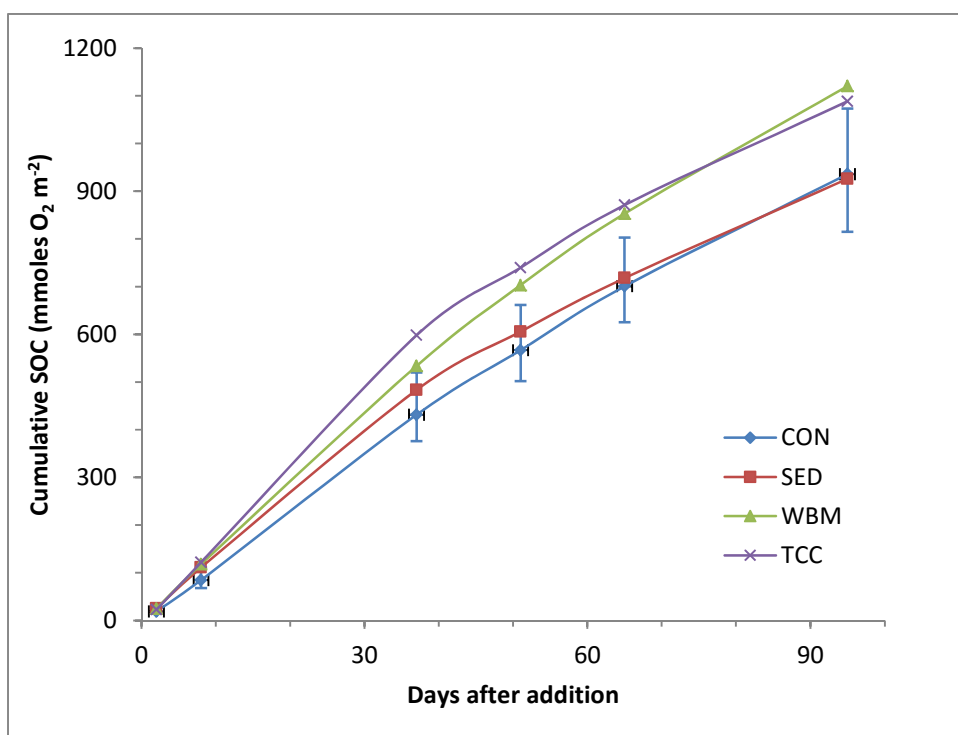


Figure 12. Cumulative sediment oxygen consumption in each treatment. Vertical bars represent  $\pm$  SD of mean SOC in control boxes.

### 3.3 Nutrient fluxes

Samples for analyses of nitrate & nitrite, ammonium, phosphate and silicate were collected just before addition, five days after and again just before termination. Unfortunately, ammonium was not analyzed in the samples collected at the end of the experiment. All data are displayed in Figure 13 and Figure 14. The three factor model run for oxygen was also run for the nutrient species on the two sets of post-treatment data. The results are given in the appendix (6.2.4).

Nitrate is consumed in the sediment by heterotrophic bacteria using this compound rather than oxygen as terminal electron acceptor for organic carbon degradation. As expected, before additions, the range and median values were relatively similar in all boxes (Figure 13). After addition the flux tended to decrease in control and SED treatment and increase in TCC and WBM. Because nothing was added to the control boxes, the decrease of the nitrate flux to almost to zero on Dec. 14<sup>th</sup> was not understood. The three-factor model run on the measurements taken after treatment (Dec. 14<sup>th</sup> and Mar. 7<sup>th</sup>), showed significant effects of treatment and time. No significant core effects and no significant interactions were recorded. The Dunnett comparison showed that the flux was significantly higher than CON in TCC ( $p < 0.0001$ ) and WBM ( $p = 0.003$ ) but not in SED ( $p = 0.274$ ). This was consistent with the SOC model which indicated increased heterotrophic activity in TCC and WBM. A question here is, however, to what extent the decrease of the nitrate consumption in the control boxes, which might indicate experimental bias, had contributed to the differences between control and cuttings treatment.

Ammonium is normally below detection limits in the source water from 60 m depth in the Oslofjord. This was also the case in this study and the flux of ammonium will therefore always be zero or positively directed from the sediment to the water. Ammonium is produced in the sediments by degradation of proteins and released by respiration and decay of dead organisms. Before treatments median ammonium fluxes were small ( $17\text{-}36 \mu\text{mol m}^{-2} \text{h}^{-1}$ ) in all treatments. In one box core (SED 1), however, an efflux of  $282 \mu\text{mol m}^{-2} \text{h}^{-1}$  was observed. This was the same core as the one which also had a very high oxygen consumption and presence of a large anemone. Apparently, respiration was large in this core due to the presence of the large anemone. After the first measurements, the anemone was removed and the release of ammonium decreased from this core, but the simultaneous increase from another SED-replicate resulted in a decrease of the mean flux, but an increase of the median flux (Figure 13). TCC showed an increase of both mean and median fluxes whereas little change was observed in WBM. The few instances of increased release of ammonium from SED and TCC treatments may have resulted from stress-related increase of respiration or decay of organisms which did not survive the treatments. None of the changes observed for fluxes of  $\text{NH}_4$  were significant, however (appendix 6.2.4).

The fluxes of phosphate and silicate showed a general decrease with time (Figure 14). This probably resulted from the general decrease of the metabolic rates in the sediment as indicated by the decrease of SOC. The statistical analyses showed significant effects of treatment and time both for silicate and phosphate and the Dunnett's comparison with control showed significant lower release from TCC and WBM. Also, the release of silicate was significantly lower than control in the SED treatment. This may be due to higher presence of silica skeletons in the surface of the control sediment sustaining higher rates of biodegradation and release in the untreated sediments.

Phosphate also showed significant treatment  $\times$  core and treatment  $\times$  time interactions. The core effect may be an inherent effect of the transferred cores as indicated by relatively large differences between the treatment groups observed also before treatment (Figure 14). The treatment $\times$ time interaction is most likely rooted in the relatively large change in SED between the two times of measurements used in the statistical analyses. The two-ways time $\times$ core interaction and the three-ways interaction of the silicate fluxes are complex and not well understood.

The most consistent and important result from the statistical model of the nutrient fluxes was the significant increase of the nitrate (& nitrite) flux in TCC and WBM, which confirmed the increased bacteria activity indicated by SOC and microelectrode measurements. This finding accords well with previous experiments (Schaanning et al., 2008, Trannum et al., 2011 a). Also, the decrease of the fluxes of silicate in all treatments and phosphate in TCC and WBM indicated effects not only from the addition of particles, but also from the composition of the added particles.

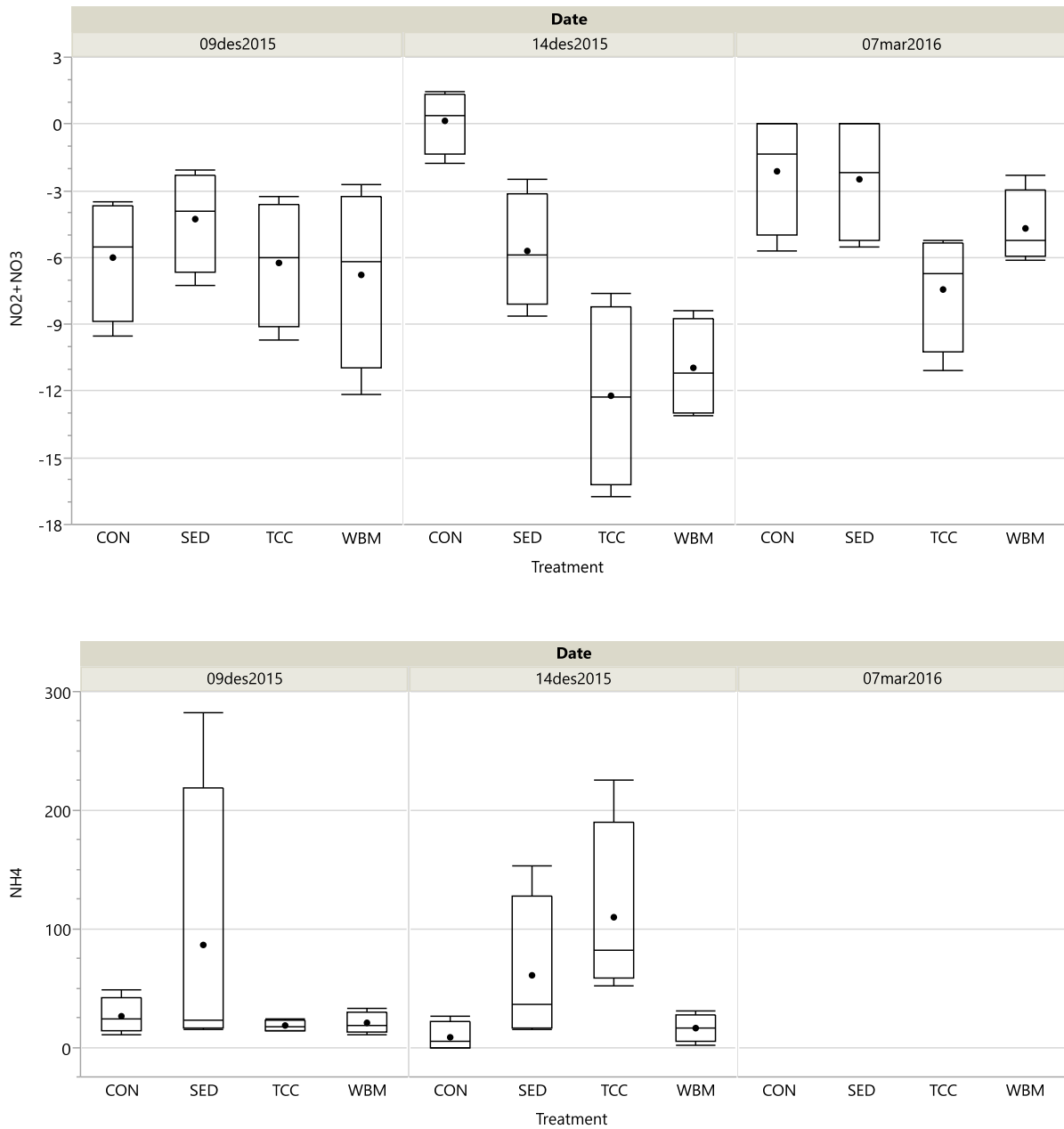


Figure 13. Box plots of fluxes of nitrate+nitrite (top) and ammonium (bottom) determined before addition on Dec. 9<sup>th</sup>, on Dec. 14<sup>th</sup>, five days after addition of sediment (SED), thermally treated cuttings (TCC) and cuttings with water based mud (WBM) and towards the end of the experimental period on Mar 7<sup>th</sup>. Unfortunately, ammonium was not determined in samples collected on Mar. 7<sup>th</sup>. The box plots show the range and median values of the four replicate cores in each treatment. The point shows the mean flux.

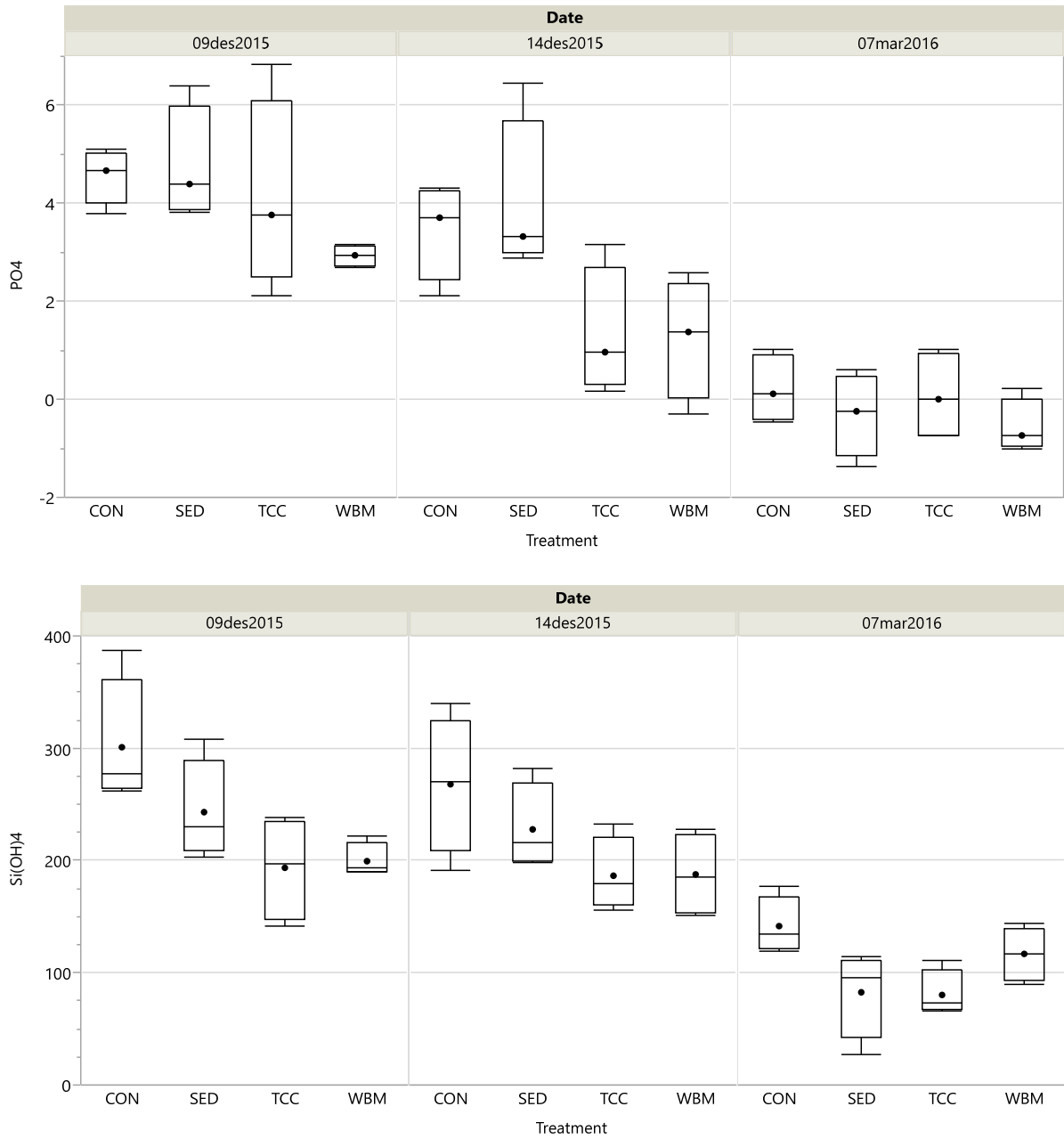


Figure 14. Fluxes of phosphate (top) and silicate (bottom) determined before addition on Dec. 9<sup>th</sup>, on Dec. 14<sup>th</sup>, five days after addition of sediment (SED), thermally treated cuttings (TCC) and cuttings with water based mud (WBM) and towards the end of the experimental period on Mar 7<sup>th</sup>. The box plots shows the range and median values of the four replicate cores in each treatment. The point shows the mean flux.

### 3.4 Faunal responses

Number of taxa, abundance, biomass and calculated biodiversity indices are presented in Table 3 and Figure 15 and Figure 16. Biomass measure is without particularly large organisms, like sea urchins and anemones, as these had a patchy distribution and dominated the weight. The parameters were analysed by ANOVA, followed by Dunnet's test.

There was a significant reduction in faunal abundance in the TCC, but not the WBM treatment, evident for both abundance, number of species and biomass. The number of species was approximately half that in the TCC-treatment compared to control. Regarding abundance, the decrease was higher, and less than 25% organisms survived. The biomass in TCC was in average 33% of the biomass in the control.

The responses of the diversity indices was less strong than the actual faunal measurements. Here it is important to be aware of the fact that there was a parallel reduction in number of species and abundance, and in such cases the indices may remain relatively unaltered. Further, in a mesocosm setup, only mortality is measured, and colonization of more tolerant taxa is not possible, and also this leads to less response of the biodiversity indices. Nevertheless, ISI<sub>2012</sub> was significantly lower in TCC than control, and Shannon-Wiender diversity (H') close to significantly lower.

Table 3. Number of species (S), abundance (N), biomass, ES<sub>50</sub>, H', NQI1, ISI<sub>2012</sub>, NSI pr. box (0.09 m<sup>2</sup>). CON=control, SED=sediment, TCC=thermally treated cuttings, WBM=water based mud.

	<b>S</b>	<b>N</b>	<b>Biomass (g)</b>	<b>ES<sub>50</sub></b>	<b>H'</b>	<b>NQI1</b>	<b>ISI<sub>2012</sub></b>	<b>NSI</b>
CON	71	782	3.65	24.00	4.86	0.78	9.14	23.14
CON	44	376	3.01	19.68	4.32	0.72	9.15	22.00
CON	45	396	4.62	18.43	4.14	0.72	9.42	21.62
CON	48	380	3.85	19.77	4.34	0.72	10.02	21.92
SED	43	202	1.26	23.90	4.69	0.75	8.55	22.23
SED	59	450	6.57	22.78	4.72	0.74	8.90	21.99
SED	47	391	3.60	21.48	4.57	0.72	9.34	22.06
SED	36	161	2.21	20.63	4.23	0.75	8.87	22.40
TCC	29	135	1.59	18.84	4.14	0.74	8.48	22.20
TCC	26	92	1.18	19.58	4.11	0.74	8.52	22.70
TCC	20	76	0.80	16.37	3.67	0.74	8.13	23.22
TCC	30	139	1.37	17.67	3.91	0.76	9.20	23.67
WMB	51	279	2.62	22.32	4.51	0.76	9.58	22.16
WMB	47	286	4.15	22.02	4.53	0.75	9.24	22.37
WMB	52	283	2.08	22.60	4.59	0.78	9.74	22.05
WMB	43	450	4.10	17.73	4.11	0.72	9.02	21.68

In a field study of Martin Linge (DNV GL, 2015) there was a reduced number of individuals and species at one of the closest station to the well (the station which tended to have the highest metal levels, ML5). There was no reduction in the diversity indices ES<sub>100</sub> and H', which again is caused by a parallel reduction in individuals and species number. It should be noted that the faunal reduction in the mesocosm was more severe than in the field. In the field, the closest stations are usually placed 250 m from the well, but closer, the effects may be more pronounced.

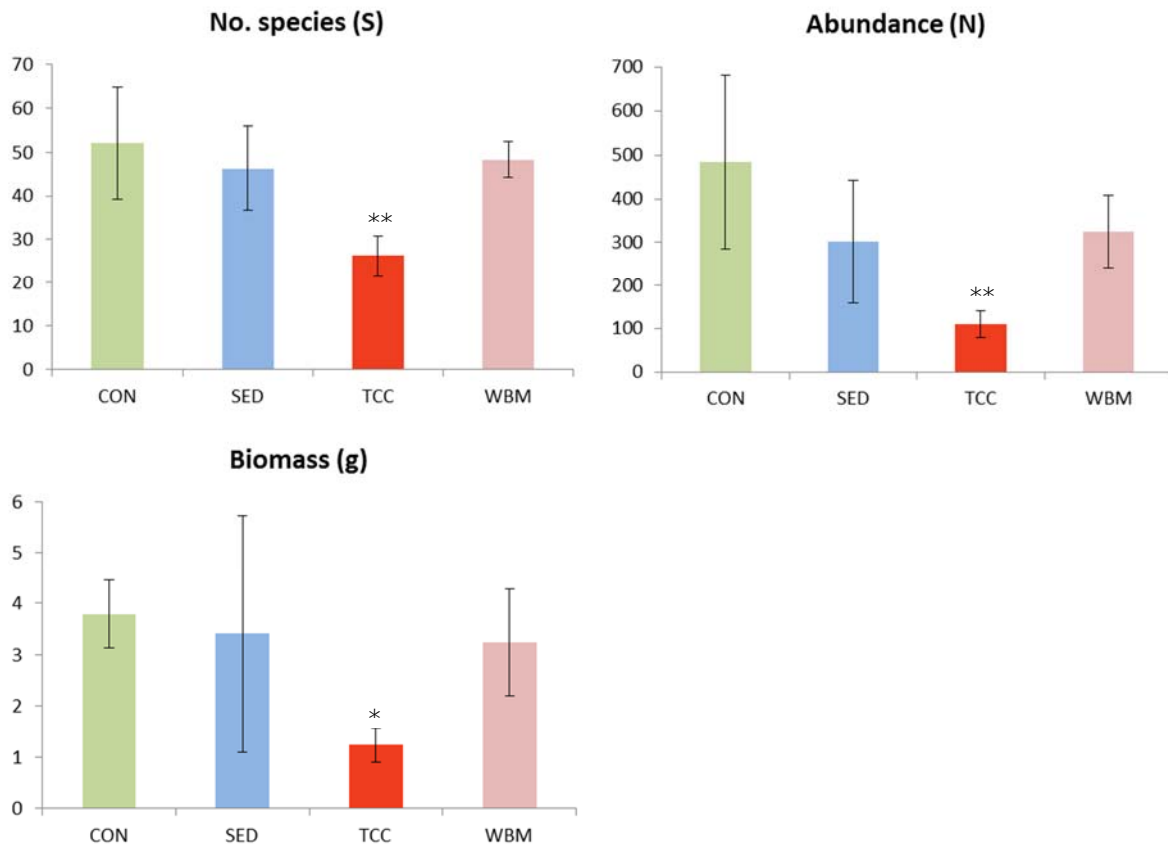


Figure 15. Mean number of species, total abundance and biomass ( $\pm$  SD) in each treatment ( $0.09 \text{ m}^2$ ). Significance levels for p values: \*\* < 0.01, \* < 0.05. CON=control, SED=sediment, TCC=thermally treated cuttings, WBM=water based mud.

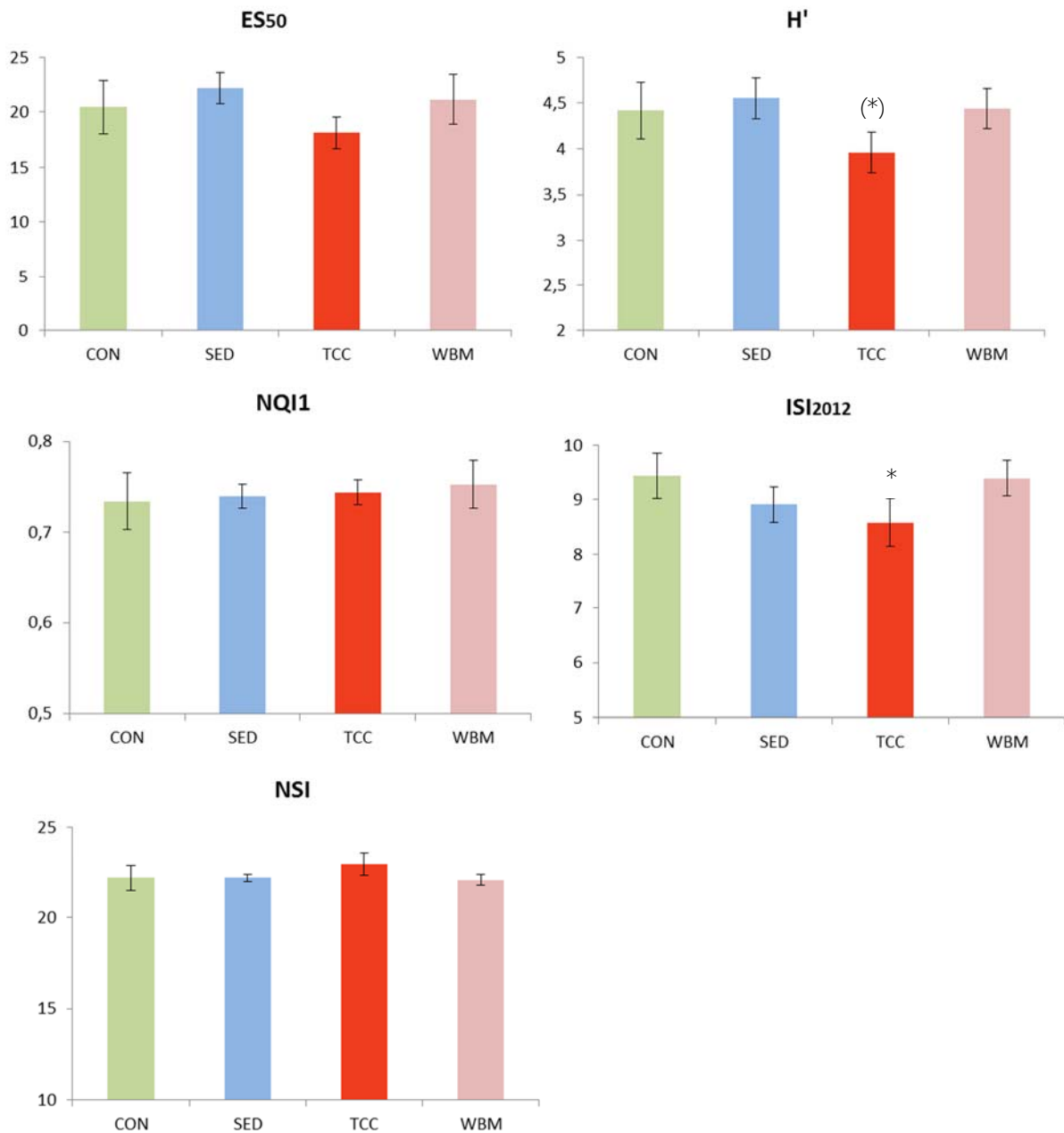


Figure 16. Mean values of biodiversity indices ( $\pm$  SD) in each treatment (0.09 m<sup>2</sup>). Significance levels for p values: \* < 0.05, (\*) close to significant (0.055). CON=control, SED=sediment, TCC=thermally treated cuttings, WBM=water based mud.

An overview of the most dominating taxa is presented in Table 3. The full species list is given in Appendix 6.3. From these tables, it is evident that the abundances in general were much lower in the TCC-treatment compared to the other treatments (12-24 individuals in the TCC-boxes vs. 34-78 in the other boxes). The bivalve *Thyasira equalis* was the most dominating species in all four treatments, but had considerable lower density in the TCC-treatment than in the other. This species lives as a subsurface deposit feeder and also hosts symbiotic chemoautotrophic bacteria. This species is generally considered quite robust, and has e.g. been found to be tolerant to sedimentation of mine tailings in a previous mesocosm experiment (Trannum et al., in prep.). Nevertheless, it responded negatively to the TCC.



Table 4. Mean number of the 20 most dominating taxa (and respective taxonomic group) pr. treatment (0.09 m<sup>2</sup>). CON=control, SED=sediment, TCC=thermally treated cuttings, WBM=water based mud.

CON			SED		
BIVALVIA	Thyasira equalis	57	BIVALVIA	Thyasira equalis	42
POLYCHAETA	Spiophanes kroyeri	50	POLYCHAETA	Spiophanes kroyeri	35
BIVALVIA	Adontorhina similis	46	BIVALVIA	Abra nitida	19
POLYCHAETA	Pseudopolydora paucibranchiata	40	POLYCHAETA	Heteromastus filiformis	17
BIVALVIA	Kelliella miliaris	28	NEMERTEA	Nemertea indet	16
BIVALVIA	Abra nitida	26	POLYCHAETA	Pseudopolydora paucibranchiata	14
BIVALVIA	Nucula sp. juvenile	24	BIVALVIA	Nucula sp. juvenile	13
BIVALVIA	Mendicula ferruginosa	19	BIVALVIA	Mendicula ferruginosa	12
POLYCHAETA	Heteromastus filiformis	12	BIVALVIA	Adontorhina similis	11
OPHIUROIDEA	Ophiuroidea juvenile	11	POLYCHAETA	Chaetozone setosa	11
POLYCHAETA	Paramphinome jeffreysii	10	BIVALVIA	Kelliella miliaris	9
BIVALVIA	Yoldiella philippiana	10	BIVALVIA	Nucula sulcate	8
BIVALVIA	Nucula sulcate	10	POLYCHAETA	Paramphinome jeffreysii	8
POLYCHAETA	Euclymeninae indet	10	POLYCHAETA	Euclymeninae indet	7
POLYCHAETA	Chaetozone setosa	9	BIVALVIA	Nucula tumidula	5
AMPHIPODA	Eriopisa elongate	8	OPHIUROIDEA	Amphilepis norvegica	5
NEMERTEA	Nemertea indet	8	BIVALVIA	Yoldiella philippiana	4
BIVALVIA	Tellimya tenella	7	POLYCHAETA	Prionospio cirrifera	4
SIPUNCULIDA	Nephasoma sp.	6	AMPHIPODA	Eriopisa elongate	4
POLYCHAETA	Exogone verugera	5	SIPUNCULIDA	Nephasoma sp.	4
WBM			TCC		
BIVALVIA	Thyasira equalis	64	BIVALVIA	Thyasira equalis	19
POLYCHAETA	Spiophanes kroyeri	30	POLYCHAETA	Paramphinome jeffreysii	14
BIVALVIA	Kelliella miliaris	23	BIVALVIA	Mendicula ferruginosa	11
BIVALVIA	Abra nitida	21	AMPHIPODA	Eriopisa elongate	8
POLYCHAETA	Heteromastus filiformis	20	BIVALVIA	Nucula sulcate	8
BIVALVIA	Nucula sp. juvenile	17	BIVALVIA	Nucula sp. juvenile	7
BIVALVIA	Mendicula ferruginosa	16	NEMERTEA	Nemertea indet	6
BIVALVIA	Adontorhina similis	14	BIVALVIA	Kelliella miliaris	5
POLYCHAETA	Pseudopolydora paucibranchiata	10	POLYCHAETA	Heteromastus filiformis	4
BIVALVIA	Nucula sulcate	10	POLYCHAETA	Chaetozone setosa	3
POLYCHAETA	Paramphinome jeffreysii	9	POLYCHAETA	Spiophanes kroyeri	3
POLYCHAETA	Chaetozone setosa	7	SIPUNCULIDA	Onchnesoma steenstrupii	3
NEMERTEA	Nemertea indet	7	POLYCHAETA	Ceratocephale loveni	2
BIVALVIA	Ennucula tenuis	6	BIVALVIA	Ennucula tenuis	2
BIVALVIA	Nucula tumidula	6	BIVALVIA	Nucula tumidula	2
SIPUNCULIDA	Nephasoma sp.	5	BIVALVIA	Tellimya tenella	2
AMPHIPODA	Eriopisa elongate	4	SIPUNCULIDA	Nephasoma sp.	1
OPHIUROIDEA	Ophiuroidea juvenile	4	BIVALVIA	Adontorhina similis	1
POLYCHAETA	Jasmineira candela	3	POLYCHAETA	Paradiopatra quadricuspis	1
SIPUNCULIDA	Onchnesoma steenstrupii	3	POLYCHAETA	Levinsenia gracilis	1

The second most dominating species in the treatments with CON, SED and WBM was the spionid *Spiophanes kroyeri*, which had a mean abundance of only three individuals in the TCC-boxes. *Pseudopolydora paucibranchiata*, another spionid, was also quite abundant in CON, SED and WBM, but clearly reduced in TCC where only one specimen was recorded in all four boxes. This tube-building species has been found to respond negatively to drill cuttings previously (Trannum et al., 2011b). The bivalve *Abra nitida* also responded very clearly, and in fact no specimen of this species survived the TCC-treatment. This species has also been found to be sensitive to drill cuttings, incl. WBM, in several previous studies (Schaanning et al., 1996, Schaanning et al., 2008, Terliezzi et al., 2005, Trannum et al., 2011a). Notably, it did not seem to

respond negatively on WBM in the present experiment. All these species live in the upper sediment mainly as surface deposit feeders, i.e. feeding on particles on the sediment surface. Surface deposit feeders have also previously been found to be particularly sensitive to drill cuttings (Trannum et al., 2010, Trannum et al., 2011a). This finding seems reasonable as they are particularly exposed to the cuttings, and thus may be affected by low nutrient content, sharpness and eventual chemicals. Furthermore, some of the species are also tube-building, like *Spiophanes kroyeri* and *Pseudopolydora paucibranchiata*. Such species may also be particularly vulnerable towards deposition of “exotic” sediments. Tube-building species have shown reduced abundances in sediments receiving high loads of drill cuttings in oil field studies (Daan et al., 1994, Olsgard and Gray, 1995, Zuvo et al., 2005, Trannum et al., 2006). At the same time it should be noted that none of the species mentioned above are considered particularly sensitive to disturbances in general, as they are often recorded in increased densities under slightly disturbed conditions, e.g. in organic enriched sediments. The negative response here may therefore possibly be related to another factor than addition of an organic compound.

The small polychaete *Paramphinoe jeffreysii* was one of the few dominant species with no tendency of a reduced abundance in the TCC-treatment. This species is generally tolerant towards disturbances, and increases in abundance e.g. in physically disturbed or organically enriched sediments. It lives as a scavenger/omnivore/carnivore, which makes it less directly exposed to the drill cuttings particles. Interestingly, this species had high abundance at the Martin Linge field in the monitoring in 2015 (DNV GL, 2015), and was in fact the most dominating species at the station which appeared slightly disturbed (ML5). Thus, these results correspond very well with each other. And also, this finding indeed shows that mesocosm results with communities taken from the Oslofjord provide highly realistic data.

Cluster- and MDS-plots are presented in

Figure 17 and Figure 18. These analyses are in line with the other findings, as the TCC-boxes were isolated from the other boxes and formed one distinct group. It can also be noted that the TCC-treatment was the treatment with most variation between the boxes. The WBM-treatment showed the least variation. Further, these boxes were placed close to the CON- and SED-boxes in the plots.

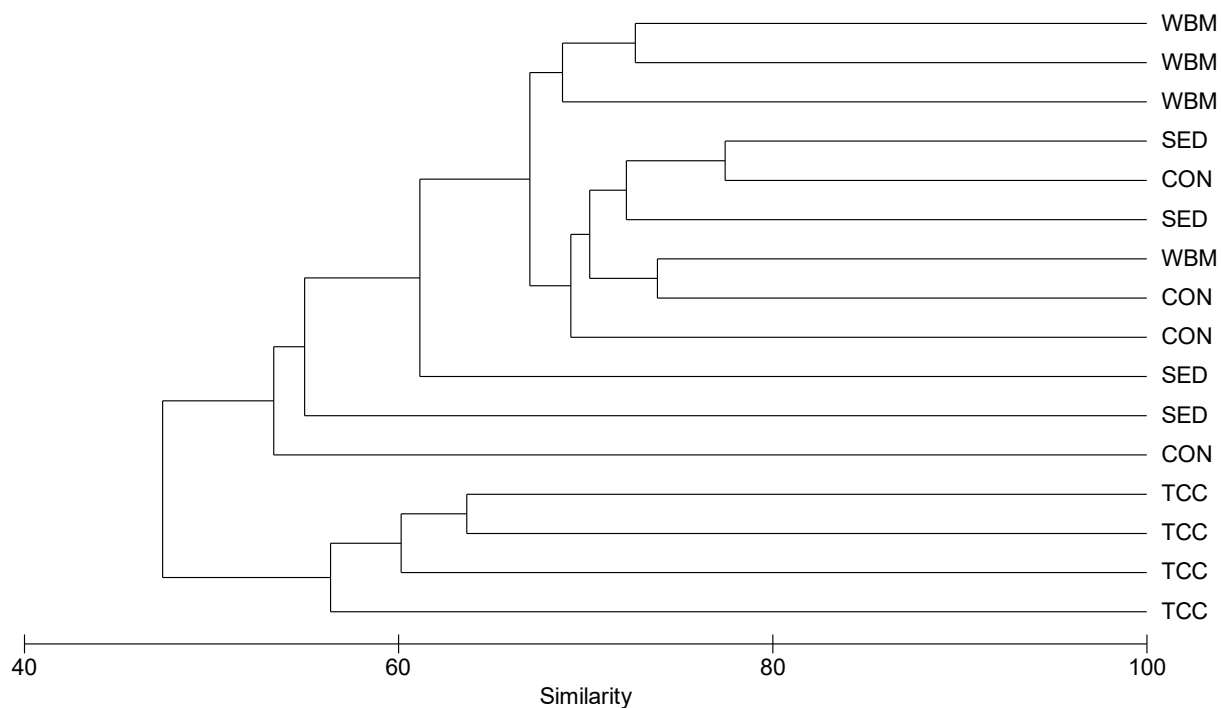


Figure 17. Cluster-plot of fauna from all experimental boxes (square root transformed data, Bray-Curtis similarity).

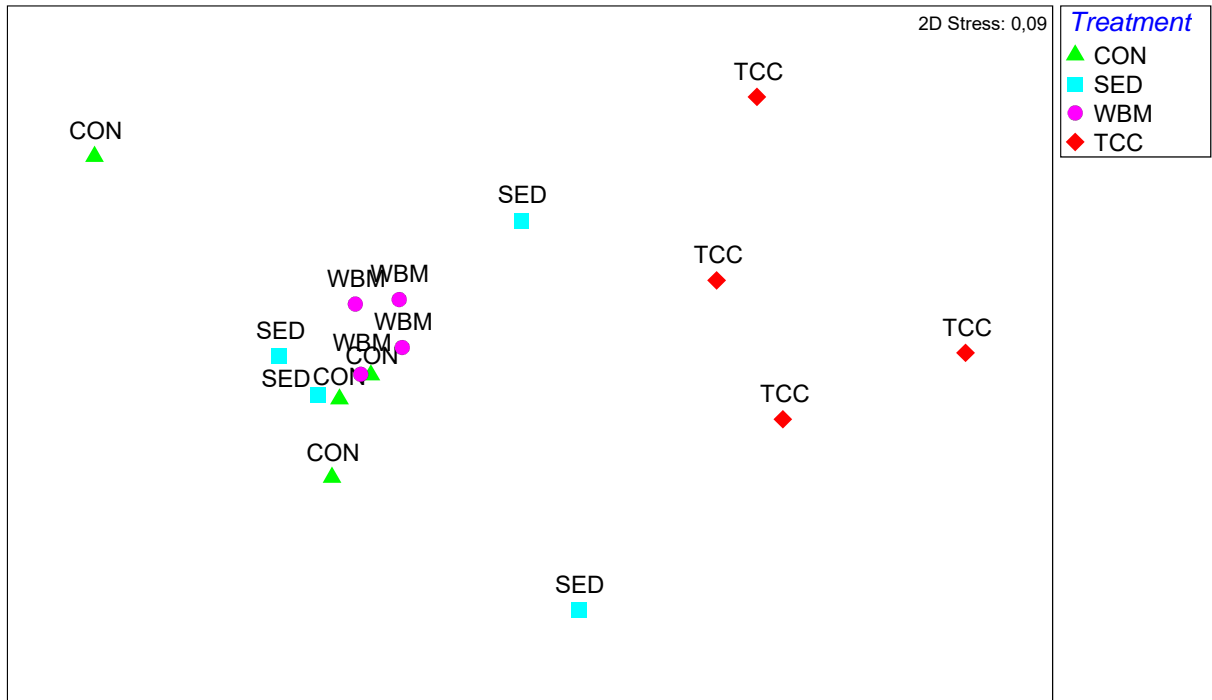


Figure 18. MDS-ordination of fauna from all experimental boxes (square root transformed data, Bray-Curtis similarity).

In order to test the multivariate pattern, PERMANOVA was performed, see Table 5. This test confirmed a significant effect of TCC, but not of WBM, on the composition of the faunal communities. The TCC-treatment was significantly different from all other treatments, while none of the other treatments showed significant differences between them.

Thus, to conclude on faunal effects, all analyses showed a very clear negative effect of TCC, but not of WBM.

Table 5. Results of PERMANOVA for faunal composition (square root transformed data, unrestricted permutation of raw data). Significance levels for p values: \*\*\*<0.001, \*\* < 0.01, \* < 0.05. P-values calculated both by permutations and Monte Carlo asymptotic distributions.

Main test	df	SS	MS	Pseudo-F	p <sub>perm</sub>	Unique perms	p <sub>mc</sub>
Treatment	3	5966	1989	2.662	0.0009***	9893	0.0039**
Residual	12	8964	747				
Total	15	14930					
Pairwise test					p <sub>perm</sub>	Unique perms	p <sub>mc</sub>
CON, SED					0.5646	35	0.4475
CON, TCC					0.0316*	35	0.0087**
CON, WBM					0.4267	35	0.3887
SED, TCC					0.0238*	35	0.0273*
SED, WBM					0.6023	35	0.4863
TCC, WBM					0.0292*	35	0.0078**

Time zero-samples were also collected, i.e. samples sieved in the field. These were collected with a standard 0.1 m<sup>2</sup> van Veen grab. The number of individuals and species were within the range of the control-boxes, but in the upper part. However, the grab has a somewhat larger bite area than the box-corer, and may sample the community a bit differently. Thus, in accordance with previous experiments, the mesocosm setup is considered to have maintained the communities intact throughout the experimental period.

### 3.5 Bottle slurry incubation

#### 1) Oxygen consumption measurements:

Organic carbon (OC) was detected in all substrates with similar content in control sediment (here denoted CS) and defaunated sediment (here denoted DS) (1.28 & 1.57 % dry weight) and lower in TCC (0.87 %) and lowest in WBM cuttings (0.4 %, Table 1). However, highest OC reactivity was recorded in WBM cuttings (Figure 19, 3-4 x higher than all other substrates, ANOVA,  $p < 0.01$ ). Reactivity of TCC-OC was also significantly higher than OC in CS & DS (ANOVA,  $p = 0.02$ ).

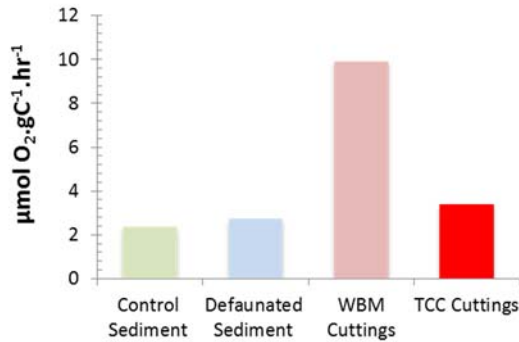


Figure 19. Relative reactivity of the organic carbon (oxygen consumption:  $\mu\text{molO}_2.\text{gC}^{-1}.\text{hr}^{-1}$ ) in the different substrates.

However, when cutting material was mixed with CS (simulating a deposit on the seafloor), only mixtures with WBM significantly increased sediment oxygen consumption (Figure 20; ANOVA,  $p < 0.01$ ). There was no significant difference between CS, DS & TCC mixtures.

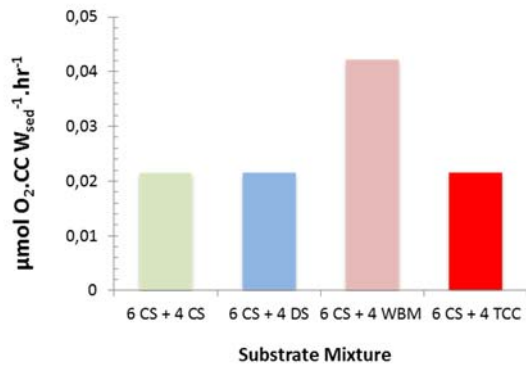


Figure 20. Impact of thin deposit (40 % cuttings) of drill cuttings on sediment oxygen consumption rates ( $\mu\text{molO}_2.\text{CCWsed}^{-1}.\text{hr}^{-1}$ ). 10 ml control sediment (CS) compared to mixtures consisting of 6 ml CS with 4 ml of defaunated sediment (DS) versus 4 ml WBM cuttings versus 4 ml TCC cuttings.

With a larger fraction of drill cuttings, again only WBM led to enhanced oxygen consumption (ANOVA,  $p=0.02$ , Figure 21). Additionally, oxygen consumption rates per CC mixture were lower with higher content of cuttings (compare Figure 21 and Figure 22). When a thicker layer of mixture is used, a larger fraction of OC degradation may occur anaerobically. Therefore, in separate incubations, CO<sub>2</sub> production rates were measured that included both oxic and anoxic processes (Figure 22).

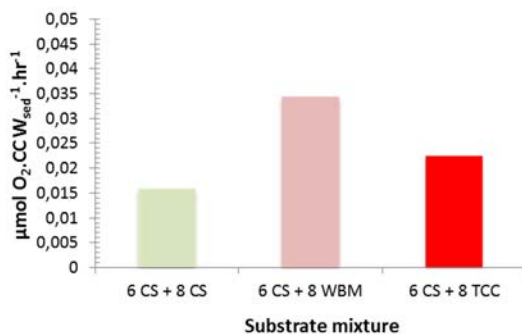


Figure 21. Impact of thick deposit (57 % cuttings) of drill cuttings on sediment oxygen consumption rates ( $\mu\text{molO}_2.\text{CCWsed}^{-1}.\text{hr}^{-1}$ ). 14 ml wet control sediment (CS) compared to mixtures consisting of 6 ml CS with 8 ml WBM versus 8 ml TCC cuttings.

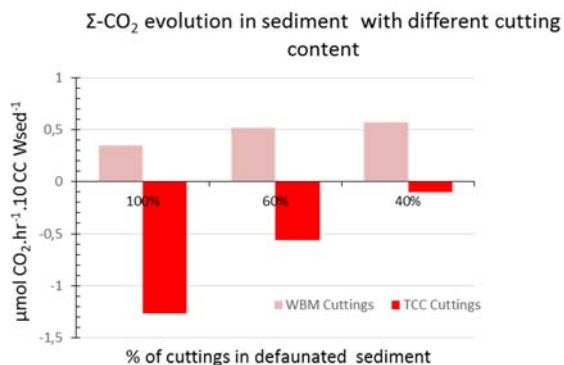
2) Evolution of  $\Sigma\text{-CO}_2$ :

Figure 22. Evolution of  $\Sigma\text{-CO}_2$  ( $\mu\text{mol } \Sigma\text{-CO}_2 \cdot \text{hr}^{-1} \cdot 10 \text{ ml wetsed}^{-1}$ ) in defaunated sediment with different contents (40 – 100 %) of WBM versus TCC cuttings.

There was a significant difference between  $\Sigma\text{-CO}_2$  evolution in WBM versus TCC mixtures (Figure 22, ANOVA,  $p = 0.01$ ); the striking difference being that there was  $\text{CO}_2$  production in WBM mixtures, which confirms trends seen in oxygen consumption rates, but surprisingly a consumption of  $\text{CO}_2$  was found in TCC mixtures. There was a strong correlation between % TCC and  $\text{CO}_2$  consumption (Figure 22; significant difference between % TCC cuttings, ANOVA,  $p = 0.04$ ) and this suggests that initial oxygen consumption measured in TCC (see Figure 20 and Figure 21) may not only reflect TCC-OC degradation, but more chemical oxygen consumption, as also concluded by AquateamCowi (2014) for several TCC treated oil-based cuttings. This would also be conforming that TCC-OC would be hydrocarbons that are not expected to degrade as rapidly as, or faster than CS & DS-OC. It is also interesting to note that oxygen saturated seawater was used in preparing all slurries and that the start dissolved oxygen concentration was indeed close to 100 % air saturation for all slurries except for TCC slurries. In TCC slurries this was always lower and lowest was found in pure TCC cutting incubations (approximately 87 % air saturation, data not shown). Before stabilizing at the lowered start value, there was a rapid decrease in oxygen content and we hypothesize that this due to oxygen going out of solution: calcium hydroxide formation is an exothermic reaction, so that with increase in temperature, partial oxygen degassing may have occurred. In any case, it is related to some characteristic of TCC. This was not the case for WBM slurries.

In WBM mixtures there is a strong correlation between  $\Delta\delta^{13}\text{C-}\Sigma\text{CO}_2$  and  $\Sigma\text{-CO}_2$  production (Figure 23A), clearly indicating degradation of OC leading to more depleted  $\delta^{13}\text{C-}\Sigma\text{CO}_2$  with higher production of  $\text{CO}_2$  (exact  $\delta^{13}\text{C}$  of OC in WBM awaits analysis). Start  $\delta^{13}\text{C-}\Sigma\text{CO}_2$  of WBM slurries was  $+0.51 \pm 0.10 \text{ ‰}$  (data not shown).

In TCC mixtures, there is also a clear correlation between  $\Delta\delta^{13}\text{C-}\Sigma\text{CO}_2$  and  $\Sigma\text{-CO}_2$  evolution, but in this case a consumption of  $\text{CO}_2$  (Figure 23B). This points to  $\text{CaCO}_3$  precipitation where inorganic precipitation results in more depleted  $\delta^{13}\text{C-}\Sigma\text{CO}_2$  in the  $\Sigma\text{-CO}_2$  left in solution (Mook, 2000). However, this carbonate precipitation would necessitate high pH in TCC slurries. Unfortunately, not anticipating this result, we did not initially measure the pH in slurry water. However, there was a set of T-0 bottles still closed in the climate room after 2 months and pH measured on an Orion pH meter confirmed indeed that pH was strongly elevated in TCC slurries and again showed a strong correlation with concentration of TCC (Figure 24B).

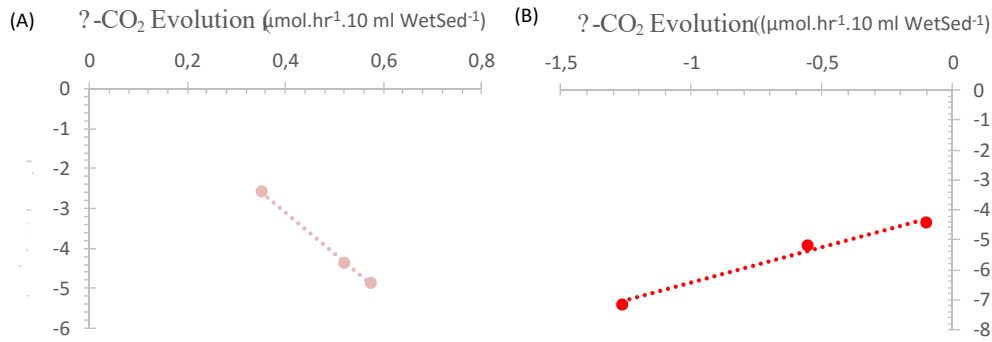


Figure 23. Relationship between  $\Delta\delta^{13}C$  of slurry water and  $\Sigma\text{-CO}_2$  evolution in (A) WBM versus (B) TCC cutting-sediment mixtures presented in Figure 22.

In case of WBM (Figure 24A), production of  $\text{CO}_2$  led to more acidic conditions after 2 months incubation. In the case of TCC, high pH still prevailed even after 2 months (Figure 24B). An independent test on pH of 10 ml TCC incubated for 3.5 days as in Figure 22 revealed a pH of 9.44 (data not shown) suggesting that initial pH in TCC slurries must have been even higher because  $\text{CaCO}_3$  precipitation would lead to drop in pH (e.g. Chave & Suess, 1970). AquateamCowi (2014) also reported elevated pH in TCC treated drill cutting solutions (pH 8.1 -9.2). This suggests that a constituent of TCC increases pH instantly and a potential candidate is CaO which is common constituent of oil-based drilling mud (evidently also in Martin Linge cuttings, see Table 6), that upon hydration forms  $\text{Ca(OH)}_2$  (e.g. Sonawane & Kulkarni 2011), that inherently has a high pH (12.5- 12.8, e.g. Athanassiadis et al., 2007). Given that CaO is formed through process called calcination ( $\text{CaCO}_3 = \text{CaO} + \text{CO}_2$ ) at temperature  $> 800^\circ\text{C}$ , calcium oxide is not produced during the TCC treatment that does not exceed  $300^\circ\text{C}$  (Kleppe, 2009). Hydrated lime or calcium hydroxide is a source of Ca and OH and is commonly added in excess to oil-based drilling fluid to maintain elevated pH that protect against corrosion due to sulfide ( $\text{H}_2\text{S}$ ) and carbon dioxide ( $\text{CO}_2$ ) gases, and prevents bacteria souring the drilling fluid (<http://petrowiki.org>). However, lime changes the carbonate balance towards higher pH and precipitates soluble carbonate ions as  $\text{CaCO}_3$  as follows:  $\text{Ca}^{2+}(\text{aqueous}) + \text{CO}_3^{2-}(\text{aqueous})$  to form  $\text{CaCO}_3$  (solid) at pH  $> 10.3$  (e.g. Sonawane & Kulkarni 2011), thereby decreasing the pH (e.g. Chave & Suess, 1970).

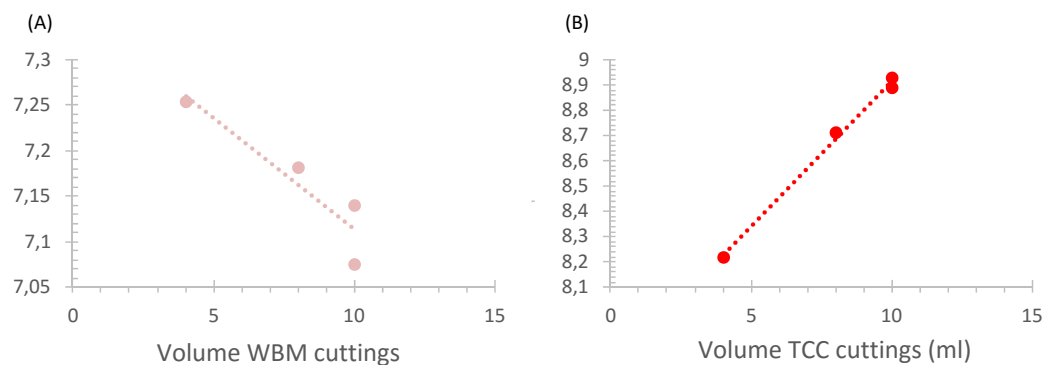


Figure 24. Check on the relationship between volume of cuttings used and measured pH of the slurry water from 2 months old samples. (A) WBM versus (B) TCC cuttings.

Table 6. Major components in the TCC cuttings quantified as oxides (% of dry sediment).

Element	ML 8 ½ inch TCC cuttings
SiO <sub>2</sub>	54.10
Al <sub>2</sub> O <sub>3</sub>	4.14
CaO	10.30
Fe <sub>2</sub> O <sub>3</sub>	3.24
K <sub>2</sub> O	0.84
MgO	0.69
MnO	0.19
Na <sub>2</sub> O	0.44
P <sub>2</sub> O <sub>5</sub>	0.04
TiO <sub>2</sub>	0.27
BaSO <sub>4</sub>	11.42
LOI	10.10
Sum Oxides	95.71

So clearly, TCC cuttings led to elevated pH in the slurries that triggered CaCO<sub>3</sub> precipitation accounting for the consumption of Σ-CO<sub>2</sub>. Initially and even after months, elevated pH prevailed and may have been detrimental for biota.

### 3) Respiration of fresh highly degradable diatom carbon

The impact on different cuttings on microbial respiration of highly degradable tracer (<sup>13</sup>C-labelled) diatoms OC was examined a separate set of slurries (Figure 25). Maximum respiration was found in water slurries without sediment (ANOVA,  $p < 0.001$ ). Here, OC is max. exposed to bacteria attack and readily respired ( $14.3 \pm 0.5$  % of added diatom carbon respired within 3.5 days, Figure 25). Diatom respiration in sediment was significantly lower than in water (due to decreased accessibility through dilution in sediment) but higher than in mixture with cuttings (ANOVA,  $p < 0.001$ ). Cuttings evidently increase dilution of diatom carbon and bacteria biomass resulting in decreased respiration of tracer OC. No significant difference was found between 40 or 60 % cutting content. However, the amount respired in TCC treatment was significantly lower than WBM treatment (Figure 25) indicating that alkaline conditions inhibited microbial functioning which may also explain the mass mortality and decrease in macrofauna biomass in TCC mesocosms (Figure 15).

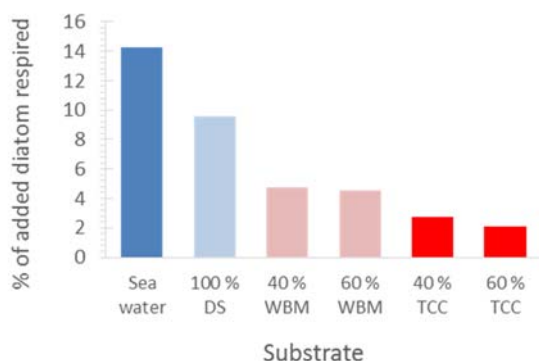


Figure 25. Respiration of tracer diatom C<sub>org</sub> after 3.5 days incubation in seawater versus in different substrates: in defaunated sediment (DS) and mixtures of defaunated sediment with 40 versus 60 % drill cuttings (WBM versus TCC,  $N=2 \pm SD$ ).

### 3.6 Mechanisms for faunal impacts

TCC was very clearly detrimental for the macrofauna. In previous work with TCC, it was concluded that the environmental risk associated with TCC-discharges was considered similar as WBM (Aquateam-COWI, 2014), but the present study does not support this.

The potential mechanisms for the adverse impacts of the TCC treatment on macrofauna are summed up below.

- Hyper-sedimentation: This factor can be eliminated as a causal factor as similar hyper-sedimentation by SED-treatment did not have any adverse impact.
- Particle properties: With the doses added, TCC cutting addition did not drastically alter granulometry of surface sediment. Particle shape was not measured, but is not assumed to have been a considerable impact factor (see below).
- Oxygen depletion: Bottom water and surface sediment oxygen depletion in TCC were not extreme and comparable to that recorded in WBM treatments that had no mass mortality of fauna.
- Starvation. Both materials contained less TOC (total organic carbon) than the natural sediment, and although TOC is not a precise estimate of available organic matter, this could be an indication of lower nutrient value in drill cuttings treatments. WBM had the lowest TOC-content, and the same time there was no significant reduction in biomass in WBM compared to controls or sediment-treatment, which does not point to starvation as an explanation.
- Toxic effects: There were no extreme high heavy metal or hydrocarbon contents in TCC cuttings to account for mass fauna mortality.
- Elevated pH: This inhibited bacteria in slurries, and similarly may explain mass mortality of mesocosm fauna.

Elimination of hyper-sedimentation as a stressor accords well with previous experiments, where layer thickness up to 24 mm of natural sediment not had any measurable effects on the fauna (Trannum et al., 2010). Regarding particle properties, grain size effects as such have not been considered to cause acute mortality of benthic species. On the other hand, physical properties such as particle sharpness may potentially be harmful. Particles of water-based drilling mud have been reported to cause damage of ciliary processes in feeding structures, gill membranes and digestive gland cells of scallops and bivalves (Cranford et al., 1999, Barlow & Kingston, 2001, Bechmann et al., 2006), and such effects have been speculated to arise from an assumed angular configuration of drill cuttings particles (Black et al., 2002; Neff, 2005). However, TCC-particles from Martin Linge have been found to have rounded forms without any sharp or pointed edges (Aquateam-COWI, 2015). The physical damages are therefore expected to be marginal.

There have been conducted standardized toxicity tests with TCC on pelagic (i.e. to predict effects in the water-column during the sedimentation process) and sediment-living species (i.e. to predict effects post-sedimentation). Some tests have been conducted with TCC-cuttings from a land-based plant (Aquateam-COWI, 2014), but there have also been conducted tests with the particular Martin Linge TCC (8 ½ inch as used in the present work) which are summed up by Aquateam-COWI (2015). When these two sets of tests were compared, there were indications of better performance of the Martin Linge TCC treatment with respect to oil removal, but higher toxicity and smaller particles in the Martin Linge TCC compared to the TCC from the land-based plant. In both sets of tests, for leachate, copper (Cu) was the substance that gave the highest contribution to the total toxicity. At the same time, Aquateam-COWI (2015) had no explanation of the higher toxicity measured for the leachate water of the Martin Linge TCC. They concluded that other components that had not been analysed could have increased the toxicity of the leachate water. pH was not discussed as an impact factor, but it is worth noting that alkaline conditions have been observed in previous tests, e.g. pH 9.18 in a test with *Calanus finmarchicus* (Biotrix, 2015).



To conclude on the present experimental work, the occasional evidence of high pH and inhibited microbial activity points to a pH-effect as the most adverse stressor in the present study. This statement should, however, be verified by particularly designed field and experimental approaches.

In previous experiments with WBM, oxygen availability in sediments decreased, and oxygen depletion was concluded to be harmful for the fauna at least with thicker layers of drill cuttings. There was also some indication of a potential toxicity evidenced by standardized bioassay toxicity tests (Trannum et al., 2011 a). Thus effects of WBM were less strong than found in a previous experiment with similar layer thickness (Trannum et al., 2011 a). The reason for this is most likely a combination of another composition/recipe of the cuttings than the one used previously in addition to the pre-treatment procedure intended to remove the water-soluble glycol. It is not possible to directly compare the cuttings as they were not subject to exactly the same analyses by NIVA. The mud used in Trannum et al. (2010, 2011 a and b) had ilmenite as the weight material, while barite was weight material in the present study. Further, the previous mud had glycol as a lubricant, which was assumed to have contributed to the increased oxygen consumption and in turn detrimental faunal effects. Trolla was drilled with KCl/polymer based mud, but the particular chemical composition, incl. the amount of glycol, was not available.

From the present experiment and previous experiments with TCC it may seem like TCC has a larger effect on water- and sediment chemistry than WBM. This not only relates to pH, but there have also been recorded reduced oxygen availability due to chemical processes rather than respiration (Torgeir Bakke, pers. com.).

## 4. Conclusions and recommendations

WBM was characterized by significantly reduced O<sub>2</sub> penetration depth in the sediments and increased sediment oxygen and nitrate consumption. At the same time OC was less in WBM than in the added sediments indicating high OC reactivity. Nevertheless WBM was characterized as non-detrimental for the macrofauna. Thus effects of WBM were less strong than found in a previous experiment with similar layer thickness (Trannum et al., 2011). The reason for this is most likely a combination of another mud recipe and different origin of the cuttings than the one used previously and the pre-treatment procedure intended to remove the water-soluble glycol.

On the other hand, addition of TCC caused a highly impoverished fauna. The TCC-boxes on average had less than one fourth number of individuals than the controls and half number of species. The most sensitive species showed almost 100% mortality. Surface deposit feeding and tube-building species seemed to be the most severely affected by the cuttings. The multivariate test PERMANOVA confirmed the significant effect of TCC, but not of WBM, on the composition of the faunal communities. In this test the TCC-treatment was significantly different from all other treatments, while none of the other treatments differed between each other.

At the start of this study, the major concern with the use of TCC drill cuttings was the potential of oil toxicity to the benthic fauna. However, it is clear that the TCC procedure applied to Martin Linge cuttings was successful in adequately cleaning the oil-based cuttings in this regard. There were not high levels of measured heavy metals or hydrocarbons. Similarly, no adverse effect of hyper-sedimentation nor detrimental oxygen depletion was evident. The effects on oxygen penetration depth and consumption of oxygen and nitrate were significant, but not different from those observed for WBM which had no severe impacts on fauna. However, there were strong alkaline conditions triggered by calcium hydroxide in TCC cuttings, which may be the causal factor of the faunal reduction.

The faunal pattern corresponded well with the field observations where one of the closest stations showed a reduction in number of individuals and species as well as dominance by the tolerant polychaete *Paraprionospio pinnata*. That species was also the only species with no tendency of impacts in the present study. Nevertheless, the effects were more severe in the mesocosm than in the field study. In the field, the closest stations are placed 250 m from the well, and thus effects can be more pronounced within this zone. On the other hand, it is important to be aware of the fact that recruitment is highly restricted in the mesocosm. Further, seawater will have a natural buffering effect which possibly may reduce the impacts in the field. And lastly, water-soluble substances may be “washed out” from the cuttings particles both in the water column and after sedimentation in the field situation. The mesocosm-results are therefore considered to represent a “worst-case scenario”.

The proposed effect of elevated pH needs to be verified by pH monitoring of pore water and overlying water after addition of cuttings in exposure studies as well as in natural seabed sediments. If it is confirmed that Ca(OH)<sub>2</sub> is the sole factor responsible for the effects on fauna, and faunal effects also prevail *in situ* at the offshore discharge locations, preventive actions such as recipe change or pre-discharge neutralisation should be considered.

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## 6. Appendices

### 6.1 Sediment analyses

Table 7. Total organic carbon (TOC, mg/kg) of box sediment (0-1 cm) (analysed by NIVA).

		TOC
CON	G18	14,5
CON	G4	12,8
CON	G10	14,2
CON	G21	10,6
SED	G1	13,4
SED	G14	11,3
SED	G3	14,5
SED	G20	11,6
TCC	G22	10,1
TCC	G23	13,8
TCC	G9	12,2
TCC	G8	9,9
WBM	G12	15,3
WBM	G16	13,9
WBM	G17	13,4
WBM	G25	11,1

Table 8. Chemical and physical analyses of the added materials and box sediment (0-5 cm) (analysed by ALS).

ELEMENT	SAMPLE	WBM	TTC	SED	17	20	25	10	16	21	8	14	22	4	3	9	12	18	1	23	
	Treatment				WBM	SED	WBM	CON	WBM	CON	TTC	SED	TTC	CON	SED	TTC	WBM	CON	SED	TTC	
Tærstoff (E)	%	76,2	99,7		66,3	45,5	52,2	54,1	45,9	45,6	55,1	54,3	50,6	45,2	50,3	55,4	51,4	47,7	56	52,4	57,3
As (Arsen)	mg/kg TS	5,11	3,41	6,49	9,14	5,45	6,13	7,79	8,98	5,2	5,09	6,39	9,72	6,52	6,29	4,93	6,66	7,24	8,42	7,2	
Ba (Barium)	mg/kg TS	2700	8270	63,2	690	115	358	91	932	61,6	7490	59,9	5800	108	75,4	3580	319	66,5	69	7420	
Be (Beryllium)	mg/kg TS	0,565	0,2	0,706	0,692	0,634	0,63	0,654	0,682	0,598	0,501	0,645	0,678	0,667	0,754	0,626	0,652	0,717	0,753	0,617	
Cd (Kadmium)	mg/kg TS	0,17	0,15	<0,10	<0,10	0,11	<0,10	<0,10	<0,10	<0,10	0,15	<0,10	0,18	<0,10	0,1	0,14	<0,10	0,15	0,23	0,21	
Co (Kobolt)	mg/kg TS	10,4	4,87	10,3	10,4	9,45	8,42	8,93	10,2	8,06	8,4	8,92	10,4	10	10,8	9,39	9,75	10,1	10,2	9,19	
Cr (Krom)	mg/kg TS	32,3	26,3	24,2	26	21,6	21,4	22,7	26,4	20,5	22,7	22,1	26,4	23	25,7	22,9	23,6	26,8	25,7	23,6	
Cu (Kopper)	mg/kg TS	13,1	27,5	19,4	20,6	16,2	15,9	18,4	19,5	16,5	18,4	17,2	23	18,3	20,3	18,2	18,4	20,1	20,5	20,4	
Fe (Jern)	mg/kg TS	23400	12600	21700	24400	18600	19300	20900	24600	17900	17200	19900	22200	19500	22200	19200	20600	21800	22700	19600	
Hg (Kvikksølv)	mg/kg TS	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20	<0,20
Mn (Mangan)	mg/kg TS	287	1160	411	426	316	293	283	620	262	541	301	544	390	304	396	379	373	319	364	
Mo (Molybden)	mg/kg TS	0,55	7,2	0,6	0,47	0,41	<0,40	<0,40	0,56	<0,40	2,1	<0,40	1,34	0,43	<0,40	0,7	0,51	0,74	0,45	1,1	
Ni (Nikkel)	mg/kg TS	23,2	23	21	22,1	19,3	18,4	19,7	22,7	18,3	19,8	19,4	22,2	19,9	22,3	20	20	24,7	23,7	22,2	
P (Fosfor)	mg/kg TS	594	94	799	727	558	642	662	713	589	437	630	731	718	715	541	714	666	702	519	
Pb (Bly)	mg/kg TS	11,2	9,1	31,3	30,6	26,7	23,7	28,7	26,4	26,3	21	28,5	30,5	31,3	32,9	27,2	28	32,9	34,5	28,1	
Sr (Strontium)	mg/kg TS	150	739	67,4	95,3	66,3	78,6	66,9	99,7	60,6	349	64,8	280	68,7	64,9	188	75,7	67	69,3	294	
V (Vanadium)	mg/kg TS	37	10,9	44,5	43,9	37,3	35	40,8	43,3	34,1	30,5	38,8	44,8	40,2	47,5	37,3	42	44,6	47,1	39,2	
Zn (Sink)	mg/kg TS	54,2	14,9	83,2	78,8	72,1	66,3	74,9	78,8	68,1	56,5	74,8	80,8	79,6	86,4	71,1	74,9	82,9	84,1	67,9	
Li (Lithium)	mg/kg TS	42,6	7,4	38	40,9	32,7	34,1	37	42,9	31,6	28,1	35	38	34,6	40,2	34,2	36,8	37,5	39,1	32,6	
Ti (Titan)	mg/kg TS	749	32,2	477	506	415	434	441	549	403	313	426	429	443	501	419	470	463	484	366	
Fraksjon >C10-C12	mg/kg TS	<2,0	6,7	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	<2,0	
Fraksjon >C12-C16	mg/kg TS	16,3	34,1	<3,0	5,4	<3,0	<3,0	<3,0	5,7	<3,0	9,4	<3,0	4,3	<3,0	<3,0	<3,0	3,4	<3,0	<3,0	4,4	
Fraksjon >C16-C35	mg/kg TS	18	41	53	72	79	70	86	64	68	59	85	88	87	96	79	87	734	80	71	
Sum >C12-C35	mg/kg TS	34,3	75,1	53	77,4	79	70	86	69,7	68	68,4	85	92,3	87	96	79	90,4	734	80	75,4	
Fraksjon >C35-C40	mg/kg TS	<5,0	11	11,9	17,3	23,1	19,2	26,9	15,5	17,4	13,9	23,4	22,2	20,7	28,8	23,8	23,7	41,4	17,4	14,4	
Fraksjon >C10-C40	mg/kg TS	39	93	66	96	104	93	116	86	87	83	111	116	111	128	107	115	780	99	90	
Kornstørrelse 31,5-63 mm	%	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010
Kornstørrelse 16-31,5 mm	%	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010
Kornstørrelse 8-16 mm	%	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010
Kornstørrelse 4-8 mm	%	0,379	<0,010	1,49	<0,010	0,102	1,85	0,198	0,593	<0,010	<0,010	1,46	<0,010	<0,010	<0,010	<0,010	0,644	<0,010	<0,010	1,58	
Kornstørrelse 2-4 mm	%	0,59	0,367	1,22	1,16	0,305	1,66	0,33	1,12	0,239	0,217	0,348	0,248	0,354	0,335	0,191	1,74	0,378	0,314	0,416	
Kornstørrelse 1-2 mm	%	1,81	0,367	0,927	1,35	0,339	2,36	0,429	1,71	0,62	0,248	0,253	0,355	0,902	0,224	0,414	3,67	0,24	0,27	0,582	
Kornstørrelse 0,5-1 mm	%	1,43	2,76	1,12	1,39	1,19	2,7	0,66	1,32	1,36	1,61	1,17	1,35	0,483	1,17	1,37	2,8	1,2	1,17	1,37	
Kornstørrelse 0,25-0,5 mm	%	1,94	19,7	4,9	4,31	7,02	6,57	2,44	5,66	6,8	7,6	5,57	9,18	6,05	5,62	11,5	5,53	6,56	5,48	7,24	
Kornstørrelse 0,125-0,25 mm	%	4,59	25,6	23,4	13,8	30,3	20,6	31,2	21,4	27,8	23,6	23,4	17,7	24	21,3	25,1	18,2	22,6	23,4	25,3	
Kornstørrelse 0,063-0,125 mm	%	9,64	15,6	11,2	10,3	13,2	11,6	10,4	12	13,6	12,2	12	10,2	12,9	11,1	12,4	11,3	11,5	12,2	13	
Kornstørrelse 0,032-0,063 mm	%	9,46	6,73	4,04	5,4	3,64	4,64	4,17	4,53	4,1	4,32	4,63	4,51	4,72	4,14	4,83	5,18	4,05	4,32	4,34	
Kornstørrelse 0,016-0,032 mm	%	15,3	4,43	8,56	11,3	7,61	8,56	8,99	9,16	7,53	8,63	9,24	10,7	9,2	9,75	7,68	9,74	9,64	9,67	8,07	
Kornstørrelse 0,008-0,016 mm	%	19,8	5,46	15,9	19,8	14	15,3	16,6	17,5	14,5	15,8	17,1	18,5	16,4	18,3	13,6	16,6	17,7	17,5	15	
Kornstørrelse 0,004-0,008 mm	%	13,6	4,38	13,5	15,3	11,3	11,9	12,7	13,4	11,7	12,6	13	13,8	12,7	14,4	10,9	12,5	13,6	13,4	11,8	
Kornstørrelse 0,002-0,004 mm	%	12,2	6,95	9,28	10,5	7,36	7,98	8,03	7,83	7,82	8,61	8,05	8,91	8,21	9,26	7,67	7,99	8,54	8,39	7,56	
Kornstørrelse > 63 mm	%	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010	<0,010
Kornstørrelse < 0,002 mm	%	9,3	7,63	4,5	5,45	3,57	4,17	3,84	3,69	3,86	4,45	3,76	4,52	3,96	4,42	4,28	4	3,9	3,89	3,64	

## 6.2 Oxygen and nutrient fluxes

### 6.2.1 Microelectrode measurements

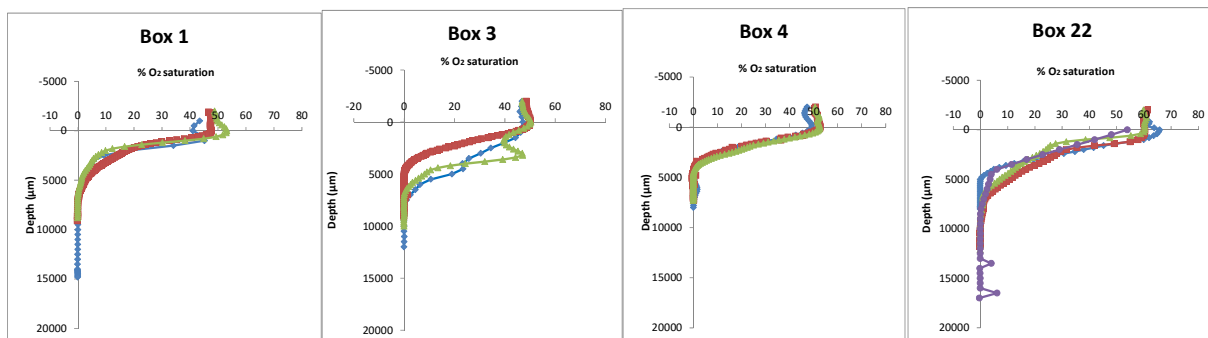


Figure 26. O<sub>2</sub> in sediments before treatment.

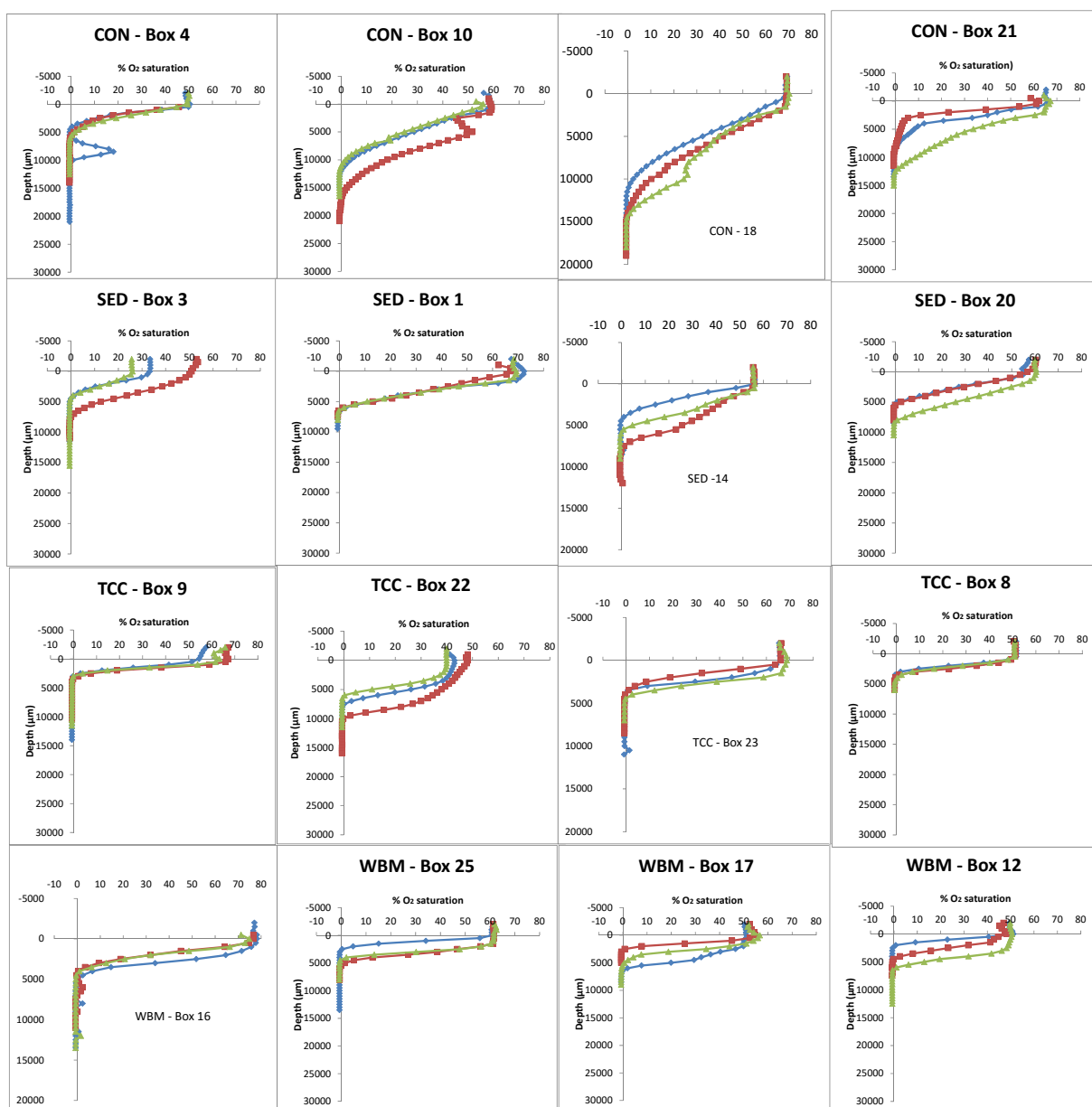


Figure 27. O<sub>2</sub> in sediments 17.12.2015.

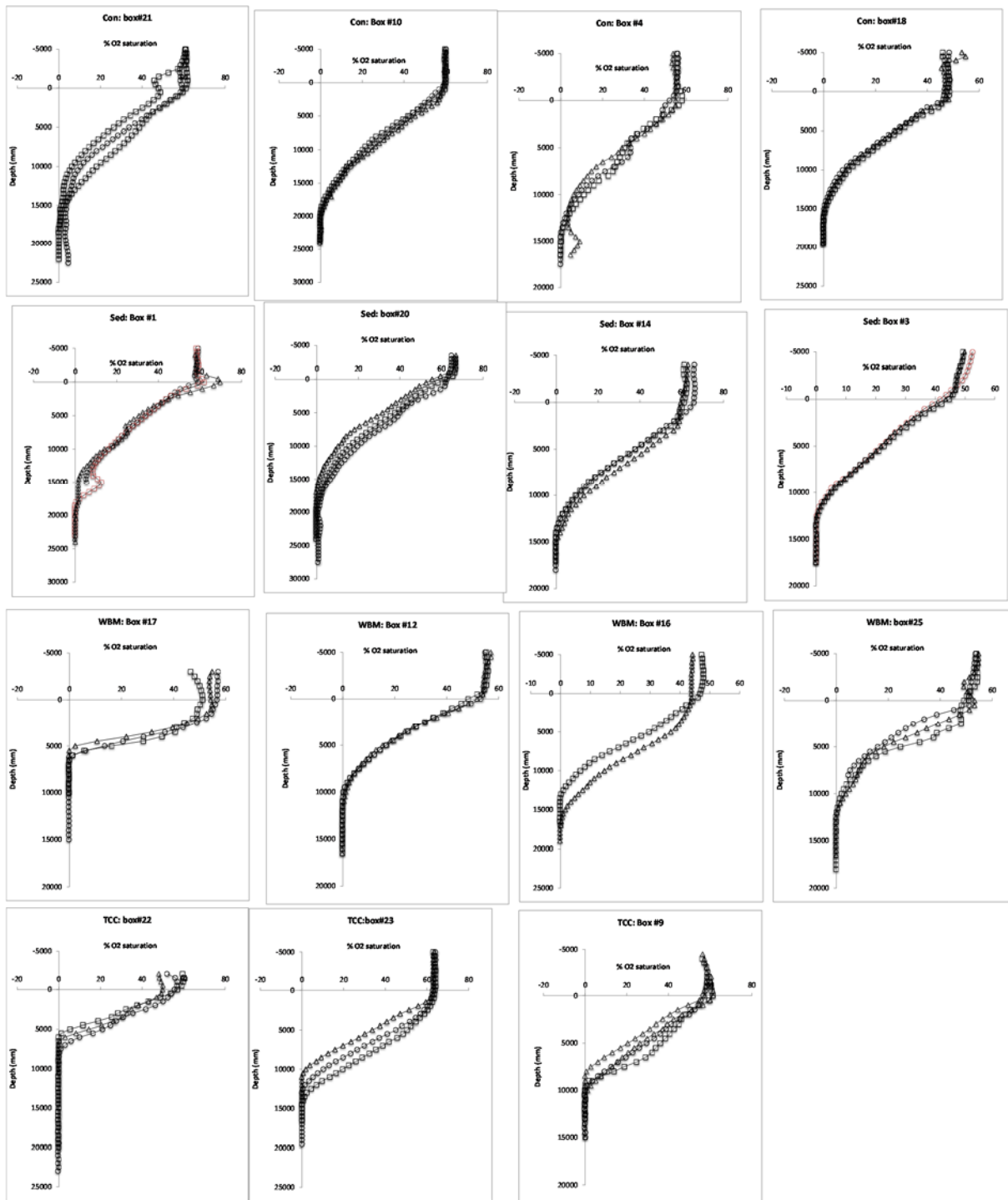
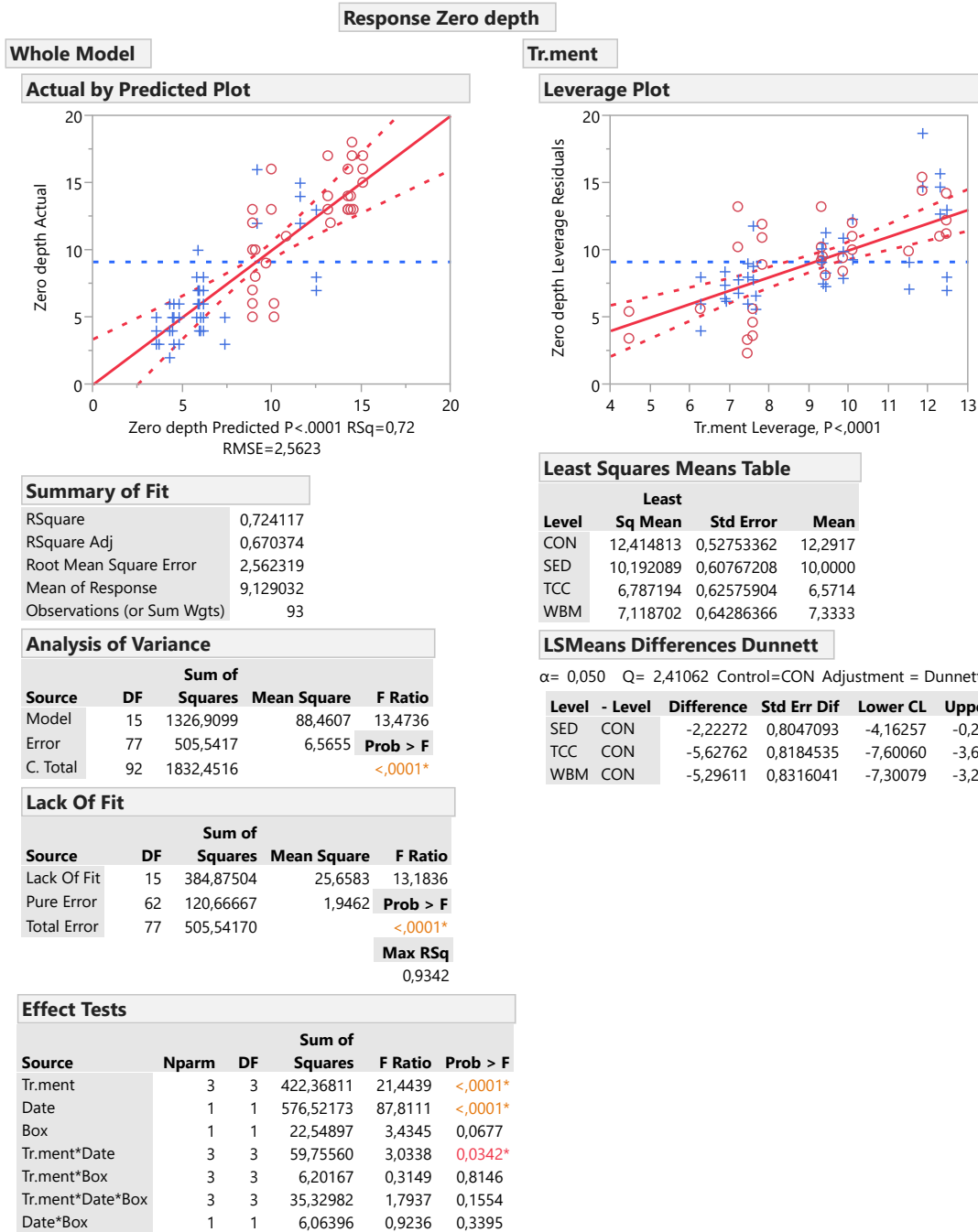


Figure 28. O<sub>2</sub> in sediments 01.03.2016.



### 6.2.2 Statistical analyses on O<sub>2</sub> penetration depth

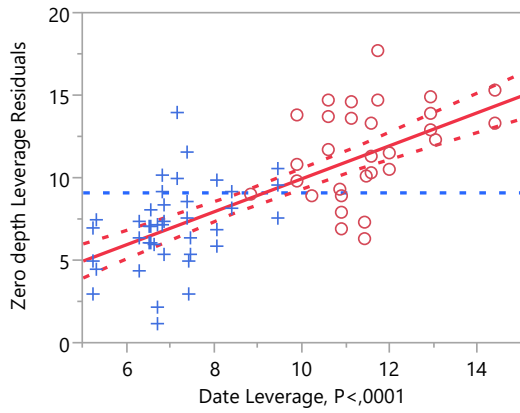


Response Zero depth.... continued

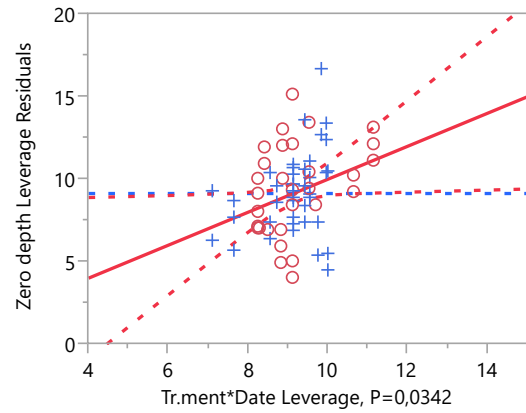
Date

Tr.ment\*Date

Leverage Plot



Leverage Plot



Least Squares Means Table

Level	Least		
	Sq Mean	Std Error	Mean
01.mar	11,951510	0,44158492	12,0889
05.jan	6,304889	0,41000493	6,3542

Least Squares Means Table

Level	Least		
	Sq Mean	Std Error	Mean
CON,01.mar	14,398225	0,74604520	
CON,05.jan	10,431401	0,74604520	
SED,01.mar	14,478648	0,85937810	
SED,05.jan	5,905530	0,85937810	
TCC,01.mar	9,052882	0,99914680	
TCC,05.jan	4,521505	0,75365986	
WBM,01.mar	9,876284	0,90914651	
WBM,05.jan	4,361121	0,90914651	

LSMeans Differences Tukey HSD

$\alpha = 0,050$   $Q = 3,11583$

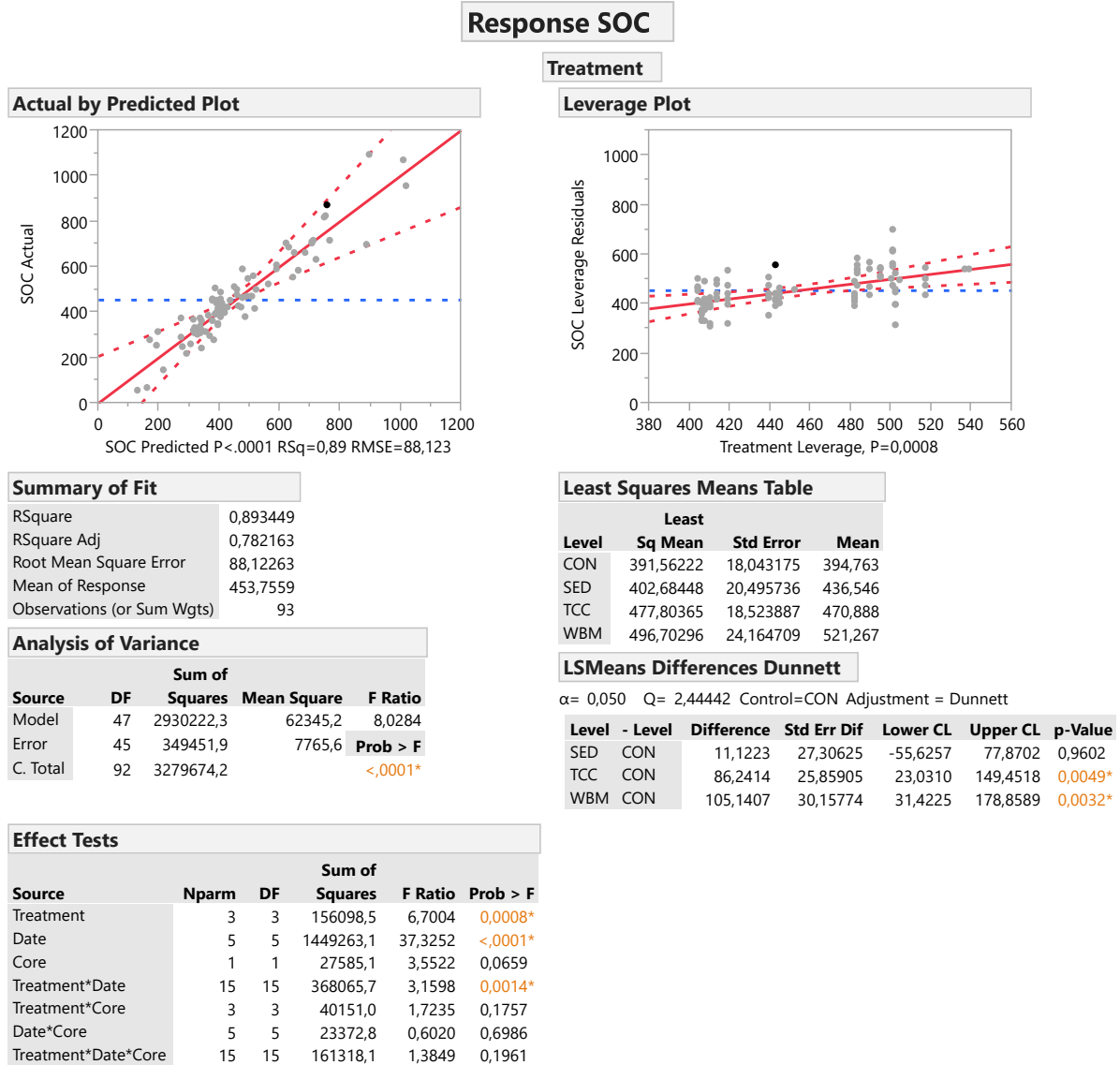
Level	Least	
		Sq Mean
SED,01.mar	A	14,478648
CON,01.mar	A	14,398225
CON,05.jan	B	10,431401
WBM,01.mar	B	9,876284
TCC,01.mar	B C	9,052882
SED,05.jan	C D	5,905530
TCC,05.jan	D	4,521505
WBM,05.jan	D	4,361121

Levels not connected by same letter are significantly different.

**NB! Date 05. jan. is not correct, should be 17.dec (has no impact on stat. analyses).**

### 6.2.3 Statistical analyses of sediment oxygen consumption rates (after addition)

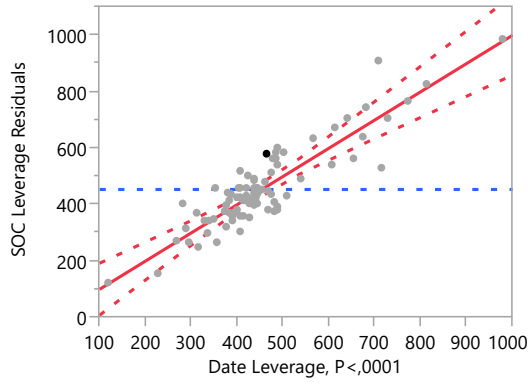
Three observations omitted due to pump failure (pump mixed air into overlying water which resulted in low or negative SOC): Day 8, box 16 and Day 95 box 25 and 16.



Response SOC....continued

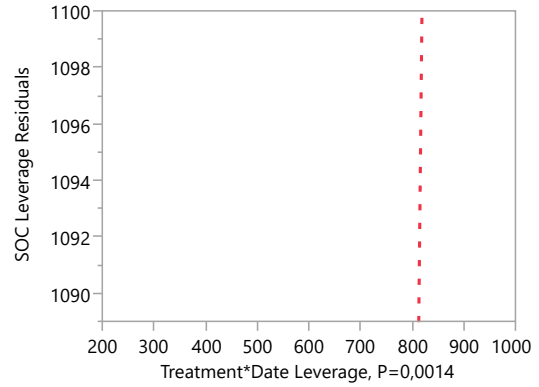
Date

Leverage Plot



Treatment\*Date

Leverage Plot



Least Squares Means Table

Level	Sq Mean	Std Error	Mean
2	475,55024	24,603380	491,594
37	426,18042	24,603380	434,144
51	395,15960	24,603380	411,844
65	365,48553	24,603380	373,963
8	728,93529	25,900787	743,280
95	261,81888	25,911706	261,814

LSMeans Differences Tukey HSD

$\alpha = 0,050$  Q= 2,97598

Level	Least Sq Mean
8	A 728,93529
2	B 475,55024
37	B C 426,18042
51	B C 395,15960
65	C D 365,48553
95	D 261,81888

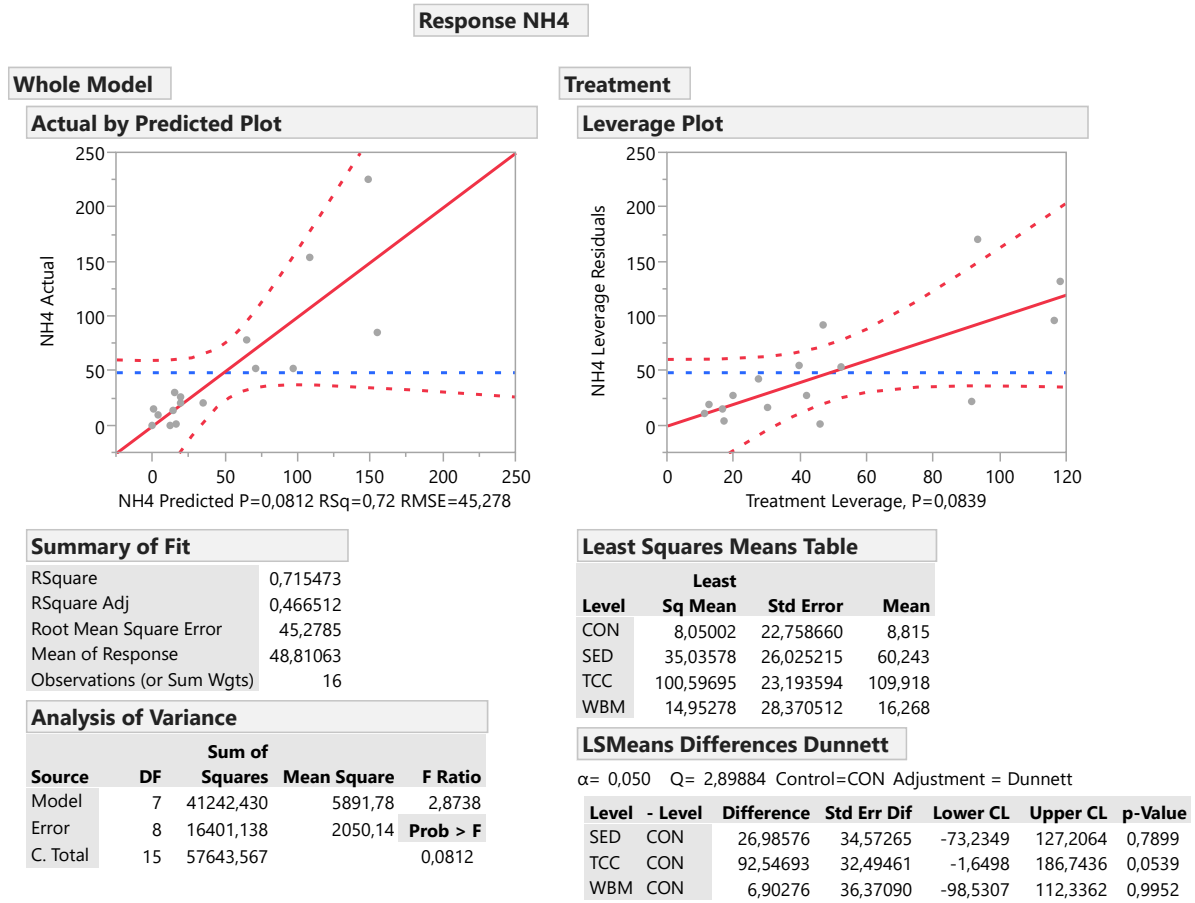
Levels not connected by same letter are significantly different.

Least Squares Means Table

Level	Sq Mean	Std Error
CON,2	318,89736	44,196573
CON,37	406,91642	44,196573
CON,51	396,25982	44,196573
CON,65	400,08534	44,196573
CON,8	578,59267	44,196573
CON,95	248,62170	44,196573
SED,2	520,97245	50,204095
SED,37	367,97989	50,204095
SED,51	314,29855	50,204095
SED,65	324,60737	50,204095
SED,8	687,49980	50,204095
SED,95	200,74882	50,204095
TCC,2	414,38191	45,374071
TCC,37	436,59487	45,374071
TCC,51	442,59724	45,374071
TCC,65	363,45838	45,374071
TCC,8	938,37803	45,374071
TCC,95	271,41146	45,374071
WBM,2	647,94924	56,148255
WBM,37	493,23050	56,148255
WBM,51	427,48277	56,148255
WBM,65	373,79103	56,148255
WBM,8	711,27067	64,815259
WBM,95	326,49355	64,885048

### 6.2.4 Statistical analyses of nutrient fluxes (after addition)

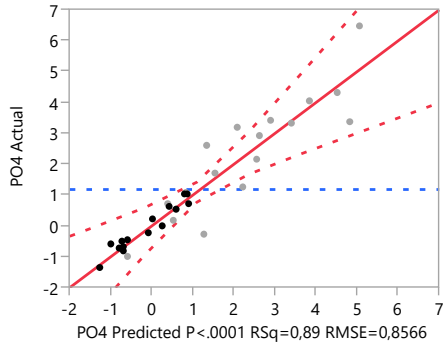
Data from Dec. 14<sup>th</sup> only. Ammonium was not analysed on March 7<sup>th</sup>.



Response PO4

Whole Model

Actual by Predicted Plot



Summary of Fit

RSquare	0,893244
RSquare Adj	0,79316
Root Mean Square Error	0,85656
Mean of Response	1,179688
Observations (or Sum Wgts)	32

Analysis of Variance

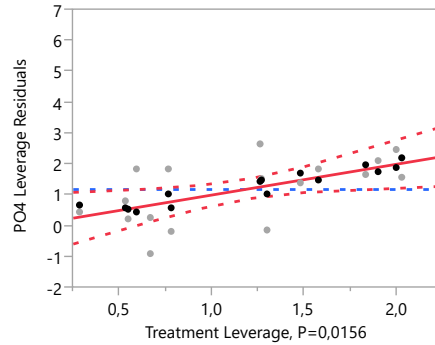
Source	DF	Sum of		F Ratio	Prob > F
		Squares	Mean Square		
Model	15	98,22278	6,54819	8,9249	
Error	16	11,73912	0,73369		Prob > F
C. Total	31	109,96190			<,0001*

Effect Tests

Source	Nparm	DF	Sum of		F Ratio	Prob > F
			Squares	Mean Square		
Treatment	3	3	10,323317	4,6901	0,0156*	
Core n..	1	1	0,000880	0,0012	0,9728	
Date	1	1	46,960587	64,0056	<,0001*	
Treatment*Core n..	3	3	11,166114	5,0730	0,0117*	
Treatment*Date	3	3	7,759884	3,5255	0,0392*	
Core n..*Date	1	1	2,538877	3,4604	0,0813	
Treatment*Core n..*Date	3	3	2,372806	1,0780	0,3864	

Treatment

Leverage Plot



Least Squares Means Table

Level	Least		
	Sq Mean	Std Error	Mean
CON	1,8264183	0,30443702	1,83625
SED	1,3560408	0,34813293	1,84000
TCC	0,5107900	0,31025503	0,69250
WBM	0,3127739	0,37950540	0,35000

LSMeans Differences Dunnett

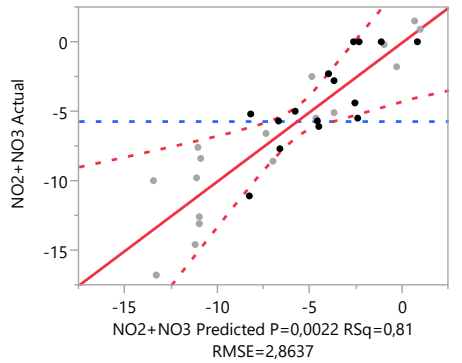
$\alpha = 0,050$   $Q = 2,60678$  Control=CON Adjustment = Dunnett

Level - Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
SED CON	-0,47038	0,4624699	-1,67594	0,735181	0,6363
TCC CON	-1,31563	0,4346724	-2,44872	-0,182532	0,0216*
WBM CON	-1,51364	0,4865247	-2,78191	-0,245380	0,0182*

Response NO2+NO3

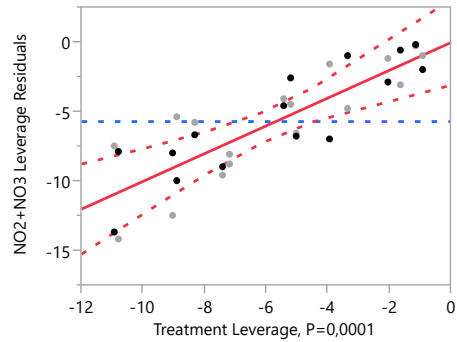
Whole Model

Actual by Predicted Plot



Treatment

Leverage Plot



Summary of Fit

RSquare	0,811649
RSquare Adj	0,63507
Root Mean Square Error	2,863748
Mean of Response	-5,69344
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	15	565,44492	37,6963	4,5965	
Error	16	131,21681	8,2011		<b>0,0022*</b>
C. Total	31	696,66172			

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
CON	-1,073428	1,0178281	-1,0038
SED	-3,630964	1,1639172	-4,0950
TCC	-9,796651	1,0372795	-9,8350
WBM	-7,561605	1,2688051	-7,8400

LSMeans Differences Dunnett

α= 0,050 Q= 2,60678 Control=CON Adjustment = Dunnett

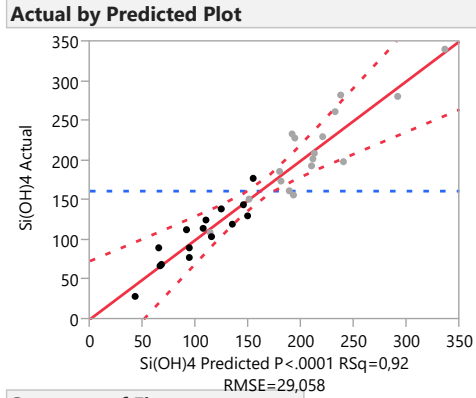
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
SED	CON	-2,55754	1,546181	-6,5881	1,47302	0,2742
TCC	CON	-8,72322	1,453246	-12,5115	-4,93493	<b>&lt;,0001*</b>
WBM	CON	-6,48818	1,626604	-10,7284	-2,24797	<b>0,0030*</b>

Effect Tests

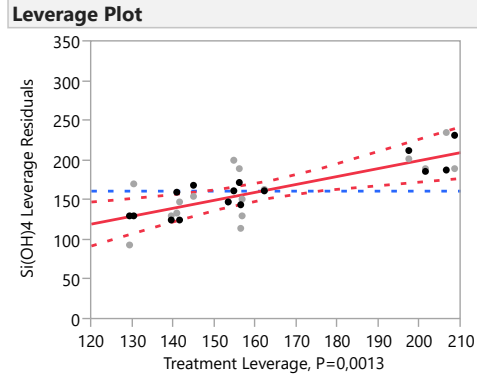
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	3	3	338,26796	13,7490	<b>0,0001*</b>
Core n..	1	1	0,76141	0,0928	0,7645
Date	1	1	48,93046	5,9664	<b>0,0266*</b>
Treatment*Core n..	3	3	10,30297	0,4188	0,7420
Treatment*Date	3	3	79,19410	3,2189	0,0509
Core n..*Date	1	1	4,31440	0,5261	0,4787
Treatment*Core n..*Date	3	3	24,68578	1,0034	0,4168

Response Si(OH)4

Whole Model



Treatment



Summary of Fit

RSquare	0,916016
RSquare Adj	0,837282
Root Mean Square Error	29,05768
Mean of Response	161,3806
Observations (or Sum Wgts)	32

Analysis of Variance

Source	DF	Sum of		
		Squares	Mean Square	F Ratio
Model	15	147350,32	9823,35	11,6342
Error	16	13509,58	844,35	<b>Prob &gt; F</b>
C. Total	31	160859,91		<b>&lt;,0001*</b>

Least Squares Means Table

Level	Least		
	Sq Mean	Std Error	Mean
CON	202,59344	10,327631	204,526
SED	143,75787	11,809958	155,539
TCC	132,94895	10,524999	133,739
WBM	153,43025	12,874228	151,719

LSMeans Differences Dunnett

α= 0,050 Q= 2,60678 Control=CON Adjustment = Dunnett

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
SED	CON	-58,8356	15,68869	-99,733	-17,9386	<b>0,0049*</b>
TCC	CON	-69,6445	14,74570	-108,083	-31,2057	<b>0,0007*</b>
WBM	CON	-49,1632	16,50472	-92,187	-6,1390	<b>0,0238*</b>

Effect Tests

Source	Nparm	DF	Sum of		
			Squares	F Ratio	Prob > F
Treatment	3	3	21756,163	8,5889	<b>0,0013*</b>
Core n..	1	1	2278,443	2,6985	0,1199
Date	1	1	95945,652	113,6327	<b>&lt;,0001*</b>
Treatment*Core n..	3	3	3035,152	1,1982	0,3421
Treatment*Date	3	3	2425,387	0,9575	0,4366
Core n..*Date	1	1	6471,284	7,6642	<b>0,0137*</b>
Treatment*Core n..*Date	3	3	8276,153	3,2673	<b>0,0488*</b>



## 6.3 Macrofauna species list

STA	GRUPPENAVN	FAMILIENAVN	GYLDIG_SYNONYM_WoRMS_sp	G10	G18	G21	G4
CON	ANTHOZOA	Cerianthidae	Cerianthus lloydii		1		
CON	ANTHOZOA	Edwardsiidae	Paraedwardsia arenaria	1			2
CON	PLATYHELMINTHES		Platyhelminthes indet			1	
CON	NEMERTEA		Nemertea indet	2	3	21	6
CON	POLYCHAETA	Amphinomidae	Paramphinome jeffreysii	4	3	20	14
CON	POLYCHAETA	Polynoidae	Gattyana cirrhosa				2
CON	POLYCHAETA	Polynoidae	Harmothoe extenuata				2
CON	POLYCHAETA	Sigalionidae	Neoleanira tetragona		3	2	2
CON	POLYCHAETA	Phyllodocidae	Chaetoparia nilssoni	2		1	
CON	POLYCHAETA	Phyllodocidae	Sige fusigera				1
CON	POLYCHAETA	Pholoidae	Pholoe baltica			1	
CON	POLYCHAETA	Pholoidae	Pholoe pallida		1	2	16
CON	POLYCHAETA	Hesionidae	Oxydromus flexuosus				1
CON	POLYCHAETA	Pilargidae	Pilargis sp.			1	
CON	POLYCHAETA	Syllidae	Exogone (Exogone) verugera				20
CON	POLYCHAETA	Syllidae	Parexogone hebes				7
CON	POLYCHAETA	Syllidae	Syllides longocirratius				3
CON	POLYCHAETA	Nereidae	Ceratocephale loveni	4		6	3
CON	POLYCHAETA	Nephtyidae	Aglaophamus pulcher	1			
CON	POLYCHAETA	Sphaerodoridae	Sphaerodoropsis disticha				1
CON	POLYCHAETA	Goniadidae	Goniada maculata	2			
CON	POLYCHAETA	Onuphidae	Paradiopatra quadricuspis		2		
CON	POLYCHAETA	Dorvilleidae	Ophryotrocha sp.				7
CON	POLYCHAETA	Paraonidae	Levinsenia gracilis	1	2	3	1
CON	POLYCHAETA	Paraonidae	Paradoneis eliasoni				6
CON	POLYCHAETA	Paraonidae	Paradoneis lyra			1	
CON	POLYCHAETA	Spionidae	Prionospio cirrifera		1	3	14
CON	POLYCHAETA	Spionidae	Prionospio dubia	2	1	4	
CON	POLYCHAETA	Spionidae	Prionospio fallax				19
CON	POLYCHAETA	Spionidae	Pseudopolydora paucibranchiata	43	54	54	8
CON	POLYCHAETA	Spionidae	Spiophanes kroyeri	62	67	56	16
CON	POLYCHAETA	Cirratulidae	Chaetozone setosa	9	5	4	16
CON	POLYCHAETA	Cirratulidae	Macrochaeta polyonyx				1
CON	POLYCHAETA	Cirratulidae	Tharyx killariensis				4
CON	POLYCHAETA	Flabelligeridae	Diplocirrus glaucus	1	3	1	8
CON	POLYCHAETA	Scalibregmidae	Scalibregma inflatum				1
CON	POLYCHAETA	Opheliidae	Ophelina cylindricaudata				4
CON	POLYCHAETA	Opheliidae	Ophelina modesta				5
CON	POLYCHAETA	Opheliidae	Ophelina norvegica			1	
CON	POLYCHAETA	Capitellidae	Heteromastus filiformis	7	13	14	14
CON	POLYCHAETA	Capitellidae	Notomastus latericeus				1
CON	POLYCHAETA	Maldanidae	Chirimia biceps biceps		1		
CON	POLYCHAETA	Maldanidae	Euclymene droebachiensis			1	
CON	POLYCHAETA	Maldanidae	Euclymeninae indet	15	7	16	
CON	POLYCHAETA	Maldanidae	Praxillura longissima			1	
CON	POLYCHAETA	Maldanidae	Rhodine loveni		1		1
CON	POLYCHAETA	Oweniidae	Galathowenia oculata	2			
CON	POLYCHAETA	Pectinariidae	Lagis koreni		1		1
CON	POLYCHAETA	Ampharetidae	Anobothrus gracilis	1			
CON	POLYCHAETA	Terebellidae	Paramphitrite tetrabanchia	1			
CON	POLYCHAETA	Terebellidae	Streblosoma intestinale			2	
CON	POLYCHAETA	Trichobranchidae	Terebellides stroemii	5	2	4	1

CON	POLYCHAETA	Trichobranchidae	Trichobranchus roseus				2
CON	POLYCHAETA	Sabellidae	Chone sp.	6		1	3
CON	POLYCHAETA	Sabellidae	Euchone papillosa	3			
CON	POLYCHAETA	Sabellidae	Jasmineira candela		5	4	5
CON	OLIGOCHAETA		Oligochaeta indet				10
CON	OPISTHOBANCHIA	Diaphanidae	Diaphana minuta				1
CON	OPISTHOBANCHIA	Retusidae	Retusa sp.		1	1	3
CON	OPISTHOBANCHIA	Philineidae	Philine sp.				1
CON	CAUDOFOVEATA		Caudofoveata indet	3			3
CON	BIVALVIA	Nuculidae	Ennucula tenuis	1	2	1	6
CON	BIVALVIA	Nuculidae	Nucula sp. juvenil	15	12	13	55
CON	BIVALVIA	Nuculidae	Nucula sulcata	7	10	8	15
CON	BIVALVIA	Nuculidae	Nucula tumidula	1	1	5	1
CON	BIVALVIA	Nuculanidae	Yoldiella lucida	1		1	
CON	BIVALVIA	Nuculanidae	Yoldiella nana	1		1	1
CON	BIVALVIA	Nuculanidae	Yoldiella philippiana	8	5	3	25
CON	BIVALVIA	Limidae	Limatula subauriculata		1		
CON	BIVALVIA	Pectinidae	Delectopecten vitreus	2			
CON	BIVALVIA	Thyasiridae	Adontorhina similis	13	7	10	152
CON	BIVALVIA	Thyasiridae	Axinulus croulinensis		2		2
CON	BIVALVIA	Thyasiridae	Mendicula ferruginosa	20	14	21	19
CON	BIVALVIA	Thyasiridae	Thyasira equalis	51	63	52	61
CON	BIVALVIA	Thyasiridae	Thyasira flexuosa	1			
CON	BIVALVIA	Thyasiridae	Thyasira sarsii				3
CON	BIVALVIA	Lasaeidae	Kurtiella bidentata				1
CON	BIVALVIA	Lasaeidae	Tellimya tenella		7		21
CON	BIVALVIA	Cardiidae	Parvicardium minimum		1		
CON	BIVALVIA	Scrobiculariidae	Abra nitida	29	29	2	43
CON	BIVALVIA	Kelliellidae	Kelliella miliaris	20	36	16	41
CON	BIVALVIA	Cuspidariidae	Cuspidaria obesa			1	1
CON	BIVALVIA	Cuspidariidae	Tropidomya abbreviata	2	3	1	6
CON	OSTRACODA	Cypridinidae	Philomedes (Philomedes) lilljeborgi		2		
CON	CUMACEA	Leuconidae	Eudorella cf. truncatula	1			
CON	CUMACEA	Diastylidae	Diastylis cornuta	1			
CON	TANAIDACEA	Parathanidae	Tanaidacea indet				6
CON	ISOPODA	Parasellidae	Desmosoma sp.				8
CON	ISOPODA	Parasellidae	Ilyarachna sp.	1			
CON	AMPHIPODA	Melitidae	Eriopisa elongata	9	8	8	8
CON	AMPHIPODA	Phoxocephalidae	Harpinia crenulata			1	1
CON	AMPHIPODA	Phoxocephalidae	Harpinia sp.		1		7
CON	AMPHIPODA	Corophiidae	Neohela monstrosa				1
CON	AMPHIPODA	Ischyroceridae	Ischyroceridae			1	
CON	SIPUNCULIDA		Golfingiida indet	6			3
CON	SIPUNCULIDA		Nephasoma sp.	6	5	2	10
CON	SIPUNCULIDA		Onchnesoma steenstrupii steenstrupii	3	4	2	1
CON	SIPUNCULIDA		Phascolion (Phascolion) strombus strombus				1
CON	OPHIUROIDEA		Ophiuroidea juvenil		1	2	41
CON	OPHIUROIDEA	Amphilepididae	Amphilepis norvegica		3	3	3
CON	ECHINOIDEA	Brissidae	Brissopsis lyrifera		1		3
CON	ASCIDIACEA	Molgulidae	Molgula sp.		1		

STA	GRUPPENAVN	FAMILIENAVN	GYLDIG_SYNONYM_WoRMS_sp	G1	G14	G20	G3
SED	ANTHOZOA	Cerianthidae	Cerianthus lloydii			1	
SED	ANTHOZOA	Edwardsiidae	Paraedwardsia arenaria		2	1	
SED	NEMERTEA		Nemertea indet	15	30	2	17

SED	POLYCHAETA	Amphinomidae	Paramphinome jeffreysii	14	6	4	6
SED	POLYCHAETA	Polynoidae	Gattyana cirrhosa		1		
SED	POLYCHAETA	Polyodontidae	Panthalis oerstedii				1
SED	POLYCHAETA	Sigalionidae	Neoleanira tetragona		1		2
SED	POLYCHAETA	Phyllodocidae	Chaetoparia nilssoni		1		2
SED	POLYCHAETA	Pholoidae	Pholoe baltica		1		2
SED	POLYCHAETA	Pholoidae	Pholoe pallida	5	3		6
SED	POLYCHAETA	Nereidae	Ceratocephale loveni	3	5	1	2
SED	POLYCHAETA	Nephtyidae	Nephtys hystricis	1			1
SED	POLYCHAETA	Goniadidae	Goniada maculata			1	
SED	POLYCHAETA	Onuphidae	Paradiopatra quadricuspis			1	
SED	POLYCHAETA	Dorvilleidae	Ophryotrocha sp.				1
SED	POLYCHAETA	Paraonidae	Levensenia gracilis	2	4		3
SED	POLYCHAETA	Paraonidae	Paradoneis eliasoni				1
SED	POLYCHAETA	Paraonidae	Paradoneis lyra	1			
SED	POLYCHAETA	Spionidae	Prionospio cirrifera		13		3
SED	POLYCHAETA	Spionidae	Prionospio dubia	1	1		1
SED	POLYCHAETA	Spionidae	Prionospio fallax	1			3
SED	POLYCHAETA	Spionidae	Pseudopolydora paucibranchiata	1	25		31
SED	POLYCHAETA	Spionidae	Spiophanes kroyeri	8	54	8	70
SED	POLYCHAETA	Cirratulidae	Chaetozone setosa	5	15	13	9
SED	POLYCHAETA	Cirratulidae	Tharyx killariensis		4		1
SED	POLYCHAETA	Cossuridae	Cossura longocirrata				1
SED	POLYCHAETA	Flabelligeridae	Brada villosa			1	
SED	POLYCHAETA	Flabelligeridae	Diplocirrus glaucus	2	5	5	2
SED	POLYCHAETA	Opheliidae	Ophelina norvegica		2	1	1
SED	POLYCHAETA	Capitellidae	Heteromastus filiformis	9	21	6	30
SED	POLYCHAETA	Maldanidae	Chirimia biceps biceps	1			
SED	POLYCHAETA	Maldanidae	Euclymeninae indet	2	13	2	9
SED	POLYCHAETA	Maldanidae	Maldanidae indet			1	
SED	POLYCHAETA	Maldanidae	Rhodine loveni	1			
SED	POLYCHAETA	Oweniidae	Galathowenia oculata	2		1	
SED	POLYCHAETA	Oweniidae	Owenia sp.			1	
SED	POLYCHAETA	Pectinariidae	Amphictene auricoma		1		
SED	POLYCHAETA	Ampharetidae	Anobothrus gracilis				1
SED	POLYCHAETA	Ampharetidae	Melinna cristata		1	1	1
SED	POLYCHAETA	Terebellidae	Proclea graffii		1		
SED	POLYCHAETA	Trichobranchidae	Terebellides stroemii		6	1	5
SED	POLYCHAETA	Sabellidae	Chone sp.		4	2	5
SED	POLYCHAETA	Sabellidae	Euchone papillosa			1	4
SED	POLYCHAETA	Sabellidae	Jasmineira candela		1		1
SED	POLYCHAETA	Sabellidae	Sabellidae indet				1
SED	OLIGOCHAETA		Oligochaeta indet	4			4
SED	OPISTHOBANCHIA	Diaphanidae	Diaphana minuta	1			
SED	OPISTHOBANCHIA	Retusidae	Retusa sp.	1			1
SED	CAUDOFOVEATA		Caudofoveata indet	2	2	1	
SED	BIVALVIA	Nuculidae	Ennucula tenuis	3	1		1
SED	BIVALVIA	Nuculidae	Nucula sp. juvenil	7	25	4	17
SED	BIVALVIA	Nuculidae	Nucula sulcata	8	6	13	5
SED	BIVALVIA	Nuculidae	Nucula tumidula	5	5		10
SED	BIVALVIA	Nuculanidae	Nuculana sp. juvenil	1			
SED	BIVALVIA	Nuculanidae	Yoldiella nana	3			3
SED	BIVALVIA	Nuculanidae	Yoldiella philippiana			6	11
SED	BIVALVIA	Arcidae	Bathyarca pectunculoides			1	
SED	BIVALVIA	Pectinidae	Palliolum sp. juvenil				2
SED	BIVALVIA	Thyasiridae	Adontorhina similis	6	17	5	16

SED	BIVALVIA	Thyasiridae	Axinulus croulinensis		2		1
SED	BIVALVIA	Thyasiridae	Mendicula ferruginosa	8	20	3	17
SED	BIVALVIA	Thyasiridae	Thyasira equalis	34	46	35	51
SED	BIVALVIA	Thyasiridae	Thyasira obsoleta	1			
SED	BIVALVIA	Thyasiridae	Thyasira sarsii	1			
SED	BIVALVIA	Lasaeidae	Tellimya tenella	2			5
SED	BIVALVIA	Cardiidae	Parvicardium minimum		1	2	
SED	BIVALVIA	Scrobiculariidae	Abra nitida	16	5	20	36
SED	BIVALVIA	Kelliellidae	Kelliella miliaris	4	13	5	14
SED	BIVALVIA	Hiatellidae	Hiatella sp.				2
SED	BIVALVIA	Thraciidae	Thracia devexa		1		
SED	BIVALVIA	Cuspidariidae	Tropidomya abbreviata		2		3
SED	OSTRACODA	Cypridinidae	Philomedes (Philomedes) lilljeborgi		1		
SED	CUMACEA	Leuconidae	Eudorella emarginata			1	2
SED	TANAIDACEA	Parathanidae	Tanaidacea indet				3
SED	AMPHIPODA	Stegocephalidae	sp.	1			
SED	AMPHIPODA	Melitidae	Eriopisa elongata	5	7	2	1
SED	AMPHIPODA	Phoxocephalidae	Harpinia crenulata		1		
SED	AMPHIPODA	Phoxocephalidae	Harpinia sp.	1			
SED	SIPUNCULIDA		Golfingiida indet				4
SED	SIPUNCULIDA		Nephasoma sp.	2	8		5
SED	SIPUNCULIDA		Onchnesoma steenstrupii steenstrupii	1	2	3	1
SED	SIPUNCULIDA		Thysanocardia procera				1
SED	OPHIUROIDEA		Ophiuroidea juvenil	5	2		3
SED	OPHIUROIDEA	Amphilepididae	Amphilepis norvegica	5	3	5	7
SED	ECHINOIDEA	Brissidae	Brissopsis lyrifera	2			1

STA	GRUPPENAVN	FAMILIENAVN	GYLDIG_SYNONYM_WoRMS_sp	G22	G23	G8	G9
TCC	ANTHOZOA	Edwardsiidae	Paraedwardsia arenaria				1
TCC	NEMERTEA		Nemertea indet	2	4	11	5
TCC	POLYCHAETA	Amphinomidae	Paramphinoe jeffreysii	14	25	8	9
TCC	POLYCHAETA	Polynoidae	Harmothoe sp.	1			
TCC	POLYCHAETA	Pholoidae	Pholoe baltica		1		
TCC	POLYCHAETA	Pholoidae	Pholoe pallida				2
TCC	POLYCHAETA	Nereidae	Ceratocephale loveni			2	7
TCC	POLYCHAETA	Onuphidae	Paradiopatra fiordica			1	
TCC	POLYCHAETA	Onuphidae	Paradiopatra quadricuspis	1	1	1	
TCC	POLYCHAETA	Lumbrineridae	Abyssoninoe hibernica			1	
TCC	POLYCHAETA	Paraonidae	Levinsenia gracilis	1	1	1	
TCC	POLYCHAETA	Spionidae	Prionospio cirrifera	1		1	
TCC	POLYCHAETA	Spionidae	Pseudopolydora paucibranchiata				1
TCC	POLYCHAETA	Spionidae	Spiophanes kroyeri	1	2	7	1
TCC	POLYCHAETA	Chaetopteridae	Spiochaetopterus typicus	1			
TCC	POLYCHAETA	Cirratulidae	Chaetozone setosa		2	5	6
TCC	POLYCHAETA	Flabelligeridae	Diplocirrus glaucus		1		1
TCC	POLYCHAETA	Scalibregmidae	Scalibregma inflatum			1	
TCC	POLYCHAETA	Capitellidae	Heteromastus filiformis	1		9	6
TCC	POLYCHAETA	Maldanidae	Rhodine loveni			2	
TCC	POLYCHAETA	Oweniidae	Galathowenia oculata			1	1
TCC	POLYCHAETA	Pectinariidae	Lagis koreni			1	
TCC	POLYCHAETA	Ampharetidae	Anobothrus gracilis			1	
TCC	POLYCHAETA	Ampharetidae	Melinna cristata		1		
TCC	POLYCHAETA	Trichobranchidae	Terebellides stroemii		2		
TCC	POLYCHAETA	Sabellidae	Chone sp.		1	1	
TCC	POLYCHAETA	Sabellidae	Jasmineira candela		1		
TCC	OPISTHOBANCHIA	Retusidae	Retusa sp.		1	1	1

TCC	CAUDOFOVEATA		Caudofoveata indet		1		1
TCC	BIVALVIA	Nuculidae	Ennucula tenuis	1	1	5	2
TCC	BIVALVIA	Nuculidae	Nucula sp. juvenil	5	7	12	2
TCC	BIVALVIA	Nuculidae	Nucula sulcata	5	12	9	4
TCC	BIVALVIA	Nuculidae	Nucula tumidula		7		
TCC	BIVALVIA	Nuculanidae	Yoldiella philippiana				1
TCC	BIVALVIA	Arcidae	Bathyarca pectunculoides		1		
TCC	BIVALVIA	Thyasiridae	Adontorhina similis	3	1		
TCC	BIVALVIA	Thyasiridae	Axinulus croulinensis			1	
TCC	BIVALVIA	Thyasiridae	Mendicula ferruginosa	9	19	10	6
TCC	BIVALVIA	Thyasiridae	Thyasira equalis	12	23	24	15
TCC	BIVALVIA	Lasaeidae	Tellimya tenella		5		2
TCC	BIVALVIA	Kelliellidae	Kelliella miliaris	4	6	8	
TCC	BIVALVIA	Cuspidariidae	Tropidomya abbreviata			1	
TCC	SCAPHOPODA	Dentaliidae	Antalis sp.		1		
TCC	TANAIDACEA	Parathanidae	Tanaidacea indet				1
TCC	AMPHIPODA	Melitidae	Eriopisa elongata	9	7	5	10
TCC	AMPHIPODA	Phoxocephalidae	Harpinia pectinata		1		
TCC	AMPHIPODA	Phoxocephalidae	Harpinia sp.				1
TCC	SIPUNCULIDA		Golfingiida indet	1	1		
TCC	SIPUNCULIDA		Nephasoma sp.	1		4	
TCC	SIPUNCULIDA		Onchnesoma steenstrupii steenstrupii	3	2	1	4
TCC	OPHIUROIDEA	Amphilepididae	Amphilepis norvegica				1
TCC	ECHINOIDEA	Brissidae	Brissopsis lyrifera		1		1

STA	GRUPPENAVN	FAMILIENAVN	GYLDIG_SYNONYM_WoRMS_sp	G12	G16	G17	G25
WMB	ANTHOZOA	Edwardsiidae	Paraedwardsia arenaria	1	1	1	
WMB	PLATYHELMINTHES		Platyhelminthes indet	1	1	2	
WMB	NEMERTEA		Nemertea indet	3	9	11	4
WMB	POLYCHAETA	Amphinomidae	Paramphinome jeffreysii	6	13	4	14
WMB	POLYCHAETA	Aphroditidae	Aphrodita aculeata	1			1
WMB	POLYCHAETA	Sigalionidae	Neoleanira tetragona	1	3	1	2
WMB	POLYCHAETA	Phyllodocidae	Sige fusigera		1		
WMB	POLYCHAETA	Pholoidae	Pholoe baltica			2	
WMB	POLYCHAETA	Pholoidae	Pholoe pallida		4		1
WMB	POLYCHAETA	Pilargidae	Pilargis sp.				1
WMB	POLYCHAETA	Syllidae	Exogone (Exogone) verugera		3	1	
WMB	POLYCHAETA	Nereidae	Ceratocephale loveni	3	1	3	2
WMB	POLYCHAETA	Nephtyidae	Nephtys sp.	1			
WMB	POLYCHAETA	Glyceridae	Glycera alba	1		1	
WMB	POLYCHAETA	Onuphidae	Paradiopatra quadricuspis	2			1
WMB	POLYCHAETA	Lumbrineridae	Augeneria tentaculata			1	
WMB	POLYCHAETA	Lumbrineridae	Lumbrineris sp.	1			
WMB	POLYCHAETA	Dorvilleidae	Ophryotrocha sp.	2			
WMB	POLYCHAETA	Paraonidae	Levensenia gracilis			4	
WMB	POLYCHAETA	Paraonidae	Paradoneis eliasoni			1	
WMB	POLYCHAETA	Spionidae	Prionospio cirrifera	1	3		
WMB	POLYCHAETA	Spionidae	Prionospio dubia	1	2	2	
WMB	POLYCHAETA	Spionidae	Prionospio fallax		3		
WMB	POLYCHAETA	Spionidae	Pseudopolydora paucibranchiata	3	2	14	21
WMB	POLYCHAETA	Spionidae	Spiophanes kroyeri	28	20	15	58
WMB	POLYCHAETA	Ctenodrilidae	Raricirrus beryli		2		
WMB	POLYCHAETA	Cirratulidae	Chaetozone setosa	12	6	3	7
WMB	POLYCHAETA	Cirratulidae	Tharyx killariensis		1	1	
WMB	POLYCHAETA	Flabelligeridae	Diplocirrus glaucus	2	3	1	5
WMB	POLYCHAETA	Opheliidae	Ophelina norvegica	1		1	

WMB	POLYCHAETA	Capitellidae	Heteromastus filiformis	13	27	12	27
WMB	POLYCHAETA	Maldanidae	Chirimia biceps biceps	1	1		
WMB	POLYCHAETA	Maldanidae	Euclymeninae indet	1	3	3	2
WMB	POLYCHAETA	Oweniidae	Galathowenia oculata				1
WMB	POLYCHAETA	Pectinariidae	Amphictene auricoma		1	1	
WMB	POLYCHAETA	Ampharetidae	Melinna cristata		1		
WMB	POLYCHAETA	Trichobanchidae	Terebellides stroemii	3	1		3
WMB	POLYCHAETA	Trichobanchidae	Trichobanchus roseus				1
WMB	POLYCHAETA	Sabellidae	Chone sp.	3	1		5
WMB	POLYCHAETA	Sabellidae	Euchone papillosa			1	
WMB	POLYCHAETA	Sabellidae	Jasmineira candela	3		8	1
WMB	POLYCHAETA	Sabellidae	Sabella pavonina	2			
WMB	OPISTHOBANCHA		Nudibranchia	1			
WMB	OPISTHOBANCHA	Retusidae	Retusa sp.			1	2
WMB	CAUDOFOVEATA		Caudofoveata indet	1		1	1
WMB	BIVALVIA	Nuculidae	Ennucula tenuis	8	2	8	5
WMB	BIVALVIA	Nuculidae	Nucula sp. juvenil	15	17	18	17
WMB	BIVALVIA	Nuculidae	Nucula sulcata	11	14	5	10
WMB	BIVALVIA	Nuculidae	Nucula tumidula	3	8	1	11
WMB	BIVALVIA	Nuculanidae	Yoldiella lucida			1	
WMB	BIVALVIA	Nuculanidae	Yoldiella philippiana	2	1	3	2
WMB	BIVALVIA	Arcidae	Bathyarca pectunculoides			1	
WMB	BIVALVIA	Pectinidae	Delectopecten vitreus			1	
WMB	BIVALVIA	Pectinidae	Palliolium sp. juvenil			1	
WMB	BIVALVIA	Thyasiridae	Adontorhina similis	15	8	14	19
WMB	BIVALVIA	Thyasiridae	Axinulus croulinensis	1	1		1
WMB	BIVALVIA	Thyasiridae	Mendicula ferruginosa	16	19	11	19
WMB	BIVALVIA	Thyasiridae	Thyasira equalis	65	57	54	78
WMB	BIVALVIA	Thyasiridae	Thyasira obsoleta		1		
WMB	BIVALVIA	Thyasiridae	Thyasira sarsii		2		
WMB	BIVALVIA	Lasaeidae	Kurtiella bidentata			1	
WMB	BIVALVIA	Lasaeidae	Tellimya tenella	3		4	
WMB	BIVALVIA	Cardiidae	Parvicardium minimum			1	
WMB	BIVALVIA	Scrobiculariidae	Abra nitida	8	8	3	63
WMB	BIVALVIA	Kelliellidae	Kelliella miliaris	7	6	35	42
WMB	BIVALVIA	Thraciidae	Thracia devexa		1		1
WMB	BIVALVIA	Cuspidariidae	Cuspidaria obesa	1			
WMB	BIVALVIA	Cuspidariidae	Tropidomya abbreviata	3	4	2	1
WMB	CUMACEA	Leuconidae	Eudorella emarginata				1
WMB	CUMACEA	Leuconidae	Eudorella sp.			1	
WMB	TANAIDACEA	Parathanidae	Tanaidacea indet	1	1		
WMB	AMPHIPODA		Amphipoda indet				1
WMB	AMPHIPODA	Stegocephalidae	sp.		1		
WMB	AMPHIPODA	Melitidae	Eriopisa elongata	4	5	4	4
WMB	AMPHIPODA	Phoxocephalidae	Harpinia crenulata	1			
WMB	AMPHIPODA	Phoxocephalidae	Harpinia sp.				1
WMB	SIPUNCULIDA		Golfingiida indet				2
WMB	SIPUNCULIDA		Nephasoma sp.	3	10	5	2
WMB	SIPUNCULIDA		Onchnesoma steenstrupii steenstrupii	4	2	2	4
WMB	OPHIUROIDEA		Ophiuroidea juvenil	6	2	5	4
WMB	OPHIUROIDEA	Amphiuridae	Amphiura sp.	1		2	
WMB	OPHIUROIDEA	Amphilepididae	Amphilepis norvegica	1	3	3	2
WMB	ECHINOIDEA	Brissidae	Brissopsis lyrifera	1	1	1	

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