FAUNAL COLONIZATION OF SUBMARINE MINE TAILINGS:

An Intertidal Experiment to Investigate the Influence of Sediment Organic Carbon Content

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Front page: Top left; *Hediste diversicolor* (32x magnification) (pers. photography), bottom right; *Streblospio shrubsolii* (55x magnification) (pers. photography), top right and bottom left; SEM imaging of mine tailings (80x magnification) taken at the SEM facility at UEA, UK.

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Table of Contents

1 Abstract

Current financial estimates of mineral mining in Norway are approximately 12 billion NOK per year. Most of the industry is located close to the coastline and the inert waste produced ('tailings', granulometrically similar to sand) is currently deposited in adjacent fjords as Submarine Tailings Placements (STPs). Deposition of STPs smothers the local resident biological assemblages and observations at current sites indicate that colonization, and therefore ecosystem recovery, is slow. This is hypothesized to result from the lack of organic carbon within such deposits.

To test this hypothesis, and to determine the optimum concentration of organic carbon to enhance the colonization process, I conducted an intertidal experiment in the Crouch Estuary, Essex (UK) from April 2012 to April 2013. The experiment comprised tailings treatments ranging in organic carbon concentrations from 0 to 5%. Samples for fauna, sediment grain size, and carbon and nitrogen content were collected and redox potentials measured at T = 0, 45, 115, 180 and 365 days. Univariate indices and community structure was investigated by looking at different aspects of the collected benthic macrofauna. The data revealed that a concentration of 0.5% organic carbon was the optimum concentration to enhance macrofaunal colonization in this study, and after one year the majority of the univariate indices indicated recovery in the mine tailings with a low concentration of organic carbon. However, the macrofaunal communities functioned differently and had a far less total production than the ambient sediments. This indicates that factors other than organic carbon are important when it comes to colonization of mine tailings, and these factors are discussed. The use of diversity indices in comparison to other more elaborate methods to determine the ecological state of the benthic community is also discussed.

2 Introduction

Mining has been in operation since the 1500's in Norway due to the country's richness of metals and minerals (Kvassnes and Iversen, 2013). It is still an important industry in Norway with an annual turnover of 12.4 billion NOK in 2011 (NGU and Direktoratet, 2012), and 13 billion NOK in 2012 (Kvassnes and Iversen, 2013). The Norwegian Geological Survey has estimated known mineral resources to be worth approximately 1400 billion NOK (Boyd *et al.*, 2012), and as the demand for minerals and metals is increasing, the mining industry in Norway is likely to continue to grow very rapidly in the future.

One of the biggest environmental challenges related to mining is proper management of waste material (Miljødirektoratet, 2010). Mining involves production of waste rock (coarse-grained material) and tailings (fine-grained material granulometrically similar to sand), the latter being the main waste product (Kvassnes *et al.*, 2009; Miljødirektoratet, 2010). The minerals and metals only accounts for a small percentage (2-5%) in the ore that is extracted, and thus the resulting waste volume is substantial. It is therefore a pressing issue to develop long-term environmental solutions to better manage this waste (Miljødirektoratet, 2010).

2.1 Submarine Tailing Placements and Related Environmental Issues

Tailings have traditionally been deposited in artificial dams or natural lakes, but for the last 100 years it has been common practice in Norway to place it on the seabed in the fjords (Kvassnes and Iversen, 2013; Miljødirektoratet, 2010). These waste deposits are commonly called Submarine Tailings Placements (STPs) (Ellis, 2008), which entails that the disposal is carried out using pipelines to discharge the tailings to the seabed to avoid any reaction with the euphotic zone (Kvassnes *et al.*, 2009; Miljødirektoratet, 2010). This method is regulated

by several Norwegian laws, and is currently allowed in Norway under the *OSPAR* Convention, Annex II, Article 3, 2(b) as it is classified as one of the exceptions under the ban of waste dumping in the marine environment (Kvassnes and Iversen, 2013). However, internationally this method is highly controversial (Ellis, 2008; Kvassnes and Iversen, 2013).

STPs are known to cover large areas of the fjord seabed (sometimes the whole length of the fjord) with deposits often 50 m thick (Kvassnes and Iversen, 2013). During deposition, the seabed is subject to hyper-sedimentation of inert, organically sterile tailings, which is considered the main environmental problem regarding STPs due to the related impacts on benthic ecosystems (Kvassnes and Iversen, 2013; Miljødirektoratet, 2010).

Benthic invertebrates are able to withstand slow sedimentation by employing different behavioral strategies (Ellis, 2008), however recovery from different burial-depths (i.e., how deep down the organism has been buried under the sediments) is species-specific and functionally dependent on, among others, motility and tolerance to anoxic conditions. In addition, it depends on the characteristics of the deposit, such as particle size and density (Bolam, 2011; Burd, 2002). Ellis (2001) has stated that shallow-water benthos may be able to withstand the effects of depositions up to 30 to 40 cm $year^{-1}$, while Bolam (2011) found that some polychaetes will not survive a 6 cm of overburden. However, in regards to STPs in Norwegian fjords the burial depths will in most cases be > 50 m where the benthos will be more sensitive to sedimentation, and the rates of deposition will most likely be more than 40 cm y^{-1}. This, in addition to the constantly changing environment during deposition will almost certainly result in smothering of the benthic community leaving the seabed sterile and unproductive (Kvassnes and Iversen, 2013; Kvassnes *et al.*, 2009; Miljødirektoratet, 2010).

There are currently seven active mines in Norway which perform STPs, and the amount of tailings deposited ranges from 300.000 tones y^{-1} to 4 million tones y^{-1} (Kvassnes and Iversen, 2013). Permits are today given on a case-by-case basis, and as many of the known mineral ores are located along the coastline, STPs will be considered as a method for waste disposal well into the future (Miljødirektoratet, 2010; Skei, 2013).

Following closure of a mine (and the subsequent ceasing of tailings discharge) it is important that the seabed affected by the former active STP regains a diverse and productive benthic community as rapidly as possible. In 2015 a new EU Water Framework Directive (WFD, 2000/60/EC) will come into effect that will require a classification of 'Heavily Modified Water Body' (HMWB) for most STPs (Kvassnes and Iversen, 2013; Kvassnes *et al.*, 2009). Along with this classification follows the criteria to have the status 'good ecological potential' implying that the state of the fjord (i.e., the HMWB) should be returned to a 'moderate ecological state' soon after STP closure (Kvassnes *et al.*, 2009).

2.2 Previous Studies on STPs and Current Knowledge on STP Recovery

Several monitoring programs and studies have been conducted to assess the impacts of STPs on the benthic community and recovery rates following closure (Burd *et al.*, 2000; Burd, 2002; Miljødirektoratet, 2010). Abundance, biomass, and biodiversity indices often indicate recovery after three to four years post closure, but the species assemblages remain distinct from non-affected areas (Burd *et al.*, 2000; Burd, 2002; Miljødirektoratet, 2010). The latter is however expected as the tailings usually create a more homogeneous habitat than the original.

Due to the lack of studies identifying which mine tailing characteristics (e.g., particle size, organic matter, shape) affect colonization, predicting the recovery time after STP closure is impossible.

The slow colonization of terminated STPs shows that these deposits have a persistent negative effect on the benthic invertebrates, and could also continue to have detrimental effects on other trophic levels (e.g. zooplankton and fish). This recovery may however be unnecessarily slow (Kvassnes *et al.*, 2009). Sediment organic matter is an important food source for marine benthos, and is thereby assumed to be an important factor when it comes to colonization (Hyland *et al.*, 2005; Miljødirektoratet, 2010). Fertilization of the sterile tailings by the addition of organic matter has been suggested as a possible strategy to increase the rate of colonization (Kvassnes *et al.*, 2009). Therefore, in this study I will assess the effect of different organic carbon concentrations on the colonization processes of benthic macrofauna. To achieve this, mine tailings will be mixed with an organic carbon source and filled in trays, which will then be set up on an intertidal mudflat.

Increasing sediment organic content is assumed to have positive effects on first species richness (number of different species present) and biomass (Figure 1a), then abundance, until it reaches a point where a decrease in all three starts due to the increased levels of toxic by-products associated with biodegradation of organic matter (e.g. sulphide and ammonia) and reduced oxygen levels (Hyland *et al.*, 2005) (Figure 1b). The optimum concentration of organic carbon (C_{org}, between point a and b in Figure 1) will vary across depth and location, and between species due to differing tolerance levels (Hyland *et al.*, 2005). At the experimental site of this study, the natural C_{org} concentration is 1.5-1.7% (Bolam *et al.*, 2004), and the optimum C_{org} for colonization of mine tailings is therefore hypothesized to be similar.

Based on a previous study (Bolam *et al.*, 2004), I also hypothesize that a C_{org} concentration > 2.5% will result in too low oxygen levels and highly toxic conditions, and this concentration is therefore proposed to be the 'cut-off' point for colonization of macrofauna in this study.

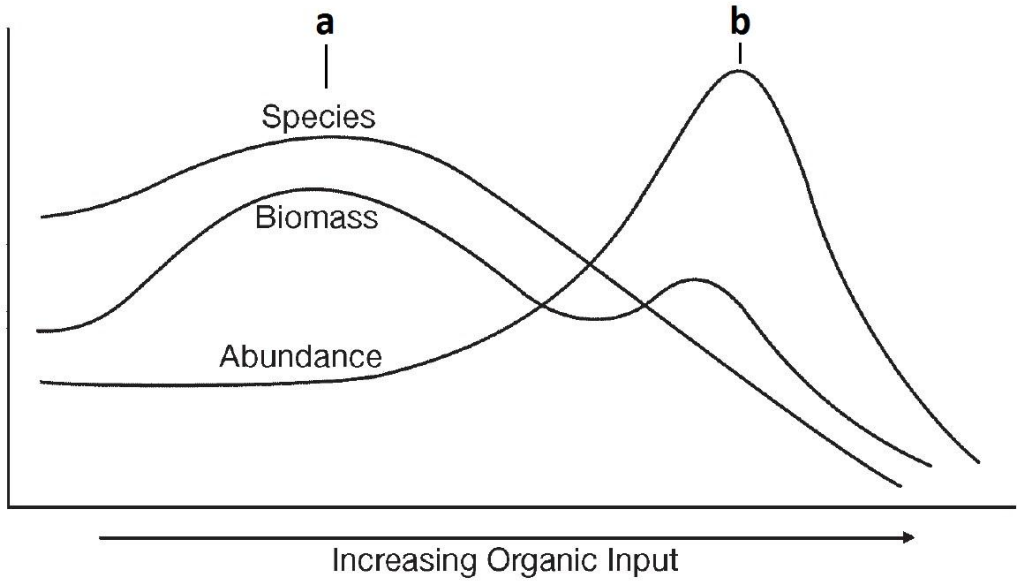


Figure 1: *Conceptual model of the response in abundance, species richness and biomass to increased organic content in benthic communities, where a represents the highest organic carbon concentration for species richness and biomass, and b represents the 'cut-off' point (reproduced from Hyland et al., 2005).*

2.3 Methods to Investigate Ecological State

When investigating the ecological state of a benthic environment, univariate measures such as total abundance and a range of different indices are commonly used (Clarke and Warwick, 2001; Gray, 2000; Salas *et al.*, 2006). The Shannon-Wiener diversity index and Pielou's evenness index are among the recommended indices to assess species richness and equitability (how evenly distributed the individuals are among the species) (Gray, 2000). Multivariate analysis of abundance and species data has become a standard method, and a

powerful tool, when studying community structure and the impacts on the benthic ecosystems (Rumohr and Karakassis, 1999), thereby leading to improved management.

The benthic system plays an essential role in nutrient cycling, decomposition and (arguably the most important) secondary production by providing food for the next trophic level (Bolam, 2012; Hyland *et al.*, 2005; Reiss and Kröncke, 2005; Snelgrove, 1998). It is therefore highly important to also assess functional community structure to obtain the full picture of the ecological state of a benthic environment post-disturbance (Bolam *et al.*, 2010; Bolam, 2012). For this reason, preservation of ecosystem functioning has received increased attention in marine conservation within the last few years (Bremner, 2008). There is limited knowledge on how the function of the benthic ecosystem recovers within STPs, which has prevented predictions regarding long term impacts on higher trophic levels. To describe, or let alone quantify, ecosystem functioning is difficult due to complex interactions between many biological, chemical and physical factors (Bremner, 2008). Two indices that have been used to study functionality are biological traits and seafloor productivity (Bolam, 2012; Bremner, 2008).

When a significant alteration of the environment has occurred, the species assemblage is likely to change as some species may be more adapted to inhabit the 'new' environment than the former species (Cooper *et al.*, 2008). It is expected that the mine tailings used in this *in situ* experiment will represent a different habitat compared to the ambient sediments, and therefore be colonized by a slightly different species assemblage. In comparison, biological trait analysis utilizes the traits of the specific species (e.g., longevity, feeding mode, mobility, reproduction mode) to indicate function. Different species can exhibit the same traits, and the function of the environment is therefore not dependent on the presence of one specific

species, and does not necessarily change if another species exhibiting the same traits is present instead (Bremner, 2008). Biological trait analysis is therefore better equipped to detect functional changes within an ecosystem (Bremner, 2008). A similar biological trait assemblage is expected where the C_{org} concentration is lower than the cut-off concentration.

Seafloor productivity can be measured in secondary production, which is a quantitative measure of energy available for the next trophic level (Brey, 2012; Cooper *et al.*, 2008). The influences of environmental conditions and biological factors on both individual growth and mortality of the population are combined in this estimate, and can therefore reflect functional changes in macrofaunal assemblages (Bolam *et al.*, 2010; Brey, 2012; Cooper *et al.*, 2008). Community production to biomass ratio (*P:B*) expresses the turnover rate of a population, i.e. how rapid one individual may be replaced by another, and may indicate if a community is physically stressed by natural or anthropogenic disturbance (Bolam *et al.*, 2010). A relatively high community *P:B* ratio is therefore expected to be found within the macrofaunal community that colonizes the mine tailings compared to the background. The total secondary production of the community should also be fairly similar given that the function reflected by the biological trait analysis is similar.

The aim of this study is to determine the optimum C_{org} concentration for colonization of mine tailings. To achieve this, an intertidal experiment will be conducted to assess different concentrations of organic carbon on the rate of macrofaunal colonization. The mine tailings will be enriched with four different concentrations of organic carbon; 0.5, 1, 2.5, and 5%, and one treatment with 0% will also be included. Sediment samples will be collected on five occasions over 1 year and macrofaunal abundance and biomass of the colonists determined. Univariate diversity indices will be analyzed to assess the rate of colonization, and compared

to structural and functional community structure, together with the total production of the community. Raw mine tailings will also be analyzed to gain knowledge on the different characteristics such as particle size, metal content, and angularity to put colonization rate in context.

3 Materials and Methods

3.1 Experimental Site

The Crouch Estuary is situated north of the Thames Estuary in Essex, South East England (Figure 2). It stretches about 45 km and is affected by tidal flushing 29 km upstream (Waldock *et al.*, 1999). The volume of freshwater input is generally low and the estuary is therefore more correctly referred to as a sea inlet rather than an estuary (Bolam *et al.*, 2004; Waldock *et al.*, 1999). The experiment was set up mid-way up the sea inlet within the tidal zone on a mudflat on the western end of Bridgemarsh Island (51°38'22N, 00°42'39E) (Bolam *et al.*, 2004; Waldock *et al.*, 1999).

Figure 2: *Map of the SE coast of England showing the experimental site situated in River Crouch in Essex (reproduced from Bolam et al., 2004).*

The experimental area was naturally sheltered from strong tidal currents, allowing a stable and non-dynamic mudflat. The sediments at the site are characterized as fine with a silt/clay content >90% and an organic content between 1.5 and 1.7% (Bolam *et al.*, 2004). The epifauna is dominated by the gastropod *Hydrobia ulvae*, and the infauna by tubificid oligochaetes and *Tharyx* polychaetes (Bolam *et al.*, 2004).

3.2 Experimental Design and Set Up

The experimental design used was a randomized complete block design consisting of five sampling blocks and one replacement block. Each of the sampling blocks (blocks 1-5) had seven experimental plots: five mine tailings treatments modified with different concentrations of organic carbon (C_{org}) and two controls. One of the controls was termed sampling control (SC) to account for natural variability, as the experiment stretched over four seasons, and to recognise possible effects caused by natural disturbance. The other control was termed procedural control (PC) and was used to assess potential impacts to the mudflat caused by disturbance related to the experiment set up and sampling (Figure 3). The replacement block (block 6) consisted of the five tailings treatments and was used to replace sediments in blocks 1 to 5 when sampled in order to maintain the C_{org} concentration. Block 6 did not include SC or PC as replacement of these was considered unnecessary.

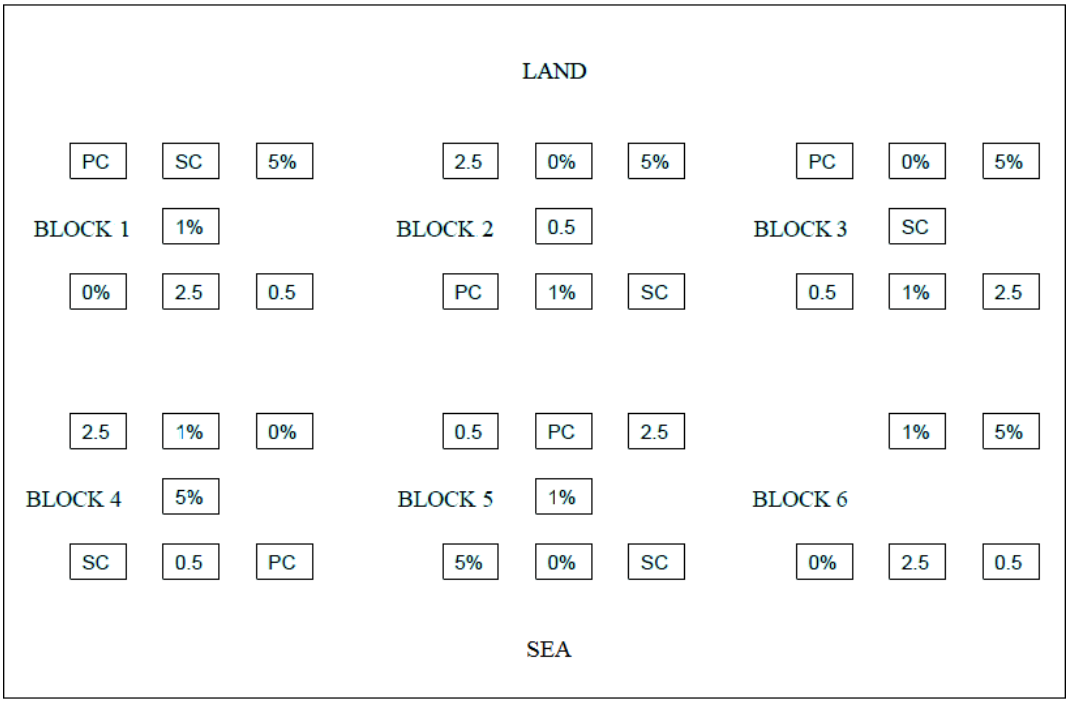


Figure 3: *Showing the layout of the five sampling blocks (Block 1-5) and the replacement block (Block 6) at the experimental site. Note: The blocks were set up adjacent to each other and parallel to the shore. Block 6 did not include PC or SC.*

The blocks were set up adjacent to each other along the shore on the mudflat to reduce potential block effects caused by the progress of tidal flushing in the estuary. The blocks were separated by 15 meters to reduce interdependence between the replicates. Block 1 was placed furthest to the south and block 6 furthest to the north with the other blocks falling numerically in between. The locations of the plots within each of the six blocks were randomized (Figure 3). Plastic freezer trays ($0.25m^2$ x 10cm with tailings treatments) were used as plots as these would withstand the environmental conditions at the site. In addition, this would only allow colonization from the above water column and migrating epibenthos, not from lateral migration of endobenthos, thereby simulating the colonization process of STPs.

Mine tailings were obtained from Rana Gruber AS, a mining company extracting iron ore resources in Mo i Rana (Norway). Ground up fish food pellets were used as the C_{org} source to attain C_{org} concentrations of 0.5%, 1%, 2.5%, and 5%. The pellets were ground using

common electronic food processors. To obtain the correct concentration of C_{org} in the treatments the plastic tray was first filled with tailings to assess the volume then combined with a weighed amount of ground pellets in a brand new cement mixer. Once a homogenous mix was obtained, the tray was refilled. The same procedure was also followed for the 0% treatments without adding any food pellets, in case the mixing of the tailings in the cement mixer had an effect. To ensure that both the correct concentration and a homogeneous mix were obtained, the procedure was conducted separately for each tray, mixing the 0% treatments first and the highest C_{org} concentration (5%) last.

The blocks were set up by excavating mud where the plot with the tailings treatments were to be positioned, then placing the tray in the hole. As larval settlement is known to be affected by near-bottom water flow it was ensured that the trays were flush with the mudflat to reduce hydrodynamic artifacts (Snelgrove *et al.*, 1995). The mud at the location for the control plots (SC and PC) was not modified in any way.

3.3 Sampling Procedure

The experiment was initiated on the 26th of April, 2012 (T = 0 days) and completed on the 29th of April, 2013 (T = 368 days). Samples from the five tailings treatments were collected on five occasions, at T = 14, 45, 115, 180 and 368 days. The SC was sampled at the beginning of the experiment (T = 0 days) and on all subsequent occasions, while the PC was only sampled at T = 0 and T = 368 days.

A 0.0078 m^2 perspex corer was used to collect sediment samples to a depth of 10 cm to collect macrofauna, and a plastic syringe (diameter = 2 cm) was used to collect samples for sediment total organic carbon (TOC) and total nitrogen (TN). Cores from the same treatment

in the replacement block were then inserted into the hollowed sediment immediately after sampling. Samples collected for TOC and TN were not replaced. Redox potential values were measured on all five occasions at 1, 2 and 4 cm depths using a Russell RL100 Redox Meter with a calomel probe.

3.4 Sample Processing and Data Acquisition

3.4.1 Sediment Chemistry Analyses

To gain better understanding of the chemistry and characteristics of the mine tailings, several analyses were conducted (see Appendix A for detailed methods). Sediment chemistry analyses performed at CEFAS laboratories (UK) by trained personnel included mineralogy, particle size distribution and shape analysis, metal content, total organic carbon (TOC) and total nitrogen (TN). SEM imaging was performed at the University of East Anglia's SEM facility. Particle characterization analysis was performed offsite at Melbourn Scientific laboratories (UK) using the Malvern Morphology G3.

3.4.2 Macrofaunal Abundance and Biomass

Sediment samples were fixed in 4% buffered formaldehyde and stored in plastic containers at room temperature. These were later washed over a 500 µm mesh sieve and stained with Rose Bengal to separate the macrofauna from the sediments. Taxa generally considered as meiofauna (i.e., nematodes and ostrocodes) were retrieved on the sieve and thus also included in the study. Enumeration and identification was conducted using dissecting and compound microscopes. Abundance was determined from head counts whereas fragments were only included for biomass estimates. Of the collected specimens, individuals belonging to the taxonomic groups Polychaeta, Oligochaeta and Gastropoda were identified to species level

whereas other specimens were identified to the lowest possible taxonomic groups (eg. Nematoda, Ostrocoda). The invertebrates were preserved in 95% ethanol. Samples collected from block 1, 3, and 5 were processed first, and taxa accumulation curves (see results) indicated that three replicates from each treatment at each sampling time was sufficient to sample the majority of the community. Using three blocks that were separated by the greatest spatial distance (compared to using 1, 2, and 3 which were spatially closer) also reduced the interdependency of the replicates (Green, 1993).

Wet biomass was measured following the methods outlined in the Clean Seas Environment Monitoring Manual's (CSEMP) Green Book (2012). Specimens were blotted and wet weight was recorded to the nearest 0.0001 g, after first being immersed in water to rinse off as much preservative as possible. Individuals belonging to the same taxonomic group and collected from the same sample were weighed collectively. Information regarding the condition of the shell (i.e. approximate percent of intact shell) of calcareous species was noted in addition to their weight to be used when later converting biomass values to energy values for the secondary production estimates.

3.4.3 Biological Trait Analysis

An array of species biological traits have been recognized and described, however, the number of traits assessed is often limited by the difficulty and time-consuming work of obtaining information on all taxa in a study (Bremner, 2008; Munari, 2013). For this study, 10 biological traits that were believed to have a functional effect were selected to describe the morphology, behavior and life history of all taxa (see Appendix B for trait table). Each trait was subdivided into categories (termed "modalities" by Bremner, 2008) for all possible variations within the trait, e.g. the trait "morphology" was subdivided into "soft", "tunic" and

"exoskeleton/shell", giving a total of 45 categories (see Appendix B for all traits and categories).

The approach used to acquire data for use in multivariate analysis was adapted from Bolam and Eggleton (2014). Information regarding all traits were collected from two sources to compile a taxon-by-trait matrix; Stefan Bolam (pers. comm.) provided the majority of the data which had been collected using various methods (Bolam and Eggleton, 2014) and the remaining was obtained from published literature (e.g. Bremner, 2005). As taxa can show intraspecific variation for a trait, a "fuzzy coding" procedure which allows coding for affinity for a category within a trait was appropriate (Chevene *et al*., 1994). Discrete affinity scores from 0 to 3 were used where 0 denoted no affinity, 1-2 denoted partial affinity, and 3 denoted total affinity (Munari, 2013). The codes in the taxon-by-trait were converted to proportions so each taxon-by-trait row became a sum of 1. A sample-by-taxon matrix based on abundance data could then be calculated by multiplying each category-code for a given taxon by its abundance m-2 in that specific sample. This was repeated for all 96 samples.

3.4.4 Secondary Production Estimates

Estimates for secondary production (*P:B* ratio (y^{-1}) and total production (kJ m^{-2} y^{-1})) were obtained following a step-by-step approach described by Bolam *et al.* (2010) and Bolam (2012). Raw abundance and biomass data were first standardized to per m^2 by dividing the raw data with the area (0.00785 m^2) of the perspex corer (i.e. area of sampled seabed). Biomass values (g WM m^{-2}) were subsequently converted to energy values (Joule m^{-2}) using conversion factors provided by Stefan Bolam (pers. comm.) that had been assembled from various sources (Bolam *et al.*, 2010). The conversion factor for the lowest available taxonomic group for each species was used. For Priapulida and Foraminifera, no information

was available in published sources, therefore a conversion factor was obtained by averaging the conversion factors of polychaeta and oligochaeta for the former, and of taxa with an exoskeleton/shell for the latter (A. Sweetman pers. comm.). Although this method might lack accuracy, these taxa were only present in a few samples (four in total) and in very few numbers (Foraminifera: 4, Priapulida: 1) thereby unlikely to influence the final results.

For invertebrates with damaged or dissolved shells (the latter due to long storage times in formaldehyde buffered seawater, the energy value for the animal, had the shell been intact, was calculated from the difference between shell and shell-free conversion factors (S. Bolam pers. comm.). For example, if the conversion factor for an individual with shell was 2.56993 and without shell was 3.554, the conversion factor for an individual with 50% intact shell would be 3.0619.

Calculated energy values were then converted to *P:B* ratios using a spreadsheet available online at http://www.thomas-brey.de/science/virtualhandbook/navlog/index.html (Brey, 2001). This self-learning artificial network model, estimates annual *P:B* ratio by pooling five trained ANNs, and subsequently gives the mean *P:B* ratio together with 95% confidence intervals (CI) (Brey, 2012). The parameterization of the network is as follows:

$$\log(P/B) = a_0 + a_1 \times H_1 + a_2 \times H_2$$

with

$$H_1 = \tan H(b_0 + b_1 \times \log(M) + b_2 \times 1/T + b_3 \times \log(D) + b_4 \times \text{Mollusca} .... + b_{20} \times \text{Exploited}$$

$$H_2 = \tan H(c_0 + c_1 \times \log(M) + c_2 \times 1/T + c_3 \times \log(D) + c_4 \times \text{Mollusca} .... + c_{20} \times \text{Exploited}$$

where a_0 is the intercept and a_1 and a_2 are the estimated coefficients, all of which differ for the five trained ANNs. Expression *P* is production, *B* is biomass, *M* is the mean individual body mass (J), *T* is mean annual bottom water temperature (°C) at the experimental site, and *D* is water depth (m) at the experimental site. Terms b_4 to b_{20} are categorical parameters providing taxonomic and lifestyle information. The categories are grouped in taxon (b_4:Mollusca, b_5:Annelida, b_6:Crustacea, b_7:Echinodermata, b_8:Insecta), mobility (b_9:infauna, b_{10}:sessile, b_{11}:crawler, b_{12}: facultative swimmer), feeding (b_{13}:herbivore, b_{14}:omnivore, b_{15}:carnivore), and habitat (b_{16}:lake, b_{17}:river, b_{18}:marine, b_{19}:subtidal, b_{20}:exploited) (Brey, 2012).

To obtain productivity estimates, I downloaded the file 'ProductivityANN01' from the website and then input the mean individual body mass (J) for each taxon, and the temperature and depth, together with the value '1' for the correct category in each group and 0 for the remaining categories within the same group (except for 'habitat' which can have more than one positive value if the population is exploited) (Table 1). The estimated *P:B* ratio and 95% CI were subsequently given for each taxon on separate data rows. Production estimates for each taxon were then derived by multiplying the *P:B* ratio (y^{-1}) by the measured biomass (kJ m^{-2}) of each taxa in that specific sample. Fragments were not included in this analysis as individual body mass was required as an input.

Table 1: *The downloaded spreadsheet 'Productivity ANN01' with input values and P:B ratio output for three species (Brey, 2001).*

	Data Input			TAXON						MOBILITY			
Taxon	Body Mass (J)	Temp (°C)	Depth (m)	Mollusca 1 or 0	Annelida 1 or 0	Crustacea 1 or 0	Echino-dermata 1 or 0	Insecta 1 or 0	Infauna 1 or 0	Sessile 1 or 0	Crawler 1 or 0	Facult. Swimmer 1 or 0
Hydrobia ulvae	7.56028817	16.00	0.5	1	0	0	0	0	0	0	1	0
Macomas Tellinid juv.	2.42681084	16.00	0.5	1	0	0	0	0	1	0	0	0
Retusa obtusa	5.95934183	16.00	0.5	1	0	0	0	0	1	0	0	0

FEEDING			HABITAT					Data Output (Mean of 5 ANNs) Production-to-Biomass Ratio		
Herbivor 1 or 0	Omnivor 1 or 0	Carnivor 1 or 0	Lake 1 or 0	River 1 or 0	Marine 1 or 0	Subtidal 1 or 0	Exploited 1 or 0	Mean P/B Ratio 1/y	95% Confidence Limits (lower)	(upper)
0	1	0	0	0	1	0	0	3.7729	2.8850	4.9339
0	1	0	0	0	1	0	0	4.7278	3.3084	6.7561
0	0	1	0	0	1	0	0	3.3518	2.4983	4.4970

Artificial Neural Networks (ANNs) have shown to perform significantly, though only slightly, better than multiple regression models. The latter have primarily been used to predict production the last few years as ANNs have not been available in a general applicable format until recently (Brey, 2012). It is important to note that the data acquired from this empirical model are estimates, and though ANNs are a powerful tool in ecology, they are not as accurate as direct methods (Brey, 2001). When looking at single species populations the estimates are associated with high errors and should therefore be interpreted with great care (Brey, 2012). However, all analyses in this study are done at a community level, which greatly reduces this error (Brey, 2012). Most of the error is presented as an underestimation of the *P:B* ratio and the calculated production (on average 10% underestimation at the community level) (Brey, 2012).

Also, the ANN used requires input of annual individual body mass, but as this study is looking at the changes in the community through one year, this would be meaningless to

input. We therefore assume that the measured body mass of the community are averages for the year. Appreciating that this assumption is most likely violated, this leads to a source of error in the production estimates. Conclusions drawn regarding the effect of organic carbon on production are therefore only valid for the present study and should not be compared to others.

3.5 Data Analyses

3.5.1 Biodiversity indices

Diversity indices (Shannon Wiener diversity index (*H'*), Pielou's evenness index (*J'*), and species richness (*S*)) were obtained for each sample using the DIVERSE program in PRIMER, version 6.1.11 (Clarke and Gorley, 2006). *S* is simply the total number of taxa in the sample, and the other indices are calculated using the equations shown below (1, 2) (Clarke and Warwick, 2001):

$$H' = -\sum_i p_i \ln(p_i) \quad (1)$$

where p_i is the proportion of individuals belonging to the *i*th species,

$$J' = H' / H'_{max} = H' / \log S \quad (2)$$

where H'_{max} is the maximum possible value of *H'* obtained if all species are present in the same abundance.

Values closer to one for *J'* indicate that species are present in approximately even numbers while values closer to zero indicate the opposite (Clarke and Warwick, 2001). Increasing

stress tends to decrease *H'*, *S*, and *J'*, and these indices can therefore be used as indicators of stress in a marine environment (Clarke and Warwick, 2001). Samples with no individuals found were omitted as calculating *H'* and *J'* is mathematically impossible for zero species. A taxa accumulation curve was constructed for all treatments at T = 368 days. The curve is constructed by adding the number of new species found in each replicate to the number found in the former replicate. A flattening of the curve indicates that the number of replicates is satisfactory for representing the diversity of the sampled community (Clarke and Warwick, 2001).

3.5.2 Statistical analyses

Excel for Windows was used to calculate mean and 95% confidence intervals (CI) and create plots (column and line charts) for graphical presentation of the data. Statistical analysis of univariate indices (*H'*, total abundance, biomass, total production) was conducted using the statistical package SigmaPlot (2008), Version 11.0. As the samples were collected from the same plots over time the macrofauna present at one point in time would most likely have an effect on the abundance at a later sampling time and could therefore not be treated as true replicates but rather as pseudoreplicates. The model used was therefore a Two-Way Repeated Measures (RM) ANOVA, where time and treatment were treated as the main effects and block as the subject being repeatedly sampled. Data was standardized to m^2 and square root transformed to meet assumptions of normal distribution and homogeneity of variance. These assumptions are automatically tested in SigmaPlot when running an ANOVA, using the Kolmogrov-Smirnov test for normality and the Levene Median test for homoscedacity (Radu, 2011).

When the ANOVA presented a non-significant interaction, but a significant difference within the main effects (with an α-level set to 0.05), a Sidak-Holm post hoc test was conducted. Sidak-Holm calculates p-values for all pairs and subsequently ranks them from lowest to highest. The p-values are then compared to a critical level (CL), which depends upon the significance of the test, the rank of the p-value and the total number of pair-wise comparisons, where a p-value lower than the CL is considered to be significant (Radu, 2011). It is therefore a more powerful test than the Bonferri and Tukey's post hoc tests. When a significant interaction between time and treatment is found, interpreting results from multiple comparison within the main effects may be misleading and was therefore avoided (Radu, 2011). The PC was not included in the 2-way RM ANOVA as it was only sampled on two occasions.

Non-metric multi-dimensional scaling (MDS) ordination was applied to Bray-Curtis similarity values calculated from abundance data to investigate the degree of difference between all treatments using the PRIMER package, version 6.1.11 (Clarke and Gorley, 2006). Prior to any analyses the data was square-root transformed, so rare taxa would exert an effect, and samples with a total abundance of zero were omitted as calculating a difference between samples with no result is not viable (Clarke and Warwick, 2001). Ordination plots (2-dimensional) were applied to the Bray-Curtis similarity matrix to graphically present the similarity in community structure, and clusters from dendograms were superimposed to further investigate the degree of similarity between treatments. Superimposing clusters allows the adequacy of the MDS to be assessed and checks that degenerate solutions have not occurred (Clarke and Warwick, 2001). The relative secondary production and trait composition in the communities was investigated using the same analysis, with the exception of using Euclidean distance matrix to perform MDS for the secondary production data.

Differences between treatments were statistically tested using the non-parametric one-way ANOSIM in PRIMER with the *a priori* treatments set as the factor. ANOSIM presents a test-statistic (*R*) deemed as important (or even more) than the p-value presented, hence the former was used to assess significant difference in this study while taking the p-value into consideration (Clarke and Warwick, 2001). ANOSIM computes a test statistic R (ε[-1,1], termed 'global R' in ANOSIM) of the distribution of the differences between the treatments within the factor, and a distribution of all possible R values for the specific dataset by conducting 999 permutations independent of the *a priori* set factor. A significant difference is subsequently found when the global R-value is higher than all possible calculated R-values. A p-value is also computed where a small p-value reflects a lower chance of the true R-value to be within the range of the possible R-values, and the number of all possible permutations found to be higher than the global R is also reported. All levels within the *a priori* set factor are also compared pair wise and R-values and p-values for each pair reported in the output.

For pair wise comparisons, R-values over 0.75 indicate samples that are significantly different with no overlapping taxa and/or a large differences in values denoted to the taxa, values over 0.5 indicate some overlapping but still significantly different, and values less than 0.25 indicate highly overlapping samples that are not significantly different (Clarke and Warwick, 2001).

To test if potential disturbance, caused by the core-sampling over time, had had a significant impact on the macrofauna community, four different aspects of the data was analyzed by comparing the PC sampled at T = 0 days with the PC sampled at T = 368 days. A t-test was conducted on the total production using SigmaPlot (2008), Version 11.0. Assumptions were

tested as in 2-way RM ANOVA. The degree of similarity in community structure was analyzed as described for all treatments over time. MDS ordination and ANOSIM was conducted on abundance data, secondary production data, and biological trait data.

4 Results

4.1 Sediment Results

The tailings were successfully manipulated to obtain different concentrations of C_{org}, and the changes in total organic carbon (TOC) and total organic nitrogen (TN) over time is seen below (Figure 4; Figure 5). The level of C_{org} in the 5% treatment was maintained for the first 14 days, after which it decreased gradually until it reached a concentration close to the ambient sediments, most likely due to microbial degradation. The same gradual decrease was observed in the 2.5% treatment. The treatments with a $C_{org} \geq 0.5\%$ showed a peak in TN at T = 14 days. The organic content in the 0.5% decreased to the same level as measured in the 0% at T = 115 days, and these two treatments maintained very similar carbon concentrations to the end of the experiment. Interestingly, the 0% treatment had a slightly higher carbon content (C_{org} = 0.3%) at T = 368 days than the 0.5% (C_{org} = 0.2%). Microbial mats and microphytoplankton had colonized the treatment which was most likely the cause for this increase (pers. observation).

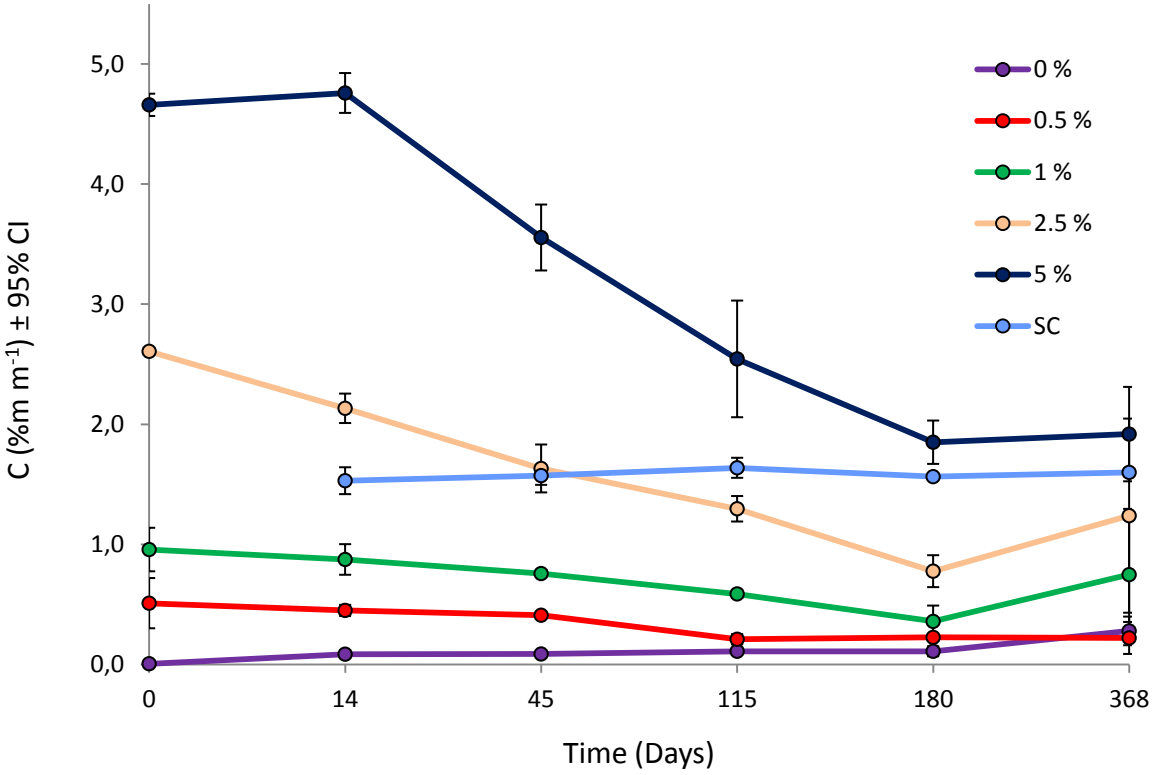


Figure 4: *Sediment TOC content (mean %m/m ± 95% CI, n = 3) in tailings treatments and the sampling control (SC) throughout the experiment.*

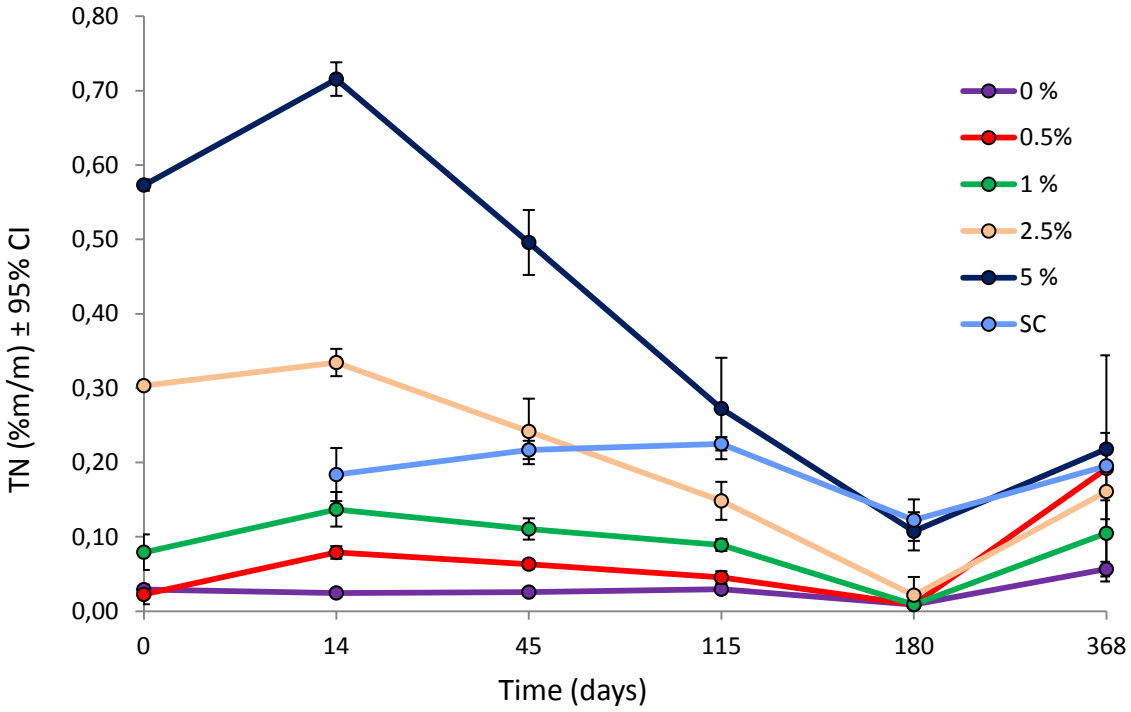
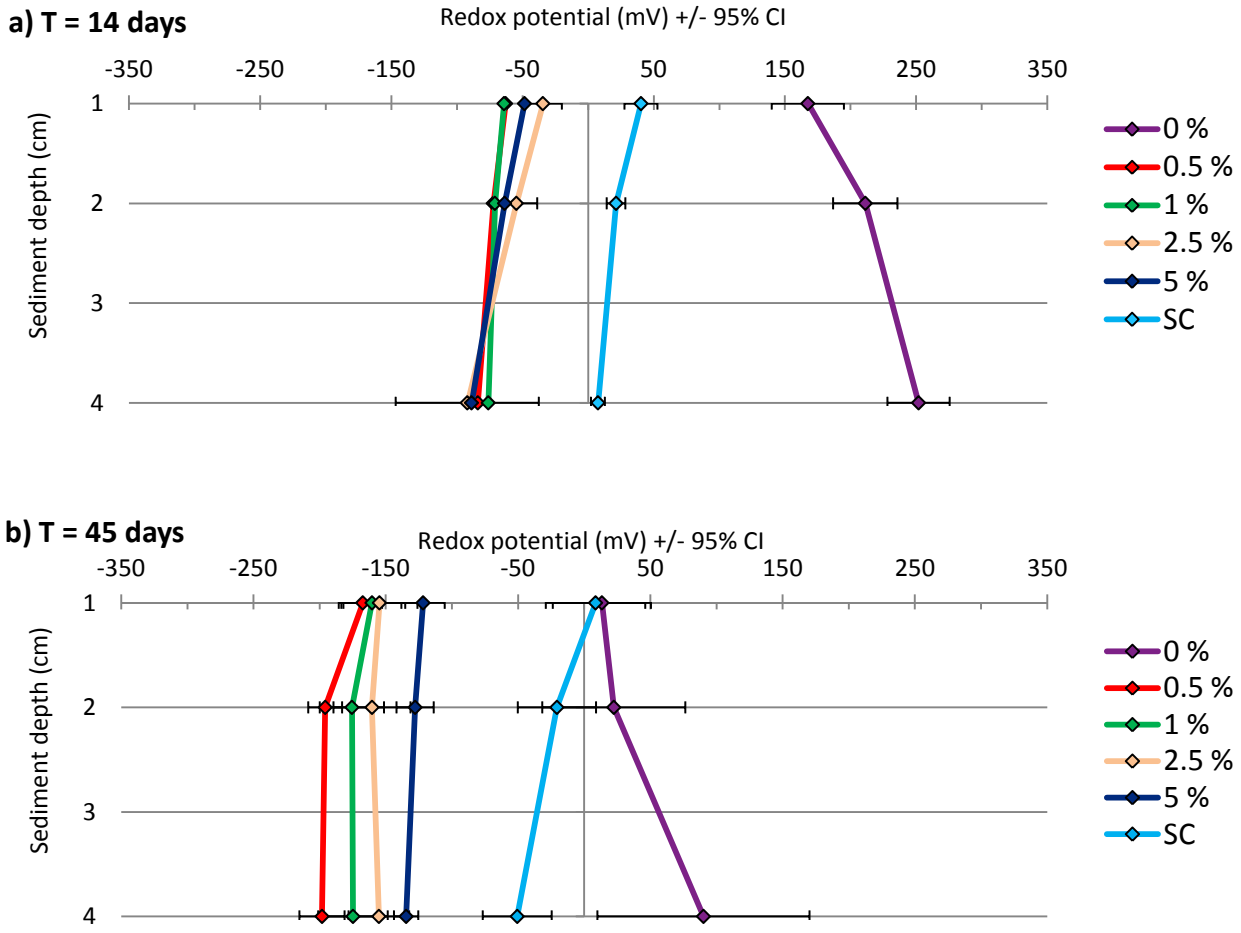


Figure 5: *Sediment TN content (mean %m/m ± 95% CI, n = 3) in tailings treatments and the sampling control (SC) throughout the experiment.*

Similar redox potentials were observed in all treatments with $C_{org} \geq 0.5\%$ down the sediment profile at all sampling events (Figure 6a-e). The reducing conditions in these treatments increased over time, and also increased down the sediment profile to a higher extent after T = 115 days. Interestingly, the condition was more reducing in the 0.5% than the 5% treatment at T = 45 days. The 0% showed higher positive values down the sediment profile until T = 180 days, which was unusual. Nevertheless, the conditions in the 0% treatment were more similar to that of the SC than in any of the other treatments.



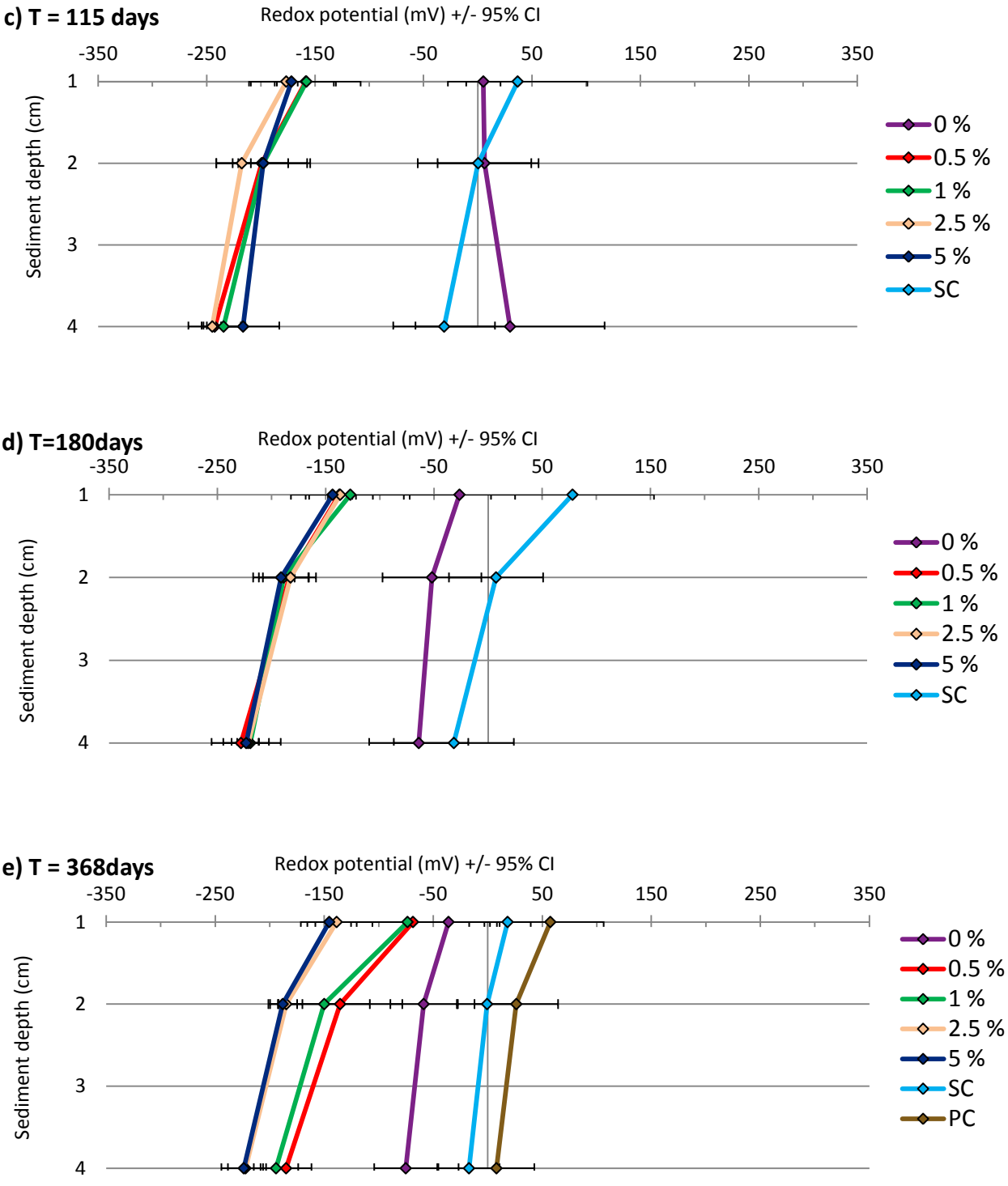


Figure 6(a-e): *Sediment redox potential profiles (mean mV ± 95% CI, n = 3) in tailings treatments and the sampling control (SC) throughout the experiment. Procedural control (PC) included for T = 368 days.*

Particle size analysis showed that the tailings clearly contained a higher volume of larger particles than the natural sediments at the experimental site (Figure 7). The average particle size in tailings had a phi unit of 2, about 30 times larger than that of the natural sediments (phi unit = 7.4). This could have affected the colonization as the tailings represented a different habitat than the ambient sediments. After 368 days, the volume of larger particles in the tailings had decreased slightly and the volume of smaller particles had increased. This was most likely a results of natural sediments being mixed in with the tailings, and not weathering process as this takes place on a much larger time scale (Russel, 1939).

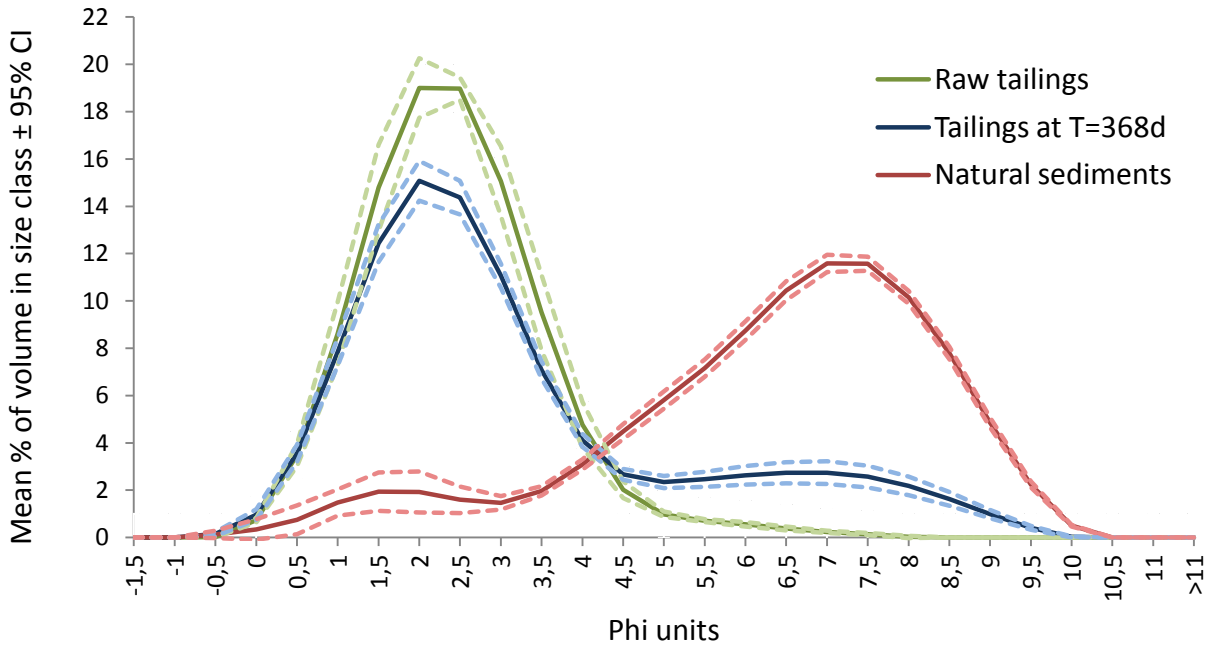


Figure 7: *Particle size distribution of raw mine tailings (green), tailings treatments at T = 368 days (blue) and natural sediments (SC and PC) at T = 368 days (red). Distribution presented in mean percent (%) of volume in each size class ± 95% CI.*

Particle shape analysis revealed that 47 % of the particles in the tailings were classified as ‘slightly elongate’, while 22 % were ‘moderately elongate’ and 27 % were ‘not elongate’ based on classification by Zingg (1935). SEM imaging showed that the tailings particles were highly angular with more sharp edges than the natural sediments (Figure 8). This high angularity is a result from crushing of the rock when extracting minerals and metals. Analysis of metals in the tailings revealed that none were present at a level which could be toxic (see Appendix C for levels).

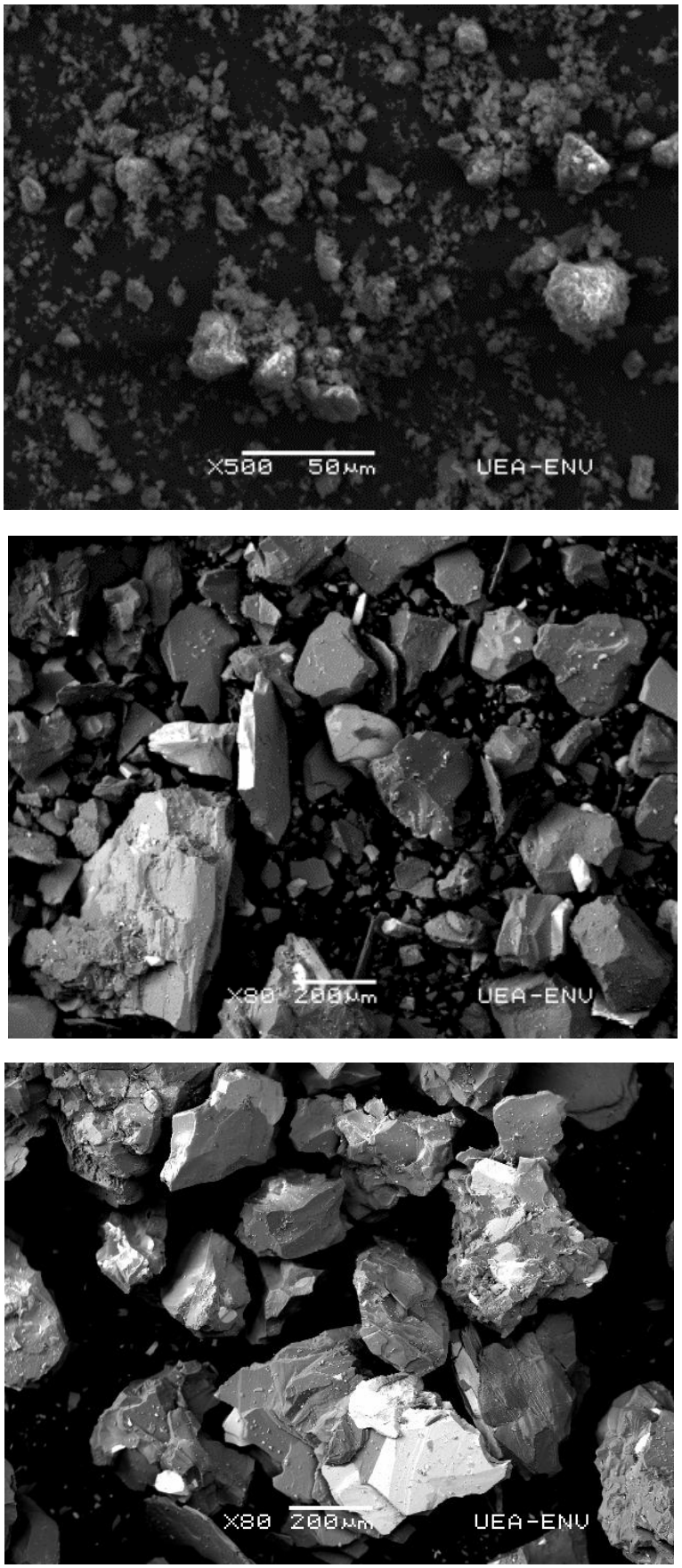


Figure 8: *SEM imaging of natural sediments (top, magnification x500) and tailings (middle and bottom, magnification x80).*

4.2 Biodiversity Indices

Diversity heterogeneity was investigated using the indices Shannon Wiener (*H'*), Pielou's evenness (*J'*) and species richness (*S*) (Figure 9, Table 2). Up to T = 180 days the variation of *H'* within the tailings treatments was very large proving it difficult to interpret how similar or dissimilar the diversity was between the tailings treatments and in comparison to the control (see Appendix C for mean ± 95% CI for all sampling events). There was no significant interaction between time and treatment from T = 115 to T = 365 days (2-way RM ANOVA, $p = 0.508$), but a significant difference within time and within treatment (2-way RM ANOVA, $p < 0.019$). At T = 115 days the treatments with a $C_{org} \geq 1\%$ were significantly different from the SC (2-way ANOVA, $p < 0.003$, CL < 0.004), while the 0% and 0.5% were not significantly different (2-way ANOVA, $p > 0.038$, CL < 0.013) (see Appendix D for all p-values and CL). At T = 180 and T = 368 days there were no significant differences between any of the pair-wise comparisons (2-way ANOVA, $p > 0.006$, CL < 0.003). However, all tailings treatments had a lower mean *H'* than the controls throughout the experiment though this was not always statistically significant.

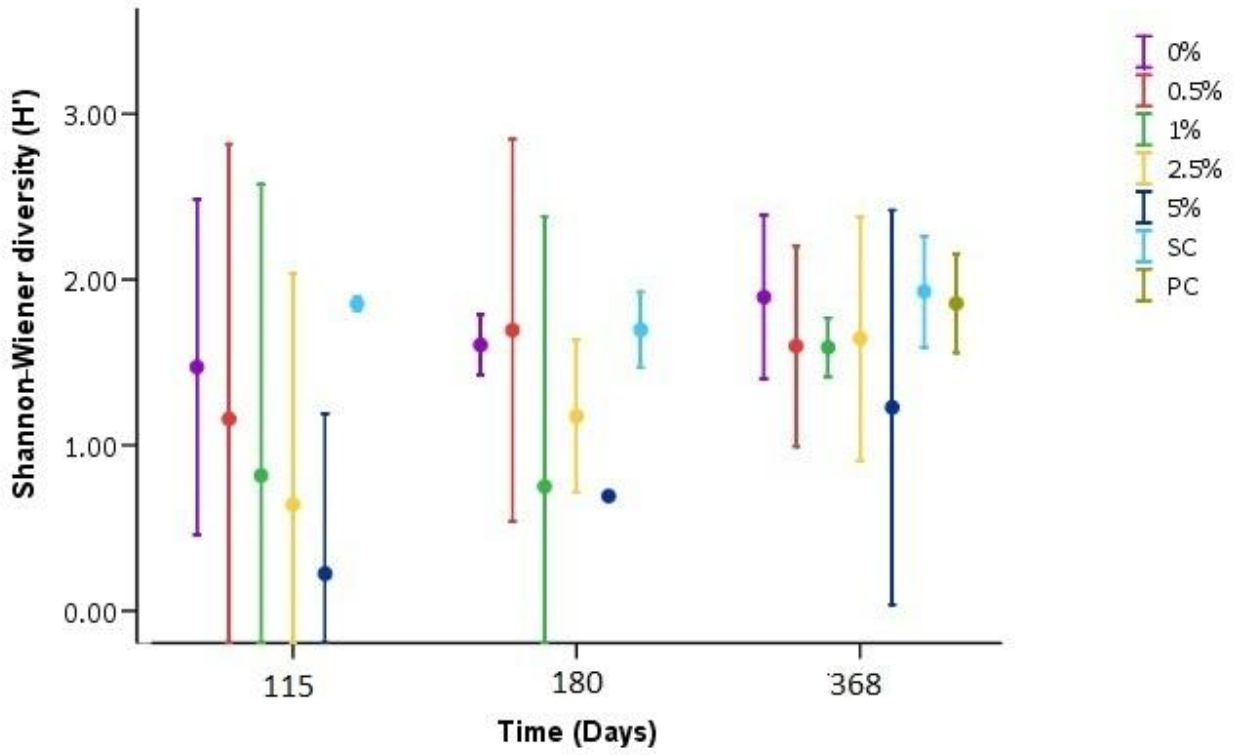


Figure 9: *Shannon Wiener diversity (mean H'± 95% CI, n=3 except for 5% at T=180; n=1) over time in tailings treatments and SC from T = 115 days to T = 368 days. PC included for T = 368 days.*

The evenness values observed in treatments with $C_{org} \geq 1\%$ were high throughout the experiment indicating that taxa were present in very similar numbers (Table 2). This could be interpreted as a community not under stress, however, the species richness was very low compared to the control which indicates the opposite (Clarke and Warwick, 2001). The evenness values were also much higher than in the control. This could be a result of the very low number of species and individuals observed up to T = 368 days in these treatments, resulting in the indices having a too small dataset to give values that truthfully represented these aspects of the community. The mean species richness in the 0% and 0.5% treatments was comparable to that in the control after 115 days, though the evenness reflected that the environment was dominated by a few taxa implying that the community was under stress (Clarke and Warwick, 2001). After T = 180 days the evenness was similar in the $C_{org} \leq 0.5\%$

compared to the control, but after 368 days the 0% had increased to a level higher than the controls, similar to the high C_{org} treatments.

Table 2: *Mean Pielou's evenness (J') and mean species richness (S) for all tailings treatments and SC over time. PC included for T = 368 days. Number of replicates stated if deviated from 3.*

Pielou's evenness (*J'*)							
	0%	**0.50%**	**1%**	**2.50%**	**5%**	**SC**	**PC**
T14	0.861 *(2)*	1.000 *(2)*	-	1.000 *(1)*	-	0.790	-
T45	0.789	0.702 *(2)*	1.000 *(1)*	0.857 *(2)*	0.982 *(2)*	0.819	-
T115	0.678	0.559	0.823 *(2)*	0.874 *(2)*	0.971 *(1)*	0.823	-
T180	0.774	0.799	0.911 *(2)*	0.982	1.000 *(1)*	0.791	-
T368	0.818	0.746	0.928	0.852	0.890	0.778	0.748
Species richness (*S*)							
	0%	**0.50%**	**1%**	**2.50%**	**5%**	**SC**	**PC**
T14	2	1	0	1	1	11	-
T45	4	2	2	3	2	12	-
T115	9	8	3	2	1	10	-
T180	8	9	3	3	1	9	-
T368	10	9	6	7	5	12	12

Species accumulation curves were constructed using the species richness (*S*) values of the samples collected at T = 368 days (Figure 10). The highest number of species of 17 was found in the controls, while the 0% and 0.5% treatments were colonized by a total of 14 and 13 species, respectively. The lowest number of a total of six species was observed in the 5% treatment. The curves for the tailings treatments flattened after two replicates indicating that three replicates was a satisfactory number of replicates to analyze for this study (Clarke and Warwick, 2001). The curves for the controls leveled after two replicates, but to a lesser extent than the tailings treatments.

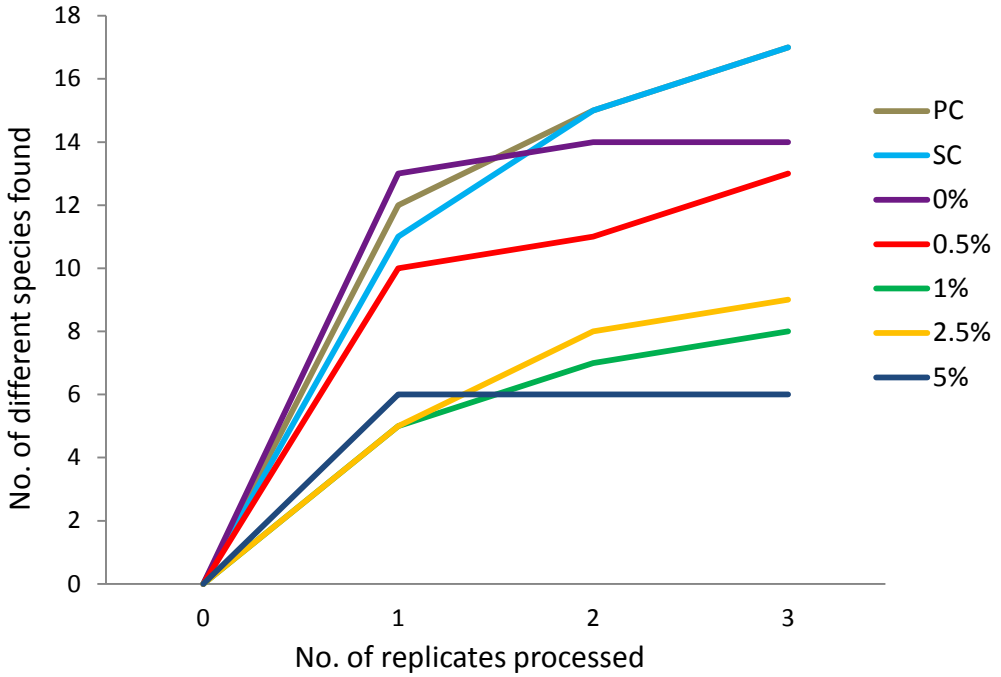


Figure 10: *Accumulated number of species identified in samples collected at T = 368 days (n=3).*

4.3 Macrofaunal Abundance and Biomass

A total of 4045 macrofaunal invertebrates from 25 taxa were sampled during the experiment. Statistical testing of the PC collected at T= 0 days and T = 368 days showed that there was no significant difference between the beginning (T = 0 days) and the end (T = 369 days) in terms of species assemblage (ANOSIM, R = 0.185, p = 0.1), trait assemblage (ANOSIM, R = 0.296, p = 0.1) or total production (t-test, p = 0.102, df = 4) (see Appendix D for results). Contribution by taxa to total production showed some separation, though the null hypothesis could not be rejected by ANOSIM due to low significance level and observation of high within-time variation (R = 0.519, p = 0.1).

In the 0%, 0.5% and SC, 19 taxa were identified, and 10, 13 and 8 taxa were found in the 1%, 2.5% and 5% treatments after 368 days. The most abundant taxa found in the 0% were *Hydrobia ulvae* (19%), Nematoda (19%), and the polychaete *Streblospio shrubsolii* (13%). In

the 0.5% *Paranais litoralis* (45%), Nematoda (17%) and *H. ulvae* (7%) were the most abundant, while Nematoda (21%) and *H. ulvae* (15%) were the dominant colonizers of the 1% and 2.5% treatment. In the 5% *T. benedii* accounted for 27% of the individuals and nematodes 20%. The most abundant taxa in the SC were *S. shrubsolii* (25%) and *T. benedii* (22%).

Macrofaunal colonization over 1 year is shown below (Figure 11). Statistical analysis revealed that there was a significant interaction between time and treatment (2-way RM ANOVA, $p < 0.001$), and statistical differences between treatments within time was therefore not assessed (see Appendix D for all p-values and CL). All tailings treatments showed a very low colonization after 45 days, but from 115 days to 368 days the 0% and 0.5% treatments showed an increased colonization in comparison to treatments with $C_{org} \geq 1\%$, indicating that the rate of colonization was higher in these treatments. At T = 115 and T = 180 days the abundance was highest in the 0.5% treatments, while the 0% treatment showed the highest abundance after 1 year. This grouping trend was also seen in the statistical analysis as the 0% and 0.5% were not significantly different throughout the experiment (2-way ANOVA, $p = 0.937$, CL = 0.05), while they were both significantly different to all $C_{org} \geq 1\%$ treatments (2-way ANOVA, $p < 0.001$, CL > 0.005). At T = 180 days there was a drop in abundance in all treatments, including the SC, before it increased again at T = 368 days in all treatments. The abundance in all tailings treatments was significantly less than the SC over the whole sampling period (2-way RM ANOVA, $p < 0.001$, CL > 0.003), indicating that recovery had not occurred.

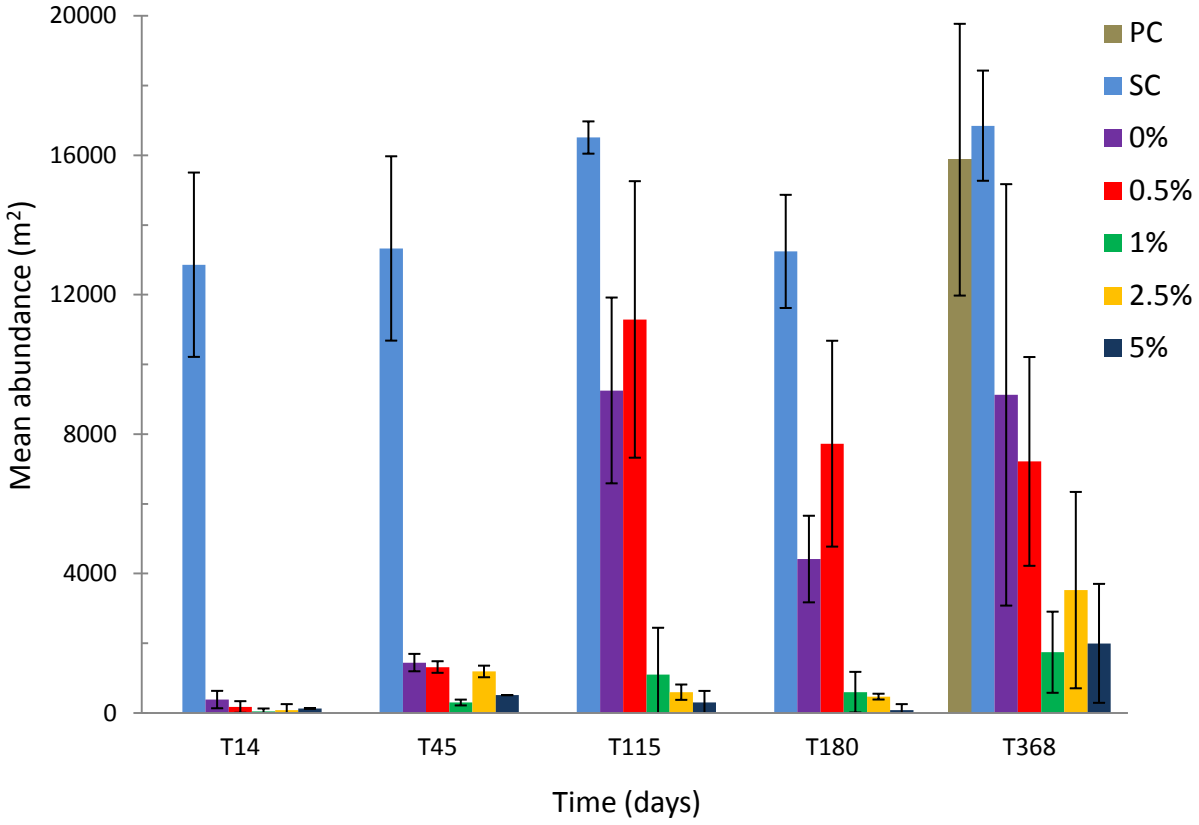


Figure 11: *Changes in macrofaunal abundance (mean ± 95% CI, n=3) over time in tailings treatments and the sampling control (SC) throughout the experiment. Procedural control (PC) included for T = 368 days.*

A slight increase in biomass was observed in the 0% treatment after 45 days, while other treatments had a very low biomass (Figure 12). Some interaction between time and treatment on biomass was occurring though this was not found to be significant (2-way RM ANOVA, $p = 0.053$) (see Appendix D for all p-values and CL). Both time and treatment were found to be significant when testing for main effects (2-way RM ANOVA, $p < 0.002$). The lack of significant interaction was most likely related to the very low biomass observed in the two highest C_{org} treatments over the course of the experiment, and also by the lack of trend over time. After 115 days an increase in total biomass was observed in treatments with $C_{org} \leq 1\%$, and statistical analysis revealed that the 0.5% and 0% treatments were not significantly different from the SC (2-way RM ANOVA, $p > 0.007$, CL < 0.006), indicating a level of

recovery in biomass. However, the 0% and 0.5% was not found to be significantly different from any of the $C_{org} \geq 1\%$ treatments either, indicating that the variance within these two treatments was very high. After 180 days the 0.5% was significantly different to the $C_{org} \geq 1\%$ treatments (2-way RM ANOVA, $p < 0.004$, CL > 0.004). At T = 368 days the highest biomass was observed in the 0% and the 2.5%, though it was not significantly higher than the biomass in the other tailings treatments (2-way RM ANOVA, Appendix D). All tailings treatments had a significantly lower biomass than the SC (2-way RM ANOVA, $p < 0.001$, CL > 0.003), indicating a lack of recovery. The total biomass was highly driven by the presence of one individual of *Macoma balthica* in both the SC and PC at T = 368 days. The high total biomass in the PC and the increase in biomass observed in SC at T = 368 days compared to the other sampling events is related to this one individual.

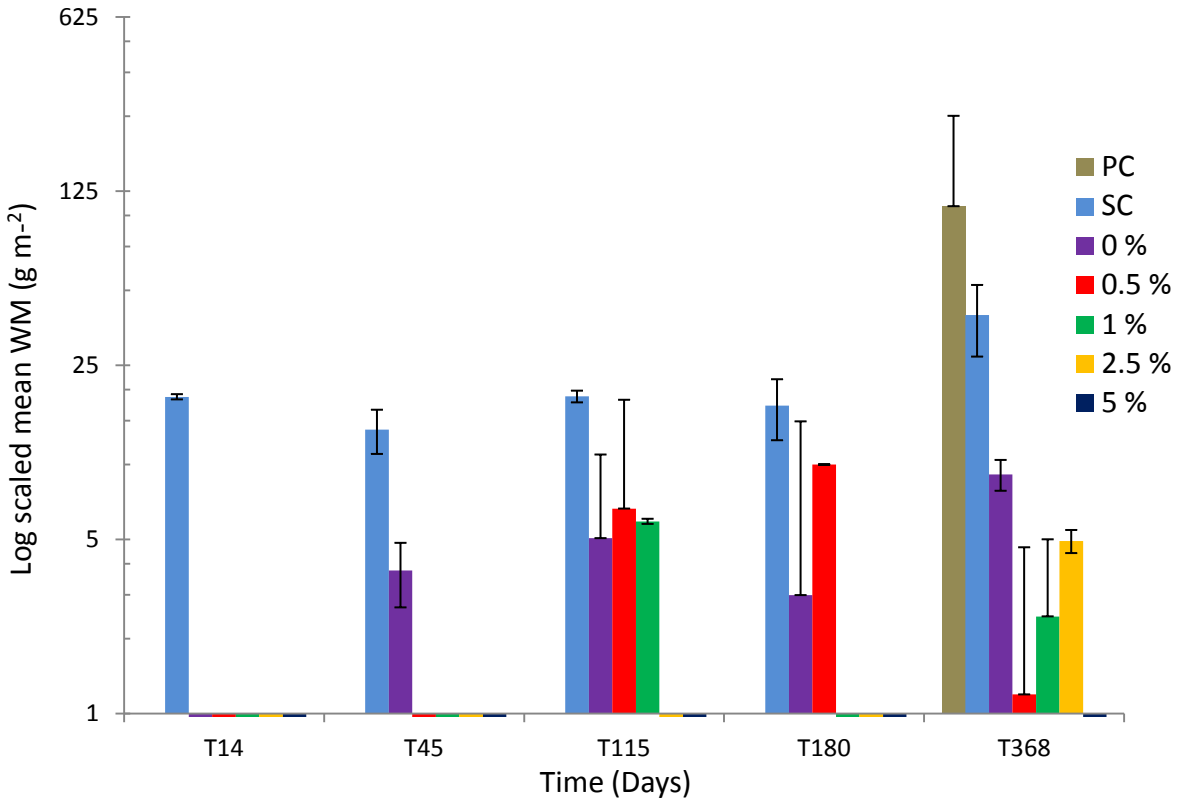


Figure 12: *Changes in log scaled macrofaunal biomass (mean WM ± 95% CI, n=3) over time in tailings treatments and the sampling control (SC) throughout the experiment. Procedural control (PC) included for T = 368 days.*

4.4 Species and Trait Assemblages

Multi-dimensional scaling (MDS) of the abundance data was conducted to investigate the similarities in the community structure of the different treatments. Statistical testing revealed a global R-value higher than would be expected by chance for all separate sampling events (ANOSIM, $R > 0.524$) and the null hypothesis that all treatments were equal was rejected at the 0.002 level (see Appendix D for all global and pair wise R values). The MDS showed an increased grouping of the tailings treatments from the start of the experiment to T = 115 days indicating that the species assemblages became more and more similar over time (Figure 13a-c). At T = 115 days the 0% treatments clustered with the SC (40% similar), while the treatments with $C_{org} \geq 1\%$ appeared to cluster together. Statistical analysis showed that the latter three tailings treatments (1, 2.5, and 5%) were not significantly different from each other (ANOSIM, R = -0.074 to -0.315, $p > 0.5$), where the negative R-values were believed to signify that the variation was higher between the replicates than the treatments (Chapman and Underwood, 1999).

ANOSIM showed different results than what was indicated by the MDS; some overlapping of species was present between the 0% and 5% (ANOSIM, $R = 0.407$, $p = 0.1$) at T = 115 days, while the other tailings treatments were significantly different from the 0% treatments (ANOSIM, $R > 0.593$, $p = 0.1$) (Figure 13c). The 0.5% had some overlapping in community structure with the 1% and 5% (ANOSIM, $R < 0.444$, $p = 0.1$), whereas the other tailings treatments were significantly different (ANSOIM, $R > 0.815$, $p = 0.1$). All tailings treatments were clearly different from the SC (ANOSIM, $R > 0.667$, $p = 0.1$) at T = 115 days except for the 5% which had a similar community structure (ANOSIM, $R = 0.333$, $p = 0.1$).

The community structures appeared to change from T = 115 to T = 368 days, with 0% and 0.5% becoming more clustered and more similar to the treatments with $C_{org} \geq 1\%$ (Figure 13c-e). There was however still a significant difference between the 0.5% and the $C_{org} \geq 1\%$ treatments (ANOSIM, $R > 0.556$, $p = 0.1$), and while the 0% was clearly different from all the $C_{org} \geq 1\%$ treatments (Figure 13e), a significant difference was only found from the 1% and 5% (ANOSIM, $R > 0.593$, $p = 0.1$). Community structure between the three high org treatments ($\geq 1\%$) was overlapping and not significantly different (ANOSIM, $R < 0.444$, $p > 0.1$). Statistical analysis revealed that all tailings treatments were significantly different from the two controls (ANOSIM, $R > 0.667$, $p = 0.1$), and that the 0% and 0.5% were still clearly different though not significantly different in terms of species assemblages (ANOSIM, $R = 0.481$, $p = 0.1$).

Stress levels in 2-dimensional ordination matrices measures how well the rank order of the similarities between the observed data points matches the predicted ones (Quinn and Keough, 2001). Stress levels > 0.1 could pose problems when trying to interpret the details of the plots (Clarke and Warwick, 2001). However, by superimposing cluster groups the chance of misinterpreting the plots is highly reduced, and as the stress levels observed here were only slightly higher than 0.1 and no distortion of the clusters were observed, misinterpretation was not an issue of concern (Clarke and Warwick, 2001).

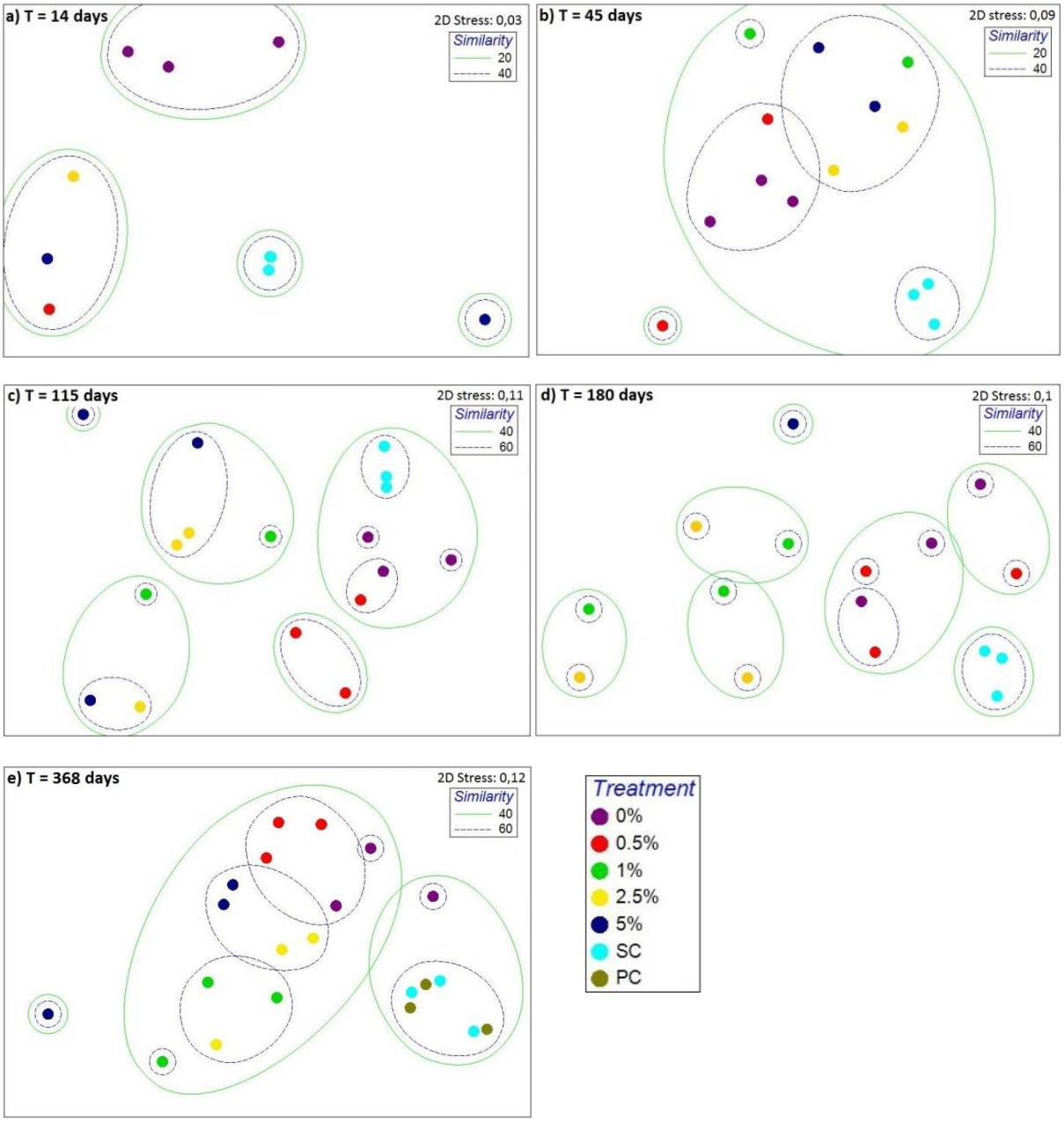


Figure 13(a-e): *Non-metric MDS of macrofaunal community structure in all tailings treatments and the SC throughout the experiment. PC included for T = 368 days. Based on Bray-Curtis similarity matrix on square-root transformed abundance data with all possible replicates. With superimposed clusters from dendograms based on same matrix (see Appendix D for dendograms).*

ANOSIM computed an R test statist higher than 0.512 for all sampling events, except T = 14days, rejecting the null hypothesis at the 0.001 level (see Appendix D for all global and pair wise R values), thus indicating that there was a significant difference between treatments after T = 45 days and throughout the experiment.

MDS of the trait composition showed that the assemblages in the tailings treatments were 60% similar after 45 days while they were significantly different from the control (ANOSIM, $R > 0.833$, $p = 0.01$) (Figure 14b). At T = 115 days the traits of the species found in the tailings treatments with $C_{org} \leq 0.5\%$ appeared more similar to that found in the control than to tailings treatments with $C_{org} \geq 1\%$, resulting in clustering of two groups (Figure 14c). Statistical analysis confirmed that the tailings treatments with $C_{org} \geq 1\%$ formed a group in which no pairs were found significantly different (ANOSIM, $R < 0$, $p > 0.4$), rather showed a higher variation between the treatments than within, and that all $C_{org} \geq 1\%$ treatments were clearly different from the 0% and the 0.5% (ANOSIM, $R > 0.556$, $p = 0.1$). ANOSIM did however reject a high similarity between the SC and the 0% and 0.5% (ANOSIM, $R > 0.556$, $p = 0.01$).

The same clustering was observed after 180 days, and it was revealed that the difference between the two clusters was still clearly different (ANOSIM, $R > 0.630$, $p = 0.25$), while the differences between the tailings treatments within the groups were not significantly different (ANOSIM, $R < 0.111$, $p > 0.7$).

After 368 days the two groups had converged to one where all samples (except for four) independent of C_{org} concentration were 60% similar in trait composition (Figure 14e). However, ANOSIM rejected the hypothesis that all treatments had the same trait assemblage ($R = 0.516$, $p = 0.001$). The largest overlapping of trait composition was found between the

two treatments with the lowest C_{org} (0% and 0.5%) and between the two treatments with the highest C_{org} (2.5% and 5%) (ANOSIM, $R = 0.074$, $p > 0.4$). Between these four tailings treatments, overlapping tended to decrease with increasing difference in C_{org}, where the lowest similarity in traits of the species present was observed between 0% and 5% (ANOSIM, $R = 0.481$, $p = 0.1$). The 1% treatment was highly similar to the higher C_{org} treatments (ANOSIM, $R < 0.111$, $p > 0.2$), while significantly different from the two lower C_{org} treatments (ANOSIM, $R > 0.741$, $p = 0.1$). Pair wise comparison between the tailings treatments and the two controls showed that the trait composition was significantly different (ANOSIM, $R > 0.556$, $p = 0.1$), except between PC and 0% (ANOSIM, $R = 0.370$, $p = 0.1$), indicating that the benthic ecosystems functioned significantly differently.

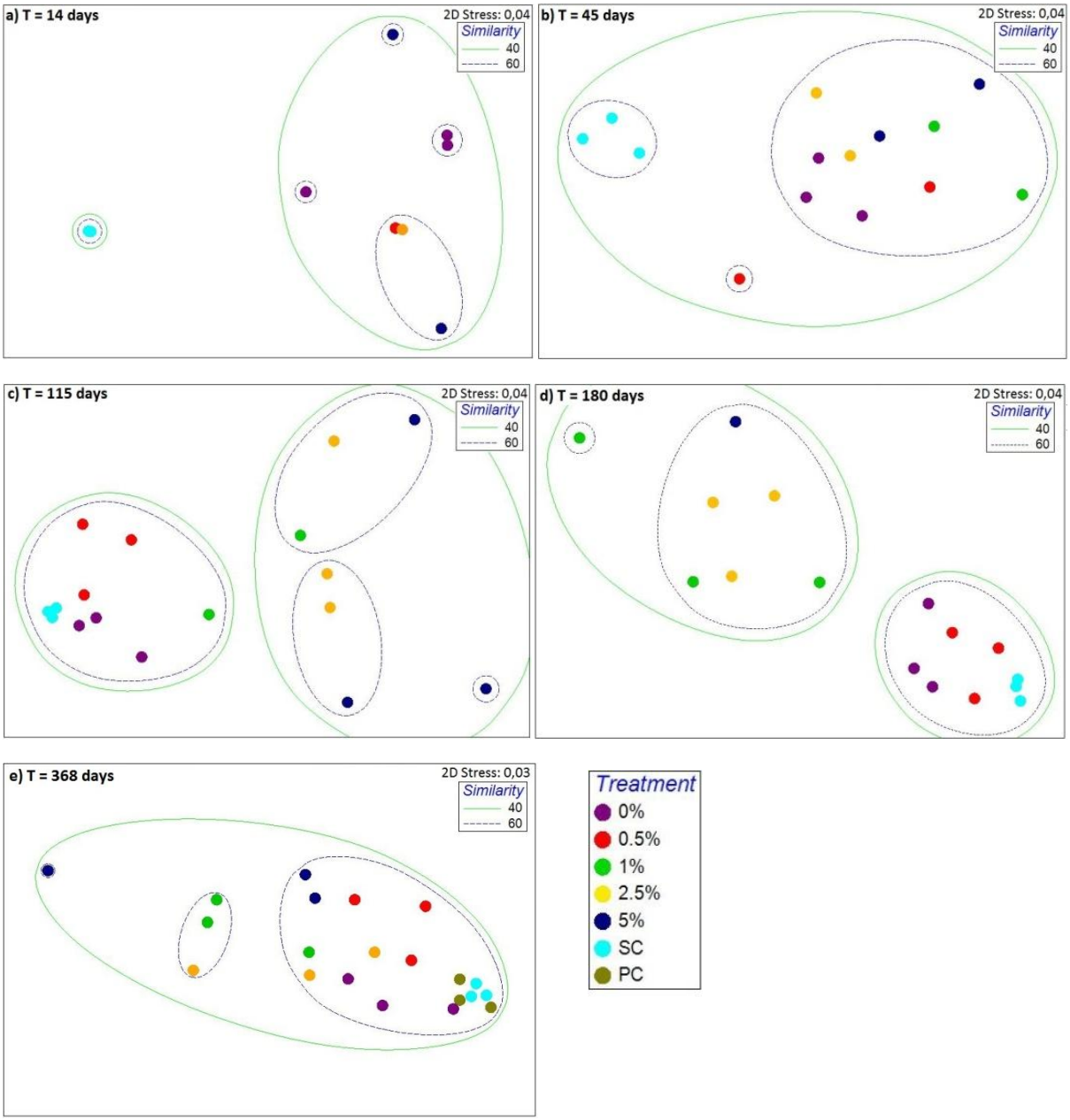


Figure 14(a-e): *Non-metric MDS of trait assemblages based on abundance data in all tailings treatments and the SC at all sampling events. PC included for T = 368 days. Based on Bray-Curtis similarity matrix on square-root transformed data. With superimposed clusters from dendograms based on same data matrix (see Appendix D for dendograms).*

4.5 Secondary Production, Total Production and *P:B* ratio

Functional community structure in terms of secondary production by the different species was compared between all treatments with 2-dimensional ordination (Figure 15a-e). Superimposed clusters indicated a clear difference in secondary production between the tailings treatments and the sampling controls throughout the experiment. Strong clustering within the tailings treatments was also observed until, and including T = 115 days with a stress level of 0 for the ordinations (Figure 15a-c). This can indicate that the groups have collapsed to the same point even though they are not 100% similar, which is most commonly caused by similarities within the groups being higher than or equal to the similarities between the groups (Clarke and Warwick, 2001).

Independent statistical testing of all tailings treatments showed that there was a significant difference between treatments at T = 115 (ANOSIM, R = 0.424, p = 0.001) (see Appendix D for all global and pair wise R values). This was most likely related to the significant difference found between all pair wise comparisons between tailings treatments and the SC (ANOSIM, R = 1, p = 0.1), as none of the tailings pairs could be clearly distinguished from each other (ANOSIM, R < 0.370, p > 0.1). The exception was the 1% and 5% which showed less overlapping (ANOSIM, R = 0.417, p = 0.2).

At T = 180 days less clustering was observed between the tailings treatments. The global R was 0.326 with a significance level of 0.014, and the null hypothesis could not be rejected with great certainty. This was most likely related to the high occurrence of negative (and a few non significant) R-values reported for almost all pairs within the tailings treatments (ANOSIM, R < 0.296, p > 0.1). This implied that the within-treatment variation was higher or equal to the between-treatment variation, and subsequently the hypothesis that the *a priori*

factor had an effect was rejected, implying that C_{org} concentration was not a significant factor governing the secondary production of the different taxa.

After 368 days the test statistic for all treatments showed a significant difference (ANOSIM, R = 0.511, p = 0.001). Pair wise comparisons revealed that all tailings treatments were significantly different to the SC (ANOSIM, R > 0.926, p = 0.1) and PC (ANOSIM, R > 0.889, p = 0.1), though 0% showed some overlapping compared to the latter (ANOSIM, R = 0.519, p = 0.1). All pair wise comparisons of tailings treatments showed strongly overlapping patterns in secondary production (ANOSIM, R < 0.333, p > 0.1) implying the same taxa contributed equally to the secondary production independent of the tailings treatment it inhabited, and thus that C_{org} content did not have a significant effect on secondary production (Figure 15e). The exception was the 2.5% which was different to the 5% (ANOSIM, R = 1, p = 0.01) and the 0.5% (ANOSIM, R = 0.741, p = 0.01).

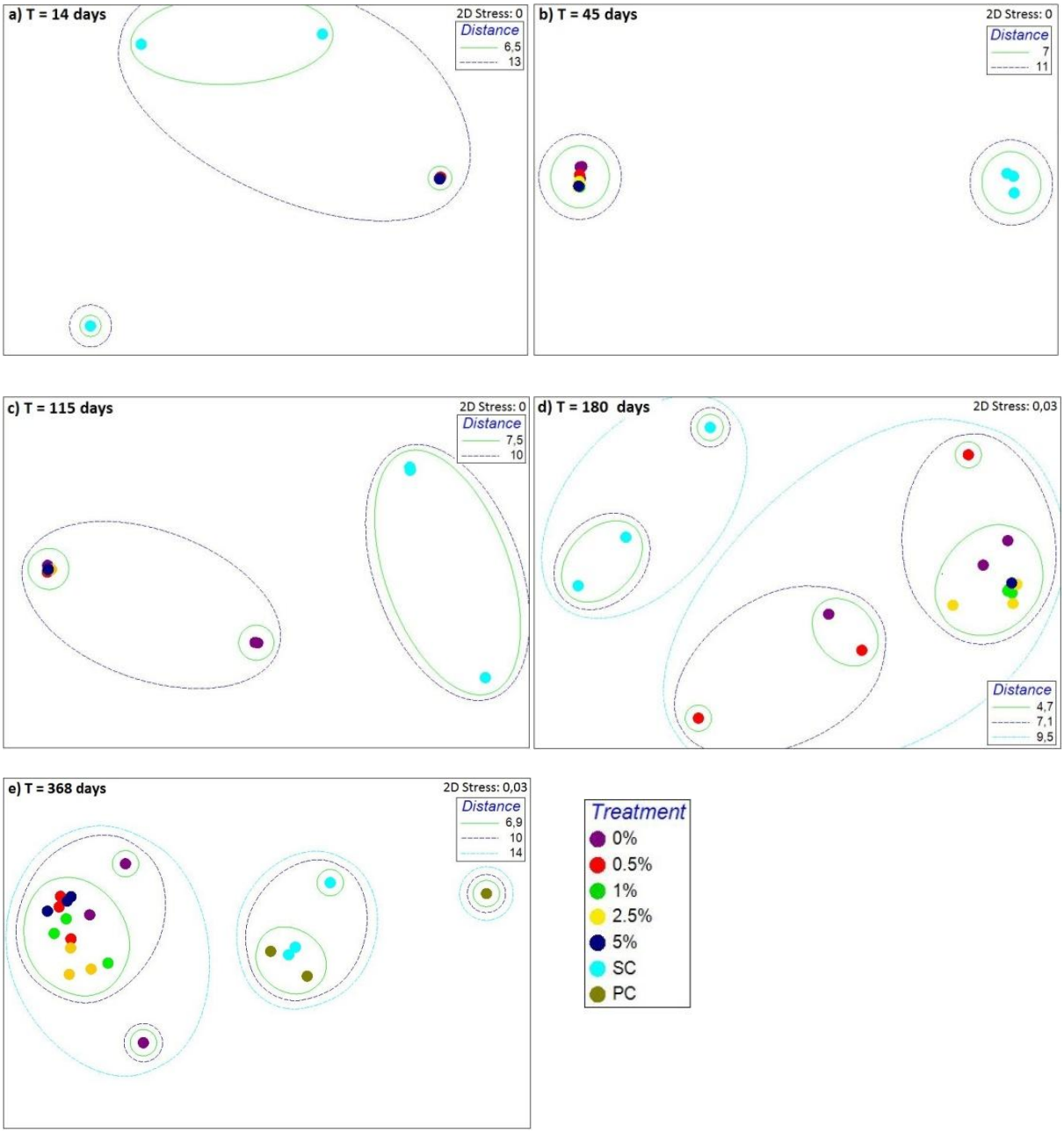


Figure 15(a-e): *Non-metric MDS showing macrofaunal secondary production in all tailings treatments and the SC at all sampling events. PC included for T = 368 days. Based on Euclidean distance matrix on square-root transformed estimates of production data. With superimposed clusters from dendograms based on same data matrix (see Appendix D for dendograms).*

The mean total production values calculated for each treatment at each sampling event showed the same trends as total biomass (Figure 16). There was little relation to the abundance, indicating that the tailings had predominately been colonized by larvae and/or juveniles. Statistical analysis revealed that there was an interaction between treatment and time, in contrast to the biomass data (2-way RM ANOVA, $p = 0.002$), and only differences between treatments over the whole year was therefore statistically assessed (see Appendix D for all p-values and CL).

The total production in the 0% showed little variation over time from T = 45 and attained the highest total production of all tailings treatments after 1 year with a mean of 86 kJ m^{-2} y^{-1} (Figure16). Statistical analysis showed that the 0% was significantly different to the 1% and %5 over time (2-way RM ANOVA, $p < 0.002$, $CL > 0.0005$), but not to the two other tailings treatments (2-way RM ANOVA, $p > 0.013$, $CL < 0.0009$). The 0.5% had the highest total production at T = 180 days, but this had declined after 1 year. The total production in the treatments with a $C_{org} \geq 1\%$ remained low over time, but increased at T = 368 days, and a significant difference was not found between any of the pairs (2-way RM ANOVA, $p > 0.195$, $CL < 0.0013$). Statistical analysis showed that all tailings had a significantly lower total production than the SC throughout the experiment (2-way RM ANOVA, $p < 0.001$, $CL > 0.003$). The large variation observed in PC and increase in SC at T = 368 days (from 174 kJ m^{-2} y^{-1} at T = 180 to 323 kJ m^{-2} y^{-1}) is related to the previously mentioned individuals of *M. balthica.*

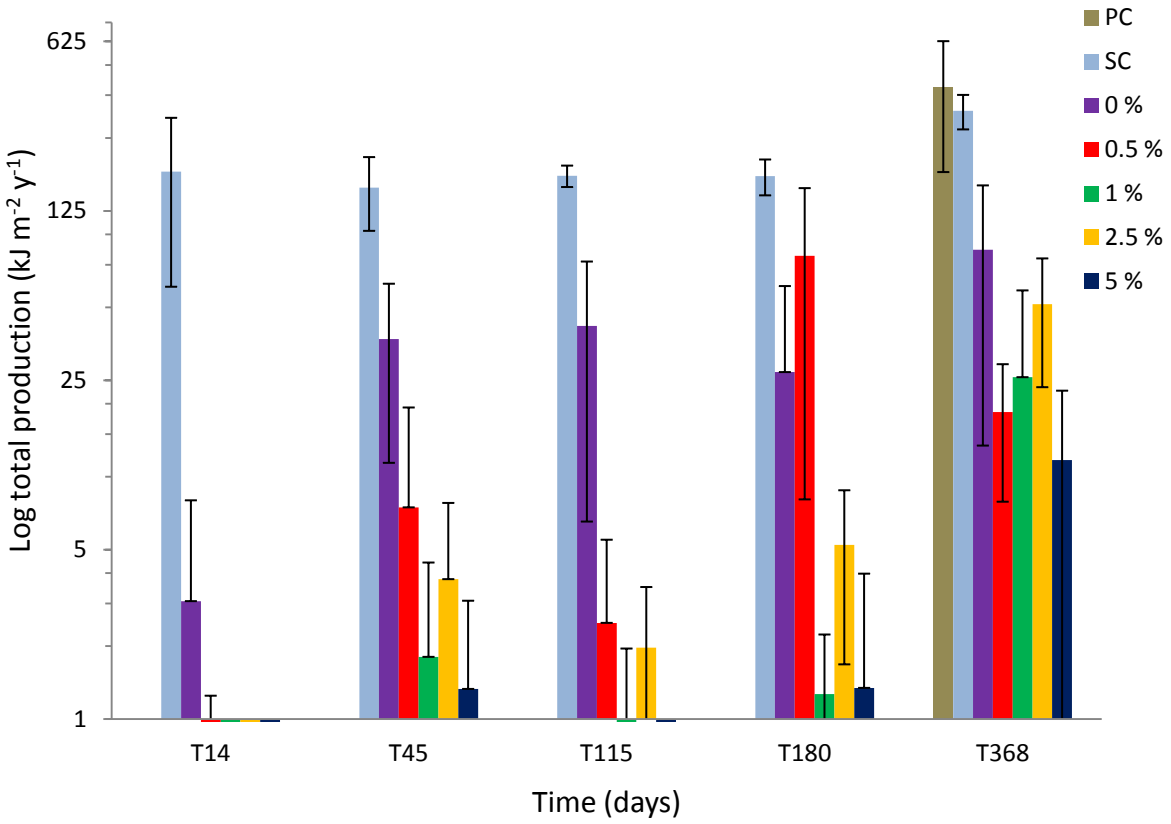


Figure 16: *Log scaled total production (log mean ± 95% CI, n=3) over time in tailings treatments and the sampling control (SC) throughout the experiment. Procedural control (PC) included for T = 368 days.*

Average *P:B* ratio of the community differed between the tailings treatments in the beginning of the experiment, ranging from 7.9 to 14.3 (Figure 17). The *P:B* ratio in the 2.5% and 5% increased until T = 115 days where it had the highest ratio of the treatments, indicating that the proportion of small bodied compared to large bodied animals was larger in these than the other tailings treatments (Bolam, 2012). It subsequently dropped to below the SC before it increased again at T = 368 days. The *P:B* ratio of the 0.5% and 1% showed an overall decline over time, indicating colonization of larger fauna over time. At T = 368 days the average *P:B* ratio in all tailings treatments was similar to the control, ranging between 9.2 and 11.1,

indicating that the turn-over rate after one year was similar in each benthic community. The *P:B* ratio in the SC remained steady between 7.9 and 9.3 throughout the experiment signifying that the macrofauna in the natural sediments have a stabile turn-over rate throughout the year.

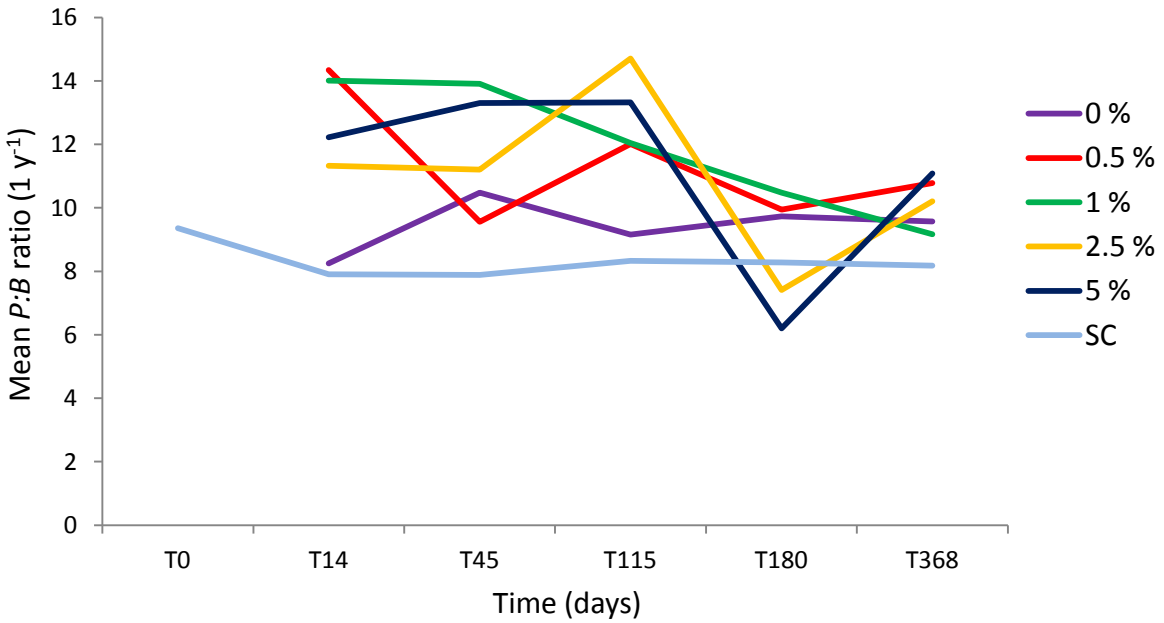


Figure 17: *Average community P:B ratio over time in tailings treatments and the sampling control (SC) throughout the experiment.*

5 Discussion

This study aimed to investigate the effect of different organic carbon concentrations on macrofaunal colonization of mine tailings (as the lack of organic carbon was believed to be one of the main factors for the observed slow colonization of STPs), and to determine the optimum concentration to enhance the colonization process. The results showed that the rate of colonization in terms of abundance and biomass was enhanced during the first season (T = 0 to T = 180 days) when enriching mine tailings with 0.5% organic carbon (Figure 11, Figure 12), indicating that this was an optimum concentration out of those tested. However, the colonization in the 0% treatment was superior to that of treatments with $C_{org} \geq 1\%$, and hence not in agreement with my predictions regarding the positive effect of increasing organic carbon up to 2.5%.

Before proceeding with detailed interpretation of the results it is important to note that the procedural control (PC) showed that possible disturbance related to core-sampling over time had exerted no significant on the environment. Some differences were observed in contribution by taxa to total production, though this was most likely related to variation within time.

5.1 Effect of Organic Carbon Total Abundance and Biomass

Previous studies have found correlations between reducing conditions and macrofaunal colonization (Bolam *et al.*, 2004; Diaz and Rosenberg, 1995) and reducing conditions and abundance (Pearson and Rosenberg, 1978) and thus the low oxygen conditions observed in the high C_{org} ($\geq 1\%$) treatments could have had a negative effect on colonization. High C_{org} in sediments may cause a drastic increase in oxygen demand as heterotrophic bacteria consume

the organic matter (Bolam *et al.*, 2004; Pearson and Rosenberg, 1978). The resulting anoxic conditions and build-up of toxic by-products (especially sulphides and ammonia) from bacterial metabolism, both stress marine organisms (Diaz and Rosenberg, 1995; Sutherland *et al.*, 2007). Stress could negatively affect colonizers by increasing their energy input for survival instead of growth and reproduction, by resulting in casualties, and could also deter fauna from colonizing. Reducing conditions increased with sediment depth were in this study observed in treatments with a $C_{org} \geq 1\%$ from T = 45 (-60mV to -170mV at surface, Figure 6), and high nitrogen values were observed in 2.5% and 5% in the beginning, which could reflect high levels of ammonia and/or large concentration of bacteria with rich nitrogenous cell walls (Figure 5).

However, the redox potentials in the 0.5% treatment were as reducing or more as the high C_{org} treatments throughout the experiment while the abundance in this treatment was the highest up to T = 180 days (Figure 6; Figure 11). This indicates that reducing conditions was not the only driver for low abundance. The 0.5% treatment was dominated by *P. litoralis* at all sampling events, and was thus the main contributor to the high abundance levels observed in this treatment. *P. litoralis* reproduces asexually through paratomy (budding of zooids), which was consistently observed in the samples, and can therefore increase dramatically in abundance over short time periods before crashing again (Gamenick *et al.*, 1996; Levinton *et al.*, 1984). This 'peak and crash' was observed at T = 115 and T = 180 days, respectively. This naidid oligochaete is generally tolerant towards low oxygen levels, but sensitive towards sulphidic conditions (Gamenick *et al.*, 1996). As the organic carbon concentration was low in the 0.5% treatments, and continued to decrease over time (Figure 4), it is likely that even though anoxic conditions were present (redox potential: - 62 to - 167 mV at surface, Figure 6), the amount of organic matter was not sufficient to cause sulphidic conditions (Berner,

1981), thus allowing the colonization of more species than the high C_{org} treatments, which was reflected by the species richness, and consequently represented the optimum C_{org} concentration for tailings colonization.

The species that were the dominant colonizer in the high C_{org} treatments, although present in low numbers, were Nematodes and *H. ulvae*. Nematodes generally have a high tolerance to low oxygen conditions and have been found to inhabit sediments which are highly reducing (Josefson and Widbom, 1988; Mazzola *et al.*, 2000; Sweetman *et al.*, 2014). This taxa also includes sulphide-tolerant genera (Sutherland *et al.*, 2007). *H. ulvae* has been found to not be affected by high organic content (Bolam *et al.*, 2004), probably because it is a mobile surface grazer, and thus stays out of the sediments. Interestingly, Bolam *et al.* (2004) found that *T. benedii* was negatively affected by high organic content, but this was the most dominant species in the 5% treatment in this study. However, *T. benedii* did not become dominant until the last sampling event at T = 368 days, when the organic content in this treatment had been reduced to a level similar to the control (Figure 4), which coincides with the result of Bolam *et al.* (2004). The evenness index showed that the species that were present, structured evenness similarly (Table 2).

This implies that colonization of the high C_{org} treatments was species-specific as it was dependent on the tolerance-level of the species, to both anoxic conditions and toxic bacterial by-products. The low species richness observed in the high C_{org} treatments (Table 2) indicates that the general tolerance among the species in the ambient sediments was low. The low number of species being able to cope with these conditions could explain the observed low abundance.

The temporal variation observed in the SC in this study concurred with previous studies (Zajac and Whitlatch, 1982); abundance decreased from late summer (August, T = 115) to early winter (November, T = 180) and subsequently increased again in late spring (April, T = 368) (Figure 11). The same temporal variation was observed in the tailings treatments indicating that colonization was dependent on the abundance of fauna in the ambient sediments and/ or larvae in the water column (Zajac and Whitlatch, 1982). Interestingly, the mean faunal abundance in the 0% treatments had increased more than the 0.5% treatments at T = 368 days, and concurrently, the C_{org} in the 0% treatments had increased to 0.3% while the C_{org} in the 0.5% treatments had decreased to 0.2% (Figure 4). Microbial mats and growth of microphytic and macrophytic algae were observed on the 0% tailings treatments, and was most likely the cause for this observed increase. This strengthens the statement that a concentration of C_{org} close to 0.5% enhances the colonization process compared to other C_{org} concentrations.

No distinct patterns in succession were observed in the tailings treatments; colonization occurred through a more gradual increase in numbers of ambient species. Bolam *et al.* (2004), who conducted a colonization experiment at the same site, found equivalent results and proposed that this was due to the natural opportunistic traits of the species inhabiting the area. As the environment in intertidal estuaries is potentially stressful for benthic invertebrates the species have developed strategies for survival through fast colonization, resulting in the community remaining at a early stage of succession consisting of predominately small bodied, short-lived and fast growing species, termed '*r*-selected' species (Bolam *et al.*, 2004).

In a disturbed environment the abundance will increase faster than the biomass as the first stage of colonization is dominated by smaller bodied opportunists (Diaz-Castañeda *et al.*,

1993). The increase in total biomass over time in the tailings treatments could clearly be related to the increase in total abundance, and some lag-effect in biomass could be observed to various degrees in the high C_{org} treatments, but especially in the 5% (Figure 12). One exception was the 1% treatment after 115 days, which in contrast to the abundance showed a total biomass almost at a similar level as the 0% and 0.5%, indicating a higher average individual body mass. This increase in biomass was related to the presence of 11 individuals of *Hediste diversicolor*; a predacious polychaete with a tolerance towards organically polluted sediments (Pearson and Rosenberg, 1978). The presence of a large bodied polychaete would cause an increase in the total biomass while having a smaller effect on the abundance (Pearson and Rosenberg, 1978). Another exception was the 0.5% which after 1 year showed a sudden decrease in biomass at T = 180 days, in contrast to the abundance. This decline could also be related to *H. diversicolor*, as this species was suddenly absent after being previously present.

The results suggest that when rehabilitating a seabed by the addition of nutrients, the pre-disturbance concentration might not be the optimum concentration for colonization. In this study an addition of a C_{org} concentration (1%) less than the natural C_{org} concentration (1.5%-1.6%) was too high, and rather than optimizing colonization led to apparently high microbial activity (observed from the redox values) and subsequent anoxic conditions, which prevented colonization, and thus also prevented biological bioturbation which could have reduced the anoxic conditions and increased the interstitial oxygen level in the sediments (Bolam *et al.*, 2002). The use of plastic trays as plots could have promoted reducing conditions both directly, through preventing flow of oxygenated water into the tailings, and indirectly, by preventing lateral colonization of bioturbators, to a greater degree than if a fine mesh had been used as used by Bolam *et al.* (2004); the redox potential in his treatments with 0.9% C_{org}

was much less reducing than in this study's 0.5% and 1% treatments. In addition, fish food pellets contain highly bioavailable organic carbon compared to detritus, and powdered *Ascophyllum nodosum* which have been used in carbon enrichment studies (Bolam *et al.*, 2004).

The use of trays and fish food pellets were however deliberate. Trays restricted colonization to take place from the above water column, which would be the mode of colonization of an azoic STP, while a mesh would also have allowed infauna to colonize horizontally through the sediments. The use of a mesh could be beneficial in future studies, but to prevent horizontal colonization the aperture would need to be smaller than the larvae and/or juvenile stage of the group of organisms colonizing the sediment. Fish food pellets were used instead of *A. nodosum* as this was an experiment on fertilization of sediments, and not natural organic enrichment. The highly bioavailable organic carbon probably increased microbial activity to a higher degree, and thereby promoted anoxic conditions, than the less bioavailable organic carbon source *A. nodosum* would have.

It is well-established that sediment organic carbon is an important food source for macrofauna, and that its presence is important for colonization is assumed (Hyland *et al.*, 2005). The high number of species that colonized the 0% treatments was therefore surprising and not in agreement with my predictions. The concentration in the 0% treatments increased to 0.1% within the first 115 days, so these mine tailings did not remain entirely barren, but the concentration was still at a level believed to be too low to encourage colonization. However, to the best of my knowledge, no studies have investigated the mechanisms, or rate of, colonization of sediments with zero initial organic carbon.

5.2 Effect of Organic Carbon on Species and Trait Assemblages

Similar to the effect of organic carbon on total abundance, there appeared to be an effect of organic carbon on the community structure; seen through a separation between the high C_{org} ($\geq$ 1%) and the low C_{org} ($\leq$ 0.5%) after 115 days (Figure 11a-c). There was therefore a difference in species assemblages and the abundance between these communities. This was most likely driven by the differing tolerance-levels towards sulphidic and anoxic conditions between taxa (Pearson and Rosenberg, 1978). The similarity between the tailings treatments increased towards the end of the experiment (T = 368 days), both in regards to community structure and diversity (Figure 9; Figure11e), and also evenness (Table 2), and could be an effect of the concurrent increased resemblance in the C_{org} and nitrogen concentrations between tailings treatments (Figure 4; Figure 5). However, there were still significant differences between the highest and the lowest C_{org} treatments, implying that there was a prolonged effect of the initial C_{org} content on the colonization process. The difference in community structure as an effect of organic content is similar to results found in previous studies (Bolam *et al.*, 2004; Pearson and Rosenberg, 1978; Weston, 1990). Bolam *et al.* (2004) showed that the species assemblages were distinctly different after one year in sediments modified with 0.9% and 2.8% organic carbon. Weston (1990) found a distributional shift at the community level as a result of organic content increasing from 0.2% to 1.26%, and related this to a change in feeding mode.

Small scale patches are usually colonized by a community with a similar community structure as the ambient sediments. Exceptions occur if the environment has been significantly altered (Bolam *et al.*, 2004). Mine tailings usually represent a more homogeneous habitat and subsequently with fewer niches than natural seabeds (Miljødirektoratet, 2010). As expected, it was found that the species' assemblages between the tailings treatments and the ambient

sediments were significantly different. In addition, the difference in particle size and angularity further altered this habitat. The function of the ecosystem does however not necessarily have to change, as one species can be replaced by another which exhibits the same traits and therefore provides the same function (Cooper *et al.*, 2008).

The trait assemblages in the tailings treatments showed a similar pattern as the species assemblages after 115 days; a distinction between the low C_{org} and high C_{org} implied that the concentration of C_{org} was an influential factor for the colonization of species with different traits (Figure 14c). Benthic populations in organically polluted areas are associated with the loss of larger, longer living species and an increase of more tolerant, smaller, deposit feeders (Pearson and Rosenberg, 1978) i.e. species with different traits. These traits were found among species inhabiting an organically enriched lagoon (Marchini *et al.*, 2008), supporting the theory of Pearson and Rosenberg (1978), and the distinct trait compositions in this study is therefore most likely a reflection of the small bodied, anoxic-tolerant species observed in the high C_{org} treatments.

The trait assemblage in the 0% treatment after one year was equivalent to the trait assemblage in one of the controls (Figure 14e), indicating that even though the species assemblage was not equal, the services provided by the species were the same, e.g. same level of bioturbation, life span and cycling of nutrients (Bremner, 2006; Bremner 2008). The trait assemblages in all other mine tailings treatments and the ambient sediments had become more similar over time, but were still significantly different. In a fjord system, this could have significant impacts as the traits of the benthic invertebrates determine how the species affect ecosystem processes (Bremner, 2006). For example, if the presence of species which burrow is lower, the

sediments will become less oxygenated, and a lower amount of nutrients will be transported up to the sediment surface, where it is available for other species (Bremner, 2006).

This difference in functioning was also observed between the tailings treatments, and the dissimilarity increased with increasing differences in initial organic carbon (Figure 14e). This indicated that even though the environmental conditions were becoming more similar, as could be observed in relation to carbon and nitrogen content (Figure 4, Figure 5), and sediment grain size (Figure 7), the initial C_{org} concentration was still impacting the benthic communities. As the trait assemblages were based on abundance, the low total abundance observed in the tailings would exert an effect on the analysis. The difference was thus not necessarily caused by the presence of different traits, but the presence of a lower number of species exhibiting the different traits.

Species diversity on estuarine mudflats is generally low, which results in a low number of species exhibiting the same traits (Levin *et al.*, 2001a). The chance of one species being able to replace another is therefore reduced. In other words, the system has 'low redundancy' (Cooper *et al.*, 2008; Levin *et al.*, 2001a). The redundancy in fjord systems is higher, as species richness is much higher (Holte *et al.*, 2004; Levin and Smith, 1984), and the chance of a more similar trait assemblage between STPs and non-impacted sites is therefore higher. Nevertheless, benthic recovery to disturbance occurs at a slower rate at deeper depths, where STPs are deposited, than in the intertidal (Levin and Smith, 1984), and a high redundancy will not increase the rate of colonization. Therefore, the function of the benthic ecosystem in STPs will most likely remain low over a long period of time (> 1 year), until the abundance is recovered.

5.3 Effect of Organic Carbon on Production and *P:B* ratio

While the trait analysis is only a qualitative method to assesses the ecological function of a benthic ecosystem, secondary production allows for a more quantitative assessment of the community function (Bolam and Eggleton, 2014). Where the species and trait assemblages were influenced by the concentration of C_{org} up to 115 days, C_{org} appeared to have no evident effect on the secondary production of the different taxa throughout the experiment, and no distinct grouping was observed (Figure 15a-c). The total production in the communities did however differ; the 0% treatment had a higher total production than the other tailings treatments at all but one sampling event (T = 180 days), though this was not always significant (Figure 16).

After 1 year the secondary production by the different taxa was more or less equal across all tailings treatments, except for the 2.5% which was found different compared to the 1% and 0.5% treatments (Figure 15e). This difference was also reflected in the total production, where the 0.5% and the 1% treatments had the lowest total community production, while the 2.5% had increased (Figure 16). The highest total community production was found in the 0% treatment, which could be related to the total biomass, and also supports my hypothesis of a low C_{org} concentration being optimum for colonization in this study (as the organic carbon in this treatment had increased). However, the total production in the 0% treatment was still barely one-third of the total production in the ambient sediments (Figure 16). This implies that the functioning, and the ecosystem services, the community in the tailings treatments provided was much lower than that of the ambient sediments. The general trend was however an increasing total production in the tailings treatments (throughout the year), even though the contribution of the different taxa was still different from the control. Therefore, the species contributing to an increasing total production in the tailings treatments were not the same as

the species that were contributing to the total production in the ambient sediments. This indicates that the importance of a species for community production, and therefore community function, depends on the environment.

Effects on higher trophic levels as a result of a degraded benthic community post-deposition of mine tailings have not been greatly studied, the focus has been on the benthic environment. In a Norwegian fjord that was subject to an active STP, discoloring of the gills of shrimp and fish was observed due to resuspension of mine tailings particles (Miljødirektoratet, 2010). However, a drastic decrease in benthic production will most likely have an impact on higher trophic levels as the amount of available prey decrease. Furthermore, the area covered by a STP becomes more spatially homogeneous (Miljødirektoratet, 2010), and this will result in a less stable predator-prey relationship (Gilinsky, 1984).

High *P:B* community ratios are generally found in physically-stressed communities (e.g. intertidal estuaries) dominated by *r*-species (Bolam *et al*., 2010), and was observed in the ambient sediments of this study (Figure 17). In general, the tailings treatments had a higher *P:B* ratio than the ambient sediments, implying a higher turnover rate (Bolam *et al.*, 2010). The two highest C_{org} treatments showed a general trend of exhibiting the highest *P:B* ratio and also varied equally over time, while the 0% and 0.5% treatments had the lowest *P:B* ratio. This indicates that the lower C_{org} treatments had a lower turnover rate than the higher C_{org} treatments, therefore the energy available for the next trophic level was lower (Bolam *et al.*, 2010). In contrast, the 0% treatment had the highest total production, indicating that factors other than the turn-over rate affect the total production of a community (Bolam *et al.*, 2010). Estuarine mudflats are some of the most productive areas in the world with high secondary production and *P:B* ratio due to the rich input of organic matter (Levin *et al.*, 2001a). It is

therefore surprising that the total production in the tailings treatments remained at such a low level.

5.4 Potential Effects of Angularity and Particle Size on Colonization

Overall the communities in the tailings treatments was far less productive than the ambient sediments throughout the experiment, indicating that even though the abundance was enhanced the functions provided by the species present were much lower. These results add to the notion that the lack of organic carbon might not be the only governing factor explaining the slow rehabilitation of STPs, but that a factor that was not tested for in this study has a high impact. The particle size of the mine tailings was much larger than the ambient sediments (Figure 7), and a great difference was also observed in the angularity between the tailings and that of the mudflat (Figure 8). The sharp edges and elongation of the mine tailings particles could have a negative effect on the digestive system of deposit feeders. I therefore propose that the low level of function in terms of total production, in addition to the slow colonization-process generally observed for STPs, is not solely the result of a lack of organic carbon but rather an effect of multiple stressors caused by low organic carbon, particle sizes and abnormal angularity of the mine tailings.

Skei and Rygg (1989) acknowledged the sharp-edged characteristics of mine tailings particles, but made no further remarks regarding potential impacts. Later, Olsgard and Hasle (1993) also observed these sharp-edged characteristics, and stated that it could cause problems for deposit feeders and infauna, and thereby reducing the diversity in STPs as less species were able colonize. In marine sediments tentaculate feeders constitute one of the major functional groups; this feeding mechanism allows for significant selective abilities (Lopez and Levinton, 1987). Selective feeding of specific particle sizes by deposit feeders has

been shown by numerous studies (Levin *et al*., 2001b), where most prefer finer particles (Lopez and Levinton, 1987). This selection could be due to the higher microbial mass as finer particles have a higher surface area to volume ratio. In an environment where the particle sizes are larger, such as the mine tailings, the microbial mass would be inherently lower, and this could have a negative effect on colonization.

Very few studies have however looked at selective feeding and the influence of particle angularity. Hughes (1975) observed that angular silica particles were partially rejected by the bivalve *Abra tenuis*, while rounded particles were favoured, independent of associated food particles. In another study it was found that the deposit feeding polychaete *Pectinaria gouldii* preferred encrusted mineral particles (Whitlatch, 1974), which Lopez and Levinton (1987) later interpreted as an affinity to angular particles. Neither the term 'angular' nor 'encrusted' were however properly described in these three studies, making these ambiguous findings difficult to compare to the current study. Nevertheless, to the best of my knowledge no studies have looked at the potential effects of particle angularity on the digestive system of deposit feeders, and this could be a potential negative effect through shredding of the gut.

Giere (2009) stated that particle shape is an indirect factor determining meiobenthic colonization due to effects on water content and permeability. This may also be true for benthic macrofauna. Sediments consisting of predominately angular particles have smaller pore space, due to a tighter packing, and thus also lower permeability and lower water content (Figure 18). The smaller pore space in mine tailings deposits may prevent large individuals from colonizing as the ability to move freely is restricted, and may explain the low biomass and high *P:B* ratio observed in all the tailings treatments. Williams (1972) observed a general

relationship was observed between pore space and the diameter of the species inhabiting the sediments, although this was only seen in some phylogenetic groups.

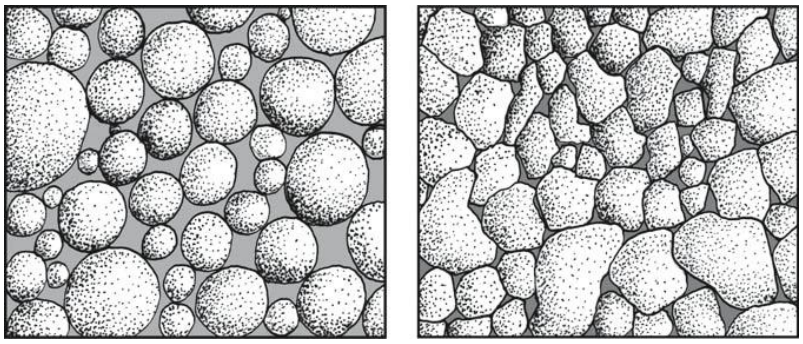


Figure 18: *Showing the difference in pore space as a results of different packing between more spherical versus more angular particles (Giere, 2009).*

5.5 Univariate Indices and Their Potential for Determining Ecological State

The biodiversity index (*H'*) showed that there was no significant difference between the tailings treatments and the ambient sediments after one year (Figure 9), indicating that recovery of all plots had occurred. The evenness index (*J'*) and the species richness implied the same, especially between the low C_{org} treatments and the ambient sediments (Table 2). The *H'* and *J'* indices can however be significantly influenced by habitat type or complexity, and may in some cases increase with stress, and therefore do not truly represent the ecological state of the disturbed environment (Clarke and Warwick, 2001; Gray, 2000; Salas *et al.*, 2006). Cooper *et al.* (2008) has suggested that these indices may not realistically reflect recovery, especially when considering an altered environment, and Salas *et al.* (2006) demonstrated that the Shannon-Wiener index was not appropriate to indicate the ecological status of an intertidal area. The same was found in this study; the total production of the benthic community in the tailings treatments was significantly less compared to the ambient

sediments, thereby implying that the ecological status was very poor, while the Shannon-Wiener index indicated a better status.

This emphasizes the importance of looking beyond the structural integrity of a community when assessing impacts on the benthic ecosystem; by not doing so could result in false reassurance regarding the ecological status. Furthermore, seabeds can be highly dynamic systems undergoing continuous change which in turn affects the species available for colonizing, thereby preventing a community to reach a 'maximum' or 'equilibrium' state (Cooper *et al.*, 2008). Gray (2000) previously urged that other methods in addition to indices should be used when investigating environmental change. Cooper *et al.* (2008) suggested that also looking at the function of an ecosystem would be of interest in addition to the species' range and proportion. The current guidelines for environmental impacts by offshore activity on the seabed includes assessment of Shannon-Wiener diversity index, Pielou's evenness index and the proportions of dominant species (Miljødirektoratet, 2011). The former two indices have shown to not be ideal for determining ecological state post-disturbance (Gray, 2000; Salas *et al.*, 2006). Benthic community structure is assessed in these guidelines using similar methods as in this study. Neither biomass nor functional community structure, although shown in this study to be vital in assessing the environmental state of an ecosystem, are included. Based on this, it is hard to avoid raising the question if we really understand the impact our activities have on the seabeds along the Norwegian coast.

Assessing secondary production of species, let alone a whole community, using classical methods is both expensive and time-consuming (Cusson and Bourget, 2005), but the increasing availability and accuracy of empirical models has made this an easier and less time-consuming task. Secondary production estimates and biological trait analysis can

significantly add to the knowledge about the benthic ecosystem, as shown in this study. It is therefore recommended that not only methods looking at the structural integrity, but also the functional integrity, are included in monitoring programs in the future.

6 Conclusions and Recommendations for Future Research

The randomized complete block design used in this study was found to be an appropriate method to address the effect of organic carbon on macrofaunal colonization, and is recommended for future experimental studies.

I conclude that 0.5% was the optimum organic carbon concentration to stimulate macrofaunal colonization in this study. The initial concentration of organic carbon showed to have a prolonged effect on both the species assemblages and trait assemblages, which was most likely the result of an unfavorable environment in the higher organic carbon treatments due to anoxic and toxic conditions. Organic carbon was therefore concluded to be an influential factor in mine tailings for colonization of different taxa, and also for the colonization of different traits. Organic carbon concentration did however have no evident influence on the contribution by different taxa to total production, and other factors were therefore concluded to govern the production.

In terms of the ecological state of the benthic communities in the tailings treatments, there were high inconsistencies between the results. Shannon-Wiener diversity index, Pielou's evenness and species richness indicated recovery of the benthic communities in the 0% and 0.5% organic carbon treatments after 1 year. Analysis of biological trait assemblages in the mine tailings, showed that the macrofaunal community was functioning differently than the community in the ambient sediments, which could be related to the low redundancy of the benthic community at the study site (Levin *et al.*, 2001a). Furthermore, contribution to total production was significantly different in the tailings treatments compared to the ambient sediments after 1 year. The services provided by the community in terms of energy to higher trophic levels were thus lower. The conclusion is therefore that an addition of organic carbon

alone does not significantly enhance the rehabilitation rate of the mine tailings. A general trend of increasing total production in the tailings treatments was however observed. This indicates that the importance of a taxon for community function depends on the environment.

The low level of function in terms of total production in this study, in addition to the slow colonization-process generally observed for STPs, are proposed to be an effect of multiple stressors caused by low organic carbon, particle sizes and the abnormal angularity of mine tailings.

The need for more knowledge regarding the long-term effects of mine tailings deposits on the function of the benthic ecosystem is evident. The gap in current research regarding the possible effects of particle angularity on benthic macrofauna should be addressed. Research on possible effects of multiple stressors related to lack of organic content, angularity, and particle size need to be conducted in order to develop better solutions for management of STPs. It is also highly recommended that environmental directives are revised, as the current methods used to assess impacts on the benthic ecosystem may not reflect the true ecological state.

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Appendix A

Sediment Chemistry Analysis

Five samples of raw mine tailings were analyzed for mineralogy. Particle size analysis was conducted using the Malvern Mastersizer 2000, with 2000G accessory. Optical mineralogy was conducted by preparing smear slides which were examined using a petrographic microscope.

Five raw mine tailings samples were analyzed for metals following a method UKAS accredited to ISO 17025:2005. A sub-sample was partially digested by nitric acid using enclosed vessel microwave. Analysis was then performed by ICP-MS and ICP-AES by external calibration with Indium as internal standard. Each batch contained a procedural blank and a certified reference material for quality control purpose.

Particle size distribution (volume weighted) was completed by laser diffraction using a Malvern Mastersizer 2000. A total of 24 samples were analyzed; three which were raw mine tailings, and 21 collected at T = 368 days from all plot types in block 1, 3 and 5. Certified reference material and in-house standards were used for quality control purposes.

Quantitative shape analysis was conducted on one raw mine tailings sample. This entailed calculating the elongation index using two measurements as follows: width (intermediate length) / length (longest axis). The particles were then categorized into extreme, very, moderately, slightly and not elongate (Zingg, 1935).

Samples analyzed for TOC and TN were: three replicates collected at T = 0, 14, 45 and 115 days (from block 1, 3, 5) from each plot except PC, and five replicates collected at T = 180

and 368 days from all plots except PC. Subsamples were sent to a subcontractor for analysis TOC and TN. Subsamples were obtained by grounding up collected sediment samples post freeze-drying and removal of any material > 2 mm. A sulphur acid digest was used to remove any inorganic carbon, and a hydrochloric acid test conducted to test all inorganic carbon was removed, before the TOC was measured using an elemental analyzer. For quality control purposes certified reference materials were measured with every batch submitted to the subcontractor, repeats (1 in 10) were completed, and every batch of 10 samples contained a procedural blank and a Sulphanilamide check standard.

SEM imaging was conducted on five samples, where two were collected from the PC in block 4 and 5 at T = 368 days and three were of raw mine tailings. A total of 48 SEM images were taken with magnification ranging from x200 to x2500 for PC sediments, and from x80 to x1200 for raw mine tailings samples.

Appendix B

Biological Traits And Conversion Factors

Table 3: *Description of traits and categories used in the biological trait analysis for all taxa.*

Trait	Category	Description
Size range (mm)	<10 11-20 21-100 101-200 201-500 >500	*Size range (height or length) in mm*
Morphology	Soft	*External tissue soft and not covered by any form of protective casing*
	Tunic	*Body covered by a protective outer tissue made up of, for example, cellulose, e.g., tunicates*
	Exoskeleton/shell	*Body covered or encased in either a thin chitinous layer or calcium carbonate shell*
Longevity	<1 3-10 >3-10 >10	*The maximum lifespan of the adult stage (y)*
Larval development location	Planktotrophic (pelagic)	*Larvae feed and grow in the water column*
	Lecithotrophic (pelagic)	*Larvae feed on yolk reserves*
	Direct (benthic)	*Larval stage missing (eggs develop into juvenile forms) or larvae are limited to the bed*
Egg development location	Asexual/Budding	*Species can reproduce asexually, either by fragmentation, budding, epitoky, etc.*
	Sexual-shed eggs (pelagic)	*Eggs are released into the water column*
	Sexual-shed eggs (benthic)	*Eggs are released onto/into the bed, either free or maintained on bed by mucous or other means*
	Sexual brood eggs	*Eggs are maintained by adult for protection, either within parental tube or within body cavity*
Living habit	Tube-dwelling	*Tube may be lined with sand, mucus or calcium carbonate*
	Burrow-dwelling	*Lives within a permanent or temporary burrow*
	Free-living	*Not limited to any restrictive structure at any time, able to move freely within and/or on the sediments*
	Crevice/hole/under stone	*Adults are typically cryptic, predominantly found inhabiting spaces made available by coarse/rock substrate and/or tubes made by biogenic species or algal holdfasts*
	Epi/endo zoic/phytic	*Live on other organisms*
	Attached to substratum	*Attached to larger, stable boulders or rock*

Table 3 continued:

Trait	Category	Description
Sediment position	Surface	*Found on or just above the seabed*
	Infauna: 0-5cm	*Species whose bodies are found almost exclusively below sediment surface between 0 and 5cm*
	Infauna: 6-10cm	*Species whose bodies are partly or exclusively found below sediment surface at a depth generally between 5 and 10 cm*
	Infauna: >10cm	*Species whose bodies are partly or exclusively found below sediment surface at a depth greater*
Feeding mode	Suspension	*The removal of particulate food taken from the water column, generally via filter-feeding*
	Surface deposit	*Active removal of detrital material from the sediment surface, includes scraping and/or grazing algal matter from surfaces*
	Subsurface deposit	*Removal of detrital material from within the sediment matrix*
	Scavenger/opportunist	*Species which feed upon dead animals*
	Predator	*Species which actively predate upon animals (including the predation on smaller zooplankton)*
Mobility	Sessile	*Species in which the adults have no, or very limited, mobility to a either because they are attached or are limited (semi-)permanent tube or burrow*
	Swim	*Species in which the adults actively swim in the water column (many usually return to the bed when not feeding)*
	Crawl/creep/climb	*Capable of some, generally limited, movement along the sediment surface or rocky substrata*
	Burrower	*Infaunal species in which adults are capable of active movement within the sediment*
Bioturbators	Biodiffusers	*Vertical and/or horizontal movement of sediment and/or particulates*
	Surface deposition	*Deposition of particles at the sediment surface resulting from e.g. defecation or egestion (pseudofaeces) by for example, filter and surface deposit feeding organisms*
	Upward conveyor	*Translocation of sediment and/or particulates from depth within the sediment to the surface via defecation by head-down vertical oriented species*
	Downwards conveyer	*The subduction of particles from the surface to some depth by feeding or defecation*
	Regenerator	*Excavation of holes/ digging that results in a transfer of sediment from depth to the surface*
	None	*Do not perform any of the above and/or not considered as contributing to any bioturbative capacity*

Table 4: *Conversion factors for converting biomass (WMmg) to energy (J) for P:B ratio and production estimates for all taxa identified.*

Species	Conversion factor J $mgWM^{-1}$ (with shell if applicable)	without shell
Hydrobia ulvae	2.56993	3.554
Macoma balthica	1.011171183	1.66617876
Macomas Tellinid juv.	1.011171183	1.66617876
Retusa obtusa	1.191868367	1.33648233
Pygospio elegans	3.284148718	
Streblospio shrubsolii	3.284148718	
Polydora sp.	3.284148718	
Spionidae sp.	3.284148718	
Capitella capitata	3.284148718	
Hediste diversicolor	2.590038657	
Manayunkia aestaurina	3.284148718	
Eteone flava	2.590038657	
Tharyx spp.	3.284148718	
Cirratulidae juv.	3.284148718	
Paranais litoralis	3.284148718	
Tubificoides benedii	3.284148718	
Tubificoides pseudogaster	3.284148718	
Oligochaeta sp.	3.284148718	
Enchytraeidae	2.3968	
Ampharete sp.	3.667385896	
Platyhelminth	4.00112	
Nemertea	4.001116239	
Ostrocoda	1.103821525	1.25469
Nematoda	4.001116239	
Foraminifera	1.472833891	
Polynoidae sp.	2.507505316	
Priapulida	3.25825	
Mytilidae juv.	1.949041088	3.38019539

Appendix C

Results From Metal Analysis

Table 5: *Results from metal analysis carried out by ICP-MS and ICP-AES on five raw mine tailings samples. Samples were analysed for chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg).*

Sample	Cr (mg/kg)	Ni (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Hg (mg/kg)
1	3,7	8,8	15	27	2,6	0,04	1,5	<0.02
2	2,7	7,2	23	22	2,0	0,05	1,2	<0.019
3	5,4	14	46	38	4,6	0,06	2,3	<0.02
4	3,3	10	18	28	3,9	0,04	1,2	<0.02
5	2,9	7,7	69	23	2,8	0,04	1,2	<0.019

Shannon-Wiener Diversity Index

Table 6: *Mean Shannon-Wiener diversity index ± 95% confidence intervals for all treatments throughout the experiment. Number of replicates stated if deviated from 3, 95% confidence intervals only calculated when 3 replicates.*

	0%	0.5%	1%	2.5%	5%	SC	PC
T = 14	0.40 ± 0.4	0.69 *(2)*	0 *(1)*	0.69 *(1)*	0 ± 0	1.87 ± 0.17	-
T = 45	1.07 ± 0.53	0.77 ± 0.43	0.69 *(2)*	1.29 *(2)*	1.08 *(2)*	2.03 ± 0.18	-
T = 115	1.47 ± 0.46	1.16 ± 0.76	0.82 ± 0.80	0.64 ± 0.64	0.22 ± 0.44	1.85 ± 0.02	-
T = 180	1.60 ± 0.08	1.69 ± 0.53	0.75 ± 0.74	1.17 ± 0.21	0.69 *(1)*	1.70 ± 0.10	-
T = 368	1.89 ± 0.22	1.60 ± 0.28	1.59 ± 0.08	1.64 ± 0.34	1.23 ± 0.54	1.93 ± 0.15	1.86 ± 0.14

Appendix D

Comparison PC T = 0 days vs. PC T = 368 days

ANOSIM and T-test Output

Abundance data

Global Test
Sample statistic (Global R): 0,185
Significance level of sample statistic: 10%
Number of permutations: 10 (All possible permutations)
Number of permuted statistics greater than or equal to Global R: 1

Secondary Production data

Global Test
Sample statistic (Global R): 0,519
Significance level of sample statistic: 10%
Number of permutations: 10 (All possible permutations)
Number of permuted statistics greater than or equal to Global R: 1

Biological Trait data

Global Test
Sample statistic (Global R): 0,296
Significance level of sample statistic: 10%
Number of permutations: 10 (All possible permutations)
Number of permuted statistics greater than or equal to Global R: 1

Total Production

t = 2.112
Df = 4
p = 0.102

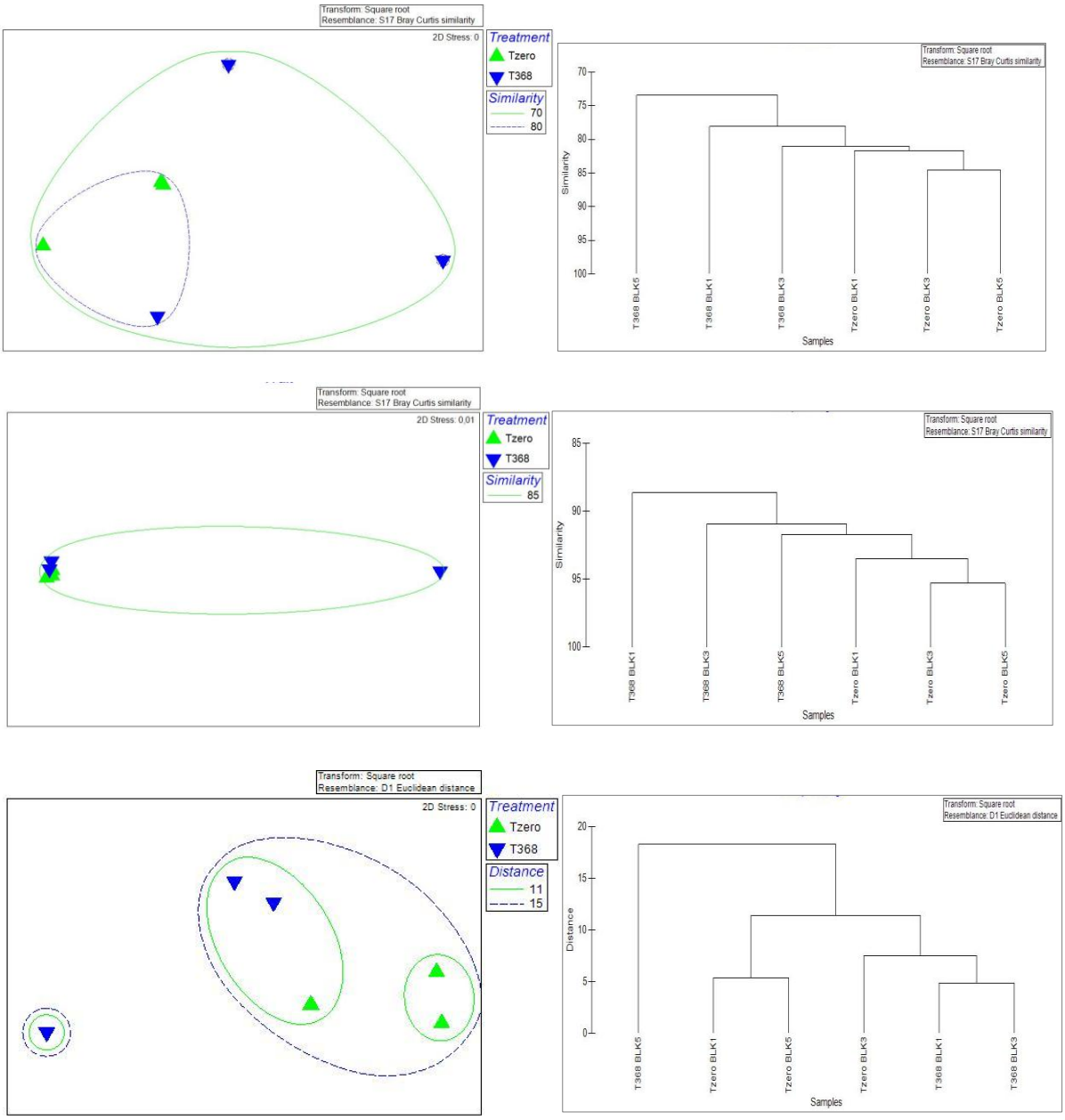

Figure 19: *Non-metric MDS (left) with superimposed clusters from dendograms (right) of community structure in PC at T = 0 days T = 368 days (n = 3). Based on: Bray-Curtis similarity matrix on square-root transformed abundance data (top), Bray-Curtis similarity matrix on square-root transformed trait data (middle), Euclidean distance matrix on square-root transformed estimates of production data (bottom).*

Appendix E

Output from statistical analyses

This appendix comprises of output from the 2-way repeated measures ANOVA for all datasets, output from ANOSIM from all datasets, and dendograms from cluster analysis which were superimposed on MDS.

2-way Repeated Measures ANOVA

Table 7a-c: *Output from 2-way Reapeated Measures ANOVA on Shannon-Wiener index values. Showing results from Holm-Sidak post hoc, comparison between treatments within time.*

a) Comparisons for factor: **Treatment within T = 115 days**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 5%	1,629	5,111	<0,001	0,003
0% vs. 5%	1,248	3,916	<0,001	0,004
SC vs. 2,5%	1,213	3,805	<0,001	0,004
SC vs. 1%	1,036	3,252	0,003	0,004
0,5% vs. 5%	0,934	2,930	0,007	0,005
0% vs. 2,5%	0,832	2,610	0,015	0,005
SC vs. 0,5%	0,695	2,180	0,038	0,006
0% vs. 1%	0,655	2,057	0,050	0,006
1% vs. 5%	0,592	1,859	0,074	0,007
0,5% vs. 2,5%	0,518	1,624	0,116	0,009
2,5% vs. 5%	0,416	1,306	0,203	0,010
SC vs. 0%	0,381	1,195	0,243	0,013
0,5% vs. 1%	0,341	1,071	0,294	0,017
0% vs. 0,5%	0,314	0,986	0,333	0,025

b) Comparisons for factor: **Treatment within T = 180 days**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 1%	0,945	2,966	0,006	0,003
0,5% vs. 1%	0,943	2,958	0,006	0,004
0% vs. 1%	0,854	2,680	0,012	0,004
SC vs. 5%	0,987	1,850	0,075	0,004
0,5% vs. 5%	0,984	1,845	0,076	0,005
0% vs. 5%	0,895	1,679	0,105	0,005
SC vs. 2,5%	0,522	1,637	0,113	0,006
0,5% vs. 2,5%	0,519	1,629	0,115	0,006
0% vs. 2,5%	0,430	1,351	0,188	0,007
2,5% vs. 1%	0,423	1,329	0,195	0,009
2,5% vs. 5%	0,465	0,872	0,391	0,010
SC vs. 0%	0,0914	0,287	0,776	0,013

0,5% vs. 0%	0,0887	0,278	0,783	0,017
1% vs. 5%	0,0414	0,0777	0,939	0,025

c) Comparisons for factor: **Treatment within T = 368 days**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 5%	0,699	2,195	0,037	0,003
0% vs. 5%	0,666	2,091	0,046	0,004
2,5% vs. 5%	0,416	1,305	0,203	0,004
0,5% vs. 5%	0,370	1,161	0,256	0,004
1% vs. 5%	0,362	1,135	0,266	0,005
SC vs. 1%	0,338	1,059	0,299	0,005
SC vs. 0,5%	0,329	1,033	0,311	0,006
0% vs. 1%	0,305	0,956	0,348	0,006
0% vs. 0,5%	0,296	0,930	0,361	0,007
SC vs. 2,5%	0,284	0,890	0,381	0,009
0% vs. 2,5%	0,251	0,786	0,439	0,010
2,5% vs. 1%	0,0540	0,170	0,867	0,013
2,5% vs. 0,5%	0,0457	0,143	0,887	0,017
SC vs. 0%	0,0330	0,104	0,918	0,025

Table 8: *Output from 2-way Reapeated Measures ANOVA on abundance data. Showing results from Holm-Sidak post hoc, comparison between treatments with time as an effect.*

Comparisons for factor: **Treatment**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 5%	101,945	17,108	<0,001	0,003
SC vs. 1%	98,143	16,470	<0,001	0,004
SC vs. 2,5%	93,107	15,625	<0,001	0,004
SC vs. 0%	57,919	9,720	<0,001	0,004
SC vs. 0,5%	57,438	9,639	<0,001	0,005
0,5 vs. 5%	44,506	7,469	<0,001	0,005
0% vs. 5%	44,026	7,388	<0,001	0,006
0,5% vs. 1%	40,705	6,831	<0,001	0,006
0% vs. 1%	40,224	6,750	<0,001	0,007
0,5% vs. 2,5%	35,669	5,986	<0,001	0,009
0% vs. 2,5%	35,188	5,905	<0,001	0,010
2,5% vs. 5%	8,837	1,483	0,169	0,013
2,5% vs. 1%	5,036	0,845	0,418	0,017
1% vs. 5%	3,801	0,638	0,538	0,025

Table 9a-e: *Output from 2-way Reapeated Measures ANOVA on biomass data. Showing results from Holm-Sidak post hoc, comparison between treatments within time.*

a) Comparisons for factor: **Treatment within T = 14 days**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 1%	126,508	5,375	<0,001	0,003
SC vs. 2,5%	126,016	5,354	<0,001	0,004
SC vs. 0,5%	124,334	5,283	<0,001	0,004
SC vs. 5%	124,130	5,274	<0,001	0,004
SC vs. 0%	115,416	4,904	<0,001	0,005
0% vs. 1%	11,092	0,471	0,640	0,005
0% vs. 2,5%	10,600	0,450	0,654	0,006
0% vs. 0,5%	8,918	0,379	0,706	0,006
0% vs. 5%	8,714	0,370	0,713	0,007
5% vs. 1%	2,379	0,101	0,920	0,009
0,5% vs. 1%	2,175	0,0924	0,927	0,010
5% vs. 2,5%	1,886	0,0801	0,936	0,013
0,5% vs. 2,5%	1,682	0,0715	0,943	0,017
2,5% vs. 1%	0,493	0,0209	0,983	0,025

b) Comparisons for factor: **Treatment within T = 45 days**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 5%	109,460	4,651	<0,001	0,003
SC vs. 1%	107,498	4,567	<0,001	0,004
SC vs. 2,5%	102,021	4,335	<0,001	0,004
SC vs. 0,5%	97,442	4,140	<0,001	0,004
SC vs. 0%	57,604	2,447	0,018	0,005
0% vs. 5%	51,855	2,203	0,032	0,005
0% vs. 1%	49,893	2,120	0,039	0,006
0% vs. 2,5%	44,416	1,887	0,065	0,006
0% vs. 0,5%	39,838	1,693	0,097	0,007
0,5% vs. 5%	12,018	0,511	0,612	0,009
0,5% vs. 1%	10,056	0,427	0,671	0,010
2,5% vs. 5%	7,439	0,316	0,753	0,013
2,5% vs. 1%	5,477	0,233	0,817	0,017
0,5% vs. 2,5%	4,578	0,195	0,847	0,025

c) Comparisons for factor: **Treatment within T = 115 days**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 5%	132,542	5,631	<0,001	0,003
SC vs. 2,5%	127,864	5,433	<0,001	0,004
SC vs. 1%	88,884	3,776	<0,001	0,004
0,500 vs. 5%	69,688	2,961	0,005	0,004
0% vs. 5%	66,742	2,836	0,007	0,005
SC vs. 0%	65,800	2,796	0,007	0,005
0,5% vs. 2,5%	65,010	2,762	0,008	0,006
SC vs. 0,5%	62,854	2,671	0,010	0,006
0% vs. 2,5%	62,064	2,637	0,011	0,007
1% vs. 5%	43,659	1,855	0,070	0,009

1% vs. 2,5%	38,981	1,656	0,104	0,010
0,5% vs. 1%	26,029	1,106	0,274	0,013
0% vs. 1%	23,083	0,981	0,332	0,017
2,5% vs. 5%	4,678	0,199	0,843	0,025

d) Comparisons for factor: **Treatment within T = 180 days**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 1%	124,794	5,302	<0,001	0,003
SC vs. 5%	124,272	5,280	<0,001	0,004
SC vs. 2,5%	110,884	4,711	<0,001	0,004
SC vs. 0%	85,633	3,638	<0,001	0,004
0,5% vs. 1%	84,901	3,607	<0,001	0,005
0,5% vs. 5%	84,379	3,585	<0,001	0,005
0,5% vs. 2,5%	70,991	3,016	0,004	0,006
0,5% vs. 0%	45,739	1,943	0,058	0,006
SC vs. 0,5%	39,893	1,695	0,097	0,007
0% vs. 1%	39,161	1,664	0,103	0,009
0% vs. 5%	38,640	1,642	0,107	0,010
0% vs. 2,5%	25,251	1,073	0,289	0,013
2,5% vs. 1%	13,910	0,591	0,557	0,017
2,5% vs. 5%	13,388	0,569	0,572	0,025

e) Comparisons for factor: **Treatment within T= 368 days**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 5%	177,182	7,528	<0,001	0,003
SC vs. 0,5%	165,626	7,037	<0,001	0,004
SC vs. 1%	154,389	6,560	<0,001	0,004
SC vs. 2,5%	128,793	5,472	<0,001	0,004
SC vs. 0%	114,170	4,851	<0,001	0,005
0% vs. 5%	63,012	2,677	0,010	0,005
0% vs. 0,5%	51,456	2,186	0,034	0,006
2,5% vs. 5%	48,389	2,056	0,045	0,006
0% vs. 1%	40,219	1,709	0,094	0,007
2,5% vs. 0,5%	36,833	1,565	0,124	0,009
2,5% vs. 1%	25,596	1,087	0,282	0,010
1% vs. 5%	22,793	0,968	0,338	0,013
0% vs. 2,5%	14,623	0,621	0,537	0,017
0,5% vs. 5%	11,556	0,491	0,626	0,025

Table 10: *Output from 2-way Reapeated Measures ANOVA on total production data. Showing results from Holm-Sidak post hoc, comparison between treatments with time as an effect.*

Comparisons for factor: **Treatment**				
Comparison	**Diff of Means**	**t**	**Unadjusted P**	**Critical Level**
SC vs. 5%	12,799	13,554	<0,001	0,003
SC vs. 1%	12,453	13,187	<0,001	0,004
SC vs. 2,5%	11,486	12,163	<0,001	0,004
SC vs. 0,5%	10,587	11,212	<0,001	0,004
SC vs. 0%	8,618	9,126	<0,001	0,005
0% vs. 5%	4,181	4,427	0,001	0,005
0% vs. 1%	3,835	4,061	0,002	0,006
0% vs. 2,5%	2,868	3,037	0,013	0,006
0,5% vs. 5%	2,211	2,342	0,041	0,007
0% vs. 0,5%	1,969	2,085	0,064	0,009
0,5% vs. 1%	1,865	1,975	0,076	0,010
2,5% vs. 5%	1,313	1,390	0,195	0,013
2,5% vs. 1%	0,967	1,024	0,330	0,017
0,5% vs. 2,5%	0,898	0,951	0,364	0,025

ANOSIM Output – Abundance Data

T = 14 days

Global Test
Sample statistic (Global R): 0,524
Significance level of sample statistic: 0,2%
Number of permutations: 999 (Random sample from 1201200)
Number of permuted statistics greater than or equal to Global R: 1

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	1	10	10	10	1
SC, 0.5%	1	10	10	10	1
SC, 1%	1	25	4	4	1
SC, 2.5%	1	25	4	4	1
SC, 5%	0,333	20	10	10	2
0%, 0.5%	1	10	10	10	1
0%, 1%	1	25	4	4	1
0%, 2.5%	-0,111	75	4	4	3
0%, 5%	0,667	10	10	10	1
0.5%, 1%	1	33,3	3	3	1
0.5%, 2.5%	1	33,3	3	3	1
0.5%, 5%	0,167	60	10	10	6
1%, 5%	-0,333	100	4	4	4
2.5%, 5%	-0,111	50	4	4	2

Failed Pairwise Tests

Groups	Error
1%, 2.5%	At least one level must be larger than 1 in size

T = 45 days

Global Test
Sample statistic (Global R): 0,669
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from 3153150)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	1	10	10	10	1
SC, 0.5%	0,833	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	1	10	10	10	1
0%, 0.5%	0,25	20	10	10	2
0%, 1%	0,917	10	10	10	1
0%, 2.5%	0,583	10	10	10	1
0%, 5%	1	10	10	10	1
0.5%, 1%	0	66,7	3	3	2
0.5%, 2.5%	0,25	33,3	3	3	1
0.5%, 5%	0,5	33,3	3	3	1
1%, 2.5%	0	100	3	3	3
1%, 5%	0	66,7	3	3	2
2.5%, 5%	-0,5	100	3	3	3

T = 115 days

Global Test
Sample statistic (Global R): 0,54
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from 190590400)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	0,852	10	10	10	1
SC, 0.5%	1	10	10	10	1
SC, 1%	0,667	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	0,333	10	10	10	1
0%, 0.5%	0,815	10	10	10	1
0%, 1%	0,593	10	10	10	1
0%, 2.5%	1	10	10	10	1
0%, 5%	0,407	10	10	10	1
0.5%, 1%	0,444	10	10	10	1
0.5%, 2.5%	0,926	10	10	10	1
0.5%, 5%	0,37	10	10	10	1
1%, 2.5%	-0,185	90	10	10	9
1%, 5%	-0,315	100	10	10	10
2.5%, 5%	-0,074	50	10	10	5

T = 180 days

Global Test
Sample statistic (Global R): 0,602
Significance level of sample statistic: 0,2%
Number of permutations: 999 (Random sample from 22422400)
Number of permuted statistics greater than or equal to Global R: 1

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	0,815	10	10	10	1
SC, 0.5%	0,593	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	1	25	4	4	1
0%, 0.5%	-0,148	70	10	10	7
0%, 1%	0,741	10	10	10	1
0%, 2.5%	0,704	10	10	10	1
0%, 5%	1	25	4	4	1
0.5%, 1%	0,63	10	10	10	1
0.5%, 2.5%	0,63	10	10	10	1
0.5%, 5%	1	25	4	4	1
1%, 2.5%	-0,204	80	10	10	8
1%, 5%	0,778	25	4	4	1
2.5%, 5%	-0,333	100	4	4	4

T = 368 days

Global Test
Sample statistic (Global R): 0,691
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
PC, SC	-0,074	60	10	10	6
PC, 0%	0,741	10	10	10	1
PC, 0.5%	1	10	10	10	1
PC, 1%	1	10	10	10	1
PC, 2.5%	0,926	10	10	10	1
PC, 5%	0,704	10	10	10	1
SC, 0%	0,741	10	10	10	1
SC, 0.5%	1	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	0,926	10	10	10	1
SC, 5%	0,667	10	10	10	1
0%, 0.5%	0,481	10	10	10	1
0%, 1%	1	10	10	10	1
0%, 2.5%	0,407	10	10	10	1
0%, 5%	0,593	10	10	10	1
0.5%, 1%	1	10	10	10	1
0.5%, 2.5%	0,741	10	10	10	1
0.5%, 5%	0,556	10	10	10	1
1%, 2.5%	0,148	30	10	10	3
1%, 5%	0,444	10	10	10	1
2.5%, 5%	0,259	20	10	10	2

ANOSIM Output – Biological Traits Data

T = 14 days

Global Test
Sample statistic (Global R): 0,574
Significance level of sample statistic: 1,1%
Number of permutations: 999 (Random sample from 1201200)
Number of permuted statistics greater than or equal to Global R: 10

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	1	10	10	10	1
SC, 0.5%	1	10	10	10	1
SC, 1%	1	25	4	4	1
SC, 2.5%	1	25	4	4	1
SC, 5%	1	10	10	10	1
0%, 0.5%	0,167	50	10	10	5
0%, 1%	1	25	4	4	1
0%, 2.5%	-0,333	75	4	4	3
0%, 5%	0,259	30	10	10	3
0.5%, 1%	1	33,3	3	3	1
0.5%, 2.5%	1	33,3	3	3	1
0.5%, 5%	0	60	10	10	6
1%, 5%	-0,333	100	4	4	4
2.5%, 5%	-0,333	100	4	4	4

Failed Pairwise Tests

Groups	Error
1%, 2.5%	At least one level must be larger than 1 in size

T = 45 days

Global Test
Sample statistic (Global R): 0,706
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from 3153150)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	1	10	10	10	1
SC, 0.5%	0,833	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	1	10	10	10	1
0%, 0.5%	0,417	10	10	10	1
0%, 1%	0,833	10	10	10	1
0%, 2.5%	0,417	20	10	10	2
0%, 5%	0,583	10	10	10	1
0.5%, 1%	0	100	3	3	3
0.5%, 2.5%	0	100	3	3	3
0.5%, 5%	-0,25	100	3	3	3
1%, 2.5%	0,75	33,3	3	3	1
1%, 5%	0	100	3	3	3
2.5%, 5%	0	100	3	3	3

T = 115 days

Global Test
Sample statistic (Global R): 0,523
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from 190590400)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	0,556	10	10	10	1
SC, 0.5%	0,704	10	10	10	1
SC, 1%	0,667	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	0,889	10	10	10	1
0%, 0.5%	0,63	10	10	10	1
0%, 1%	0,556	10	10	10	1
0%, 2.5%	1	10	10	10	1
0%, 5%	0,778	10	10	10	1
0.5%, 1%	0,593	10	10	10	1
0.5%, 2.5%	1	10	10	10	1
0.5%, 5%	0,815	10	10	10	1
1%, 2.5%	0	40	10	10	4
1%, 5%	-0,222	90	10	10	9
2.5%, 5%	0,074	40	10	10	4

T = 180 days

Global Test
Sample statistic (Global R): 0,62
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from 22422400)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	0,852	10	10	10	1
SC, 0.5%	0,407	10	10	10	1
SC, 1%	0,889	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	1	25	4	4	1
0%, 0.5%	-0,074	60	10	10	6
0%, 1%	0,630	10	10	10	1
0%, 2.5%	1	10	10	10	1
0%, 5%	1	25	4	4	1
0.5%, 1%	0,815	10	10	10	1
0.5%, 2.5%	1	10	10	10	1
0.5%, 5%	1	25	4	4	1
1%, 2.5%	0,037	70	10	10	7
1%, 5%	-0,111	100	4	4	4
2.5%, 5%	0,111	75	4	4	3

T = 368 days

Global Test
Sample statistic (Global R): 0,516
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
PC, SC	0,185	30	10	10	3
PC, 0%	0,37	10	10	10	1
PC, 0.5%	0,815	10	10	10	1
PC, 1%	1	10	10	10	1
PC, 2.5%	0,741	10	10	10	1
PC, 5%	0,556	10	10	10	1
SC, 0%	0,556	10	10	10	1
SC, 0.5%	0,926	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	0,704	10	10	10	1
SC, 5%	0,556	10	10	10	1
0%, 0.5%	0,074	40	10	10	4
0%, 1%	0,741	10	10	10	1
0%, 2.5%	0,111	30	10	10	3
0%, 5%	0,481	10	10	10	1
0.5%, 1%	0,889	10	10	10	1
0.5%, 2.5%	0,259	20	10	10	2
0.5%, 5%	0,37	10	10	10	1
1%, 2.5%	0	30	10	10	3
1%, 5%	0,111	20	10	10	2
2.5%, 5%	0,074	50	10	10	5

ANOSIM Output – Secondary Production Data

T = 14 days

Global Test
Sample statistic (Global R): 0,26
Significance level of sample statistic: 0,4%
Number of permutations: 999 (Random sample from 190590400)
Number of permuted statistics greater than or equal to Global R: 3

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	0,667	10	10	10	1
SC, 0.5%	0,667	10	10	10	1
SC, 1%	0,667	10	10	10	1
SC, 2.5%	0,667	10	10	10	1
SC, 5%	0,667	10	10	10	1
0%, 0.5%	0,37	30	10	10	3
0%, 1%	-0,037	100	10	10	10
0%, 2.5%	-0,037	100	10	10	10
0%, 5%	0,111	40	10	10	4
0.5%, 1%	0,222	40	10	10	4
0.5%, 2.5%	0,222	40	10	10	4

0.5%, 5%	0,259	40	10	10	4
1%, 2.5%	-0,222	100	10	10	10
1%, 5%	0	40	10	10	4
2.5%, 5%	0	40	10	10	4

T = 45 days

Global Test
Sample statistic (Global R): 0,405
Significance level of sample statistic: 0,3%
Number of permutations: 999 (Random sample from 21021000)
Number of permuted statistics greater than or equal to Global R: 2

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	1	10	10	10	1
SC, 0.5%	1	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	1	10	10	10	1
0%, 0.5%	0,083	40	10	10	4
0%, 1%	0,704	10	10	10	1
0%, 2.5%	0,5	20	10	10	2
0%, 5%	0,5	20	10	10	2
0.5%, 1%	0,25	30	10	10	3
0.5%, 2.5%	-0,25	100	3	3	3
0.5%, 5%	0,25	66,7	3	3	2
1%, 2.5%	0,25	40	10	10	4
1%, 5%	-0,333	90	10	10	9
2.5%, 5%	-0,25	100	3	3	3

T = 115 days

Global Test
Sample statistic (Global R): 0,424
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from 190590400)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	1	10	10	10	1
SC, 0.5%	1	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	1	10	10	10	1
0%, 0.5%	0,333	10	10	10	1
0%, 1%	0	50	10	10	5
0%, 2.5%	0,296	20	10	10	2
0%, 5%	0,333	20	10	10	2
0.5%, 1%	0,083	40	10	10	4
0.5%, 2.5%	0,37	10	10	10	1
0.5%, 5%	0,185	20	10	10	2
1%, 2.5%	-0,167	80	10	10	8
1%, 5%	0,417	20	10	10	2
2.5%, 5%	0,185	40	10	10	4

T = 180 days

Global Test
Sample statistic (Global R): 0,326
Significance level of sample statistic: 1,4%
Number of permutations: 999 (Random sample from 22422400)
Number of permuted statistics greater than or equal to Global R: 13

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
SC, 0%	0,926	10	10	10	1
SC, 0.5%	0,63	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	1	25	4	4	1
0%, 0.5%	-0,222	100	10	10	10
0%, 1%	0,296	10	10	10	1
0%, 2.5%	-0,037	60	10	10	6
0%, 5%	-0,778	100	4	4	4
0.5%, 1%	0,259	20	10	10	2
0.5%, 2.5%	0,259	20	10	10	2
0.5%, 5%	-0,556	100	4	4	4
1%, 2.5%	0,111	30	10	10	3
1%, 5%	1	25	4	4	1
2.5%, 5%	-0,333	100	4	4	4

T = 368 days

Global Test
Sample statistic (Global R): 0,511
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
PC, SC	-0,148	100	10	10	10
PC, 0%	0,519	10	10	10	1
PC, 0.5%	0,852	10	10	10	1
PC, 1%	0,741	10	10	10	1
PC, 2.5%	0,778	10	10	10	1
PC, 5%	0,889	10	10	10	1
SC, 0%	0,926	10	10	10	1
SC, 0.5%	1	10	10	10	1
SC, 1%	1	10	10	10	1
SC, 2.5%	1	10	10	10	1
SC, 5%	1	10	10	10	1
0%, 0.5%	0,074	30	10	10	3
0%, 1%	0,148	30	10	10	3
0%, 2.5%	0,259	30	10	10	3
0%, 5%	0,333	10	10	10	1
0.5%, 1%	0,037	40	10	10	4
0.5%, 2.5%	0,741	10	10	10	1
0.5%, 5%	0,111	40	10	10	4
1%, 2.5%	0,111	30	10	10	3
1%, 5%	0,259	20	10	10	2
2.5%, 5%	1	10	10	10	1

Dendograms – Abundance Data

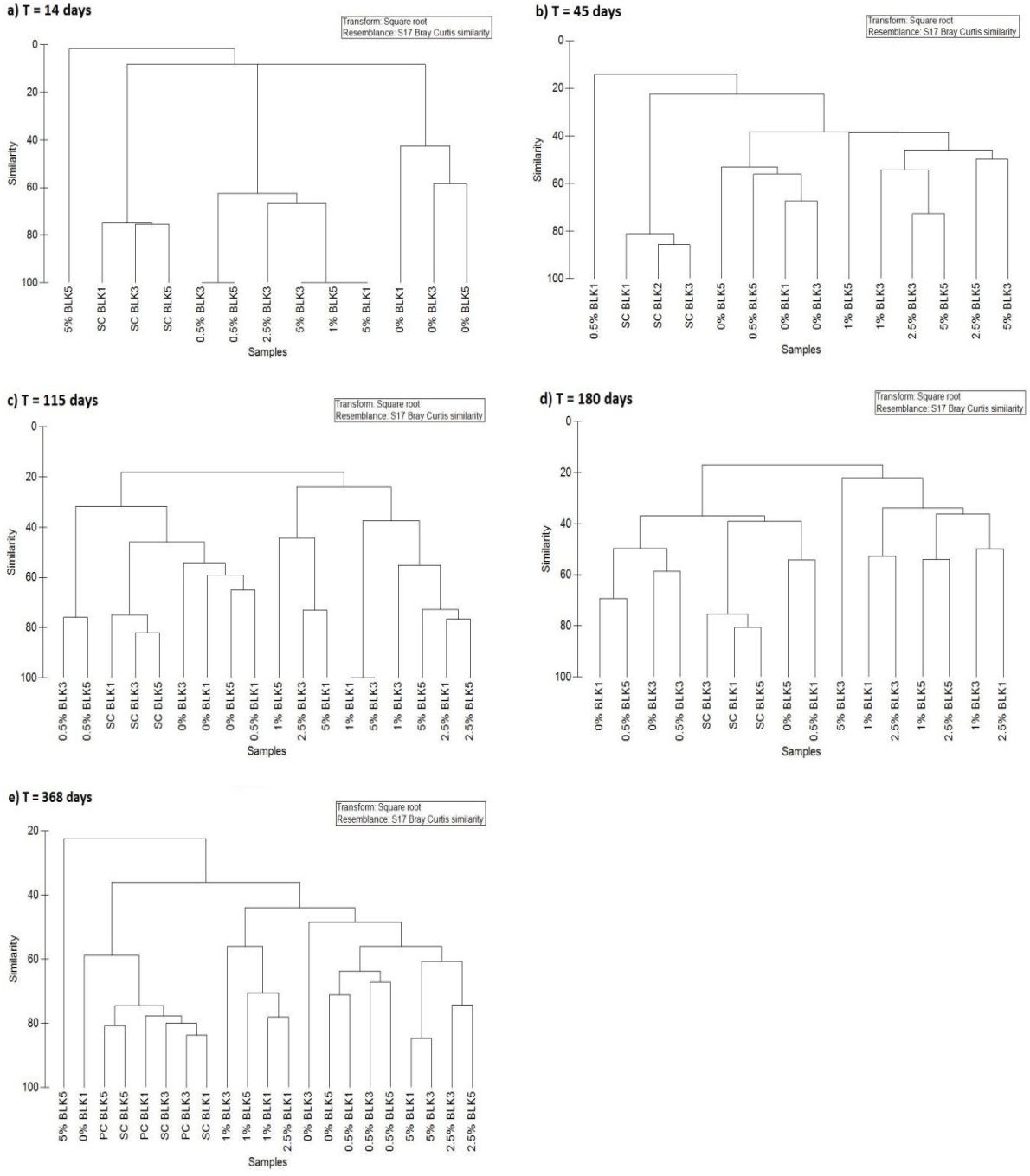

Figure 20(a-e): *Cluster analysis based on Bray-Curtis similarity matrix on square-root transformed abundance data. Groups determined from analysis were superimposed on MDS in Figure 13(a-e).*

Dendograms – Biological Trait Data

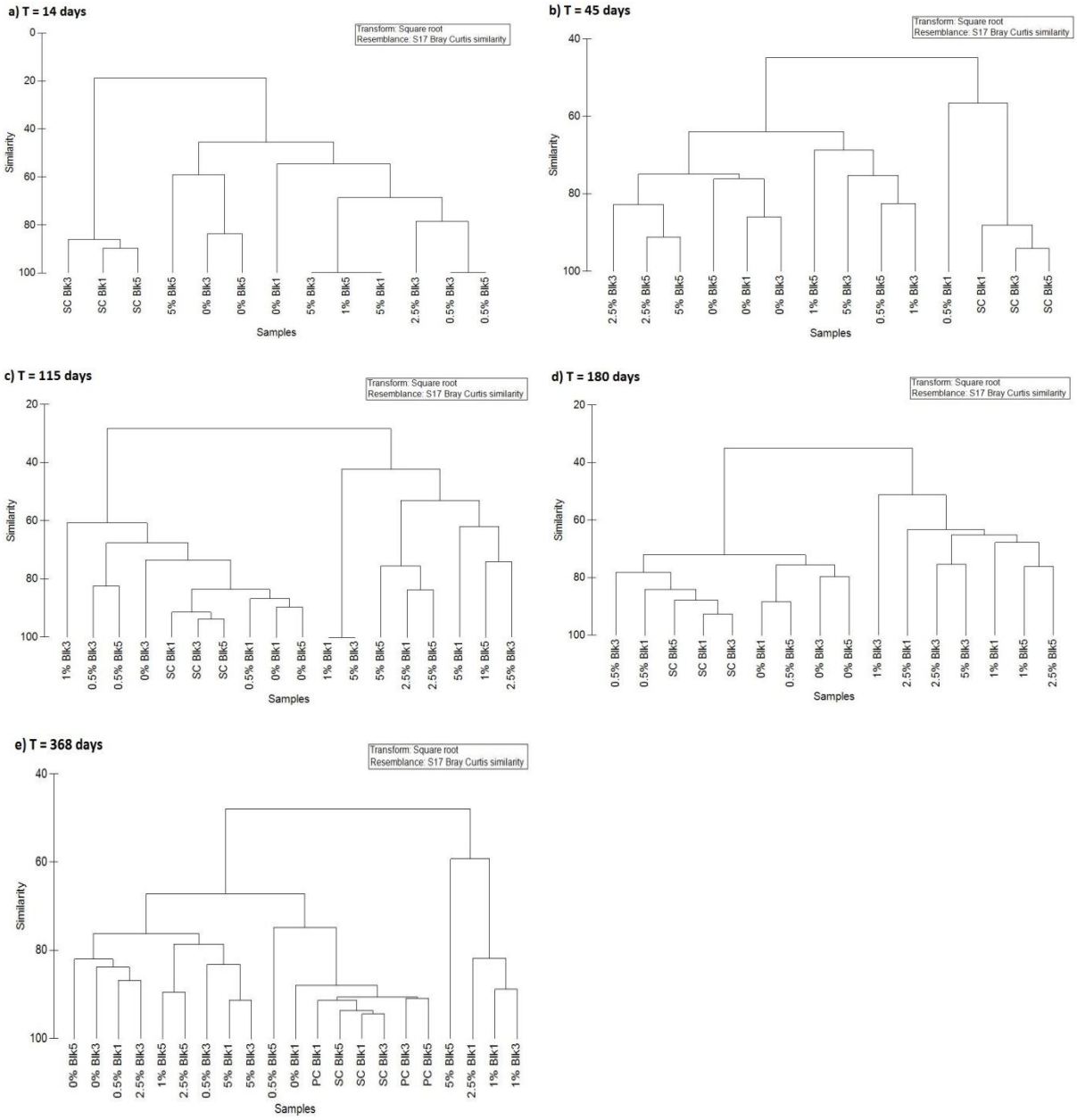

Figure 21(a-e): *Cluster analysis based on Bray-Curtis similarity matrix on square-root transformed trait assemblage data. Groups determined from analysis were superimposed on MDS in Figure 14(a-e).*

Dendograms – Secondary Production Data

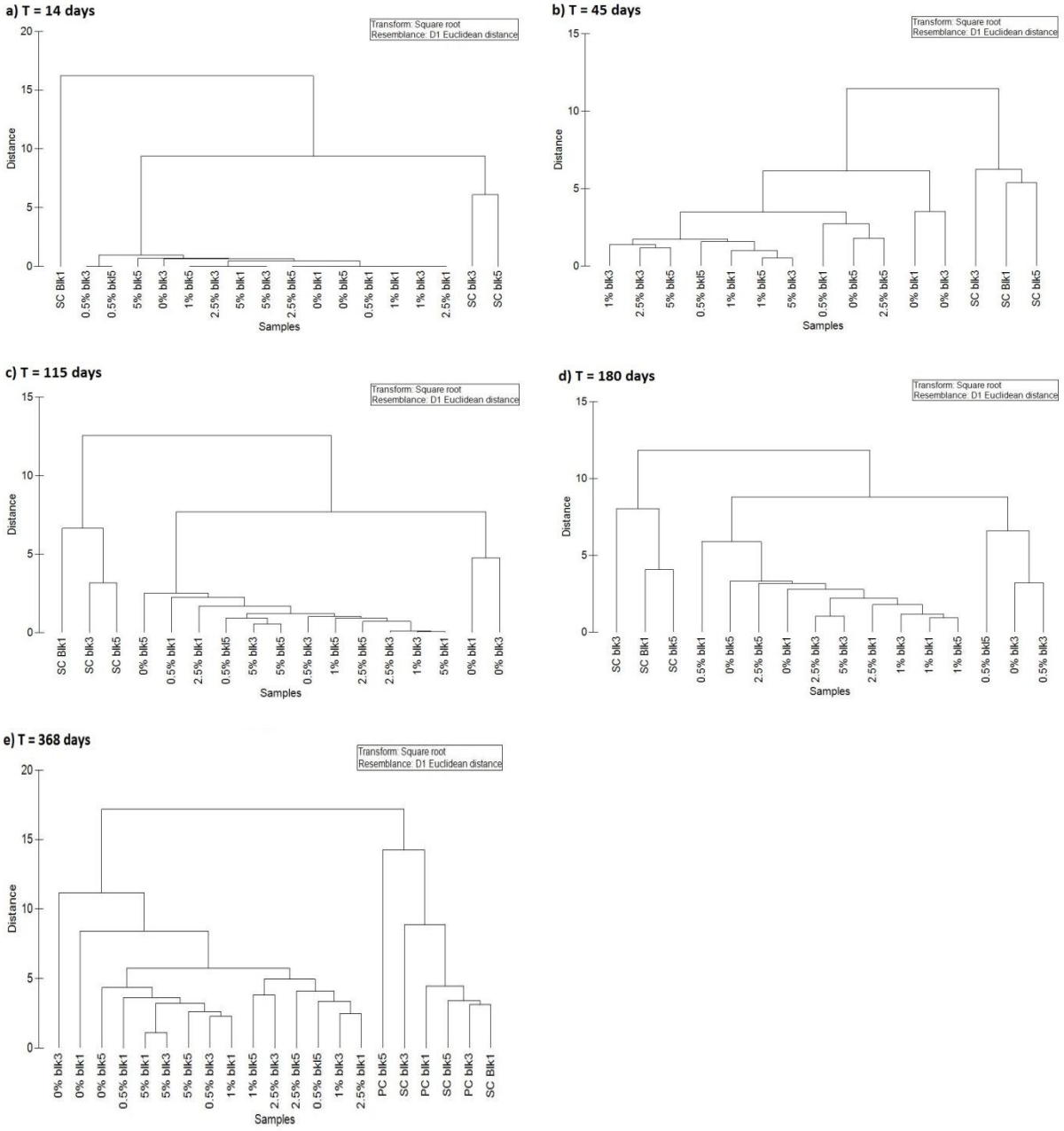

Figure 22(a-e): *Cluster analysis based on Euclidean distance matrix on square-root transformed estimates of production data. Groups determined from analysis were superimposed on MDS in Figure 15(a-e).*

Appendix F

Faunal colonization of submarine mine tailings

Barbro T. Haugland[1], Stefan G. Bolam[2], Andrew K. Sweetman[3]

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[2]Center for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft, UK
[3]Norwegian Institute for Water Research (NIVA), now at International Research Institute of Stavanger (IRIS), Stavanger, Norway

Introduction

The majority of the mining industry in Norway is located close to the coastline, using the adjacent fjords as disposal sites for inert mine waste ('tailings'). Mine tailings deposits, so-called submarine tailing placements (STPs) often comprise a 50m thick deposit at the seafloor, which initially smothers resident benthic communities leaving the seafloor azoic and organically sterile (Kvassnes *et al.*, 2009).

The mining industry in Norway and worldwide is expected to grow over the next few decades, resulting in even greater pressure exerted on deep-sea fjord ecosystems, creating a real environmental challenge. There is thus a clear and urgent need to establish methods that can be used to stimulate faunal colonization of STPs and shorten the time required for ecosystem recovery (*ibid*).

In this study, we assessed the role of organic carbon (C_{org}) in faunal colonization of mine tailings. We hypothesized that:

"The abundance of fauna will be greater in mine tailings treatments with ≤ 2.5% C_{org} compared to treatments with 5% C_{org}".

Materials and methods

The experiment was set up along an intertidal mudflat in the Crouch Estuary in Essex, UK in April 2012 (Figure 1).

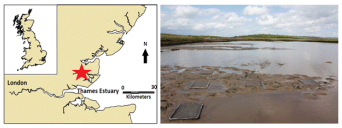

Figure 1: Sampling site shown as red star (left), plot set-up (right)

We used a complete randomized block design that consisted of 3 sampling blocks and one replacement block. Each block comprised 2 controls and 5 different mine tailings treatments with different concentrations of C_{org}: 0%, 0.5%, 1%, 2.5% and 5% (Figure 2).

Sediment cores were collected on 7 occasions from the start date (T=0) to the end (T=365). Samples were analysed for macrofauna (>500µm), sediment grain size, metal content, and carbon and nitrogen content and angularity. Reduction-oxidation potentials were also measured for each plot on each occasion. Here we report the changes in faunal abundance and community structure over the first 180 days.

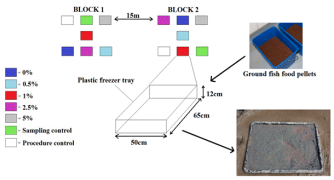

Figure 2: Basic set up of 3 sampling blocks showing the randomized placement of the different treatments within each block

Results

After 115 days faunal abundance was significantly higher ($p<0.01$) in treatments with C_{org} < 1% compared to treatments with C_{org} ≥ 1%.

The community structure in the 0.5% and 0% C_{org} treatments was strongly overlapping with the community in the control after 180 days (ANOSIM, R=0.333), while the community structure between the control and the other taillings treatments differed significantly (ANOSIM, $R \geq 0.852$, $p<0.001$) (Figure 4).

MDS analysis of untransformed vs. presence/absence transformed data showed the same structure over time, suggesting that the differences in the community structure was more related to the composition of species rather than to their abundances.

SEM imaging revealed strong differences in angularity between natural sediments and the mine tailings (Figure 5).

Mean macrofaunal abundance over time

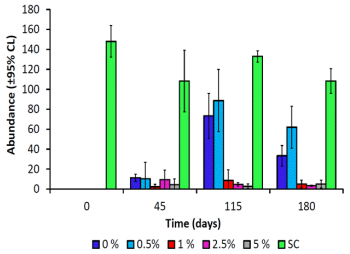

Figure 3: Mean macrofaunal abundance (± 95% confidence limits) in sediment cores (10 cm in diameter) from the different experimental treatments over time

SEM imaging of natural sediments and mine tailings

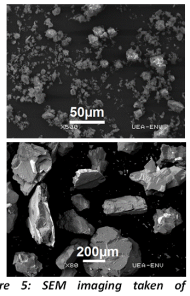

Figure 5: SEM imaging taken of natural sediments at x500 magnification (top) and mine tailings at x80 magnification (bottom)

Comparison of macrofaunal communities

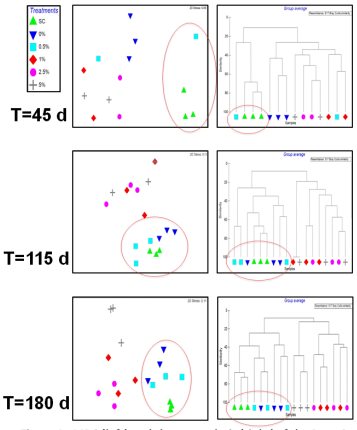

Figure 4: MDS (left) and cluster analysis (right) of the Bray-Curtis similarity index based on untransformed data looking at community structure at species level at T=45d (top), T=115d (middle) and T=180d (bottom)

Conclusions

Our experiment showed that adding little organic carbon (0.5%) to the mine tailings enhanced colonization after 115 days to a greater extent than in treatments with ≥1% organic carbon. These results therefore show that **adding organic material to inert tailings does stimulate benthic recovery.**

The poor colonization and persistent impacts on community structure seen in the higher C_{org} treatments may result from juvenile colonists being unable to cope with high levels of organic C and the increased exposure to toxic metabolic byproducts (e.g. sulfides).

It may also be an effect of multiple stressors caused by the angularity of the grains shredding the guts of deposit feeders.

Further work is now necessary to determine the generality of these findings and assess if the processes documented in this investigation are characteristic of deep-sea STPs.

Literature cited

Kvassnes, A.J.S., Sweetman, A.K., Iversen, E.R., and Skei, J., 2009. Sustainable use and Future of Submarine Tailings Placements in the Norwegian Extractive Industry. Peer-reviewed paper published in the Proceedings for the International Conference on Acid-Rock Drainage.

Acknowledgments

We would like to acknowledge the Norwegian Research Council, Norwegian Institute for Water Research, Sydvaranger Gruver AS, Rana Gruber AS, Nordic Mining ASA and Titania AS that funded SGB and AKS to carry out this work, and Claire Mason at CEFAS for help with the analytical analysis.

Figure 23: *Poster presented at the Nordic Marine Sciences Conference (NMSC) 2013 October 28-30th 2013 in Oslo, Norway.*

Faunal colonization of submarine mine tailings

Barbro T. Haugland[1], Stefan G. Bolam[2], Andrew K. Sweetman[3]

[1]University of Bergen (UiB), Bergen, Norway, email: barbro.haugland@gmail.com
[2]Center for Environment, Fisheries and Aquaculture Science (CEFAS), Lowestoft, UK
[3] International Research Institute of Stavanger (IRIS), Stavanger, Norway

Introduction

The majority of the mining industry in Norway is located close to the coastline, and uses the adjacent fjords as disposal sites for inert mine waste ('tailings'). Mine tailings deposits, so-called submarine tailing placements (STPs) often comprise a 50m thick deposit at the seafloor, which initially smothers resident benthic communities leaving the seafloor azoic and organically sterile (Kvassnes *et al.*, 2009).

The mining industry in Norway is expected to grow over the next few decades, resulting in even greater pressure exerted on deep-sea fjord ecosystems, creating a real environmental challenge. There is thus a clear and urgent need to establish methods that can be used to stimulate faunal colonization of STPs and shorten the time required for ecosystem recovery.

Because tailings are organically sterile when placed at the seafloor, we assessed the effect of organic carbon (C_{org}) enrichment on faunal colonization of mine tailings. We hypothesized that:

"The abundance of fauna will be greater in mine tailings treatments with ≤ 2.5% C_{org} compared to treatments with 5% C_{org}".

Materials and methods

The experiment was set up along an intertidal mudflat in the Crouch Estuary in Essex, UK in April 2012 (Figure 1).

Figure 1: Sampling site shown as red star (left), plot set-up (right)

We used a complete randomized block design that consisted of 3 sampling blocks and one replacement block. Each block comprised 2 controls and 5 different mine tailings treatments with different concentrations of C_{org}: 0%, 0.5%, 1%, 2.5% and 5% (Figure 2).

Sediment cores were collected on 6 occasions from the start date (T = 0 days) to the end of the experiment 1 year later (T = 365 days). Samples were analysed for macrofauna (>500µm), sediment grain size, metal content, carbon and nitrogen content and angularity. Reduction-oxidation potentials were also measured for each plot on each occasion. Here we report the changes in faunal abundance, community structure and secondary production (2^{o} production) over the 1 year experiment.

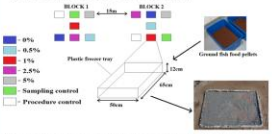

Figure 2: Basic set up of the 3 sampling blocks showing the randomized placement of the different treatments within each block

Results

After 115 days, faunal abundance was significantly higher ($P<0.05$) in the < 1% C_{org} treatments compared to the C_{org} ≥ 1% treatments (Fig. 3). After 1 year, there was no significant difference ($P>0.05$) between the 0% and the sampling control (Fig. 3). Treatments with a C_{org} content of ≥ 1% showed consistently low faunal abundances throughout the experiment (Fig. 3).

Community structure between the sampling control and the tailings treatments was significantly different after 1 year (ANOSIM, $R \geq 0.667$). However, all tailings treatments (with the exception of two replicates) were 40% similar after 1 year (Fig. 4).

In terms of total 2^{o} production after 1 year, there were no signs of recovery in any tailings treatments to the levels of total 2^{o} production in the sampling control (Fig. 5).

SEM imaging revealed strong differences in angularity between natural sediments and the mine tailings (Fig. 6).

Analysis of total organic carbon (TOC) revealed that the C_{org} content in the 2.5% and 5% treatments was similar to the C_{org} content in the sampling controls after 115 days.

Colonization by macrofauna over time

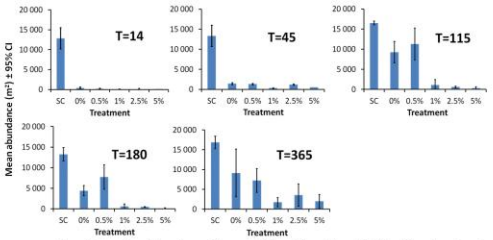

Figure 3: Macrofaunal abundance m^{-2} (mean ± 95% confidence intervals) in the different treatments over time (T=days)

Macrofaunal community structure

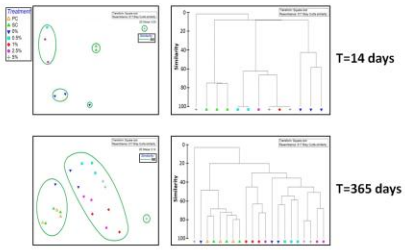

Figure 4: MDS (left) and cluster analysis (right) of the Bray-Curtis similarity index based on square root transformed data looking at community structure at species level at T=14 days (top) and T=365 days (bottom)

Comparison of total 2^{o} production between treatments

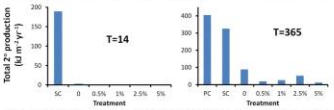

Figure 5: Mean total 2^{o}production in the different experimental treatments after T=14 days (left) and T=365 days (right). Note different y-axis scales.

SEM imaging of natural sediments and mine tailings

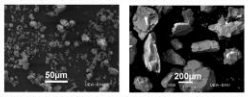

Figure 6: SEM imaging taken of natural sediments at x500 magnification (left) and mine tailings at x80 magnification (right)

Conclusions

Our experiment showed that adding little organic carbon (≤0.5%) to the mine tailings enhanced colonization after 115 days to a greater extent than adding ≥1% organic carbon.

The poor colonization initially seen in the higher C_{org} treatments (< 115 days) may result from juvenile colonists being unable to cope with high levels of organic C and the increased exposure to toxic metabolic byproducts (e.g. sulfides and ammonium). It may also be an effect of multiple stressors caused by the size and angularity of the grains shredding the guts of deposit feeders.

The difference in angularity between the sampling control and tailings treatments may also be a major cause for the difference in biodiversity seen in the MDS plots after 365 days (Fig.4).

Similar biodiversity between the tailings treatments after 1 year may relate to the decrease in organic carbon concentration of the tailings through time (especially in the 2.5% and 5% treatments), which allowed those species more intolerant to high organic levels to colonize the high C_{org} treatments.

Even though the abundance in the low C_{org} treatments (e.g. 0%) showed similar faunal abundance levels as the sampling control after 1 year, total secondary production was significantly lower in the tailings treatments ($P>0.01$) compared to the sampling control.

This ultimately highlights the need for a multi-parameter approach (e.g., assessments of abundance, secondary production, trait diversity and species biodiversity) in studies assessing colonization and recovery processes in the marine benthos.

Further work is now necessary to determine the generality of these findings, and assess if the processes documented in this investigation are characteristic of deep-sea STPs.

Literature cited

Kvassnes, A.J.S., Sweetman, A.K., Iversen, E.R., and Skei, J., 2009. Sustainable use and Future of Submarine Tailings Placements in the Norwegian Extractive Industry. Peer-reviewed paper published in the Proceedings for the International Conference on Acid-Rock Drainage.

Acknowledgments

We would like to acknowledge the Norwegian Research Council, Norwegian Institute for Water Research, Sydvaranger Gruver AS, Rana Gruber AS, Nordic Mining ASA and Titania AS that funded SGB and AKS to carry out this work, and Claire Mason at CEFAS for help with the sediment chemistry analysis. We thank the SEM facility at the University of East Anglia, UK, for carrying out the SEM imaging of the sediments

Figure 24: *Poster presented at the Ocean Sciences Meeting 2014 February 23-28th in Honolulu, Hawaii.*