Simulated mining-related sedimentation impacts on the deep-sea macrofauna of the Chatham Rise, New Zealand

by

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Abstract

With the possibility of deep-sea mining of marine mineral resources occurring in the near future, it is necessary to understand the potential impacts that mining may have on benthic communities. Previous simulated mining experiments have observed direct impacts of deep-sea mining (e.g., faunal mortality); however, indirect impacts of sedimentation were not understood. In New Zealand, there has been interest in mining the seabed of the Chatham Rise, but mining consents have been refused, partly due to the uncertainties of sedimentation impacts on benthic communities. A disturbance experiment conducted in 2019 on the Rise used a modified agricultural plough designed to create a sediment cloud that could result from mining. This disturbance was used to assess the resilience of benthic communities to sedimentation in a proposed future mining area. Macrofaunal and sediment samples were collected with a multicorer before, immediately after and one year after disturbance to assess the impact on the community and its ability to recover. Sampling events took place in disturbed (physically run over by the plough and subjected to sedimentation) and undisturbed areas (subjected to sedimentation only) at each sampling period. Macrofaunal abundance significantly decreased in disturbed areas after disturbance but not in undisturbed areas. However, community structure changed in both areas after disturbance; in disturbed areas this was mostly driven by changes in numerically dominant fauna, but in undisturbed areas by the more sensitive fauna which may provide an early warning sign for further changes under increased sedimentation. One year after disturbance, community structure had recovered in both areas. Abundance-based community structure correlated most strongly with C:N molar ratios in the sediment which increased after disturbance. Ecosystem function was measured by sediment community oxygen consumption (SCOC) which increased similarly in both disturbed and undisturbed areas after disturbance; SCOC may be a more sensitive measure than community structure in assessing sedimentation impacts. No correlations were found between SCOC and macrofaunal abundance, biomass, diversity or bacterial abundance. The results of this research are useful for managing the impacts of industries where sedimentation is an issue, such as for bottom trawl fisheries and deep-sea mining. The results highlight the importance of leaving unmined patches of seabed adjacent to or within mined areas, to aid the recovery of macrofaunal communities subjected to mining disturbance.

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I first want to acknowledge God. I thank Him for providing me with the opportunity to explore His creation and conduct my research with a team of people at NIWA that always supported me and my work.

"Those who go down to the sea in ships, Who do business on great waters,
They see the works of the Lord, And His wonders in the deep." Psalm 107:23-24 NKJV

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1. Introduction

1.1 Deep-sea mining

Interest in mineral resources in the deep sea continues to grow as land-based resources diminish and global demand for high- and green-technology increases (Petersen et al. 2016, Hein et al. 2020). Major resources (and their uses) include cobalt (hybrid and electric vehicle batteries), manganese (construction industry), silver, tin (mobile phone and laptop batteries), copper (electrical wiring), zinc (rust prevention, pharmaceuticals), phosphorus (agricultural fertilisers) and various rare earth elements (hybrid and electric vehicles, wind turbines, energy-efficient lighting) (Hein et al. 2013, Miller et al. 2018). These resources can be found in seafloor massive (polymetallic) sulfides at hydrothermal vents, cobalt-rich crusts on seamounts, manganese (polymetallic) nodules on abyssal plains, and phosphorite deposits located on continental margins (Petersen 2014) (Fig. 1).
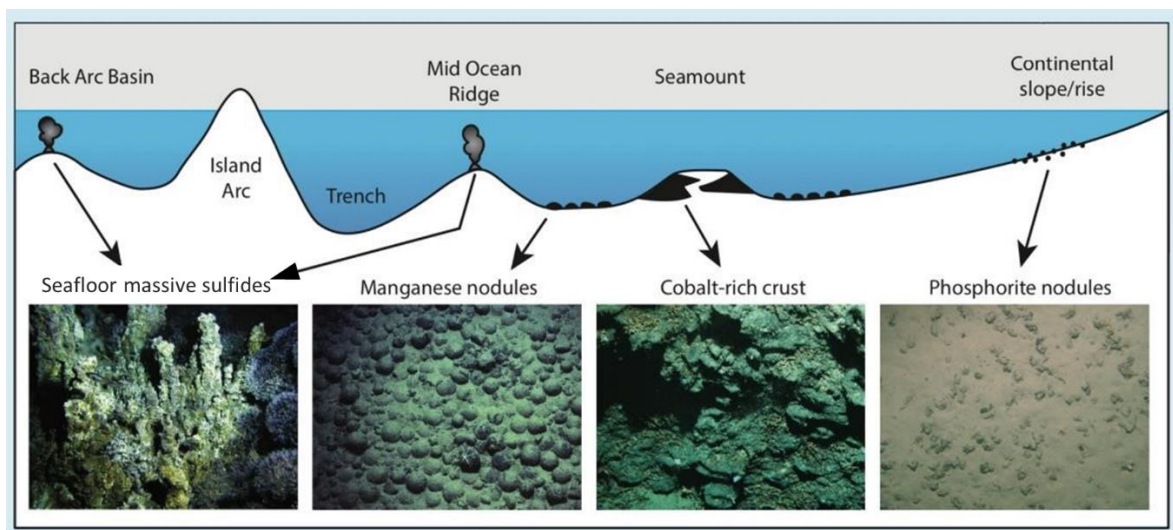
Figure 1. The main sources of seabed minerals. Image provided by IUCN/NIWA.

Extraction of these minerals in the deep sea (particularly manganese nodules) was first proposed in 1965 (Mero 1965); exploration occurred mainly in the 1970s and early 1980s, with a recent resurgence in the 21st century (Sparenberg 2019). However, large-scale commercial mining in the deep sea is yet to commence. But global demand for raw materials, along with significant economic opportunities, will likely see mining operations

begin in the near future. Currently, there are 31 exploration permits granted by the International Seabed Authority (ISA) to explore deep-sea mineral resources outside areas of national jurisdiction (ISA 2021). Due to the proposed scale of commercial mining operations, significant environmental impacts are expected to occur in what is, for the most part, a relatively stable environment (van Dover et al. 2017). Despite research having been conducted to determine the potential effects of deep-sea mining (e.g., see references in review by Jones et al. 2017), there remains a need to assess environmental impacts efficiently and accurately before the commencement of mining activities (Clark 2019a). Although scientific research is occurring before mining, the true extent of the environmental impact may only be revealed after full-scale mining operations begin (Sharma 2005). The direct contact of mining gear on the seabed will no doubt affect benthic communities (e.g., direct faunal mortality, habitat removal, modification; Foell et al. (1990), Desprez (2000)) but it is also recognised that the resuspension and deposition of sediment resulting from the physical disturbance of the seabed and sediment discharges from mining vessels (Fig. 2) will also impact both pelagic and benthic organisms (Sharma et al. 2001, Christiansen et al. 2019). The resuspension and deposition of sediment (hereafter referred to collectively as sedimentation) will likely constitute the most significant *indirect* impact of mining on deep-sea ecosystems. The effects of sedimentation on individual organisms are wide-ranging and occur along a continuum from potentially beneficial (Rosenfeld et al. 1999, Anthony 2000) to causing mortality (Flores et al. 2012, Hendrick et al. 2016). Sedimentation can reduce the function of individual organisms (Pinheiro et al. 2021), and at the wider community level, ecosystem functioning can be impaired (Mevenkamp et al. 2017). Sedimentation effects are known largely from studies of shallow-water benthic communities and laboratory experiments on shallow-water taxa. Extrapolating these results to deep-sea communities and taxa can prove unreliable; organisms are often adapted to deep-sea conditions and, therefore, may respond differently to their shallow-water relatives (Tarasov et al. 2005, Mestre et al. 2019). It is only comparatively recently that a larger focus has been given to assessing the responses of deep-sea communities and taxa to the effects of sedimentation. These studies are often driven by the need to gain a better understanding of anthropogenic impacts in the deep sea and how to manage them. Defining what constitutes "serious harm" to an ecosystem in the context of deep-sea mining is a critical step in determining conditions under which mining companies may or may not operate (Levin et al. 2016).

However, understanding the implications for biological communities affected by mining remains limited, and previous mining impact experiments (see below), although informative, have not provided a satisfactory understanding of the specific effects of sedimentation.
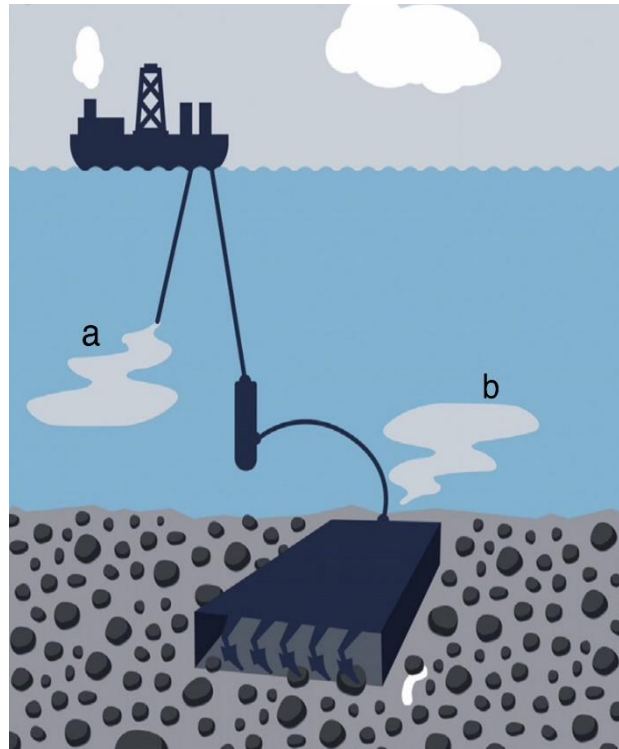
Figure 2. Sedimentation is expected to result from (a) discharges from vessels and (b) physical disturbance of the seabed. Modified from (Gollner et al. 2017).

1.2 Simulated mining experiments

1.2.1 History of simulated mining experiments

It is thought that impacts of mining for sulfide deposits at hydrothermal vent sites may be relatively low due to the high frequency of natural disturbance (volcanic or tectonic activity that 'shuts down' venting) and the resilience of biological communities at vents to these disturbances (van Dover 2014). However, it has been noted that some vent fields may be more stable than previously thought (Du Preez and Fisher 2018), and therefore, some vent communities are potentially vulnerable to mining disturbance. The feasibility of mining cobalt-rich crusts on seamounts is currently limited by the development of appropriate

technology (Du et al. 2017). Polymetallic nodules on abyssal plains are estimated to cover 38 million km^2 of seabed compared to 3.2 million km^2 and 1.7 million km^2 for polymetallic sulfides and cobalt-rich crusts, respectively (Hein et al. 2020). Therefore, sedimentation effects at polymetallic sulfide and cobalt-rich crust sites will not be discussed here, as assessment has been minimal and the proposed mining techniques (rock crushing) may not be comparable to techniques proposed for nodule mining (scooping or suctioning the seabed). Polymetallic nodules on abyssal plains form through the deposition of minerals, primarily manganese, and may grow in thickness at a rate of 10-20 mm per million years in low turbidity environments, and are typically 2-8 cm (maximum 15 cm) in diameter (Gollner et al. 2017, Kuhn et al. 2017). These nodules provide habitat for a diverse assemblage of mainly small-bodied taxa such as foraminiferans and sponges, but also larger organisms such as corals and anemones (Gooday et al. 2015, Amon et al. 2016, Vanreusel et al. 2016, Simon-Lledó et al. 2019a). The formation of phosphorite deposits is not fully understood but is thought to result from interactions between high biological concentrations, reworked sediments and mineral precipitation aided by bacterial activity (Glenn et al. 1994, Föllmi 1996, Crosby and Bailey 2012). These deposits form over hundreds of thousands to millions of years in continental margin and upwelling regions and vary from grains of 1 mm to nodules from 2 to 150 mm (Cullen 1980). A range of benthic taxa have been found in association with phosphorite deposits. These communities include a potentially specialised infauna dominated by amphipods (Leduc et al. 2015), while larger phosphorite nodules can sit exposed on the seabed surface and be colonised by animals such as corals and sponges (Dawson 1984, Kudrass and Von Rad 1984b). Mining of polymetallic and phosphorite nodules is expected to remove large amounts of seabed, and generate associated sediment plumes. Therefore, the benthic communities and taxa of abyssal plains and phosphorite deposit habitats are expected to be particularly vulnerable to the impacts of sedimentation from mining activities.

The majority of deep-sea mining experiments have been carried out in areas containing polymetallic nodules, namely, the abyssal plains, which are perhaps most appropriate for assessing sedimentation impacts. In 1970, Deepsea Ventures Inc. carried out the first test mining operation in 800 m deep water off the Florida coast (Amos et al. 1972). Further evaluation led to the central Pacific being recognised as the most likely nodule mining site,

and test mining activities in the Clarion-Clipperton Zone (CCZ) followed in 1976-78 (reviewed in Jones et al. 2017). It was not until 1989 when the next major mining experiment (and arguably the most extensive) was conducted; the German-led *Dis*turbance and Re*col*inization Experiment (DISCOL) in the Pacific Ocean, off the Peruvian coast (Foell et al. 1990). An area of 11 km^2 of seabed was disturbed using a plough system that resuspended the upper layer of sediment (hundreds of mm) which subsequently settled in a layer up to 30 mm thick within the disturbed area (Thiel et al. 2001). Six months after the disturbance some organisms were still coated in sediment, the diversity and richness of mobile fauna had decreased, and sessile organisms were almost completely absent (Foell et al. 1990). The fate of suspended sediment depends on the magnitude and direction of currents, as well as the depth at which they are released. Modelling of the DISCOL sediment plume suggested that sediment could settle up to 15 km from the disturbed area (Zielke et al. 1995) and with particle settlement of >100 g m^{-2} within 1-2 km (Jankowski et al. 1996). After a few unsuccessful attempts at a benthic disturbance experiment in the early 1990s, the US National Oceanic and Atmospheric Administration (NOAA) conducted the Benthic Impact Experiment (BIE-II) in 1993, successfully resuspending 4000 m^3 of wet sediment, with impacts assessed the following year (Trueblood 1993). The benthic disturber developed for this experiment (Deep-Sea Sediment Resuspension System (DSSRS)) (Brockett and Richards 1994) was subsequently used for most future studies of mining-related disturbance.

In 1994, the Japan Deep-Sea Impact Experiment (JET) was conducted using DSSRS to disturb the seabed; roughly 350 tons (dry weight) of sediment was resuspended, settling at a thickness of 19.5 mm (Fukushima 1995). The DSSRS was used again for two mining impact experiments over the next few years. The first (IOM BIE) was carried out in 1995 by the InterOcean Metal Joint Organization (IOM) in the CCZ (Kotlinski and Stoyanova 1998). The second experiment, the Indian Deep-sea Environmental Experiment (INDEX) was conducted in 1997 in the nodule-containing Central Indian Ocean Basin (Ingole et al. 2005). The INDEX experiment resuspended more than 6000 m^3 of wet sediment.

The experiments discussed here are the best available representations of a proposed mining operation and quantitative data relevant to sedimentation is presented here for each experiment, where possible (Table 1).

Table 1. Overview of the major mining experiments and their sediment plume characteristics. The DSSRS was used to disturb the seabed in all experiments except DISCOL (plough harrow). For each experiment, sediment concentration was only estimated at the point of disturbance where direct contact of mining gear removes biota (Yamazaki and Sharma 2001). It is therefore not possible to assess the *indirect* effect of sedimentation on benthic communities; concentration estimates were thus excluded from the table. CCZ = Clarion Clipperton Zone.

Experiment (Year) Location	Extent of disturbance	Volume of suspended sediment	Sediment concentration	Area of plume settlement	Sediment thickness at settlement	References
DISCOL (1989) Southeast Pacific off Peru	11 km^2 [a]	-	-	Modelling suggests sediment deposition 15 km away from disturbance[b]	Up to 30 mm within disturbed area[c] Maybe >100 g m^{-2} 1-2 km from disturbance area[d]	[a](Foell et al. 1990) [b](Zielke et al. 1995) [c](Thiel et al. 2001) [d](Jankowski et al. 1996)
BIE-II (1993) CCZ	49 tows over 150X3000 m [e]	4000 m^3 wet sediment[e]	-	-	-	[e](Trueblood 1993)
JET (1994) CCZ	19 tows over two parallel 1600 m tracks [f]	352 tons dry weight of sediment[f] estimated to be 1427 m^3 wet sediment[g]	-	-	Up to 19.5 mm [h]	[f](Fukushima 1995) [g](Yamazaki and Sharma 2001) [h](Fukushima et al. 2002)
IOM BIE (1995) CCZ	14 tows of 2.5 km in an area of 200X2500 m [i]	1300 m^3 wet sediment[i]	-	-	An additional 2-6 mm of sediment was found settled immediately after disturbance; however, this may be due to natural sedimentation events[j]	[i](Tkatchenko et al. 1996) [j](Radziejewska 2002)
INDEX (1997) Central Indian Ocean	26 tows in an area of 200X3000 m [k]	6023 m^3 wet sediment [k]	-	As far as 150 m from the disturbed area [l] At least 2 km from the disturbed area[m]	Averaged across study site (mg m^{-2} day^{-1}) Pre-disturbance = 43 During = 144 Post-disturbance (within 6 days) = 102 [k]	[k](Sharma et al. 2000) [l](Vanreusel et al. 2016) [m](Valsangkar 2001)

1.2.2 Biological response to simulated mining experiments

Of the aforementioned experiments, all resulted in physical disturbance of the seabed and subsequent deposition of sediment. Variation in the magnitude and duration of disturbances also occurred between experiments. A meta-analysis assessed the impacts of these experiments (16 studies published by 2011) on biological communities and found that within one year of disturbance, the density of 64 % of faunal classes had decreased significantly through both direct and indirect effects (Jones et al. 2017). Some level of recolonisation, especially by meiofauna, was observable but most faunal groups had shown little recovery over decadal time-scales. A couple of exceptions include the increase in polychaetes and isopods at the BIE-II site after 2-3 years and the recovery of meiofaunal abundance at the JET site after 17-18 years (Fukushima and Tsune 2019). Species diversity was only assessed at the DISCOL site, decreasing for all faunal groups and showing very little recovery over the next decade. As mining aims to remove hard substrate, nodule-associated fauna will be more significantly impacted, especially regarding the ability to recolonise any remaining hard substrate (Vanreusel et al. 2016). Very few faunal groups across different experiments had recovered to baseline conditions after decades, suggesting long-term detrimental effects of mining across most taxonomic groups (Jones et al. 2017, Stratmann et al. 2018a, Simon-Lledó et al. 2019b).

1.2.3 Limitations of simulated mining experiments

The mining experiments carried out over the last half century are certainly informative and provide insights into the expected effects of mining on biological communities. Although much has been gained from this work, these experiments do not provide sufficient information on specific responses to sedimentation and how those responses are ecologically meaningful at a community or ecosystem level. The following list describes some of the limitations of these experiments.

1. The majority of mining-simulation experiments did not comprehensively monitor changes to the sediment environment before and after disturbance, nor track or measure the fate of the sediment plume, as exemplified by the gaps in Table 1. For

instance, the BIE-II, JET and IOM BIE experiments provide data on the volume of sediment resuspended and the area of disturbance, but no information is given describing the settlement area or concentration of the plume. The true spatial extent of the plume effects is unknown, as is the nature of the disturbed sediment environment; thus, the true biological response is unknown and is based on limited quantitative data on the sediment plume and a potentially altered sediment environment. As the fate of the plume is subject to local environmental conditions, effects are likely much farther-reaching and could cover more than twice the area disturbed (Gjerde et al. 2016). This limitation has been recognised and, in response, a number of recent studies have modelled sediment plume dynamics from ship discharges (Rzeznik et al. 2019), laboratory experiments (Gillard et al. 2019) and in situ seabed disturbances (Kulkarni et al. 2018, Spearman et al. 2020). Whilst this modelling is a step in the right direction, these recent experiments do not assess biological responses. To satisfactorily assess the impact on biological communities requires the coupling of environmental and biological data, ideally from the same experiment.

2. The experiments do not represent the scale of an actual mining operation (see review by Sharma 2005). Commercial mining is expected to operate 300 days/yr, whereas DISCOL (2 weeks) and BIEs (18-88 hours) had much smaller operation times. The DISCOL experiment covered the largest area (11 km^2) while actual mines may cover 300 km^2 annually. The volume of resuspended sediment may only be 2-3.7 % of the amount generated by actual mining (Yamazaki and Sharma 2001). At the experimental scale, the biological response was overwhelmingly negative. Given the proposed scale of mining, the extent of the impact is expected to be significantly larger but it is unknown whether the biological response to sedimentation will scale proportionally or exponentially with mining effort. Perhaps, understanding the impacts of natural sedimentation events, such as turbidity flows and submarine landsides (e.g., Bigham et al. 2021), may provide some insights at larger spatial scales for the time being. However, it will likely be difficult to determine knowledge about the ecosystem effects of mining by sampling such proxy situations. Therefore, some local-scale studies of the influence of biodiversity loss by simulated mining on

ecosystem function will be necessary, which can be used to at least begin to understand the potential for ecosystem shifts that may occur over wider areas should mining occur (Gamfeldt et al. 2015). A "test-mining" stage is envisaged under International Seabed Authority recommendations for mining companies during exploration and may provide an opportunity to study mining effects at a larger scale, but these tests have not yet taken place and are not currently required by ISA regulations (ISA 2013).

3. Methodologies of mining experiments differed, making comparisons between studies more difficult. The DISCOL experiment, for example, used a plough system to disturb the seabed, whereas most other studies used the DSSRS. The DISCOL plough gouged the seabed more deeply than DSSRS, likely producing differences in sediment plume characteristics, such as particle size variation. Different particle sizes are known to affect organisms differently (O'Connor et al. 1976, Etter and Grassle 1992, Leduc et al. 2012); thus, comparing the effects of sedimentation between experiments is problematic. A standardised approach to future experiments would allow the generation of comparable/combinable datasets.

4. It is difficult to understand the effects of sediment plumes on pelagic species as assessment needs to be carried out in real time, due to the mobility of pelagic organisms (but see Christiansen et al. 2019).

5. Previous mining experiments have focused on before-after measurements of species density and diversity without providing explanations for the patterns observed. At the very least, future field-based simulation experiments should collect co-occurring environmental data that could potentially provide explanations for the observed faunal patterns. Ideally, laboratory experiments should also be conducted to better explain processes by which impacts are propagated in the benthic community. Overall, laboratory experiments using shallow-water taxa are much more common than studies of deep-water taxa. In order to address this limitation, sedimentation experiments on deep-sea organisms must be designed to assess biological responses, such as respiration rates or tolerances to particular sediment

concentrations or particle sizes. Identifying thresholds for particular organisms (e.g., the sediment concentration a sponge can tolerate before mortality) will help define "serious harm" and ensure that mining is managed in a way so as to remain below these thresholds (Levin et al. 2016).

1.3 Ecosystem function

Deep-sea soft-sediment ecosystems cover approximately two thirds of the surface of the Earth (Hesse and Schacht 2011), and provide ecosystem services, such as calcium carbonate dissolution and the sequestration of atmospheric carbon (Archer and Maier-Reimer 1994, Wenzhöfer et al. 2001, Sala et al. 2021). Disruptions to benthic ecosystem function could alter these services and have implications beyond mined areas of seabed. In order to understand how changes in community structure (e.g., abundance, biomass, biodiversity) resulting from seabed mining may affect the wider ecosystem, a measure of ecosystem function at a local scale is necessary. Sediment community oxygen consumption (SCOC) is a commonly used proxy for organic matter processing by benthic organisms and thus benthic metabolism (Snelgrove et al. 2018). In deep-sea sediments, benthic metabolism is strongly driven by the input of particulate organic carbon (POC) from surface waters (Smith et al. 2008), which itself is influenced by water column processes, such as hydrodynamics, recycling and remineralisation before reaching the seabed (Lampitt and Antia 1997, Turner 2002). Bacteria are mainly responsible for the processing of this organic material in deep-sea sediments (Pfannkuche 1993, Beaulieu 2002, Hubas et al. 2006), while the contribution of respiration by infauna (relatively larger organisms within the sediment) to benthic metabolism is relatively small (Glud et al. 2000, Rowe et al. 2008). However, infaunal organisms can influence bacterial activity through bioturbation and defecation (Lohrer et al. 2004, Papaspyrou et al. 2004, 2010, Bonaglia et al. 2014), thus indirectly affecting rates of SCOC. Positive relationships have been found between benthic ecosystem function and infaunal community parameters, such as abundance (Tahey et al. 1994, Braeckman et al. 2010, Papaspyrou et al. 2010, Leduc et al. 2020), biomass (Clough et al. 2005) and biodiversity (Danovaro et al. 2008, Baldrighi et al. 2017), suggesting that negative impacts on infaunal communities as a result of seabed mining could potentially lead to a loss of ecosystem function. In previous simulated-mining experiments, infaunal abundance and

diversity decreased severely after disturbance (Jones et al. 2017), but ecosystem function was not measured. Labelling a community as 'recovered' by measuring, say, abundance or diversity alone, does not necessarily mean that ecosystem function also recovers. There is a general trend across simulated mining experiments in the deep sea that, after disturbance, small individuals recover more quickly than larger individuals (Gollner et al. 2017, Jones et al. 2017). It has also been observed that the scale and intensity of disturbance can also affect the magnitude of the response by opportunistic species (Norkko et al. 2006). Therefore, abundance or diversity may quickly recover by these processes, but ecosystem function could remain impaired, especially because larger individuals are known to significantly influence benthic metabolism (Norkko et al. 2013). Using ecosystem function as an additional measure of recovery is therefore necessary to determine whether or not a benthic community has recovered from the impact of seabed mining and is able to perform the same function as in a pre-disturbed state.

1.4 Deep-sea mining in New Zealand

1.4.1 History of deep-sea mining in New Zealand

Deep-sea mineral resources contained within the Exclusive Economic Zone (EEZ) of New Zealand include manganese/cobalt-rich crusts on seamounts, seafloor massive sulfide deposits along the Kermadec Arc, polymetallic nodules south of the Campbell Plateau, and phosphorite deposits on the Chatham Rise (Glasby and Wright 1990, Lamarche and Clark 2011) (Fig. 3).
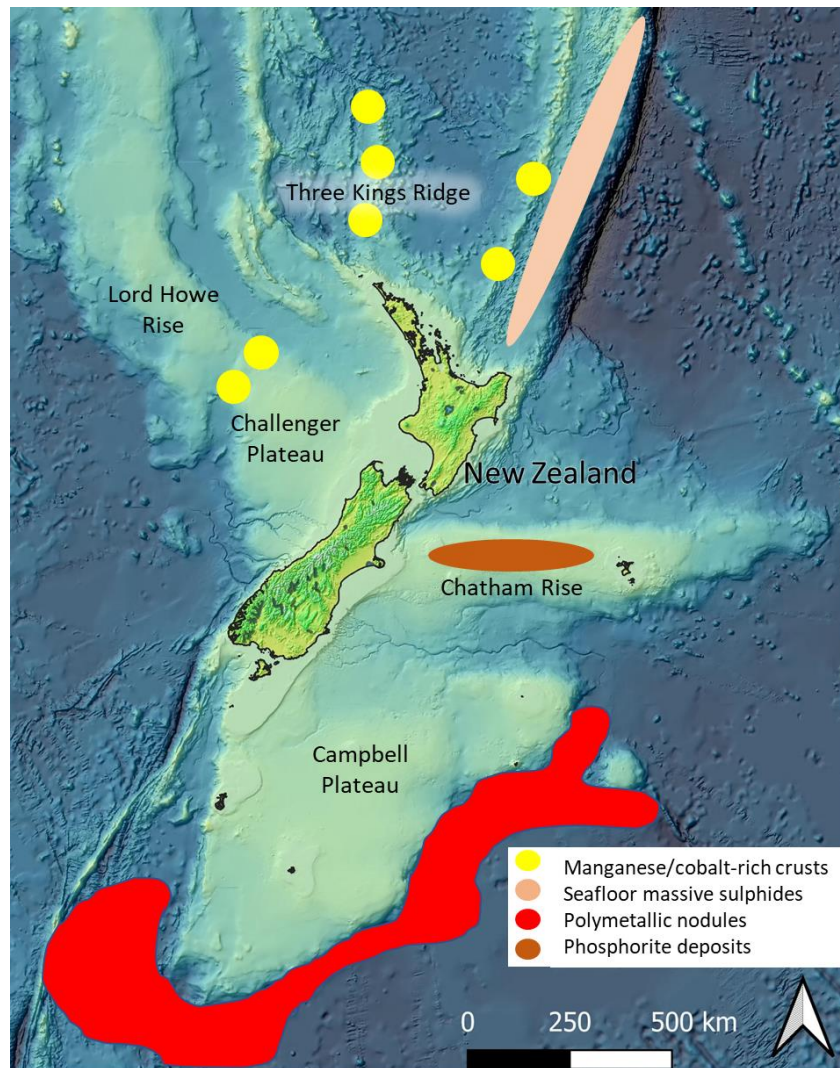
Figure 3. Potential deep-seabed resources of the New Zealand region. Map made using QGIS.

Interest in mining the deep-seabed has largely focused on two resource types: polymetallic sulfides along the Kermadec Volcanic Arc (Boschen et al. 2016), and phosphorite nodules on the Chatham Rise. Whereas activities associated with the polymetallic sulfides did not progress far, interest in phosphorite nodules was much greater since their economic potential and their potential use as a locally-sourced agricultural fertiliser was recognised (Cullen 1975, Mackay et al. 1980). However, little was known about benthic communities in this area at the time that the prospect of mining was originally proposed (Dawson 1984, Kudrass and Von Rad 1984b) and subsequent mining-related studies have also been limited to assessing baseline conditions (e.g., Leduc et al. 2015). In 2013, a Mineral Prospecting Licence was issued to Chatham Rock Phosphate (CRP) by the New Zealand government which allows CRP to conduct sampling to evaluate the economic value of phosphorite

deposits within their licence area on the Rise. But in order to begin mining, companies must first obtain a 'marine consent' from the Environmental Protection Authority (EPA) in accordance with the Exclusive Economic Zone and Continental Shelf (Environmental Effects) Act 2012. An environmental impact assessment (EIA) is required to gain this consent (among other requirements), and was provided by CRP based on numerous field and desktop studies (Chatham-Rock-Phosphate 2014). The consent to mine sought by CRP was refused in 2015 in part because the EIA did not present sufficient quantitative information on the expected impact of the proposed mining on benthic communities in the area, including the likely effects of sedimentation that would result from CRP's proposed mining plan (Fig. 4) (EPA 2015). In refusing the mining consent application, the EPA Decision Making Committee (DMC) stated that:

"Outside of the mining blocks there are likely to be additional impacts to benthic communities associated with suspended sediment and sediment deposition." … "The sensitivities of the benthic communities and individual species are largely unknown and the applicant has had to rely on information on species that do not live on the Chatham Rise and in many cases are not found in New Zealand waters. Given these uncertainties, the DMC finds that it is not possible to quantify the scale of effects on benthic communities away from the mining blocks." (EPA 2015).

Submitting thorough EIAs is particularly challenging when baseline data on ecosystems is deficient and when EIAs assess and monitor new activities in the marine environment. Recent evaluations of the EIA process highlight these difficulties and aim to make the process more effective both globally (Durden et al. 2018, Clark 2019a, Clark et al. 2020) and in New Zealand (Clark et al. 2017, Ellis et al. 2017). For the Chatham Rise ecosystem, where baseline data is limited, further investigation of sedimentation on benthic communities and their responses are required before future EIAs can be successfully conducted.
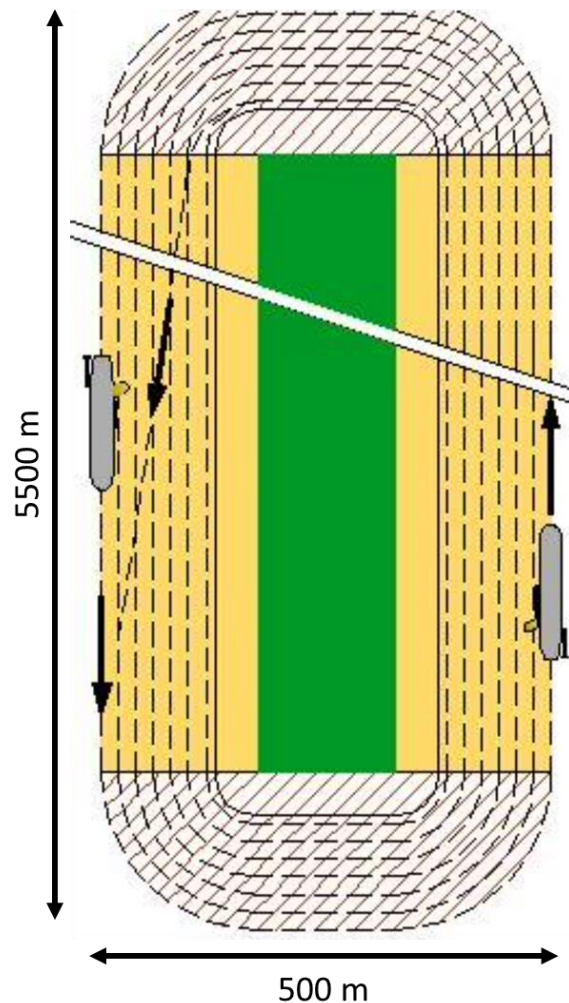
Figure 4. Chatham Rock Phosphate's proposed mining plan involves a mining vessel 'spiralling out' counter-clockwise from an unmined patch (in green) while depositing sediment in previously mined areas (in yellow). After mining becomes inefficient, a 'new area' will be similarly mined, but it is not clear how far away the new area will be from the recently mined area. Modified from van Raalte (2014).

1.4.2 The ROBES project

In 2016, the National Institute of Water and Atmospheric Research (NIWA) of New Zealand initiated a project (Resilience of deep-sea benthic communities to the effects of sedimentation (ROBES)(NIWA 2019)) to assess the response of benthic communities on the Chatham Rise to sedimentation from a disturbance designed to simulate a sediment plume that could result from mining and potentially bottom trawling, which has been shown to negatively impact benthic communities (Hinchen et al. 2021). Information from this project will provide a better understanding of benthic communities on the Chatham Rise and their

response to sedimentation, and will help ensure that future activities in this area are conducted in a way that balances resource exploitation with environmental protection. The ROBES project (2016-2021) consists of both a field study on the Chatham Rise and laboratory studies. In the field, a number of benthic disturbances were conducted (in 2018-19) in an area where there is interest in mining phosphorite deposits. The fate of the sediment plumes generated by these disturbances have been monitored up to 10 km from the areas of disturbance and the impact on benthic communities is being assessed before, immediately after, and one year after disturbance to examine community resilience and/or recovery in short to medium timeframes. In situ measurements of the sediment plume were conducted with a variety of instruments attached to "benthic landers" positioned on the seabed, as well as through the deployment of ocean gliders, CTD casts, and acoustic surveys (Clark et al. 2018, Clark 2019b). These instruments include turbidity sensors, Niskin water bottles (sediment concentration data) and sediment traps (sediment deposition data), as well as instruments for water column profiling and monitoring local oceanic conditions. Multicoring operations took place at different sites in which multiple sediment cores penetrating down to 40 cm into the seabed provided samples that were analysed for sediment characteristics and benthic fauna. The field-based experiments were coupled with a laboratory study in which the scleractinian coral, *Goniocorella dumosa*, and demosponge, *Ecionemia novaezelandiae*, were collected from the study site and exposed to different concentrations, particle size compositions and durations of sediment, based on data from the initial field disturbance survey. The exposure of these taxa to different sediment characteristics will provide information that can guide future management decisions regarding deep-sea mining. It is expected that particular sediment thresholds for corals and sponges will be incorporated into EIAs and decision makers will be better informed to assess whether a mining operation would cause serious harm to the environment or not. The coupled field and laboratory approach of the ROBES project will address many of the limitations of previous simulated mining experiments and will provide informative data not only for the deep-sea mining industry where necessary information is lacking, but also for other industries that use the Chatham Rise and disturb the seabed causing sedimentation, such as the bottom-trawl fishing industry (Pilskaln et al. 1998, De Madron et al. 2005). In discussing the limitations of simulated mining experiments (section 1.2.3), one of the considerable shortfalls was a lack of comprehensive data on sediment plume characteristics

and the sediment environment. Without such data, interpreting the effect of sedimentation on biological communities becomes problematic. Currently, the DISCOL experiment provides the best estimate of potential effects of mining by conducting a relatively larger-scale disturbance than other experiments and completing a more extensive survey of the benthic community over a number of years. However, the major weakness of the DISCOL experiment is the lack of data on the sediment environment, which precludes a robust examination of the benthic community response to sedimentation. As stated earlier, sediment plume modelling studies have been conducted in recent years, but coupling sediment data with biological data, ideally from the same experiment, will provide the most powerful assessment of sedimentation effects on biological communities. The ROBES project bridges the gap between these two historically separate datasets by simultaneously monitoring the conditions of the sediment environment and the sediment plume, and comprehensively sampling the benthic community over time, thus allowing a relatively more robust assessment to be conducted.

1.5 Relevance of study

The ROBES project investigates the impact of sedimentation on a range of benthic fauna. My research uses samples from ROBES to assess the response of macroinfauna; small-bodied organisms (typically 0.3-1 mm) that live within sediments of the seabed. Macroinfauna (hereafter referred to as macrofauna) have been sampled in a number of simulated mining experiments (DISCOL, INDEX, JET, BIE-II (Jones et al. 2017)); however, their responses have not been analysed in relation to particular sediment variables or measures of ecosystem function. Deep-sea macrofaunal communities are known to be influenced by a range of sedimentary variables at small spatial scales, such as total organic carbon/matter (Mamouridis et al. 2011), chlorophyll *a* concentrations (Tselepides and Eleftheriou 1992) and sediment grain size variation (Stora et al. 1999, Leduc et al. 2012); thus, macrofaunal responses to disturbance may be better understood by incorporating such variables into community analyses. Macrofauna are more responsive to environmental changes than larger epifauna (Ruhl and Smith 2004), and given that macrofauna live within the sediment itself, it is an appropriate place to observe any sedimentation impacts. During ROBES, macrofauna were sampled by a multicorer and a subset of the cores obtained were

incubated under natural conditions to measure rates of SCOC. SCOC measurements act as indicators of ecosystem function and when measured over time, can be used to examine how a community responds and may recover from sedimentation events. With reference to the current literature, it is difficult to say with much confidence how macrofauna will respond to a changing sediment environment and the associated side effects of sedimentation. My research aims to address this uncertainty by identifying the response and recovery of macrofauna, with respect to specific sediment variables.

1.6 Research questions

The two focal questions of my research are:

1. How is macrofaunal community structure impacted by the physical disturbance of the seabed and sedimentation?
2. Do macrofaunal communities show signs of recovery from such impacts within short to medium timeframes?

The secondary aim of my research is to investigate how the observed macrofaunal patterns may be explained by a suite of sediment variables, and what influence any apparent changes in the macrofauna community may have on benthic ecosystem function, as measured by SCOC. Together, this information will provide a possible explanation to the observed patterns in the macrofaunal response to disturbance and the likely ecosystem level impact of this disturbance, and will thereby allow more accurate predictions of the consequences of sedimentation events caused by seabed disturbance.

2. Methods

2.1 Study area

The Chatham Rise is a submarine topographic feature that extends roughly 1000 km east of South Island, New Zealand. The crest of the Rise is 300-400 m deep and slopes away northwards to the Hikurangi Plateau and southwards to the Bounty Trough to depths of more than 3000 m (McKay et al. 2005). The Chatham Rise contains a variety of geomorphic features including sloping flanks with occasional valleys, a topographically uneven, though flat-lying crest with a number of shallow banks in the west (Mernoo, Reserve and Veryan Bank), isolated clusters of seamounts (Andes and Graveyard complexes) and the emergent Chatham Islands in the east (Nodder et al. 2012).

Warm, nutrient-poor water from the north (East Cape Current) mixes with colder, nutrient-rich water from the south (Southland Current) to form the Subtropical Front above the Rise, creating conditions that support high levels of primary production (Sutton 2001, Pinkerton 2011). In turn, this production supports a diverse benthic community (Nodder et al. 2003) and deep-sea fishing industry (Francis and Fisher 1979) associated with the Chatham Rise. The Rise is also the location of phosphorite deposits that have been of seabed mining interest since the late 1960s (Summerhayes 1967, Watters 1968), but which have not yet been the subject of commercial exploitation (Fig. 5).
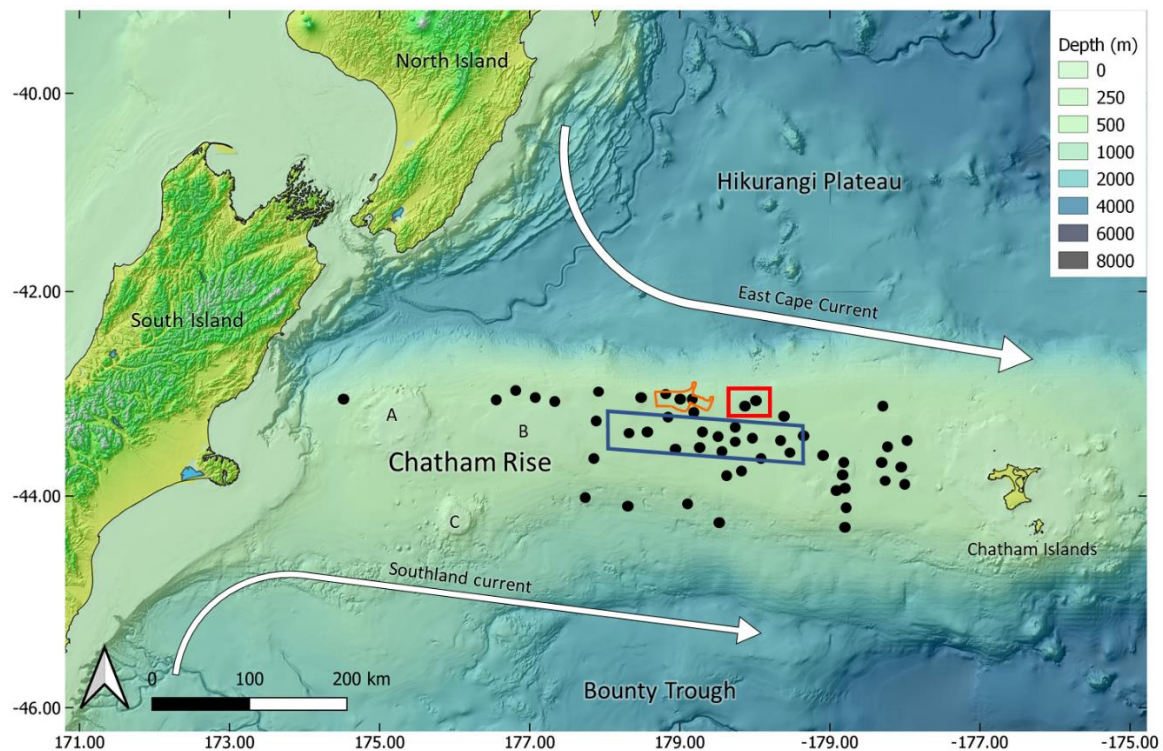
Figure 5. Chatham Rise showing the locations of (A) Mernoo, (B) Reserve and (C) Veryan Banks, the ROBES survey area (in red box), the main licence area held by Chatham Rock Phosphate (in orange box), the Mid-Chatham Benthic Protection Area (in blue box), and the main phosphorite deposits (black dots). Map made using QGIS.

The topography of the crest, where the phosphorite deposits are mainly concentrated, is highly irregular and contains sediment-filled hummocks and swales (Kudrass and Von Rad 1984a), pockmarks and iceberg scour marks (Nodder et al. 2012). Sediments covering the crest region are predominantly organic-rich, glauconitic muddy sands; terrigenous sediments are more common in western areas. Phosphorite deposits that occur on the crest were formed from Late Oligocene and Late Miocene limestone and carbonate rubble which phosphatised in the Late Miocene, as determined by the age of enclosed foraminifera in nodules (Zobel 1984, Nielsen et al. 2015). Surficial or near-surface nodules occur in a layer of sediment that ranges in thickness from a few centimetres to 70 cm deep (20-30 cm average) (Cullen 1987). Phosphorite nodules have been identified along 400 km of the crest between longitudes 177°E and 177°W, but the highest concentrations occur between longitudes 179°E and 180° (Cullen 1987).

Exposed bedrock and phosphorite nodules on the crest of the Chatham Rise provide the only hard substrate for sponges and corals (Dawson 1984). Most of the crest region contains

soft-substrate habitat for epibenthic fauna, such as crustaceans, ophiuroids and bivalves (McKnight and Probert 1997), as well as a structurally and functionally diverse infaunal community dominated by macrofaunal polychaetes, amphipods, echinoderms and molluscs (Leduc et al. 2015) and meiofaunal nematodes (Pilditch et al. 2015). Macrofaunal and meiofaunal density and biomass are highest on the crest and the upper southern slope, and decrease with increasing water depth either side of the Rise; however, this decrease is more pronounced on the northern slope (Probert and McKnight 1993, Probert et al. 1996, Grove et al. 2006, Probert et al. 2009). This relationship is likely explained by strong correlations between infaunal biomass and sediment chlorophyll *a* concentration (often decreasing with increasing water depth), revealing a close link between benthic biomass and food supply on the Chatham Rise (Berkenbusch et al. 2011, Pilditch et al. 2015). This pattern reflects the different levels of biological productivity of the Subtropical Front, and flux of organic matter from the euphotic zone to the seabed across the Rise (Nodder et al. 2003, Nodder et al. 2007).

2.2 Survey area and design

The ROBES survey area (Fig. 5) is located on the northern slope of the crest of the Chatham Rise at depths of 400-500 m, and is situated several kilometres north of the northern boundary of the Mid-Chatham Benthic Protection Area (BPA) and east of the main licence area held by CRP. The area consists of a relatively uniform sediment slope with patches of exposed bedrock and cobbles where previous seabed imagery and benthic samples indicated that coral communities could be present (Clark et al. 2018). The area was selected based on the following criteria; substrate: an area with nodule/sediment combination realistic as a substrate for potential mining (and trawling); biology: an area where the faunal composition is representative of CRP's mining license area (and trawling areas) (e.g., presence of the coral *Goniocorella dumosa*), with densities high enough to measure meaningful changes following sedimentation, but not too high as to be considered a sensitive or vulnerable habitat; trawling distribution: an area with low trawling effort to date, and likely to be untrawled in the future; clear of protected areas: an area outside of the Mid-Chatham BPA.

The survey was designed to assess the effects of sedimentation on benthic communities and their resilience and potential recovery over time. The first research voyage, TAN1805, took place in 2018 with the purpose of disturbing the seabed and creating a sediment plume. Both pre- and post-disturbance sampling events were conducted in 2018 and future monitoring surveys were planned for 2019 and 2020 (a summary of ROBES voyages is given in Table 2).

The original DSSRS (Brockett and Richards 1994), which had been used for previous deep-sea simulated mining experiments (see Jones et al. 2017), was modified for use by NIWA, and called more simply the "benthic disturber" or BDR. Unfortunately, post-disturbance camera surveys showed that the BDR had much less impact than expected; only the top few centimetres of fine sediment were being suspended as opposed to coarser sediments at 10-15 cm depth that was expected from previous operations of the DSSRS. Images also revealed a lack of sediment build up near the BDR runs and on adjacent coral communities; the very fine sediment was being swept away by near-seabed currents.

Table 2. Summary of ROBES voyages.

Voyage	Dates	Initial survey plan	Amended survey plan
TAN1805	9 May - 7 June 2018	- Baseline survey of benthic communities - Seabed disturbance - Monitor sediment plume and deposition - Post-disturbance survey of benthic communities	Data to be used to measure longer term response of benthic communities to original disturbance
TAN1903	9 June - 29 June 2019	Resample benthic communities	- Resample benthic communities from TAN1805 disturbance site - New baseline sampling at Butterknife site - Butterknife disturbance - Post-disturbance sampling at Butterknife site
TAN2005	9 June – 22 June 2020	Resample benthic communities	Resample benthic communities

Based on the experiences of the initial disturbance in the 2018 survey, it was decided that during the second voyage (TAN1903), a more intense, localised seabed disturbance would

be conducted adjacent to a seafloor feature called the "Butterknife". Backscatter data from an acoustic survey using a multibeam echo sounder (MBES) and camera footage identified that the butterknife-shaped feature consisted of a hard substrate rim of exposed bedrock and cobbles with dense coral communities and soft substrate either side of the rim (Fig. 6). Pre- and post-disturbance sampling events were planned for 2019 followed by a post-disturbance sampling event one year later in 2020, allowing the resilience and potential recovery of benthic communities to be assessed in the short to medium term. In place of the BDR, an agricultural plough, similar to that used for the DISCOL disturbance, was obtained and modified to control the depth of penetration and to direct sediment vertically during the disturbance. This device was named "Sediment Cloud Induction Plough" or "SCIP" (Fig. 7). The weight of SCIP, including the attached harrow mat, was 800 kg and the frame measured 2.5 x 3.4 x 0.8 m (length x width x height). A tickler chain was attached to the front of SCIP which first disturbed the very top layer of sediment during the disturbance event. Behind the chain, twenty-eight tynes, or 'teeth' (total length of each tyne = 67 cm), were attached to the SCIP frame in 3 rows. These tynes extended 16 cm below the frame, and were used to stir up deeper sediments. Finally, a harrow mat (which alone weighed 180 kg) was attached at the rear of the frame with the purpose of scraping the top of the sediment with smaller 'teeth' at a higher density than the more deeply-penetrating tynes (Fig. 7).
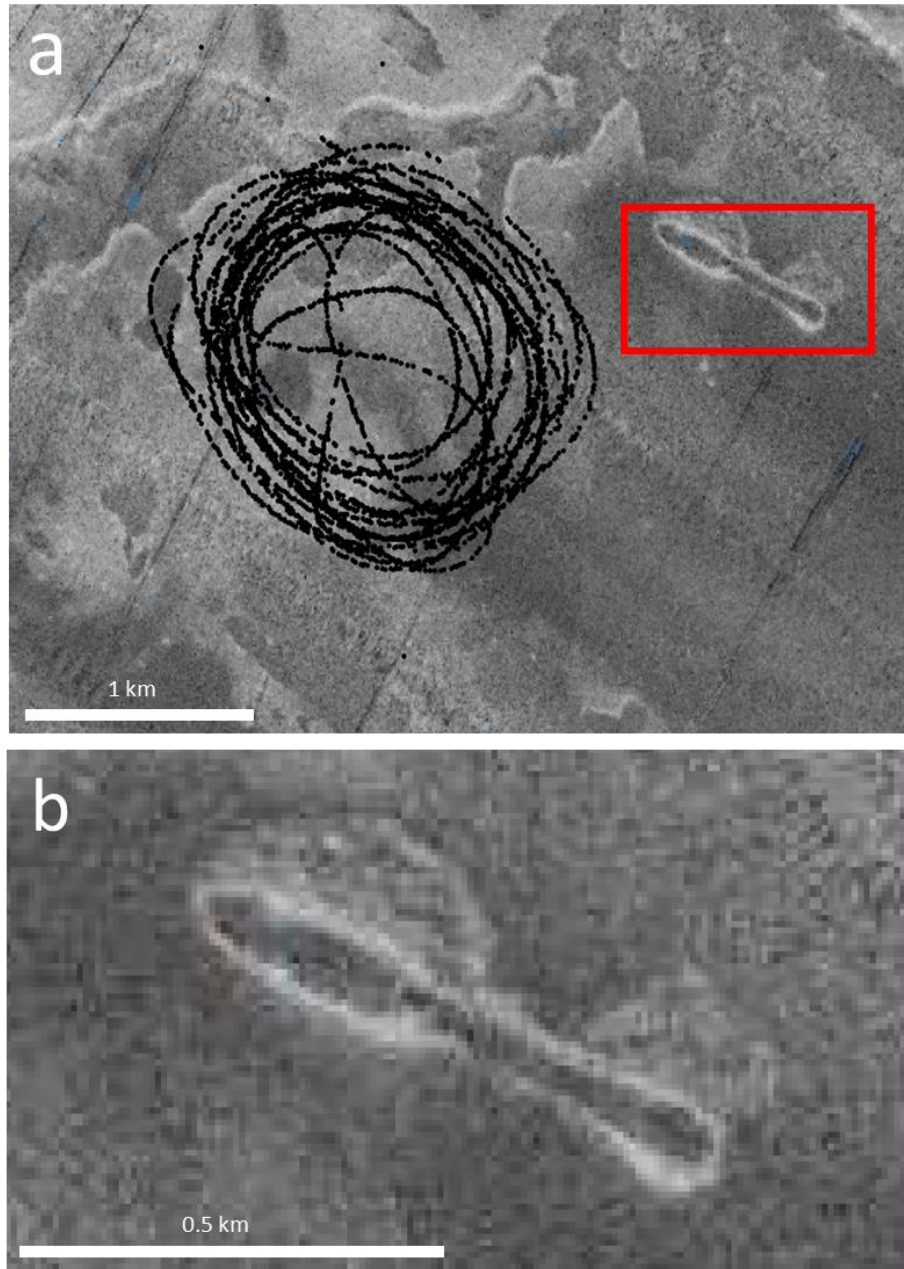
Figure 6. MBES backscatter showing (a) the TAN1805 main disturbance track (in black) in relation to the Butterknife feature (in red box), and (b) the Butterknife feature. The light grey colour indicates high reflectivity (i.e., hard substrate), dark grey indicates softer substrate.
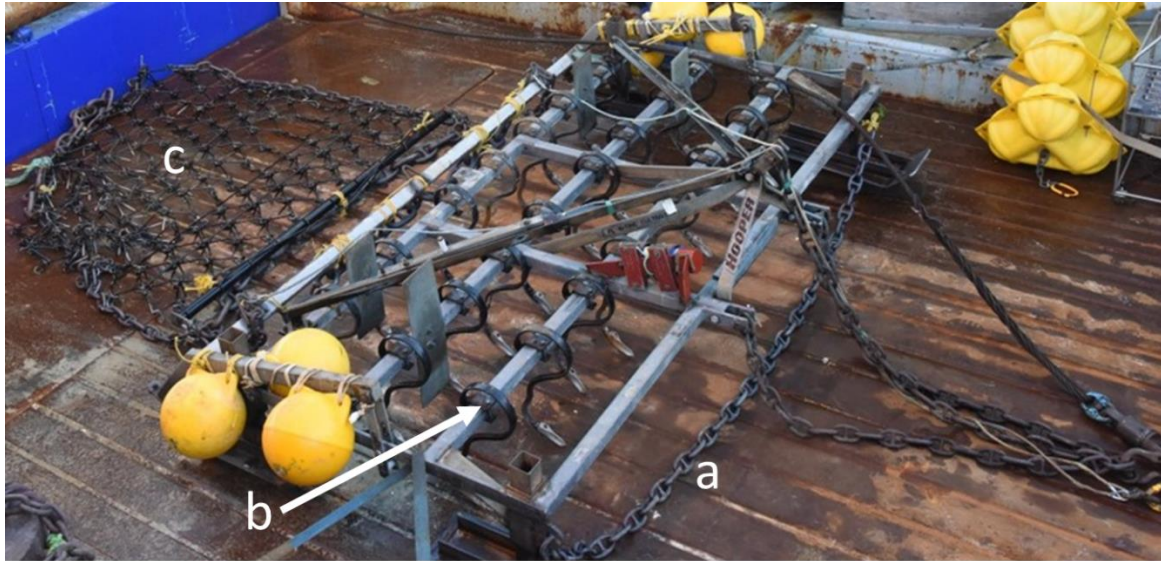
Figure 7. The Sediment Cloud Induction Plough or SCIP used during the Butterknife disturbance experiment, showing (a) tickler chain, (b) tynes, and (c) harrow mat.

After initial trials to evaluate the performance of SCIP 7-8 kilometres to the southeast of the Butterknife, the disturbance adjacent to the Butterknife took place during TAN1903 between 19-23 June 2019. The penetration depth of SCIP was set at 15 cm with towing speeds between 1.5-2.0 knots. There were three disturbance transects around the Butterknife; (1) to the south of the southern rim, (2) 'inside' the rim with shorter tows to land and haul clear of the east and west rims and (3) to the north of the northern rim. Multiple SCIP tows were conducted along each transect to more accurately simulate mining (Fig. 8).
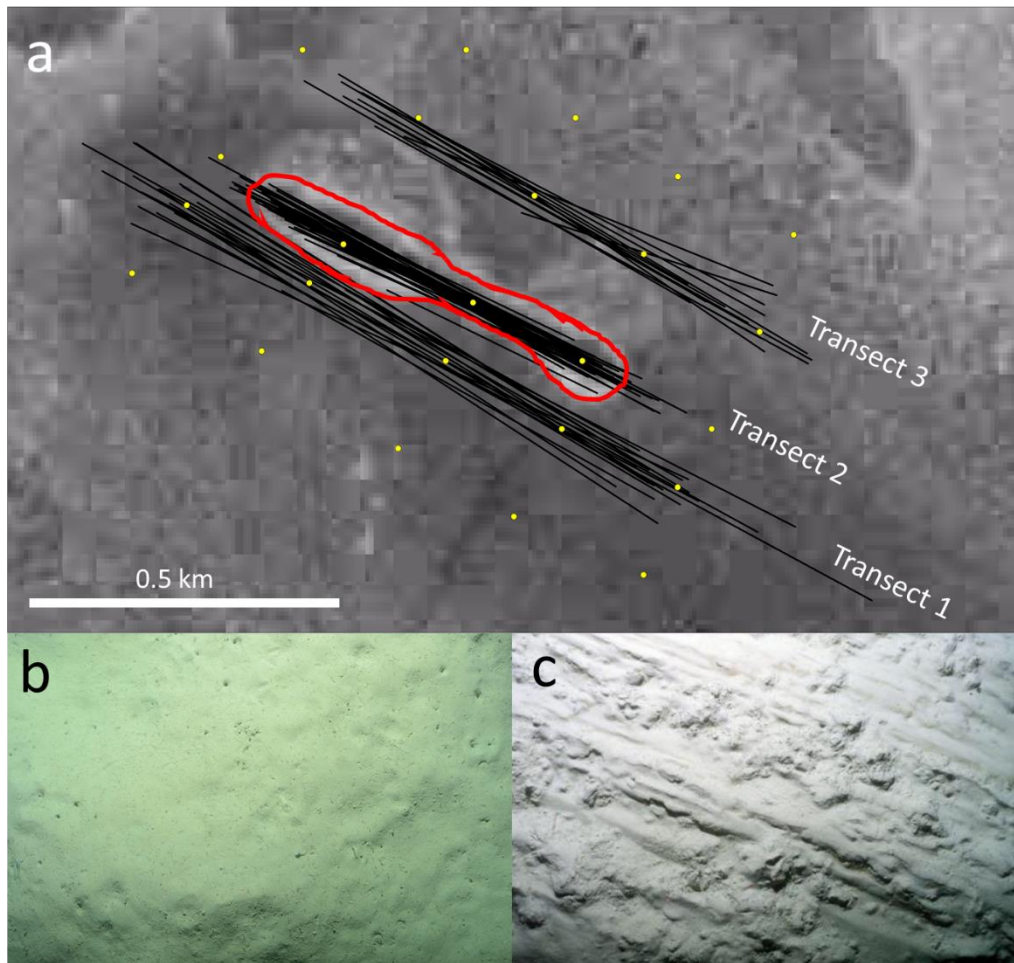
Figure 8. (a) The three SCIP transects showing where SCIP was in contact with the seabed. The Butterknife rim is outlined in red and the yellow dots are multicoring sampling locations (see section 2.3). The seabed (b) before and (c) after disturbance showing SCIP tracks.

2.3 Sampling equipment and deployment methodology

ROBES is a multi-disciplinary project, but because my research aims to answer questions pertaining to benthic ecology only, the following descriptions of the sampling equipment used during ROBES only include those relevant to my study; that is, equipment that collected data that I will relate to the samples that I analysed from the multicorer. Three benthic landers were deployed for the duration of the Butterknife disturbance and carried a range of instrumentation, as follows:

- Acoustic Doppler Current Profiler (Nortek Aquadopp 2 MHz)
- Turbidity sensors (an AQUAscat and Aquatec AQUAloggers)
- Temperature-salinity-dissolved oxygen sensor (Seabird MicroCAT)
- Camera and light

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- Niskin water bottle (5 litre)

- Sediment trap (Technicap, 12 sample bottles) to measure particle deposition

Lander 1 had the full set of instrumentation, while the other two landers did not have an AQUAscat. The landers were deployed on 15 June 2019 in an area expected to be covered by the sediment plume in order to measure particle size, density and composition of the suspended sediment. Lander 1 was positioned to the north of the northern Butterknife rim within 180 m of the disturbed area; Landers 2 and 3 were positioned to the south and southeast of the southern Butterknife rim and 180 m and 370 m to the nearest disturbed area, respectively (Fig. 9c). After the disturbance, the landers were retrieved on 25 June 2019 for analysis of the samples and data they collected.

An Oceans Instruments MC-800 multicorer was used to collect core samples of the seabed (Fig. 9a). Six core tubes (9.52 cm internal diameter) were loaded onto the multicorer frame. The multicorer was lowered from the ship and, on contact with the seabed, the core tubes were gently pushed into the seabed by a hydraulically dampened weight, preserving the sediment-water interface. The arrangement of core tubes on the multicorer ensured that samples were obtained for the range of parameters tested, including for macrofauna and sediment (Fig. 9b). A 5 X 5 sampling grid was designed to cover the Butterknife area to span the range of disturbance and non-disturbance conditions (Fig. 9c). At each sampling point, the multicorer was deployed once. The entire grid was sampled at each time period; pre-disturbance on 16-17 June 2019, post-disturbance on 24-25 June 2019 and one year after disturbance on 14-19 June 2020. A subset of these sites (15 of 25) was selected for further experimentation (see section 2.4). Multicorer deployments were also carried out at selected sites from the main area disturbed during TAN1805 (0.7 km to the south-west of the Butterknife). Three multicorer drops were performed at each of these sites, which included monitoring (MON1-9) and disturbance (DIS1, 2) sites. A reference site (REF1) situated 14.8 km to the north-west of the Butterknife disturbance area was also sampled using the multicorer (Fig. 9d).
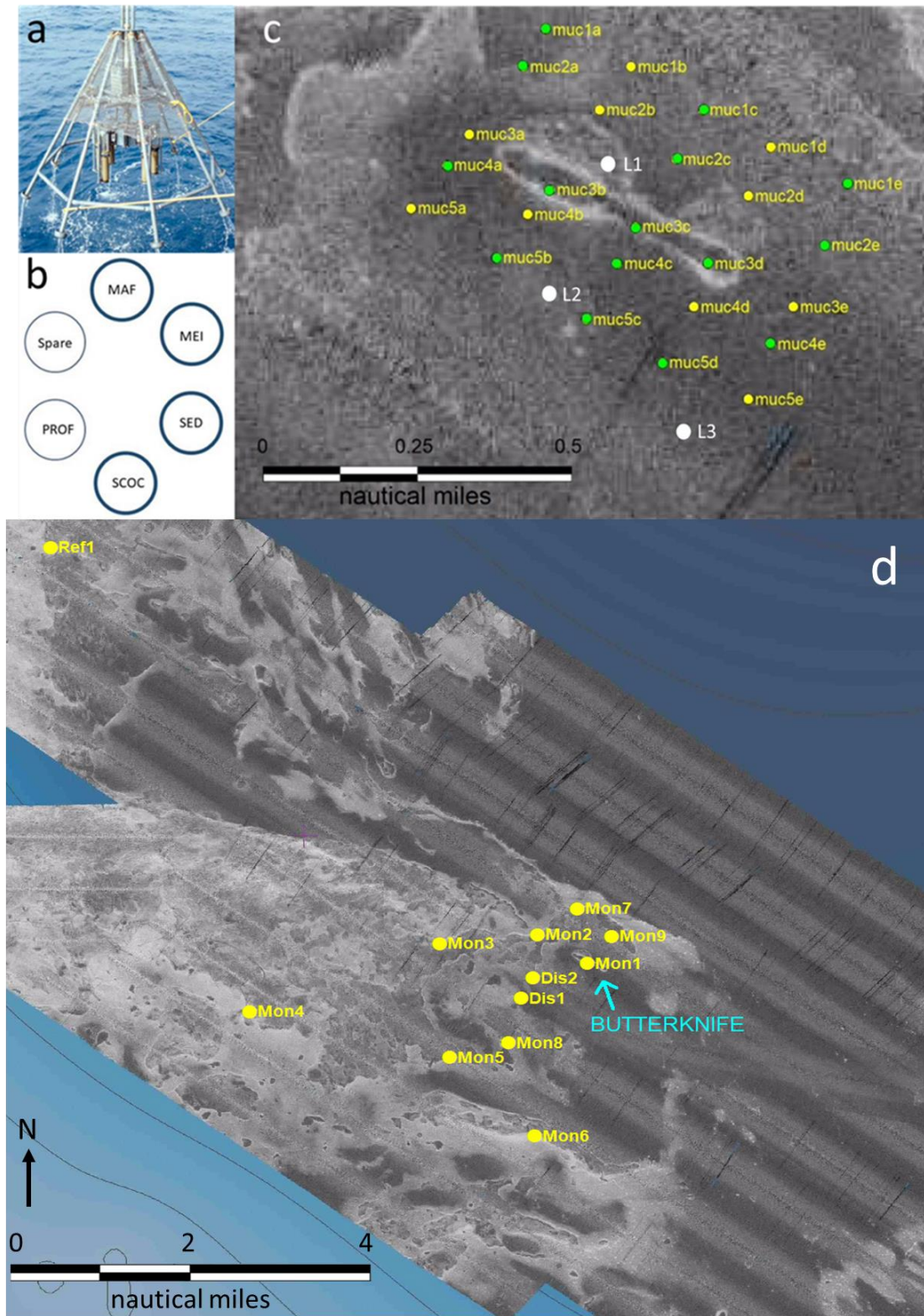
Figure 9. (a) Oceans Instruments MC-800 multicorer with core tubes. (b) the arrangement and designation of each core on the multicorer (MAF = macrofauna, MEI = meiofauna, SED = sediment parameters, SCOC = sediment community oxygen consumption, PROF = profiling). (c) The 5 X 5 sampling grid of the Butterknife area (green dots = SCOC sites, yellow dots = non-SCOC sites, white dots = benthic lander positions). (d) The positions of the Butterknife, monitoring (MON), TAN1805 disturbance (DIS), and reference (REF) sites.

2.4 Sample treatment and analysis

In order to investigate the effect of sedimentation on macrofaunal communities, I used core samples obtained from TAN1903 and TAN2005, with a particular focus on cores collected across the Butterknife sampling grid. Both macrofauna and sediment community oxygen consumption cores (hereafter referred to as MAF and SCOC cores, respectively) were processed onboard RV *Tangaroa* by others during TAN1903, and by myself and others during TAN2005. Processing of cores was as follows; MAF: each MAF core was sectioned at 0-5, 5-10 and 10-15 cm sediment depth intervals, wet-sieved at 300 μm and fixed in 10 % buffered formalin; SCOC: these cores were incubated by Dr. Rachel Hale (NIWA) to estimate sediment community oxygen consumption. The upper 13-15 cm of sediment and overlying water from each core was carefully extruded into incubation chambers. The chambers were sealed and placed in water baths at ambient bottom water temperatures (± 0.1°C) and held in the dark for 26-48 hr. Water column respiration was accounted for by an additional chamber containing only near-bottom water. Water was circulated using a magnetically driven impeller fitted to the chamber lids. Oxygen concentrations were measured with a PreSens FIBOX 3 PSt3 optode O_2 sensor approximately 6 hr after chambers were placed in the water baths. Further oxygen readings were made at 6 hr intervals until the incubations were terminated when initial oxygen concentrations had decreased by 10-20 %. Oxygen consumption by the sediment community was estimated from the decline of oxygen with time (linear regression, $r^2 > 0.9$), after controlling for water column respiration. Upon completion of the incubations, the core samples were processed for macrofauna by wet-sieving at 300 μm and fixation in 10 % buffered formalin.

After each voyage, the MAF and SCOC samples were delivered to NIWA's formalin laboratory at Greta Point, Wellington, where I treated them[1] as follows. Because SCOC cores had been processed onboard for macrofauna after incubations, their treatment in the laboratory was identical to MAF cores. The samples were stained with the biological stain

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[1] Prior to my research, I spent two weeks in August 2019 at NIWA's Greta Point site in Wellington learning how to sort macrofauna in the laboratory using core samples unrelated to my research. This training ensured I would pick out a minimum of 90 % of the macrofauna from a core, and could identify macrofauna accurately and efficiently for my research.

Rose Bengal, and left overnight. After staining, the samples were poured onto a 300 μm sieve, with formalin being funnelled into a waste container. Under a fume hood, tap water was used to wash samples and remove excess formalin and any fine particulate matter before being placed into petri dishes and examined under a Zeiss Stemi SV11 stereo microscope. For each core, macrofauna were removed from the sediment using forceps, separated into their major taxonomic groups (phylum, class, order; see Table 3 for complete list) and were preserved in labelled vials containing 80 % ethanol (except nematodes; 10 % buffered formalin). Identifying individuals to higher taxonomic levels is sufficient to assess impacts on benthic communities and loses little information when compared to species-level identification (Ferraro and Cole 1990, Gesteira et al. 2003, Wlodarska-Kowalczuk and Kedra 2007); in fact, the impact of mining waste disposal on deep-sea macrofauna has been detected at the phylum level (Hughes et al. 2015).

Individuals were identified using taxonomic guides (including online resources) when necessary, and unknown specimens were sent to taxonomic experts at NIWA where possible (see acknowledgements). To ensure abundance estimates were accurate, for those taxa with heads, only individuals with heads were counted; animal fragments were not counted as they may have belonged to an individual already counted. The following describes how organisms without obvious heads were counted; order Actiniaria: only whole anemones were counted; class Bivalvia: only bivalves with two valves and obvious stained biomass inside were counted; class Gastropoda and class Scaphopoda: only whole shells with obvious stained biomass inside were counted; phylum Bryozoa: bryozoans are colonial organisms, thus counting individuals is difficult, so, in this case, each bryozoan fragment was counted as an individual; class Ophiuroidea: only the central disc was counted, arm fragments were not; class Echinoidea: only complete tests (urchin 'shells') were counted, spines or test fragments were not; class Scyphozoa: as scyphozoans tend to live pelagically, the only individuals found were in larval tubes which attach to the seabed, so, only tubes containing obvious stained biomass were counted; class Ascidiacea: individuals with intact bodies, including inhalant and exhalent siphons were counted.

Table 3. Major taxonomic groups for macrofauna. Individuals were identified to the lowest taxonomic group presented here. No macrofaunal groups were identified for family or genus levels, hence their exclusion from the table.

Phylum	Class	Order	Species
Cnidaria	Anthozoa	Actiniaria	-
		-	Taiaroa tauhou
	Scyphozoa (larvae only)	-	-
	Hydrozoa	-	-
Nemertea	-	-	-
Nematoda	-	-	-
Mollusca	Bivalvia	-	-
	Gastropoda	-	-
	Gastropod eggs	-	-
	Scaphopoda	-	-
	Aplacophora		
Annelida	Polychaeta	-	-
Sipuncula	-	-	-
Platyhelminthes	-	-	-
Crustacea	Ostracoda	-	-
	Copepoda	-	-
	Malacostraca	Amphipoda	-
		Tanaidacea	-
		Cumacea	-
		Isopoda	-
		Mysida	-
		Leptostraca	-
Arthropoda (subphylum Chelicerata)	Pycnogonida	-	-
Bryozoa	-	-	-
Echinodermata	Ophiuroidea	-	-
	Echinoidea	-	-
	Asteroidea	-	-
	Holothuroidea	-	-
Chordata	Ascidiacea	-	-
Kinorhyncha	-	-	-
Unknown	-	-	-

The biomass of each taxon per sample was measured using a Mettler Toledo AG245 Analytical Balance. Blotted wet weight measurements were conducted whereby each taxon from the same sample was separately blotted on absorbent paper to remove any adherent

fluid, weighed to the nearest 0.00001 grams (or given a measurement of 0.000001 g for samples weighing <0.00001 g) and returned to their preservation vials. Abundance and biomass data from the MAF cores were pooled from the three depth intervals.

Sediment data generated by others at NIWA was supplied in order to investigate potential explanations for patterns observed in the macrofaunal response. One core from each multicorer drop (SED core, Fig. 9b) was sectioned at intervals of 0-1 and 1-5 cm, and a range of physical and biogeochemical parameters from the sediment environment were measured for each interval. Physical parameters calculated using the GRADISTAT computer program (Blott and Pye 2001) include mean grain size, and proportions of gravel, sand and mud, each subclassified as very coarse, coarse, medium, fine and very fine (for grain size fractions, see Table 1 in Blott and Pye 2001). Other physical parameters include sorting, porosity, void ratio, and water content (%H_2O). Biogeochemical parameters include proportions of particulate organic carbon (%POC) and particulate nitrogen (%PN), as measures of food availability, as well as their molar ratio (C:N) as a measure of food quality. Other biogeochemical parameters include total organic matter (%TOM) as an additional measure of food availability, chlorophyll *a* (Chl *a* (μg/g of dry weight sediment)) as a measure of 'fresh' phytodetrital organic matter, phaeopigments (Phaeo (μg/g of dry weight sediment)) as a measure of degraded phytodetrital organic matter, and their ratio (Chl *a*:Phaeo) as a measure of the 'freshness' of the phytodetrital organic matter, with higher values being 'fresher'. Physical parameters (except %H_2O, porosity and the void ratio) were determined by laser diffraction, while %H_2O and %TOM were determined by loss-on-ignition (4 h at 500°C) (Eleftheriou and Holme 1984). Porosity, a measure of the void spaces in the sediment, was calculated by dividing the volume of fluid (cm^3) (V_w) by the total volume of wet sediment (cm^3) (V_t) in each core sample. The void ratio (E) was calculated by dividing porosity by the volume of dry sediment (cm^3) (V_s). Chlorophyll *a* and phaeopigments were determined by standard spectrophotometric techniques subsequent to freeze-drying and extraction in 90 % acetone (Sartory 1982), and %POC and %PN were measured using a CHN analyser (CE Instruments NC2500) with an estimated machine precision of 2%.

Bacterial sampling was conducted by others during each sampling period and samples were collected as a surface scrape from meiofauna (MEI) cores (Fig. 9b) using a sterilised

stainless-steel spoon, and were subsequently placed in 50 ml Falcon tubes and frozen at -80°C before being transported to the laboratory for analysis. Flow cytometry was used to measure bacterial cell abundance (cells/cm^3) following the methods of Morono et al. (2009, 2013).

2.5 Data treatment

Prior to analysing the macrofaunal data, certain taxa were removed from the data set for the following reasons; *Acutiserolis* sp.: one single large isopod was removed because it was epibenthic, not infaunal (Dr Rachael Peart, pers. com.); gastropod eggs, Scyphozoa larvae and nauplii: larval or juvenile individuals were removed due to difficulty in identifying their taxonomic group; Bryozoa: individuals were counted as fragments rather than individual zooids, potentially misrepresenting their abundance, and they are also not often considered part of the sediment community due to their reliance on hard substrate; Nematoda, Copepoda, Kinorhyncha: these taxa are often categorised as meiofauna, not macrofauna, and only larger individuals were retained when sieving for macrofauna. These taxa will be treated as part of a separate meiofaunal analysis. Only one specimen was unable to be identified and was therefore removed.

Based on the location of the SCIP disturbance transects, multicore sampling points were allocated to one of two treatments; undisturbed or disturbed. The undisturbed samples were those from an area not physically run over by SCIP, but were likely subjected to lower levels of sedimentation as a result of the SCIP disturbance (so relatively 'undisturbed'). The disturbed samples are those from an area that was physically run over by SCIP and, being in close proximity to the disturbance transects, were likely subjected to high levels of sedimentation (Fig. 10)[2]. One of the major concerns cited by the EPA in their decision to reject CRP's mining consent application was that "it is not possible to quantify the scale of effects on benthic communities away from the mining blocks." (EPA 2015). For the SCIP

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[2] During TAN2005, the multicorer sampling point "muc2b" was accidentally sampled twice, and "muc3b" was not sampled. However, because both sampling points were allocated to the same treatment (disturbed), the two samples from "muc2b" were considered independent replicates within that treatment, thus not affecting the outcome of statistical results.

disturbance, the disturbed treatment acts as a mining block and the undisturbed treatment represents areas adjacent to the mining block, allowing the impact of disturbance on the benthic community to be quantitatively assessed under each treatment.
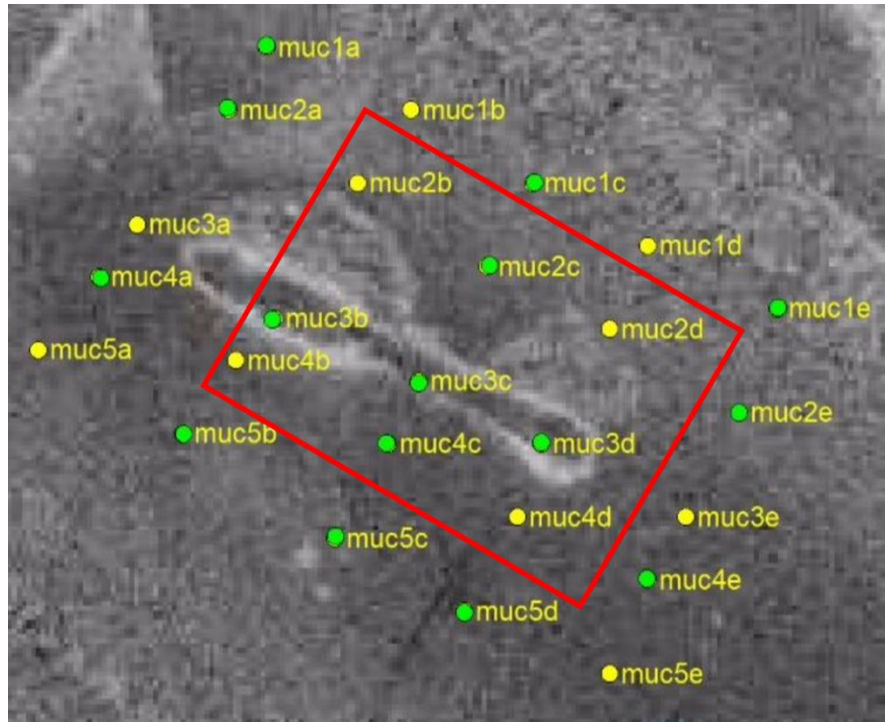
Figure 10. The Butterknife multicore sampling grid; inside the red box is the disturbed treatment (n = 9) and outside is undisturbed (n = 16). (green dots = SCOC sites, yellow dots = non-SCOC sites).

My dataset was derived from a combination of SCOC and MAF cores, which were both processed for macrofauna. In order to assess the suitability of using the two core types as part of the same analysis, I performed an initial univariate PERMANOVA test using after-disturbance total abundance data from MAF and SCOC cores for undisturbed and disturbed areas. I also performed a PERMANOVA test using after-disturbance multivariate abundance data, to test whether core type had any effect on community structure in both undisturbed and disturbed areas. Total abundance values for SCOC and MAF cores did not differ from one another for either treatment (undisturbed; t = 1.411, p = 0.1794, disturbed; t = 1.4551, p = 0.2024). Community structure was also unaffected by core type (undisturbed; Pseudo-$F_{(1, 14)}$ = 1.6997, p = 0.1109, disturbed; Pseudo-$F_{(1, 7)}$ = 1.028, p = 0.4607). Therefore, data from both MAF and SCOC cores could be combined for a single data analysis without introducing any bias associated with their different on-board treatments.

2.6 Data Analyses

Experimental designs in which measures of the same sampling points are taken at two or more time periods are considered repeated measures designs. These designs account for samples that are correlated through time and thus lack independence. The multicore sampling grid at the Butterknife feature was sampled on three separate occasions; however, due to the nature of sampling at sea, there are a number of reasons to ignore the repeated measures aspect of this design. The multicorer was deployed from RV *Tangaroa* and lowered by cable to the seabed at depths of ~450 metres. Ocean currents affect the lateral movement of the instrument as it is lowered, and therefore make it unlikely to be positioned directly below the vessel. Based on the ship's coordinate positions while sampling the same location at multiple time points, I calculated that the ship may be up to 47 metres away from where the initial sample was taken. Given that the macrofauna core samples were 10 centimetres in diameter, and that the composition of deep-sea macrofaunal communities can vary at the centimetre scale (McClain et al. 2011), it is likely that the samples were not taken from the exact location as in previous sampling events, but rather, that core samples obtained from the same site but from different deployments are technically independent, and the repeated measures aspect is not necessary to consider for subsequent statistical analyses. However, not using a repeated measures design lowers the ability to detect changes over time, so it is more conservative.

For all community analyses, sediment depth intervals (0-5, 5-10 and 10-15 cm) were pooled for each MAF core sample. The majority of the data analyses were performed using statistical routines in PRIMER v.7 (Clarke and Gorley 2015) with the PERMANOVA add-on (Anderson et al. 2008). SCOC regressions were performed using SAS Enterprise Guide 7.1.

2.6.1 Univariate community analyses

Five univariate community variables for each core were measured: total abundance, total biomass, taxa richness, diversity, and evenness. Abundance was the number of individuals per core, biomass was wet weight of each taxon per core in milligrams, and taxa richness

was the number of taxa per core. Diversity was calculated using the Simpson diversity index (Simpson 1949) using the following equation:

$$D = 1 - (\sum n_i(n_i-1)/N(N-1))$$

where N = total number of organisms of all taxa, and n_i = total number of individuals in a given taxon. The value of D ranges between 0 and 1; as the value increases, diversity increases. For evenness, I calculated the Simpson's diversity equation without the complement (1-D), and used the following equation:

$$E_{1/D} = (1/D)/S$$

where S = the number of taxa in the sample (taxa richness). The value of $E_{1/D}$ ranges between 0 and 1; as the value increases, evenness increases.

Rather than using traditional ANOVA to analyse the univariate data, I used PERMANOVA, which is a permutation-based version of ANOVA (Anderson et al. 2008). Permutation tests are suitable for "the analysis of data which do not fulfil the assumptions of traditional statistical approaches because they have too many response variables, or because one or more response variables are not normally distributed." (Anderson et al. 2008). PERMANOVA is also suitable for unbalanced sampling designs, as was the case for my dataset (at each sampling period, undisturbed n=16, disturbed n=9). Resemblance matrices were first constructed for each community variable based on Euclidean distance of untransformed data (Anderson et al. 2008). I performed a separate PERMANOVA test, based on these matrices, for each of the five univariate community variables, where p-values were obtained by 9999 permutations of the residuals under a reduced model. Each test was formatted identically with two fixed factors; Treatment (Tr) (2 levels; undisturbed (U) and disturbed (D)), and Sampling period (Sa) (3 levels; pre-disturbance (P), immediately after disturbance (A) and one year after disturbance (O)) and a significance level of 0.05. Where main PERMANOVA tests found a significant factor effect, pairwise PERMANOVA tests were performed for these factors. Where main PERMANOVA tests found a significant interaction effect, pairwise PERMANOVA tests were performed for Treatment at each level of Sampling period, and then for Sampling period at each level of Treatment. REF samples were excluded from these analyses because they were inconsistent with the Butterknife data; however, figures including the REF data are in Appendix A for visual comparison.

2.6.2   *Multivariate community analyses*

Multivariate analyses for abundance and biomass data were based on Bray-Curtis resemblance matrices (Bray and Curtis 1957) using square root-transformed data in order to downweigh the contribution of dominant taxa to the similarities between samples. PERMANOVA tests, based on these matrices, were performed, where p-values were obtained by 9999 permutations of the residuals under a reduced model. Each test was formatted identically with two fixed factors; Treatment (Tr) (2 levels; undisturbed (U) and disturbed (D)), and Sampling period (Sa) (3 levels; pre-disturbance (P), immediately after disturbance (A) and one year after disturbance (O)) and a significance level of 0.05. Where main PERMANOVA tests found a significant factor effect, pairwise PERMANOVA tests were performed for these factors. Where main PERMANOVA tests found a significant interaction effect, pairwise PERMANOVA tests were performed for Treatment at each level of Sampling period, and then for Sampling period at each level of Treatment. Bray-Curtis resemblance matrices were used to construct non-metric multi-dimensional scaling plots (nMDS plots) in order to visualise the relative dissimilarities between sample treatment groups on a 2D plane. A centroid was derived for each sample group to be presented in a subsequent nMDS plot (one each for abundance and biomass) to more clearly represent the response of each sample group to the disturbance event. REF samples were excluded from PERMANOVA tests and nMDS plots because of their inconsistency with Butterknife samples; however, nMDS plots including REF samples are in Appendix B for visual comparison. In order to compare the dispersion of each sample group, the Bray-Curtis resemblance matrices, from which the nMDS plots were derived, were also used to calculate a relative dispersion for each sample group using the MVDISP (multivariate dispersion) routine in PRIMER.

If PERMANOVA tests identified differences in community structure between sample treatment groups, the SIMPER (Similarity percentage) routine was performed on the square root-transformed data, in order to identify the contribution of each taxon to the dissimilarities between these groups. These analyses identified taxa that characterised and best discriminated between the previously identified groups. Characterising taxa are those that contribute the most to the dissimilarity between groups (>10 % contribution), while

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discriminating taxa are those who contribute relatively consistently to those differences (dissimilarity/SD ratio >1.3) (Clarke and Warwick 2001).

I investigated potential relationships between predictor variables from the sediment environment and macrofaunal community structure at the Butterknife using distance-based linear models (DistLMs) in PRIMER (Anderson et al. 2008). DistLM is a semi-parametric, permutation-based method used to examine the relationship between a multivariate species resemblance matrix and one or more predictor variables. I refined the list of predictor variables by first checking for multicollinearity between variables (r ≥ 0.95, Anderson et al. 2008) and removing covariates. I then compared the 0-1 cm, 1-5 cm and combined weighted averages (0-5 cm) of these variables for their ability to explain the macrofaunal variation using the forward selection procedure and R^2 selection criterion. From this, I found that the weighted average variables explained 15-20 % more of the variation in macrofaunal patterns than either the 0-1 and 1-5 cm variables, so the weighted average variables were retained for the main DistLMs. Even though DistLMs do not rely on normally distributed data, % TOM and C:N molar ratio were log-transformed to lessen the influence of outliers as recommended by Anderson et al. (2008). DistLMs were conducted only where the PERMANOVA results detected significant changes in multivariate abundance and biomass in disturbed areas in order to explain what environmental variables may be driving changes in macrofaunal community structure. Four DistLMs were performed; first, for multivariate abundance, between pre-disturbance and after-disturbance sampling periods to examine the impact of disturbance; second, for multivariate abundance between after-disturbance and one year after disturbance to investigate what environmental predictor variables may be influencing the recovery from that impact; third, for multivariate biomass between pre-disturbance and one year after disturbance; and fourth, for multivariate biomass between after-disturbance and one year after disturbance. Both multivariate abundance and biomass datasets were square root-transformed to reduce the influence of dominant taxa, and Bray-Curtis resemblance matrices were constructed for each. Relationships between each environmental predictor variable and macrofaunal community structure were investigated in marginal tests, and subsequently by sequential tests, using the stepwise selection procedure and Akaike information criterion with 9999 permutations, to determine which combination of factors best explains variation in

community structure. For both marginal and sequential tests, only variables significantly correlated with macrofaunal community structure are presented.

2.6.3   SCOC analyses

Resemblance matrices for the SCOC data were constructed based on Euclidean distance of untransformed data (Anderson et al. 2008), where p-values were obtained by 9999 permutations of the raw data. A PERMANOVA test was performed with two fixed factors; Treatment (Tr) (2 levels; undisturbed (U) and disturbed (D)), and Sampling period (Sa) (3 levels; pre-disturbance (P), immediately after disturbance (A) and one year after disturbance (O)) and a significance level of 0.05. A pairwise PERMANOVA test was carried out where the main test identified a significant factor effect.

Regression analyses were also performed between SCOC and macrofaunal abundance, biomass and diversity (the community parameters that have been found in previous studies to positively correlate with SCOC; Tahey et al. (1994), Clough et al. (2005), Danovaro et al. (2008), Braeckman et al. (2010), Papaspyrou et al. (2010), Baldrighi et al. (2017), Leduc et al. (2020)). I predict that these relationships will still be linearly positive across the three sampling periods despite the expectation that the macrofaunal community parameters will decrease as a result of the SCIP disturbance; i.e., for disturbed samples, abundance, biomass and diversity are expected to decrease, as will the rate of SCOC, but at higher values of these community parameters, as expected in undisturbed samples, the rate of SCOC will be higher, so the positive linear relationship should, in theory, hold true. Based on this prediction, three simple linear regression tests were conducted between SCOC and each of macrofaunal abundance, biomass and diversity using all Butterknife SCOC core samples. A final fourth regression test was performed between SCOC and bacterial abundance to investigate their relationship, which is also predicted to be linearly positive (Pfannkuche 1993, Beaulieu 2002, Hubas et al. 2006). Regression assumptions were met for all tests. Despite pooling all samples for regression tests, scatterplots were constructed by plotting samples according to their sample groups (e.g., UAfter, undisturbed samples after disturbance) to better visualise the response of macrofaunal community parameters under the various treatments.

3. Results

3.1 Environmental characteristics of the site

The turbidity sensors (AQUAscat and Aquatec AQUAloggers) contained on the benthic landers deployed around the Butterknife recorded increased turbidity in the water column during the time of multicoring operations and the main SCIP disturbance, and confirmed that the main disturbance did create a sediment plume resulting from the resuspension of seafloor sediment (Fig. 11).
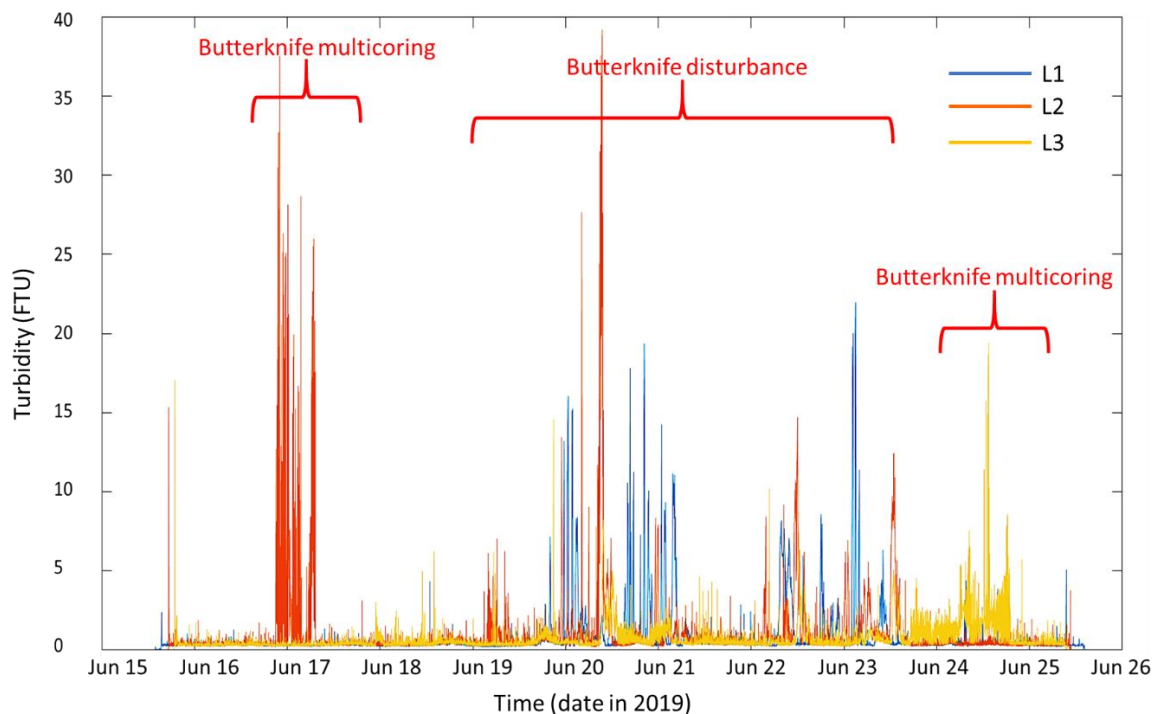
Figure 11. Turbidity measurements from three benthic landers positioned around the Butterknife. Benthic landers 1, 2 and 3 are labelled L1, L2 and L3, respectively. Lander 1 was positioned closest to the Butterknife and Lander 3 was furthest (see Fig. 9c). Red labels indicate the time at which these activities took place. FTU = Formazin Turbidity Unit.

Environmental variables derived from 75 SED core samples (25 from each sampling period and allocated to either disturbed or undisturbed treatments) from the Butterknife were analysed with each variable measured at both 0-1 and 1-5 cm depth intervals, and these variables are displayed in Figure 12 and described here. The mean grain size of sediments

ranged between 62.8 and 76.6 µm. For the 0-1 cm interval, disturbed areas had a slightly smaller mean grain size before disturbance than after (64 and 68.8 µm, respectively), while the opposite was true for undisturbed areas (65 and 62.8 µm, respectively). One year after disturbance, mean grain sizes were larger under both treatments and at both depth intervals. Sorting values ranged between 56.3 and 65.7, where increasing values equate to more poorly-sorted sediments. In general, sediments became slightly more poorly sorted over time, especially at the 1-5 cm depth interval. Porosity (measured as a fraction) ranged from 0.65 to 0.71 and was ~5 % lower at the 1-5 cm depth compared to the 0-1 cm depth. Porosity did not change at the 1-5 cm depth over time, but for the 0-1 cm depth in disturbed areas, porosity decreased from 0.71 to 0.68 after disturbance, before increasing to 0.71 one year later. The void ratio ranged from 0.29 to 0.41 and was higher at the 0-1 cm depth compared to the 1-5 cm depth. The void ratio for the 1-5 cm depth did not change much over time, but at the 0-1 cm depth for disturbed areas, this ratio decreased after disturbance and subsequently increased one year later, while the opposite pattern was observed in the undisturbed area for the same depth interval. The sediment samples were entirely composed of a mixture of mud and sand; the percentage value of one of these variables is the complement of the other, thus, they perfectly negatively correlate. Before disturbance, the sediment samples contained a roughly 60/40 split for %mud/%sand for both treatments and depth intervals. After disturbance, the mud content dropped to 56.6 % in disturbed areas at the 0-1 cm depth but remained unchanged for other areas and depths. One year after disturbance, the mud content of sediments was lower in both treatments and depths (53 % mud and 47 % sand) compared to the pre-disturbance levels, consistent with the observed increase in mean grain size. The water content of sediments (%H_2O) at the 0-1 cm depth was consistently 48 % across treatments and sampling periods except in disturbed areas after disturbance, where it dropped to 45.4 % before returning to 48 % one year later. At the 1-5 cm depth, the sediment water content was consistently 42 %, and did not change for either treatment or any sampling periods. Total organic matter (%TOM) of sediments ranged between 3.1 and 4.8 %, and before disturbance, measured ~3.5 % for both treatments and depths. After disturbance, TOM dropped to 3.1 % for the 0-1 cm depth in disturbed areas but was relatively unchanged under all other conditions. One year later, TOM increased to ~4.3 % in all but the undisturbed 1-5 cm treatment (3.6 %). Chlorophyll *a* ranged between 0.43 and 0.63 µg/g, and generally decreased over time at the 1-5 cm depth

in both disturbed and undisturbed areas. At the 0-1 cm depth, chl *a* increased steadily in undisturbed areas over the three sampling periods (from 0.49 to 0.55 to 0.56 µg/g), but in the disturbed area, chl *a* decreased after disturbance to 0.47 µg/g, before returning to its pre-disturbed level of 0.63 µg/g one year later. Phaeopigment values ranged between 3.6 and 5.6 µg/g and were higher at the 0-1 cm depth (~5 µg/g) compared to the 1-5 cm depth (~4 µg/g). For the 0-1 cm depth in disturbed areas, phaeopigment values decreased from pre- to after disturbance (0.61 to 0.47 µg/g, respectively) and then increased one year later (0.63 µg/g); the opposite pattern was observed under all other conditions. The ratio of chlorophyll *a* to phaeopigments was slightly higher in the 1-5 cm depth interval compared to the 0-1 cm interval, though it did not change noticeably under different treatments or sampling periods. Minimum and maximum values of particulate organic carbon (POC) were 0.47 and 0.68 %, respectively. At the 0-1 cm depth for the disturbed treatment, POC decreased from 0.63 % before disturbance, to 0.55 % after disturbance, and then returned one year later to 0.62 %. On the other hand, the undisturbed area at both depth intervals experienced an increase in POC after disturbance, before decreasing back to pre-disturbance levels. Particulate nitrogen (PN) values ranged from 0.043 to 0.075 %. For both disturbed and undisturbed treatments at both depth intervals, PN values decreased after disturbance, and then returned to pre-disturbance levels one year later, though this pattern was more pronounced in disturbed areas. The carbon to nitrogen molar ratio (C:N) was on average 9.99 before disturbance across both treatments and depths, but increased to 22.5 after disturbance (being slightly higher at the 1-5 cm depth interval). One year later, C:N molar ratio values returned to close to pre-disturbance levels (9.68).
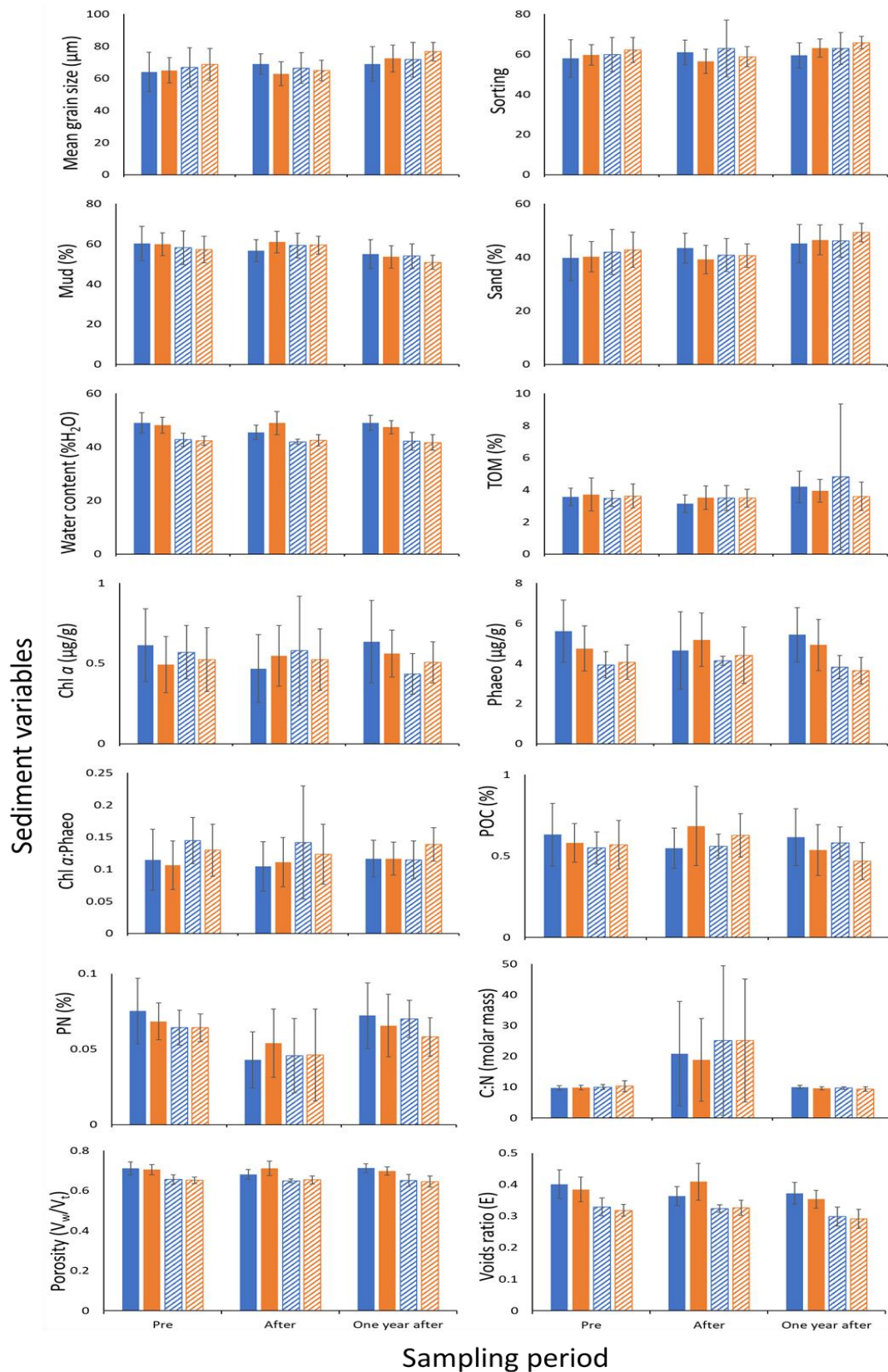
Figure 12. Means of sediment variables. Error bars are one standard deviation. Sampling periods are pre-disturbance (Pre), after disturbance (After), and one year after disturbance (One year after). Treatments are Disturbed 0-1 cm ■, Undisturbed 0-1 cm ■, Disturbed 1-5 cm ▨, and Undisturbed 1-5 cm ▨.

3.2 Biological characteristics of the site

Seventy-five macrofaunal core samples were analysed (25 from each sampling period), containing 24 different taxa. Abundances ranged from 16 to 160 individuals per core (of 71 cm^2) and biomasses from 8.98 to 504.77 mg per core. Macrofaunal abundance was dominated by polychaete worms (50-63 %), while other taxa with proportionally smaller contributions included amphipods (5-9 %), isopods (3-8 %), tanaids (5-7 %) and ostracods (3-7 %); all other taxa individually contributed <5 % to total abundance. Macrofaunal biomass was also dominated by polychaetes (50-70 %), with other major contributions from ophiuroids (1-17 %), bivalves (3-13 %) and amphipods (1-6 %); all other taxa individually contributed <5 % to total biomass. The contribution of each taxon to total abundance and biomass for each combination of factor levels is visualised in Figure 13.
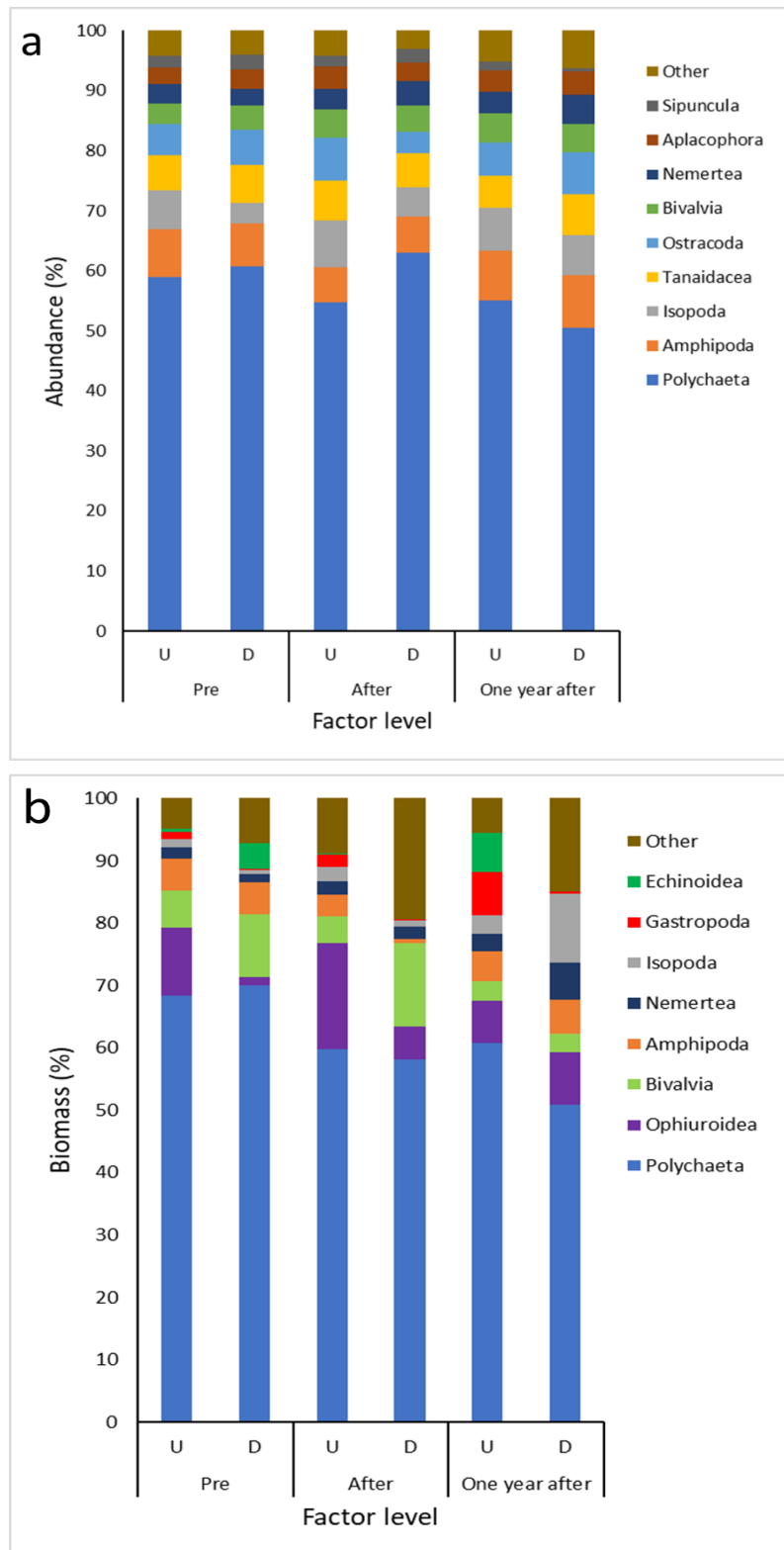
Figure 13. (a) Contribution of each taxon to total macrofaunal abundance for each combination of factor levels. Taxa that consistently contributed <2 % to the total were pooled into "Other". (b) Contribution of each taxon to total macrofaunal biomass for each combination of factor levels. Taxa that consistently contributed <3 % to the total were pooled into "Other". Factor levels are the sampling periods pre-disturbance (Pre), immediately after disturbance (After) and one year after disturbance (One year after), and treatments are undisturbed (U) and disturbed (D).

3.3 Univariate community analyses

3.3.1 Abundance

There was a significant effect of both Treatment and Sampling period on mean macrofauna abundance per core but no interaction effect (Table 4a). Abundance differed significantly between the undisturbed and disturbed treatments only immediately after the disturbance (Table 4b). Abundance did not change for the undisturbed treatment across sampling periods. For the disturbed treatment, mean abundance decreased significantly from 104.11 to 58.33 individuals per core between the pre-disturbance and immediately after disturbance sampling periods and then increased significantly to 90.44 individuals per core one year after the disturbance, at which time there was no significant difference in abundance compared to the pre-disturbance treatment (Table 4c, d, Fig. 14). Variance was relatively high in disturbed areas after disturbance (Table 4d, Fig. 14).

Table 4. For abundance, (a) main PERMANOVA test, (b) pairwise PERMANOVA test between treatments, (c) pairwise PERMANOVA test between sampling periods and (d) mean abundances (individuals per core) with standard deviation in parentheses. Factors are Treatment = Tr (with levels D = Disturbed, and U = Undisturbed) and Sampling period = Sa (with levels P = Pre-disturbance, A = Immediately after disturbance, and O = One year after disturbance). Statistically significant P values are in bold.

(a)	Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
	Tr	1	3877.2	3877.2	4.9128	**0.0321**	9813
	Sa	2	8803.7	4401.8	5.5776	**0.0055**	9929
	TrXSa	2	4469.3	2234.6	2.8315	0.066	9944
	Res	69	54455	789.2			
	Total	74	69026				

(b)	Groups	Sampling period level	t	P (perm)	Unique perms
	D, U	P	0.30749	0.7713	383
	D, U	A	2.5716	**0.0155**	498
	D, U	O	1.3865	0.1795	327

(c)	Groups	Treatment level	t	P (perm)	Unique perms
	P, A	D	2.73	**0.0168**	255
	P, O	D	1.2257	0.2456	141
	A, O	D	2.1614	**0.0474**	218
	P, A	U	0.67206	0.5018	235
	P, O	U	0.30684	0.7657	223
	A, O	U	1.0068	0.3178	232

(d)	Disturbed	Undisturbed
Pre-disturbance	104.11 (28.56)	103.19 (25.87)
After disturbance	58.33 (41.41)	94 (28)
One year after disturbance	90.44 (16.82)	103.38 (24.55)
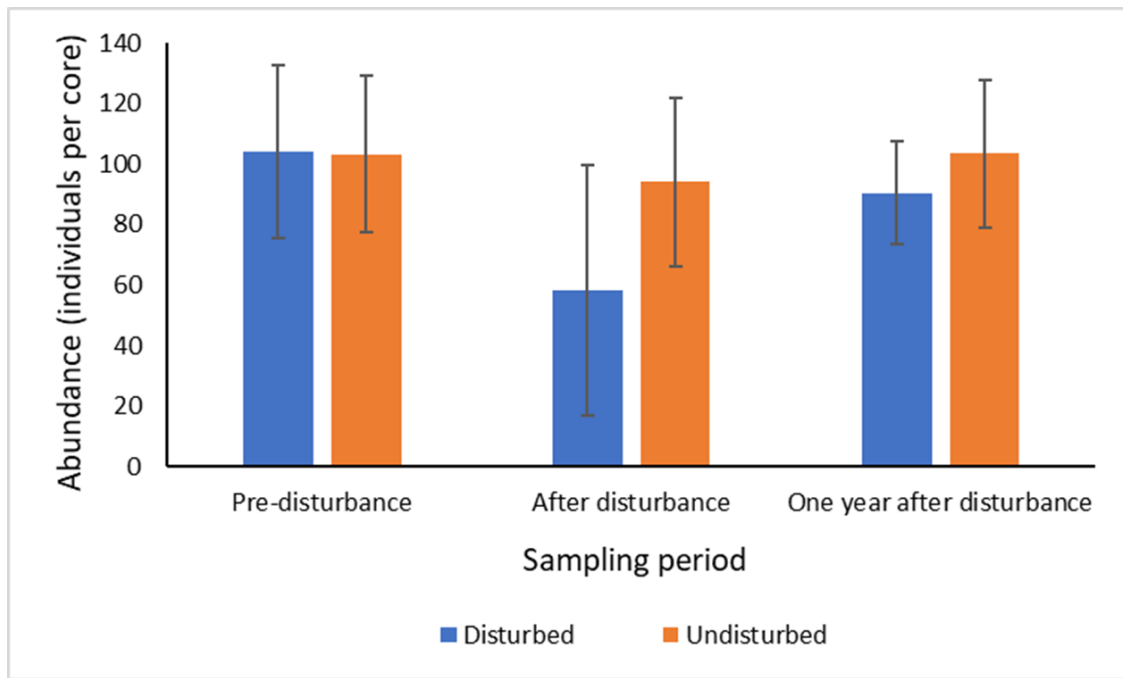
Figure 14. Mean macrofauna abundance (individuals per core of 71 cm^2) per treatment across the sampling periods. Error bars are one standard deviation.

3.3.2 Biomass

There were no significant effects of Treatment, Sampling period, or their interaction on mean macrofauna biomass per core (Table 5a, b, Fig. 15). Variance was relatively high in disturbed areas after disturbance (Table 5b, Fig. 15)

Table 5. For biomass (a) main PERMANOVA test and (b) mean biomass (mg per core) with standard deviations in parentheses. Factors are Treatment = Tr and Sampling period = Sa.

(a)	Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
	Tr	1	1857.1	1857.1	0.17274	0.684	9818
	Sa	2	16510	8254.9	0.76781	0.4668	9954
	TrXSa	2	16567	8283.7	0.77049	0.478	9957
	Res	69	7.4183E+05	10751			
	Total	74	7.7949E+05				

(b)	Disturbed	Undisturbed
Pre-disturbance	76.69 (48.74)	86.58 (75.39)
After disturbance	129.27 (169.08)	75.15 (70.35)
One year after disturbance	88.8 (95.67)	126.01 (123.93)
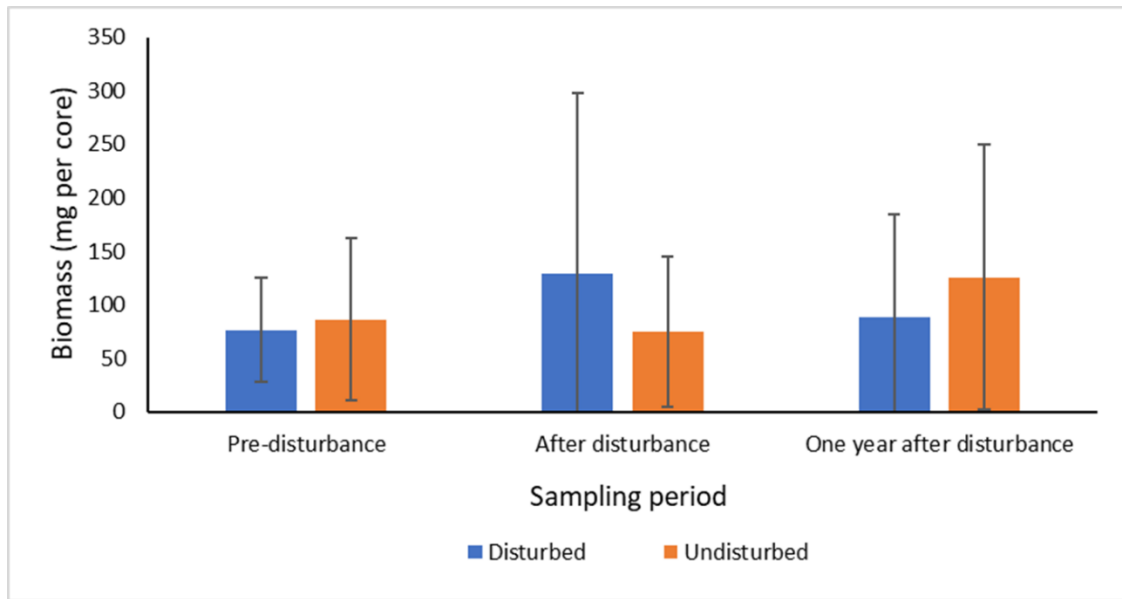
Figure 15. Mean macrofauna biomass (mg per core of 71 cm^2) per treatment across the sampling periods. Error bars are one standard deviation.

3.3.3 Taxa richness

For taxa richness, there was no effect of Treatment or interaction, but a significant effect of Sampling period (Table 6a). Taxa richness did not change for the undisturbed treatment across sampling periods. For the disturbed treatment, mean taxa richness decreased from 11.22 to 8.78 taxa $^{-core}$ between pre- and immediately after the disturbance, however, this decrease was not statistically significant. Mean taxa richness increased significantly from immediately after (8.78 taxa $^{-core}$) to one year after disturbance (11.67 taxa $^{-core}$) (Table 6b). There was no difference in taxa richness between the pre- and one year after disturbance treatments (Table 6b, c, Fig. 16). Variance was relatively high in disturbed areas after disturbance (Table 6c, Fig. 16).

Table 6. For taxa richness, (a) main PERMANOVA test, (b) pairwise PERMANOVA test between sampling periods and (c) mean taxa richness (taxa per core) with standard deviations in parentheses. Factors are Treatment = Tr (with levels D = Disturbed, and U = Undisturbed) and Sampling period = Sa (with levels P = Pre-disturbance, A = Immediately after disturbance, and O = One year after disturbance). Statistically significant P values are in bold.

(a)	Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
	Tr	1	8.3333	8.3333	2.4706	0.1205	9781
	Sa	2	43.251	21.625	6.4113	**0.0025**	9949
	TrXSa	2	19.251	9.6253	2.8536	0.0635	9944
	Res	69	232.74	3.373			
	Total	74	294				

(b)	Groups	Treatment level	t	P (perm)	Unique perms
	P, A	D	1.9503	0.0882	19
	P, O	D	0.81228	0.5511	9
	A, O	D	2.4089	**0.0381**	18
	P, A	U	Negative	-	-
	P, O	U	1.6585	0.1357	15
	A, O	U	1.8959	0.09	14

(c)	Disturbed	Undisturbed
Pre-disturbance	11.22 (1.39)	10.94 (1.91)
After disturbance	8.78 (3.49)	10.94 (1.57)
One year after disturbance	11.67 (0.87)	11.88 (1.2)
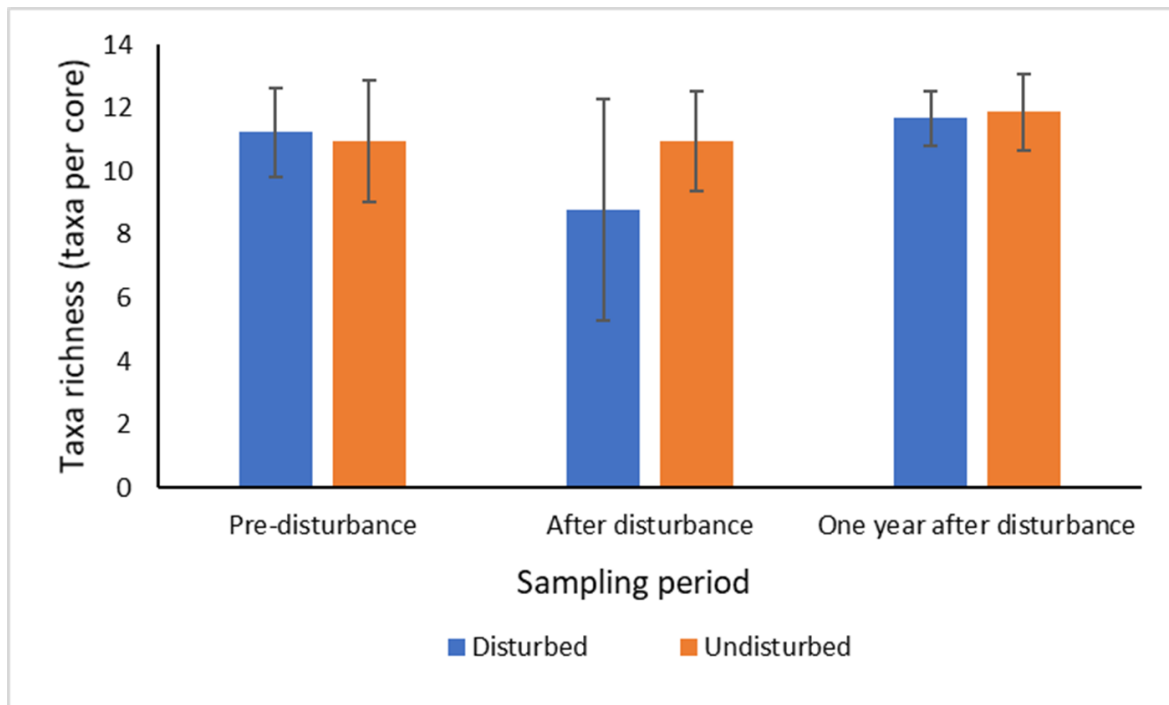
Figure 16. Mean macrofaunal taxa richness (taxa per core of 71 cm^2) per treatment across the sampling periods. Error bars are one standard deviation.

3.3.4 Diversity

For diversity there was an effect for Sampling period, but not for Treatment, and there was a significant interaction between Treatment and Sampling period (Table 7a). Diversity differed between the undisturbed and disturbed treatments only immediately after the disturbance (Table 7b). Diversity did not change for the undisturbed treatment across sampling periods. For the disturbed treatment, diversity did not change significantly between pre- and immediately after disturbance, but increased significantly from 0.5819 to 0.7057 between immediately and one year after the disturbance. Diversity between pre- (0.6056) and one year after the disturbance (0.7057), also differed significantly from one another (Table 7c, d, Fig. 17).

Table 7. For diversity, (a) main PERMANOVA test, (b) pairwise PERMANOVA test between treatments, (c) pairwise PERMANOVA test between sampling periods and (d) mean diversity with standard deviations in parentheses. Factors are Treatment = Tr (with levels D = Disturbed, and U = Undisturbed) and Sampling period = Sa (with levels P = Pre-disturbance, A = Immediately after disturbance, and O = One year after disturbance). Statistically significant P values are in bold.

(a)	Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
	Tr	1	0.010518	0.010518	1.5971	0.209	9844
	Sa	2	0.067644	0.033822	5.136	**0.0082**	9960
	TrXSa	2	0.048247	0.024124	3.6633	**0.0308**	9953
	Res	69	0.45439	0.0065853			
	Total	74	0.5654				

(b)	Groups	Sampling period level	t		P (perm)	Unique perms
	D, U	P	0.53355		0.5963	5667
	D, U	A	3.0821		**0.0055**	5609
	D, U	O	1.0769		0.2971	5579

(c)	Groups	Treatment level	t		P (perm)	Unique perms
	P, A	D	0.52345		0.6095	3485
	P, O	D	2.1422		**0.0479**	3905
	A, O	D	2.5103		**0.0302**	3978
	P, A	U	1.9551		0.0603	3824
	P, O	U	1.5829		0.1246	3987
	A, O	U	0.25941		0.7946	3194

(d)	Disturbed	Undisturbed
Pre-disturbance	0.6056 (0.0898)	0.6251 (0.0869)
After disturbance	0.5819 (0.1013)	0.6737 (0.0485)
One year after disturbance	0.7057 (0.1077)	0.6684 (0.0665)
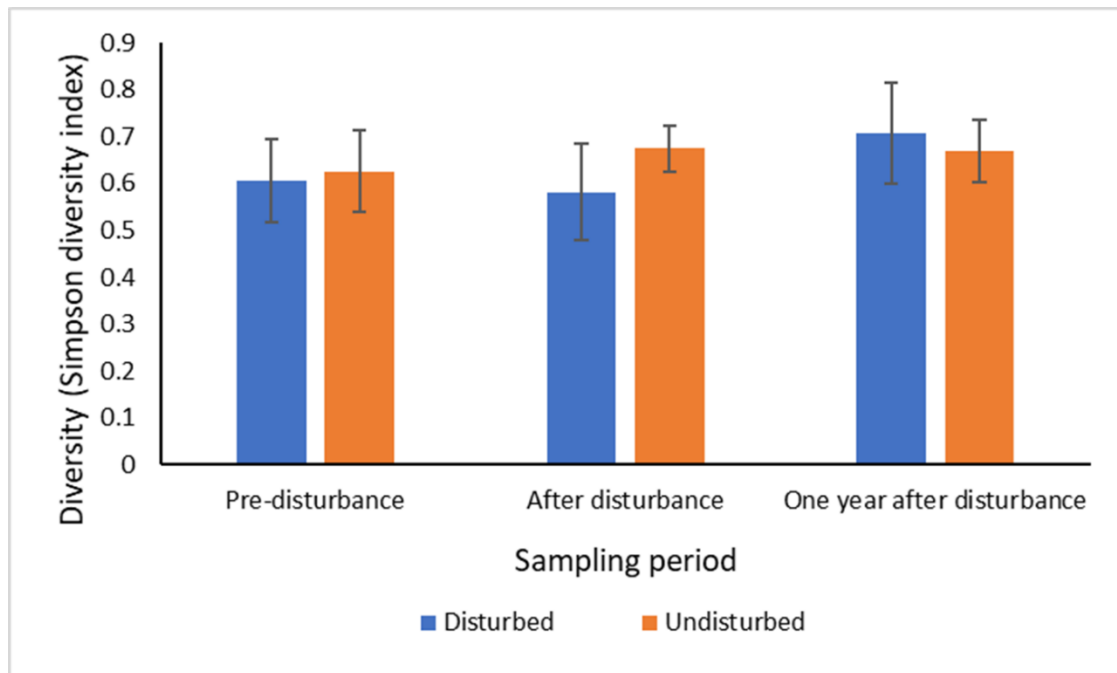
Figure 17. Mean macrofaunal diversity per treatment across the sampling periods. Error bars are one standard deviation.

3.3.5 Evenness

For evenness, there was no effect of Treatment or interaction, but a significant effect of Sampling period (Table 8a). Evenness did not change for the undisturbed treatment across sampling periods. For the disturbed treatment, evenness increased significantly between pre- (mean = 0.2377) and immediately (mean = 0.3209) and one year after (mean = 0.3129) the disturbance. There was no significant difference in mean evenness between the latter two sampling periods (Table 8b, c, Fig. 18).

Table 8. For evenness, (a) main PERMANOVA test, (b) pairwise PERMANOVA test between sampling periods and (c) mean species evenness with standard deviations in parentheses. Factors are Treatment = Tr (with levels D = Disturbed, and U = Undisturbed) and Sampling period = Sa (with levels P = Pre-disturbance, A = Immediately after disturbance, and O = One year after disturbance). Statistically significant P values are in bold.

(a)	Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
	Tr	1	0.0094813	0.0094813	2.3243	0.1273	9833
	Sa	2	0.042259	0.02113	5.1799	**0.0079**	9940
	TrXSa	2	0.018157	0.0090784	2.2256	0.1165	9955
	Res	69	0.28146	0.0040792			
	Total	74	0.34214				

(b)	Groups	Treatment level	t	P (perm)	Unique perms
	P, A	D	2.0826	**0.0482**	3448
	P, O	D	2.3372	**0.0299**	3249
	A, O	D	0.021509	0.9821	3672
	P, A	U	1.9837	0.0591	2825
	P, O	U	0.38362	0.6995	2676
	A, O	U	1.6556	0.1065	2685

(c)	Disturbed	Undisturbed
Pre-disturbance	0.2377 (0.0468)	0.2574 (0.0467)
After disturbance	0.3209 (0.1103)	0.2892 (0.0440)
One year after disturbance	0.3219 (0.0975)	0.2635 (0.0438)
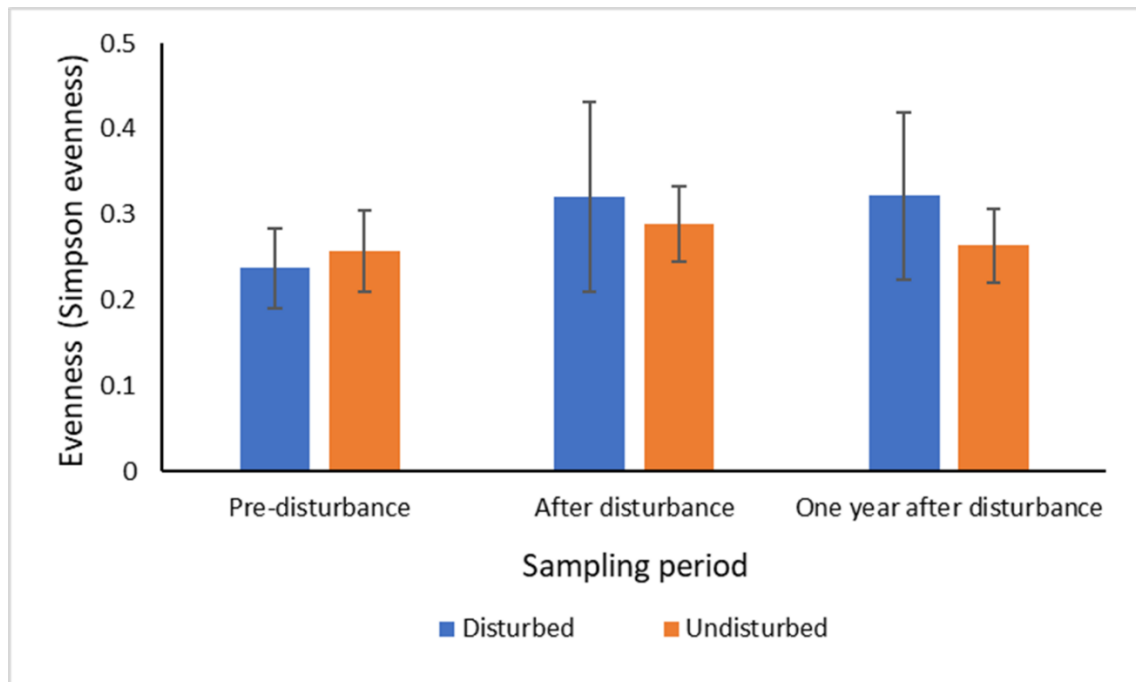
Figure 18. Mean macrofaunal evenness per treatment across the sampling periods. Error bars are one standard deviation.

3.4 Multivariate community analyses

3.4.1 Abundance-based community structure

For abundance-based community structure, there was a significant effect of Treatment and Sampling period, however, there was a significant interaction between these factors (Table 9a). The only significant difference between undisturbed and disturbed sample groups occurred immediately after the disturbance (Table 9b). For the undisturbed treatment, community structure was significantly different only between pre- and immediately after the disturbance (Table 9c). For the disturbed treatment, there were significant differences in community structure between the same sampling periods, and also between immediately and one year after disturbance (Table 9c). The nMDS ordination plot shows that immediately after disturbance, the disturbed samples are widely dispersed compared to the tightly clustered pre- and one year after disturbance samples, and that the immediately after disturbance sample cluster shows little overlap with clusters of pre- and one year after disturbance samples which closely overlap one another, indicating a recovery of the macrofaunal community that is consistent with the PERMANOVA results (Fig. 19a). Immediately after disturbance, the undisturbed samples are also dispersed more widely

(albeit to a lesser extent than for disturbed samples), and overlap less compared to pre- and one year after samples, which cluster tightly and overlap one another, indicating some level of impact from disturbance, and a subsequent recovery from that impact in undisturbed areas. An nMDS ordination plot showing sample groups averaged (centroids) demonstrates the impact and recovery of the macrofaunal community in both disturbed and undisturbed areas (Fig. 19b). A measure of relative dispersion for each sample group is also consistent with the nMDS plot in Figure 19a, and can be found in Table 10.

Table 9. For abundance-based community structure, (a) main PERMANOVA test, (b) pairwise PERMANOVA test between treatments, and (c) pairwise PERMANOVA test between sampling periods. Factors are Treatment = Tr (with levels D = Disturbed, and U = Undisturbed) and Sampling period = Sa (with levels P = Pre-disturbance, A = Immediately after disturbance, and O = One year after disturbance). Statistically significant P values are in bold.

(a)	Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
	Tr	1	957.31	957.31	2.5002	**0.0152**	9929
	Sa	2	2880	1440	3.7608	**0.0001**	9931
	TrXSa	2	1837.2	918.62	2.3991	**0.0042**	9925
	Res	69	26420	382.9			
	Total	74	31460				

(b)	Groups	Sampling period level	t	P (perm)	Unique perms
	D, U	P	0.95233	0.5284	9908
	D, U	A	2.1314	**0.0023**	9924
	D, U	O	0.58827	0.9421	9928

(c)	Groups	Treatment level	t	P (perm)	Unique perms
	P, A	D	1.8108	**0.0118**	8117
	P, O	D	1.2686	0.1035	8167
	A, O	D	1.8382	**0.0097**	8159
	P, A	U	1.4572	**0.0259**	9939
	P, O	U	0.79958	0.7744	9937
	A, O	U	1.3072	0.0842	9940
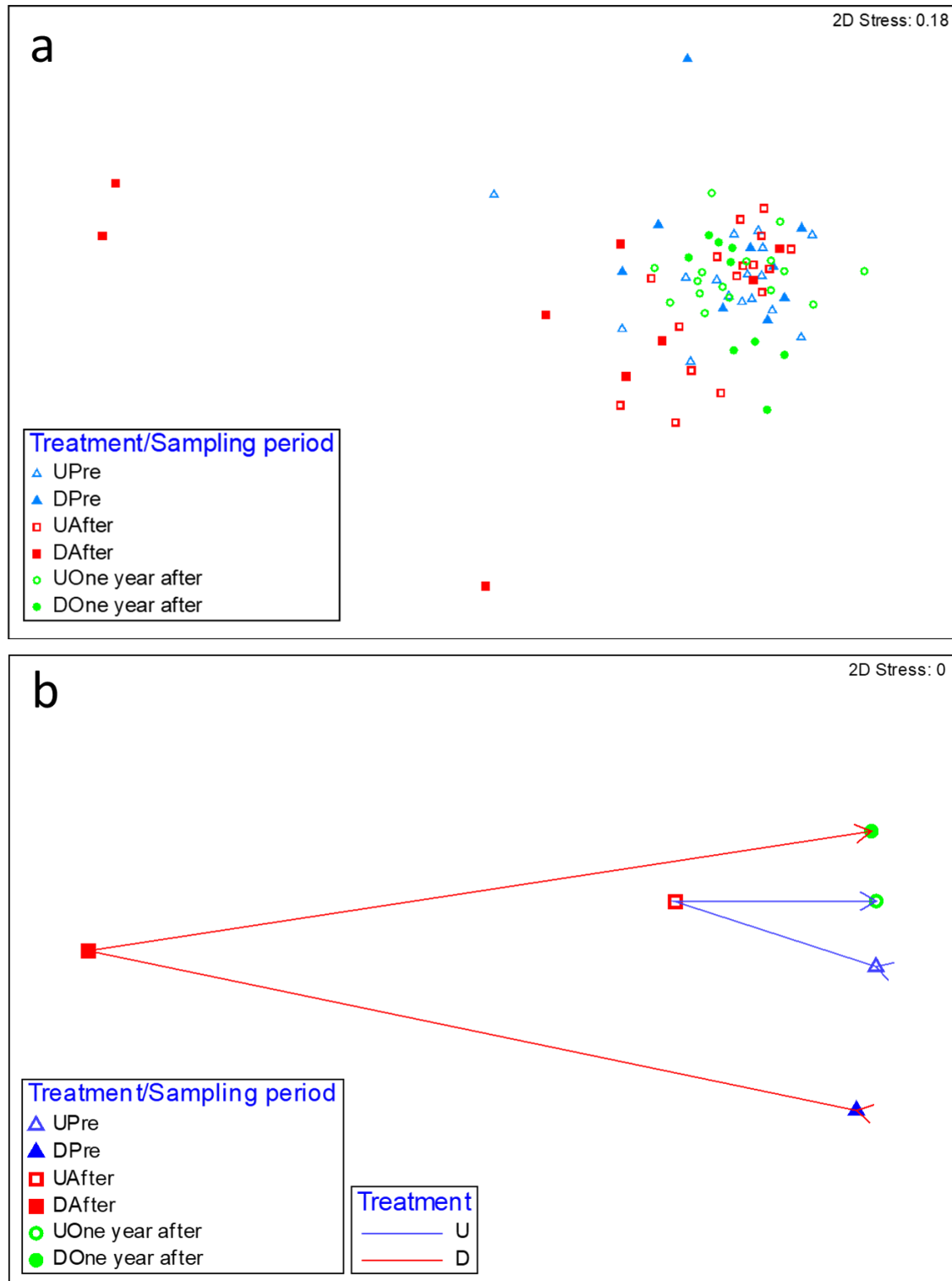
Figure 19. (a) nMDS ordination plot for abundance-based community structure. (b) nMDS ordination plot showing centroids of sample groups averaged from Fig. 19a. Symbols represent treatments (U = undisturbed and D = disturbed) and sampling periods (Pre = Pre-, After = immediately after, One year after = one year after disturbance). A 2D stress value of <0.2 is considered an adequate representation of the data (Clarke 1993).

Table 10. Relative dispersion of sample groups for abundance-based community structure. Higher values indicate sample group is more dispersed. Sampling periods are pre- (P), after (A), and one year after (O) disturbance.

Treatment	Sampling period	Dispersion
Disturbed	P	0.866
	A	1.423
	O	0.712
Undisturbed	P	1.007
	A	1.072
	O	0.921

SIMPER analyses were performed only on the sample groups that were found to be significantly different by PERMANOVA tests. Taxa contributing <5 % to the dissimilarity between groups were not presented in the SIMPER tables; tables displaying the full suite of taxa are available in Appendix C. Abundance-based community structure between undisturbed and disturbed groups immediately after disturbance had an average dissimilarity of 35.93 %, and five taxa were responsible for more than 50 % of this dissimilarity (Table 11). Both polychaetes (14.23 %) and ostracods (10.65 %) best characterised the dissimilarity between these groups, with both these taxa being more abundant in the undisturbed samples. Polychaetes and ostracods were also good discriminating taxa (Diss/SD: 1.34 and 1.51, respectively) between these sample groups. Bivalves and amphipods were also good discriminating taxa (Diss/SD: 1.43 and 1.35, respectively), and shared similar patterns regarding their differences in abundance between groups and their contribution to the average dissimilarity. After average abundance values were back-transformed from square root values (i.e., X^2), ostracods exhibited the most notable difference in abundance between undisturbed and disturbed sample groups (6.2 compared to 1.4 mean individuals per core, respectively).

Table 11. Results of SIMPER analysis for dissimilarity in abundance-based community structure between undisturbed (U) and disturbed (D) samples for the sampling period immediately after the disturbance (average dissimilarity = 35.93 %), showing taxa making an individual contribution of >5 %. Average abundances are square root-transformed. Bold values indicate characterising and discriminating taxa. Av.Abund = average abundance; Av.Diss = average dissimilarity; Diss/SD = dissimilarity/standard deviation ratio; Contrib = contribution; Cum = cumulative.

Taxon	Group U Av.Abund	Group D Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	7.08	5.64	5.14	**1.34**	**14.32**	14.32
Ostracoda	2.49	1.18	3.83	**1.51**	**10.65**	24.97
Isopoda	2.55	1.58	3.38	1.1	9.41	34.37
Tanaidacea	2.45	1.7	3.32	1.24	9.23	43.6
Bivalvia	1.94	1.36	2.82	**1.43**	7.84	51.44
Amphipoda	2.16	1.57	2.69	**1.35**	7.48	58.92
Nemertea	1.62	1.22	2.34	1.19	6.5	65.43
Sipuncula	0.92	0.67	2.23	1.13	6.22	71.65
Aplacophora	1.76	1.12	2.2	1.05	6.12	77.77
Ophiuroidea	0.87	0.46	1.91	1.25	5.32	83.09

SIMPER analysis revealed that the average dissimilarity in abundance-based community structure observed between undisturbed samples pre- and immediately after the disturbance was relatively low (25.12 %), and six taxa were responsible for just under 50 % of this dissimilarity (Table 12). The highest contributor to this dissimilarity were polychaetes (10.24 %) which were marginally more abundant pre- compared to immediately after the disturbance. Other taxa that had a similar pattern of relative abundance were sipunculids, amphipods, cumaceans, and scaphopods. Average abundance was higher immediately after compared to pre-disturbance for the remaining taxa that contributed >5 % to the average dissimilarity, however, the differences were not high. Cumaceans and ostracods were the best discriminators (Diss/SD: 1.41 and 1.32, respectively) between the sample groups, but exhibited different patterns for back-transformed average abundance; cumaceans decreased from 1 to 0.06 individuals per core, whereas ostracods increased slightly from 5 to 6.2 individuals per core from before and after the disturbance.

Table 12. Results of SIMPER analysis for dissimilarity in abundance-based community structure between pre-disturbance (Pre) and immediately after disturbance (After) samples for the undisturbed treatment (average dissimilarity = 25.12 %), showing taxa making an individual contribution of >5 %. Average abundances are square root-transformed. Bold values indicate characterising and discriminating taxa. Av.Abund = average abundance; Av.Diss = average dissimilarity; Diss/SD = dissimilarity/standard deviation ratio; Contrib = contribution; Cum = cumulative.

Taxon	Group Pre Av.Abund	Group After Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	7.71	7.08	2.57	1.29	**10.24**	10.24
Bivalvia	1.69	1.94	2.23	1.26	8.89	19.13
Sipuncula	1.14	0.92	1.97	1.26	7.86	26.99
Tanaidacea	2.33	2.45	1.9	1.19	7.55	34.54
Amphipoda	2.66	2.16	1.88	1.16	7.5	42.05
Nemertea	1.61	1.62	1.8	1.25	7.17	49.22
Cumacea	1	0.24	1.74	**1.41**	6.94	56.16
Isopoda	2.51	2.55	1.72	0.98	6.84	63
Ostracoda	2.23	2.49	1.57	**1.32**	6.25	69.25
Aplacophora	1.54	1.76	1.54	1.24	6.13	75.38
Scaphopoda	0.86	0.51	1.41	1.18	5.61	81
Ophiuroidea	0.68	0.87	1.29	1.17	5.13	86.12
Actiniaria	0.28	0.73	1.29	1.17	5.12	91.24

The average dissimilarity in abundance-based community structure between disturbed samples pre- and immediately after the disturbance was 37.66 %, and five taxa were responsible for more than 50 % of this value (Table 13a). Polychaetes were the best characterising taxon (contributing 16.56 % to the overall dissimilarity), and were less abundant immediately after the disturbance than before it. The average abundance of all the rest of the characterising taxa also decreased immediately after the disturbance. After back-transforming average abundance data, the most notable decreases between the two sampling periods were observed in polychaetes (from 61.2 to 31.8 individuals per core) and ostracods (from 5.8 to 1.4 individuals per core); both were discriminating taxa (Diss/SD = 1.41 and 1.49, respectively). Back-transformed abundance data for the other taxa revealed only a slight decrease between sampling periods, including for the other discriminating taxa (sipunculids, tanaids, and amphipods).

The SIMPER analysis indicated that the average dissimilarity in abundance-based community structure observed in disturbed samples between immediately after and one year after

disturbance was 36.76 %, with the highest contributors to the dissimilarity being polychaetes and ostracods (13.14 % and 10.32 %, respectively) (Table 13b). The five highest-contributing taxa to the overall dissimilarity between these two sampling periods were the same for the dissimilarity observed pre- and immediately after the disturbance (Table 13a), and were also the most abundant taxa at the Butterknife prior to disturbance. The average abundance of all taxa presented in Table 13b increased from immediately after to one year after the disturbance. Between these sampling periods, the most notable increases in back-transformed average abundance were observed for ostracods (from 1.4 to 6.2 individuals per core), amphipods (from 2.5 to 7.6 individuals per core) and cumaceans (from 0.1 to 0.8 individuals per core). The former two taxa were identified as discriminating taxa (Diss/SD values = 1.49 and 1.44, respectively).

One year after disturbance, the average abundance values of the five highest-contributing taxa (from Tables 13a, b) were similar to the pre-disturbance values in disturbed areas (SIMPER results in Table 3c in Appendix C), which would have contributed to the non-significant result of the PERMANOVA test between pre- and one year after. These five taxa, and their back-transformed average abundance values pre- and one year after disturbance, include polychaetes (from 61.2 to 45.4 individuals per core), amphipods (from 6.9 to 7.6 individuals per core), isopods (from 2.8 to 5.5 individuals per core), tanaids (from 6.3 to 5.2 individuals per core), and ostracods (from 5.8 to 6.2 individuals per core).

Table 13. Results of SIMPER analysis for dissimilarity in abundance-based community structure between (a) pre-disturbance (Pre) and immediately after disturbance (After) for the disturbed treatment (average dissimilarity = 37.66 %), and (b) immediately after (After) and one year after disturbance (One year after) for the disturbed treatment (average dissimilarity = 36.76 %), showing taxa making an individual contribution of >5 %. Average abundances are square root-transformed. Bold values indicate characterising and discriminating taxa. Av.Abund = average abundance; Av.Diss = average dissimilarity; Diss/SD = dissimilarity/standard deviation ratio; Contrib = contribution; Cum = cumulative.

(a)	Taxon	Group Pre Av.Abund	Group After Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
	Polychaeta	7.82	5.64	6.24	**1.41**	**16.56**	16.56
	Ostracoda	2.41	1.18	3.59	**1.49**	9.53	26.09
	Tanaidacea	2.51	1.7	3.44	**1.36**	9.13	35.22
	Amphipoda	2.62	1.57	3.09	**1.36**	8.2	43.42
	Isopoda	1.66	1.58	2.83	1.23	7.51	50.93
	Sipuncula	1.5	0.67	2.78	**1.48**	7.38	58.3
	Aplacophora	1.61	1.12	2.68	1.29	7.13	65.43
	Nemertea	1.44	1.22	2.4	1.24	6.38	71.81
	Bivalvia	1.97	1.36	2.05	1.11	5.43	77.24

(b)	Taxon	Group After Av.Abund	Group One year after Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
	Polychaeta	5.64	6.74	4.83	1.28	**13.14**	13.14
	Ostracoda	1.18	2.49	3.79	**1.49**	**10.32**	23.46
	Amphipoda	1.57	2.75	3.22	**1.44**	8.75	32.21
	Tanaidacea	1.7	2.27	3.12	1.21	8.49	40.70
	Isopoda	1.58	2.35	2.94	1.18	7.92	48.62
	Nemertea	1.22	2.04	2.51	1.24	6.84	55.46
	Aplacophora	1.12	1.58	2.28	1.12	6.2	61.66
	Bivalvia	1.36	2	2.08	1.1	5.65	67.32
	Ophiuroidea	0.46	0.83	1.84	1.19	5.01	72.32
	Cumacea	0.33	0.9	1.84	1.1	5	77.32

The DistLM analysis showed that, for pre- and after disturbance sampling periods, abundance-based community structure was significantly correlated with % PN and log C:N molar ratio in marginal tests, but only the latter in sequential tests which explained 24 % of the variation in community structure (Table 14). For after- and one year after sampling periods, community structure was significantly correlated with % coarse silt, % medium silt, % very fine sand, depth, % PN and log C:N molar ratio in marginal tests. In sequential tests,

only log C:N molar ratio and depth were retained, together explaining 36 % of the variation in community structure (Table 14).

Table 14. Results of DistLM analyses between sediment predictor variables and abundance-based macrofaunal community structure in disturbed sites at the Butterknife. Only variables with p <0.05 are shown. Sampling periods are pre-disturbance (Pre), immediately after disturbance (After), and one year after disturbance (One year after disturbance). Prop. = proportion of variation explained by variable, Cumul. = cumulative proportion, res.df = residual degrees of freedom.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
Pre, After						
Marginal tests						
% PN	1913.3	2.8110	0.032	0.1578		15
log C:N Molar Ratio	2881.5	4.6771	0.010	0.2377		15
Sequential tests						
log C:N Molar Ratio	2881.5	4.6771	0.012	0.2377	0.2377	15
After, One year after						
Marginal tests						
% Coarse Silt	1889.6	3.0282	0.022	0.1591		16
% Medium Silt	1670.4	2.6195	0.039	0.1407		16
% Very Fine Sand	2120.7	3.4790	0.021	0.1786		16
Depth (m)	1992.6	3.2265	0.025	0.1678		16
% PN	2563.1	4.4045	0.007	0.2159		16
log C:N Molar Ratio	3020.0	5.4575	0.008	0.2543		16
Sequential tests						
log C:N Molar Ratio	3020.0	5.4575	0.010	0.2543	0.2543	16
Depth (m)	1228.2	2.4159	0.042	0.1034	0.3578	15

3.4.2 Biomass-based community structure

For biomass-based community structure, there was a significant effect of Sampling period, but no Treatment effect (Table 15a). The community structure of undisturbed samples did not differ across sampling periods. For the disturbed treatment, there were significant differences in community structure between pre- and one year after the disturbance, and between immediately after and one year after disturbance (Table 15b). The nMDS ordination plot shows that undisturbed sample clusters from each sampling period overlap

one another, but the after- and one year after disturbance groups are slightly more dispersed than the pre-disturbance sample group (Fig. 20a). For the disturbed samples, the pre- and after disturbance sample groups overlap one another, with the latter being more widely dispersed, and the one year after samples cluster quite tightly and mostly overlap the other disturbed sample clusters. It is difficult to find consistency between the nMDS plot and the PERMANOVA results, likely because at least one p-value is close to the 0.05 significance level, and because the 2D stress value of the nMDS plot is right on the cut-off (<0.2) of what is considered to be a fair representation of the data. However, a simplified nMDS ordination plot showing sample groups averaged (centroids) is given in Figure 20b, and a measure of relative dispersion for each sample group to complement the nMDS plot in Figure 20a, can be found in Table 16.

Table 15. For biomass-based community structure, (a) main PERMANOVA test and (b) pairwise PERMANOVA test between sampling periods. Factors are Treatment = Tr (with levels D = Disturbed, and U = Undisturbed) and Sampling period = Sa (with levels P = Pre-disturbance, A = Immediately after disturbance, and O = One year after disturbance). Statistically significant P values are in bold.

(a)	Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
	Tr	1	1027.9	1027.9	0.92468	0.5094	9935
	Sa	2	4645.9	2323	2.0898	**0.0046**	9906
	TrXSa	2	3007.3	1503.7	1.3527	0.1402	9912
	Res	69	76698	1111.6			
	Total	74	84872				

(b)	Groups	Treatment level	t	P (perm)	Unique perms
	P, A	D	1.1894	0.173	8125
	P, O	D	1.3393	**0.0462**	8224
	A, O	D	1.4215	**0.0305**	8156
	P, A	U	1.0926	0.2977	9928
	P, O	U	1.0299	0.3813	9922
	A, O	U	1.2783	0.0925	9925
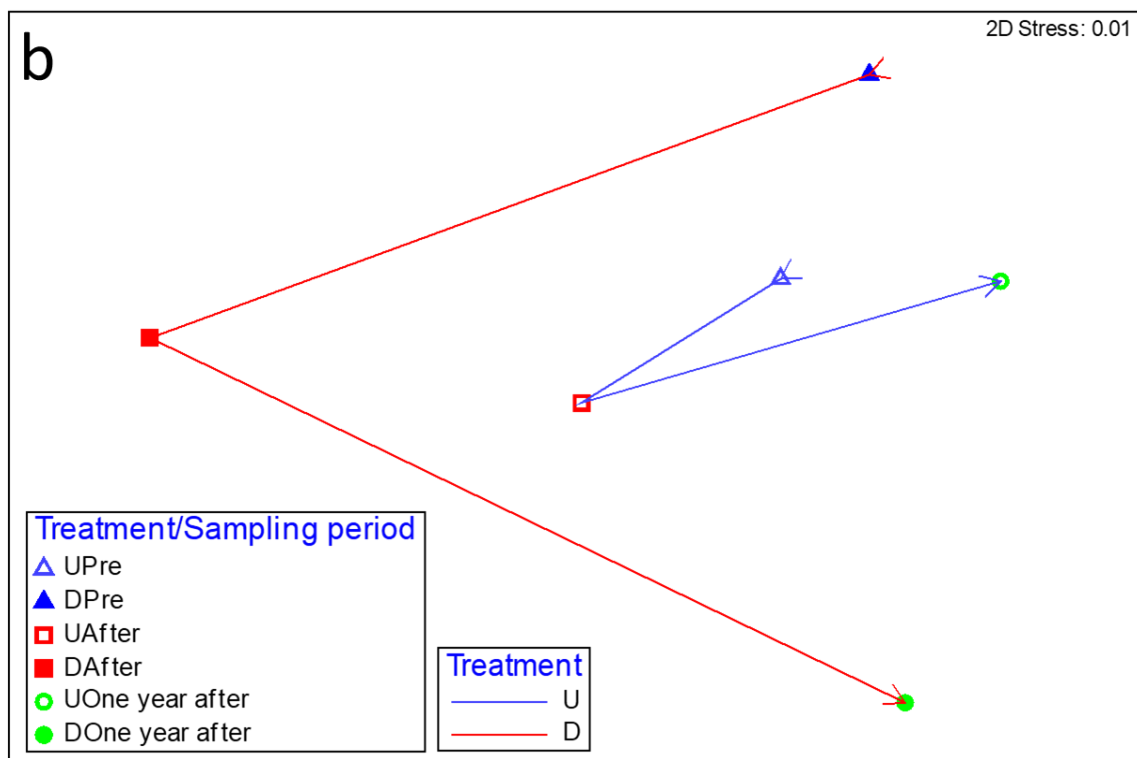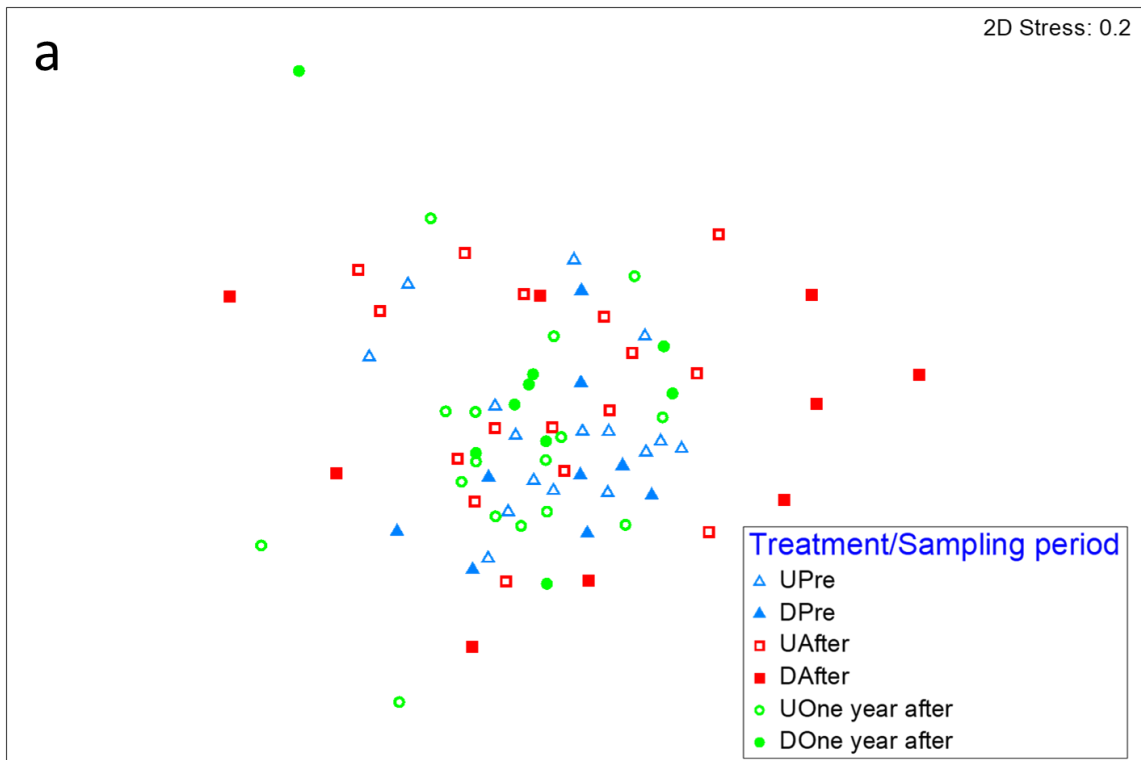
Figure 20. (a) nMDS ordination plot for biomass-based community structure. (b) nMDS ordination plot showing centroids of sample groups averaged from Fig. 20a. Symbols represent treatments (U = undisturbed and D = disturbed) and sampling periods (Pre = Pre-, After = immediately after, One year after = one year after disturbance). A 2D stress value of <0.2 is considered an adequate representation of the data (Clarke 1993).

Table 16. Relative dispersion of sample groups for biomass-based community structure. Higher values indicate sample group is more dispersed. Sampling periods are pre- (P), after (A), and one year after (O) disturbance.

Treatment	Sampling period	Dispersion
Disturbed	P	0.695
	A	1.446
	O	0.858
Undisturbed	P	0.832
	A	1.119
	O	1.049

SIMPER analyses were performed only on the sample groups that were found to be significantly different following PERMANOVA tests. The average dissimilarity of the biomass-based community structure between disturbed samples pre- and one year after the disturbance was 43.63 %, and five taxa contributed over 54 % to this dissimilarity (Table 17a). The highest individual contributors to this dissimilarity were polychaetes (15.64 %) and ophiuroids (12.89 %). The differences in average biomass between these two sampling periods was inconsistent for each taxon contributing >5 % of the dissimilarity; some taxa increased, others decreased, and some did not change. Back-transformed average biomass data shows that polychaete biomass was lower one year after the disturbance than before it (32.4 mg compared to 48.4 mg per core), whereas average biomass for ophiuroids was higher one year after than before (6.4 mg compared to 0.4 mg per core). Nemertean average biomass showed the same pattern as ophiuroid biomass (2.5 mg compared to 0.4 mg per core) and nemerteans were a good discriminating taxon (Diss/SD: 1.42), along with polychaetes (Diss/SD: 1.47) for the observed difference in biomass-based community structure between these two sampling periods.

The SIMPER analysis revealed that the average dissimilarity in biomass-based community structure for disturbed samples between immediately after- and one year after the disturbance was 56.13 %, and four taxa contributed to over 51 % of this value (Table 17b). The taxa best characterising this dissimilarity were polychaetes (contributing 18.33 % to the overall dissimilarity), ophiuroids (12.87 %), and actiniarians (12.46 %). As for the dissimilarity observed in community structure between pre- and one year after the disturbance (Table 17a), each taxon contributing >5 % of the dissimilarity displayed different patterns regarding

average biomass between immediately after and one year after the disturbance. Back-transformed average biomass data showed that biomass was notably higher one year after disturbance than immediately after for ophiuroids (6.4 mg compared to 1.6 mg per core) and amphipods (3.6 mg compared to 0.4 mg per core). Some taxa were only present immediately after (actiniarians) and one year after (ascidians) the disturbance. Amphipods (Diss/SD: 1.55) and nemerteans (Diss/SD: 1.3) were the two taxa identified as good discriminators of the differences in community structure between these two sampling periods.

Table 17. Results of SIMPER analysis for dissimilarity in biomass-based community structure between (a) pre-disturbance (Pre) and one year after disturbance (One year after) for the disturbed treatment (average dissimilarity = 43.63 %) and (b) immediately after (After) and one year after disturbance (One year after) for the disturbed treatment (average dissimilarity = 56.13 %), showing taxa making an individual contribution of >5 %. Average biomass is square root-transformed. Bold values indicate characterising and discriminating taxa. Av.Biom = average biomass; Av.Diss = average dissimilarity; Diss/SD = dissimilarity/standard deviation ratio; Contrib = contribution; Cum = cumulative.

(a)	Taxon	Group Pre Av.Biom	Group One year after Av.Biom	Av.Diss	Diss/SD	Contrib%	Cum.%
	Polychaeta	0.22	0.18	6.83	**1.47**	**15.64**	15.64
	Ophiuroidea	0.02	0.08	5.63	1.05	**12.89**	28.54
	Nemertea	0.02	0.05	3.81	**1.42**	8.72	37.26
	Amphipoda	0.06	0.06	3.68	1.15	8.43	45.69
	Bivalvia	0.06	0.03	3.64	0.88	8.34	54.03
	Ascidiacea	0	0.05	3.16	0.35	7.24	61.27

(b)	Taxon	Group After Av.Biom	Group One year after Av.Biom	Av.Diss	Diss/SD	Contrib%	Cum.%
	Polychaeta	0.19	0.18	10.29	1.25	**18.33**	18.33
	Ophiuroidea	0.04	0.08	7.22	1.14	**12.87**	31.2
	Actiniaria	0.12	0	7	0.56	**12.46**	43.66
	Amphipoda	0.02	0.06	4.55	**1.55**	8.11	51.77
	Nemertea	0.02	0.05	4.22	**1.3**	7.52	59.29
	Bivalvia	0.04	0.03	4.14	1	7.37	66.66
	Ascidiacea	0	0.05	3.34	0.34	5.96	72.62

The DistLM analysis for after- and one year after disturbance sampling periods showed significant correlations between biomass-based community structure and % coarse silt, % medium silt and % very fine sand in marginal tests (Table 18). In sequential tests, significant correlations were found for % medium silt and % fine silt, which together explained 23 % of the variation in biomass-based community structure. For sampling periods pre- and one year after disturbance, no significant correlations were found between predictor variables and biomass-based community structure in marginal or sequential tests.

Table 18. Results of DistLM analyses between sediment predictor variables and biomass-based macrofaunal community structure in disturbed sites at the Butterknife. Only variables with p <0.05 are shown. Sampling periods are pre-disturbance (Pre), immediately after disturbance (After), and one year after disturbance (One year after disturbance). Prop. = proportion of variation explained by variable, Cumul. = cumulative proportion, res.df = residual degrees of freedom.

Variable	SS(trace)	Pseudo-F	P	Prop.	Cumul.	res.df
After, One year after						
Marginal tests						
% Coarse Silt	3137.3	2.1371	0.024	0.1178		16
% Medium Silt	3645.4	2.5382	0.009	0.1369		16
% Very Fine Sand	2989.0	2.0233	0.041	0.1123		16
Sequential tests						
% Medium Silt	3645.4	2.5382	0.010	0.1369	0.1369	16
% Fine Silt	2505.3	1.8355	0.036	0.0941	0.2310	15
Pre, One year after						
No significant variables found						

3.5 SCOC analyses

PERMANOVA analysis found that there was a significant effect of Sampling period on SCOC, but no Treatment or interaction effect (Table 19a). However, the pairwise PERMANOVA tests revealed that, with one exception, SCOC did not differ significantly between pairs of sampling periods for either treatment. Significant differences in SCOC were only observed in the undisturbed areas between immediately after and one year after the disturbance (Table

19b). Table 19c and Figure 21 show that mean SCOC values increased similarly in disturbed and undisturbed areas after disturbance, and after one year were similarly below pre-disturbed levels; hence no Treatment effect. There was high variability within samples for both treatments across the sampling periods which was much higher immediately after disturbance (Table 19c, Fig. 21); hence the generally undetectable Sampling period effect.

Table 19. For SCOC, (a) main PERMANOVA test, (b) pairwise PERMANOVA test between sampling periods, and (c) mean SCOC ($\mu mol/m^2/h$) with standard deviations in parentheses. Factors are Treatment = Tr (with levels D = Disturbed, and U = Undisturbed) and Sampling period = Sa (with levels P = Pre-disturbance, A = Immediately after disturbance, and O = One year after disturbance). Statistically significant P values are in bold.

(a)	Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms
	Tr	1	1925	1925	0.054118	0.8199	9834
	Sa	2	2.8564E+05	1.4282E+05	4.0153	**0.0242**	9945
	TrXSa	2	273.29	136.64	0.0038415	0.9968	9946
	Res	37	1.3161E+06	35570			
	Total	42	1.6296E+06				

(b)	Groups	Treatment level	t	P (perm)	Unique perms
	P, A	D	0.84841	0.5122	126
	P, O	D	1.416	0.2061	125
	A, O	D	1.4075	0.202	126
	P, A	U	1.3578	0.2029	9325
	P, O	U	1.4824	0.1595	8112
	A, O	U	2.1246	**0.0485**	9325

(c)	Disturbed	Undisturbed
Pre-disturbance	230.278 (111.042)	212.442 (112.472)
After disturbance	362.741 (330.99)	344.982 (272.042)
One year after disturbance	151.381 (56.5005)	144.843 (77.8745)
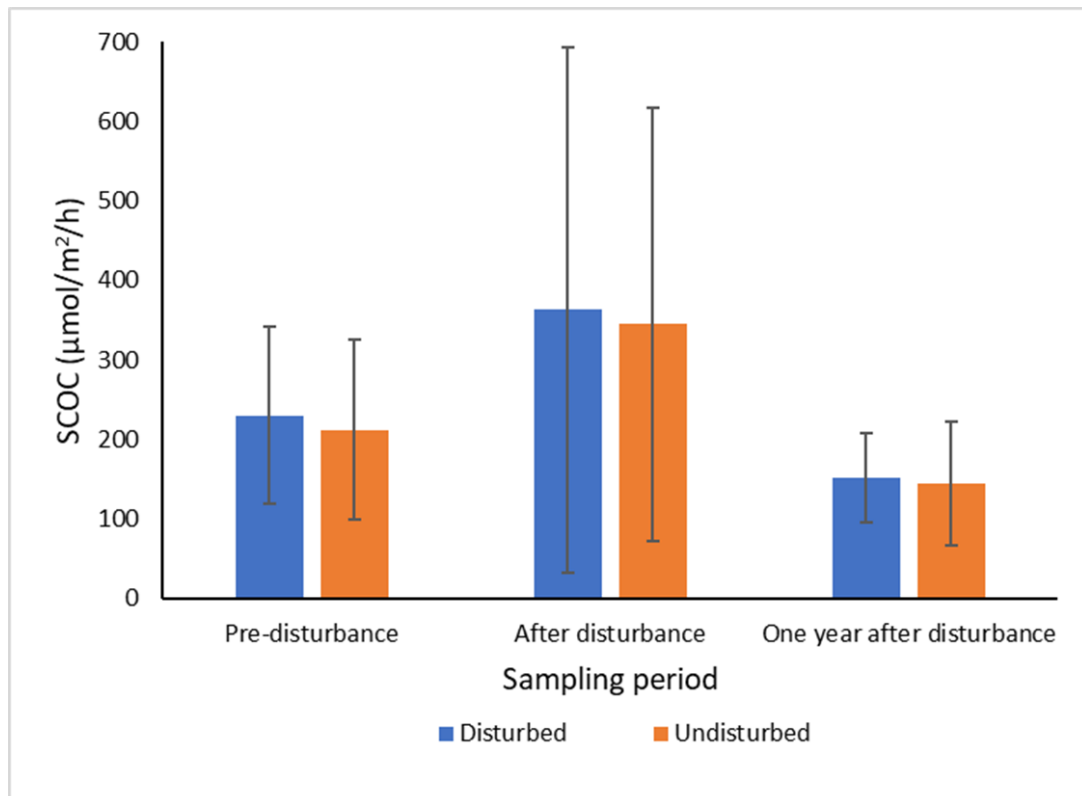
Figure 21. Mean SCOC ($\mu mol/m^2/h$) per treatment across the sampling periods. Error bars are one standard deviation.

Regression analyses found no significant relationships ($p > 0.05$) between SCOC and macrofaunal abundance, biomass, diversity, or bacterial abundance, the community variables that were predicted to be positively and linearly related to SCOC (Fig. 22).
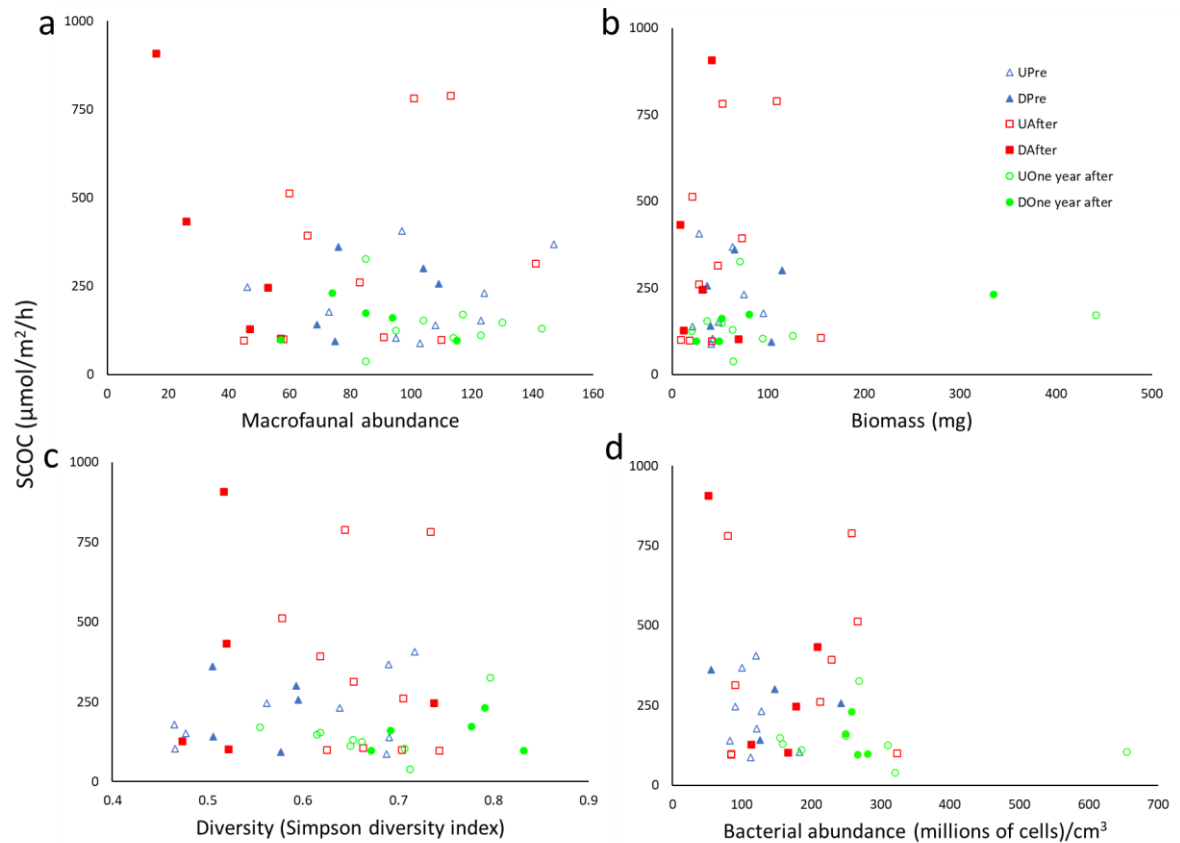
Figure 22. Scatterplots of SCOC and (a) macrofaunal abundance (individuals per core of 71 cm^2), (b) macrofaunal biomass (mg per core of 71 cm^2), (c) macrofaunal diversity (Simpson diversity index), and (d) bacterial abundance ((millions of cells)/cm^3). All sample groups were combined for regression tests. Symbols represent treatments (U = undisturbed and D= disturbed) and sampling periods (Pre = pre-disturbance, After = immediately after disturbance, One year after = one year after the disturbance).

4. Discussion

This study addresses the uncertain impacts of sedimentation on macrofaunal communities by investigating their response and recovery from a disturbance designed to produce a sediment cloud that could result from mining. The main aims were to determine how macrofaunal community structure at the Butterknife was impacted by direct and sedimentation impacts from disturbance and whether the community showed signs of recovery from those impacts in short to medium timeframes. I found community structure changed in both disturbed and undisturbed areas of the Butterknife after the SCIP disturbance and subsequently recovered after one year. The secondary aims of my research involved investigating relationships between macrofaunal community structure and sediment environment variables and how macrofaunal community parameters may relate to benthic ecosystem function. Abundance-based community structure was most strongly correlated with C:N molar ratios but no correlations were found between community parameters and benthic ecosystem function. These results are discussed in detail below with reference to previous findings. These results also have implications for the deep-sea mining industry; future mining operations must consider effects beyond mined blocks of seabed and should leave unmined areas of seabed adjacent to or within mined areas to aid the recovery of impacted macrofaunal communities.

4.1 Univariate community analyses

There are very few studies that address macrofaunal responses to sedimentation and those that have been conducted, focus on species-specific responses in a laboratory setting, (e.g., Hendrick et al. 2016), or occur in shallow-water environments, such as estuaries (Thrush et al. 2003, Anderson et al. 2004, Sweetman et al. 2020). However, one mesocosm experiment conducted in the deep sea found that macrofaunal communities were unaffected by the deposition of natural sediments (Trannum et al. 2010). The SCIP disturbance conducted during the ROBES experiment allowed a comparison of the macrofaunal community response between disturbed (physically disturbed and subjected to a high level of sedimentation) and undisturbed (not physically disturbed but subjected to relatively lower

levels of sedimentation) areas of seabed to better understand how sedimentation may affect deep-sea macrofaunal communities in situ.

The impact of the SCIP disturbance on the total abundance of the macrofaunal community across the Butterknife site was, as expected, much stronger for areas that were physically disturbed, as opposed to areas that were solely subjected to low level sedimentation. Despite being impacted in such a way, the community in the disturbed areas showed some degree of recovery, as the total abundance one year after disturbance had almost returned to a pre-disturbance level and, given the rate of recovery, may have fully recovered in the following months. The DISCOL experiment provides a good comparison with ROBES regarding the impact of a plough disturbance on macrofaunal abundance in both disturbed and undisturbed areas. Immediately after the DISCOL disturbance, average macrofaunal abundance in the disturbed sites was reduced to 39 % of the undisturbed value (Borowski and Thiel 1998). For ROBES, this corresponds to sampling period After, where the average abundance of total macrofauna for disturbed sites was reduced to 62 % of the undisturbed value. It is important to note that the DISCOL disturbance was more severe than ROBES in both duration (3 weeks versus 4 days) and areal extent (11 km^2 versus 0.316 km^2). Macrofauna were resurveyed 6 months after the DISCOL disturbance and the average abundance at disturbed sites had recovered to 73 % of the undisturbed value. For ROBES, average macrofaunal abundance at disturbed sites recovered to 88 % of the undisturbed value one year after the SCIP disturbance. The recovery trajectories in both experiments appear similar; however, changes in average macrofaunal abundance cannot inform of whether the community had recovered to its pre-disturbance structure (see discussion below on multivariate analyses).

A noteworthy feature of the total abundance data, and which is consistent for many of the univariate data, is the large increase in variance associated with the disturbed sites after disturbance. Variances for the disturbed and undisturbed treatments were similar before the SCIP disturbance took place, suggesting that increased variance in the disturbed areas was not an artefact of an unbalanced dataset. Increased variance is a potentially important indicator of stress in marine communities, and has been shown to increase with increasing levels of disturbance (Warwick and Clarke 1993). For ROBES, this is a good indication that

the SCIP disturbance impacted the benthic community of the Butterknife, and a decrease in variance one year after disturbance is indicative of a recovery from that impact.

The total biomass of the benthic community across the Butterknife did not change statistically between sampling periods or treatments. However, the variance substantially increased for the disturbed sites after disturbance, and remained relatively large one year later for both treatments. It is possible that the statistical signal-to-noise ratio is not large enough here (i.e., the large variances potentially mask changes in biomass). In saying this, the variance itself is an important consideration, as it indicates that within the disturbed area, the benthic community was impacted in different ways, which may also, to some degree, correspond to the patchy nature of the SCIP disturbance. The large variances may have also resulted from the presence of a few large infaunal individuals (two actiniarians, an ascidian and an echinoid) that also inflated the average biomass values. In order to understand the impact of the SCIP disturbance on total biomass, it may be more useful to examine the means and standard deviations displayed in Table 5b and the bar chart in Figure 15, both of which are derived from raw data. This approach requires cautious interpretation and does not warrant a complete dismissal of permutational test results, but can reveal certain patterns that were possibly masked by large variances. Between sampling periods Pre and After, average biomass for disturbed sites increased by 69 % from 76.69 mg to 129.27 mg. Relatively little change was observed in undisturbed sites for the same sampling periods; from 86.58 mg to 75.15 mg. After one year, biomass decreased to 88.8 mg in disturbed areas, but increased in undisturbed areas to 126.01 mg; though, none of these differences were statistically significant. Comparing whole-community biomass with abundance can show the impact of SCIP on the size of surviving fauna. The abundance:biomass (mg) ratio for disturbed sites for sampling periods Pre (1.36:1), After (0.45:1) and One year after (1.02:1), show that the individuals remaining after the SCIP disturbance were larger on average compared to individuals before disturbance, though this average may be inflated by the presence of a couple of anthozoans with relatively high biomass values in disturbed samples after disturbance. After one year, the abundance:biomass ratio was increasing towards the pre-disturbed value, a pattern consistent with most other univariate community parameters. There are no biomass data

from DISCOL or other simulated mining studies with which to compare the results of the ROBES study.

Patterns in Simpson's diversity are better understood when considering species evenness and richness. The SCIP disturbance only affected the biodiversity of the disturbed areas of the Butterknife. Interestingly, diversity did not change immediately after disturbance. However, because Simpson's diversity incorporates both species evenness and richness, it will often only increase when the values of both of these constituent metrics increase. Between sampling periods Pre and After for the disturbed areas, evenness increased but richness decreased. The contributions of these two metrics to Simpson's diversity offset one another and therefore diversity did not change after disturbance. For the disturbed areas, Simpson's diversity was higher one year after the SCIP disturbance than at any other sampling period. This result is due to evenness increasing after disturbance and remaining high one year later, and richness recovering to pre-disturbance conditions. Similar to abundance and biomass, both evenness and richness had large variances in the disturbed areas after the SCIP disturbance. The variance stabilised after one year for richness but remained large for evenness, suggesting that, during recovery, the benthic community was being structured by factors at a small-scale (potentially centimetres to metres). Physical and biologically-mediated disturbances have been found to create heterogeneous sediment microhabitats that favour a patchy macrofaunal distribution which can increase variances around the evenness metric (Cosson et al. 1997). In another study macrofaunal assemblages in stable sand environments were not found to differ at the 500 m scale, but in heavily bioturbated sandy mud sediments, samples taken 50 m apart showed significant differences in the structure of macrofaunal assemblages, and this difference mainly resulted from changes in dominance of the most abundant fauna (Kendall and Widdicombe 1999). It is possible that, within the disturbed area, the SCIP disturbance created heterogeneous sediment microhabitats which may have led to differences in the dominance and/or composition of the macrofaunal community that still remained in place after one year. These findings have implications for the spatial management of deep-sea mining operations; where physical disturbance of the seabed occurs, macrofaunal community structure can become highly variable at small spatial scales compared to relatively undisturbed communities. In order for impacted communities to recover faster from such disturbances,

mined sites must contain or be surrounded by sites where community structure is unaffected by disturbance and where it resembles the structure of the impacted community prior to mining. In the ROBES study, the macrofaunal community in undisturbed areas remained relatively unaffected by the level of sedimentation generated by the SCIP disturbance, and this community may have acted as a source of colonising fauna that helped the disturbed community recover more quickly in the year following disturbance.

None of the five univariate variables changed between any of the sampling periods for the undisturbed sites (i.e., in a univariate sense, the macrofaunal community was unimpacted by low levels of sedimentation). For the disturbed sites, abundance, biomass and species richness recovered to baseline levels but Simpson's diversity and species evenness remained high one year after the SCIP disturbance. The increased value of Simpson's diversity one year after disturbance was largely a consequence of elevated species evenness. These results taken together suggest that the abundance of the Butterknife macrofaunal community can recover from physical disturbance within one year, but the structure of the community (more specifically, relative taxa abundances) requires a longer period of time before reaching a state that resembles the pre-disturbance community.

There are a number of studies that assess the colonisation and recovery of macrofaunal communities after disturbance, though their experimental designs differ from that of ROBES. Two different studies investigated macrofaunal colonisation by placing trays of azoic sediment on the seabed at depths of 20 m (Arntz and Rumohr 1982), and 1800-3600 m (Grassle and Morse-Porteous 1987), and comparing tray communities with the surrounding sediment communities over a number of years. For the shallow trays, abundance, biomass and evenness took 2-3 yr to resemble the natural community, and abundance did not resemble the natural community in the deep sites after 5 years; Grassle and Morse-Porteous (1987) note that colonisation rates in the deep sea are slower than in shallow water areas. Although these experiments demonstrate that it can take a long time for a macrobenthic community to mature, the ROBES study experiment did not completely defaunate the seabed after the SCIP disturbance, and thus focuses more on recovery than colonisation of an empty patch. A different experiment, in which macrofauna were buried by artificial sediment mounds placed on the seabed, examined the recovery of macrofauna

at a depth of 1240 m (Kukert and Smith 1992). Less than one month after burial by these mounds, macrofaunal abundance had reduced by 32 %, but had recovered to baseline levels after 11 months. Despite not specifically measuring species evenness in their study, Kukert and Smith (1992) note that succession (changes in species composition over time) continued for 2 years after disturbance. They also state that the 14 dominant background species had lower abundances and constituted a smaller percentage of total abundance in the artificial mound sediments than in background sediments; species that were naturally rare were proportionally higher in artificial mounds. Their results align well with ROBES where total macrofaunal abundance had recovered after one year, but diversity and evenness remained high. It is not known how long it may take for species evenness at the Butterknife to resemble pre-disturbed conditions. These studies show that when a macrofaunal community is not entirely eliminated by a disturbance, total macrofaunal abundance can recover to pre-disturbance levels much faster than in a completely defaunated patch of seabed (~1 yr compared to >2 yr, respectively). This finding has implications for deep-sea mining; if mining is severe enough to defaunate the seabed, it may significantly increase the time required for the macrofaunal community to become as abundant as the pre-disturbed community and evenness may take longer still. When comparing the small-scale disturbance of ROBES (0.316 km^2 area disturbed over 4 days) and other mining-simulation experiments (see Table 1) to the proposed scale of commercial deep-sea mining (300 km^2 area disturbed for 300 days/yr), it is almost certain that macrofaunal communities will be more significantly impacted by mining than in these experiments, potentially to the point of seabed defaunation. As disturbance intensity and frequency increase, benthic communities are more severely impacted, impeding their ability to recover by reducing the abundance and proximity of potential colonising individuals (Rhoads et al. 1978, Thrush et al. 1998, Thrush et al. 2008). Some macrofaunal taxa have low dispersal abilities meaning recovery more heavily relies on self-recruiting populations (Shull 1997, Lundquist et al. 2004). In fact, in the artificial mound experiment (Kukert and Smith 1992), some mounds were "floored" (with a solid base) to prevent colonisation by burrowing species from below. After comparing floored and unfloored treatments, burrowing was found to be a significant dispersal mode for colonising fauna. Despite the SCIP penetration depth being set at 15 cm, the mining equipment that CRP proposes to use is capable of dredging the seabed down to 50 cm deep (Chatham-Rock-Phosphate 2014) where phosphorite nodules may be found (Cullen 1987,

Nielsen et al. 2015). While processing sediment cores collected during the ROBES experiment, I observed very few organisms present in the 10-15 cm core sections, and natural sediments recovered during this experiment had a dissolved oxygen penetration to anoxic conditions at 30-50 mm (Clark et al. 2018). It is therefore likely that macrofauna are extremely rare below 15 cm sediment depths, and dredging the seabed below 15 cm may defaunate the seabed and/or preclude recolonisation by burrowing fauna from below. Community recovery in defaunated sediments thus relies on the dispersal of individuals from neighbouring communities which may significantly favour species with good dispersal abilities, altering the community structure, and greatly increasing the time to recover, if at all, to a pre-disturbance state (Günther 1992, Whitlatch et al. 1998, Lundquist et al. 2010). Dispersal ability may be further reduced in hydrodynamically-stable deep-sea environments compared to ecosystems, such as estuaries, where dispersal is largely influenced by strong tidal currents (Valanko et al. 2010). Thus, the ability for a community to recover from deep-sea mining is much enhanced when mining operations do not completely defaunate the seabed.

One year after disturbance, the Butterknife macrofaunal community seemed to be close to recovering to its pre-disturbance state; only elevated species evenness prevented a resemblance to the pre-disturbance community. The univariate evenness metric indicated changes to the community as a whole, and the multivariate analyses discussed next identify these changes and the responses of particular taxa to the SCIP disturbance and sedimentation.

4.2 Multivariate community analyses

A major advantage of multivariate analyses is the ability to identify differences in community structure, which can be overlooked by more general univariate tests. Multivariate methods can also identify particular taxa which are mainly responsible for these differences. Because the macrofaunal community at the Butterknife is dominated by polychaetes (50-63 %), these organisms tend to have the largest influence on changes in community structure, and for all SIMPER analyses, polychaetes were the highest contributing taxa to the dissimilarities between groups. By square root-transforming the

data, the contributions of dominant taxa are down-weighted, while the contributions of less abundant taxa are still retained. The Diss/SD values in the SIMPER routine are particularly useful for identifying consistent changes in taxa with low abundances.

The PERMANOVA test on multivariate abundance showed that community structure differed between disturbed and undisturbed areas after the SCIP disturbance. In relation to deep-sea mining, this corresponds to the impact on community structure between mined blocks of seabed versus the areas surrounding the mined blocks that may also be subjected to sedimentation. All taxa were less abundant in the directly disturbed areas, providing further evidence that the physical disturbance of the seabed affects all macrofauna more severely than sedimentation alone, at least within the settled sediment rates in this study. Polychaetes and ostracods best characterised community differences and were the best indicators (along with bivalves and amphipods) of the difference in environmental conditions caused by the SCIP disturbance.

Although the univariate analyses detected no changes to the macrofaunal community in undisturbed areas, the multivariate tests found that community structure in these areas differed before and after the SCIP disturbance (albeit a weak average dissimilarity of 25.12 %), indicating an effect of low-level sedimentation. SIMPER results revealed an increase in abundance for some taxa, while others decreased. Cumaceans were consistently negatively impacted by sedimentation, almost to the point of extirpation. In a study of human impacts on soft-sediment assemblages in Antarctica, cumaceans were absent from all disturbed sites (two waste dumps, a sewage outfall and a wharf) but present in all control sites (Stark et al. 2003). Cumaceans migrate vertically from the sediment-water interface into the water column at night and are considered by some to be part of the hyperbenthos (a community of organisms living just above the seabed) (Anger and Valentin 1976, Kaartvedt 1986); thus, at the Butterknife, they may have been more affected by sedimentation than taxa that primarily live within the seabed. However, cumaceans have been recognised as early colonisers of defaunated patches of seabed (Santos and Simon 1980). In their study of macrofaunal colonisation, Santos and Simon (1980) found that colonising cumaceans were entirely composed of adult individuals (rather than larvae), indicating that their swimming activity may facilitate colonisation after disturbance. In fact, cumaceans in undisturbed

areas of the Butterknife recovered to pre-disturbance levels one year after disturbance (average abundance: Pre = 1, After = 0.06 and One year after = 0.94). With no planktonic larval stage, cumacean recovery is somewhat reliant on adult migration as they (and other peracarids) bear larvae in a brood pouch, with offspring emerging as post-larval juveniles referred to as mancae (Ax 2000). Along with cumaceans, ostracods were also good indicators of the difference in environmental conditions before and after the SCIP disturbance in undisturbed areas.

The physical disturbance of the seabed is where deep-sea mining is expected to have the greatest impact on benthic communities. The SCIP disturbance significantly changed the structure of the macrofaunal community in disturbed areas, reducing the abundance of all taxa from pre-disturbance levels. SIMPER results for disturbed areas showed that polychaetes were the largest contributor to the dissimilarity between community structure before and after disturbance, likely due to their numerical dominance in the community, as well as a significant reduction to 52 % of their initial abundance. Ostracods were also severely impacted, being reduced to 24 % of their initial abundance, but very little change in average abundance for isopods was observed. There was no positive effect of the SCIP disturbance on the macrofaunal community of the Butterknife, which is an expected response based on the results of other deep-sea simulated mining experiments (Jones et al. 2017).

A multivariate PERMANOVA showed that the community as a whole had recovered one year after the SCIP disturbance with ostracods, amphipods and cumaceans all showing a good ability to recover from very low abundance after disturbance; however, polychaete abundance was only 68 % of the pre-disturbed level. Because most other taxa resembled their pre-disturbed level of abundance, or even exceeded those levels (e.g., isopods: Pre = 2.76, After = 2.5, One year after = 5.52), polychaetes constituted a smaller percentage of the total macrofaunal abundance, thus slightly reducing their dominance in the community. This finding is also manifested in elevated species evenness values one year after disturbance (see above).

In all of the SIMPER analyses that used multivariate abundance data, ostracods were always good indicators of the difference in environmental conditions caused by the SCIP disturbance, suggesting that they are sensitive to environmental changes. Currently, assessments of human-induced disturbance on ostracods in the marine environment have focused only on pollution (Ruiz et al. 2005, and references from Table 1 within), and the impact of sedimentation on ostracods has only been examined in freshwater (Cohen et al. 1993) and estuarine environments (Hayward et al. 2004); in each case, as part of a wider community analysis. Freshwater ostracods have been recognised as excellent bioindicators of changes in environmental variables, such as bottom grain sizes, salinity, temperature, pH, dissolved oxygen and hydraulic conditions (Ruiz et al. 2013, and references therein). Ostracods are even sensitive to small-scale changes within the same estuary (Ruiz et al. 2004), and may provide an early warning of environmental shifts, particularly in relatively stable habitats, such as the deep sea. SIMPER results suggest that ostracods, more than any other taxon, have the potential to be useful indicators of deep-sea environmental changes caused by physical disturbance and sedimentation. Information on ostracod dispersal and colonisation ability is scarce, though there is some indication that swimming ability (as in cumaceans) may influence ostracod distribution more than reproductive modes (Külköylüoğlu et al. 2019).

Polychaetes, unlike most other taxa, had not fully recovered to the pre-disturbed level of abundance. Most polychaetes produce planktonic larvae (Hill et al. 2018), thus local recruitment to disturbed patches may be more sporadic than for taxa whose offspring develop directly, such as peracarid crustaceans. Santos and Simon (1980) found that most polychaetes settled by larval dispersal instead of lateral migration of adults when colonising defaunated sediments. Lateral migration may have been an important factor in the recovery of the Butterknife community given that the SCIP transects did not cover the entire seabed within the disturbed area, but rather, created small-scale patches of disturbance that did not completely defaunate sediments. Adjacent patches of undisturbed seabed may have been a source of colonising individuals that migrated laterally, supplementing the recolonisation by surviving fauna in disturbed patches. Macrofaunal recolonisation after the DISCOL experiment (which also used a plough for disturbance) was observed to be faster than originally predicted, and was attributed to lateral migration caused by the same

patchiness of disturbance that likely occurred during ROBES (Borowski and Thiel 1998). The abundance to biomass ratio for polychaetes in disturbed areas before disturbance was 1.18:1 (abundance: biomass (mg)). After disturbance this ratio was 0.66:1, suggesting that polychaetes surviving the SCIP disturbance were larger adults. The ratio was 1.34:1 one year after disturbance; polychaetes at this sampling period were smaller on average than they were before disturbance, indicating that the recovery of the polychaete assemblage was being driven by small individuals (juveniles and/or smaller adults, smaller species), that had colonised over the year. This finding is a good indication that the polychaete assemblage is still recovering, but also suggests that persistent disturbance may not allow sufficient time for large individuals/species to establish, which could have implications for the functioning of benthic communities (Norkko et al. 2006, Norkko et al. 2013). Around the Butterknife, the polychaete assemblage has the potential to eventually restore its dominance to the pre-disturbed level of abundance, and in so doing, would cause species evenness to decrease to its baseline level, though a timeframe for this is difficult to predict.

After polychaetes, peracarid crustaceans constitute the second largest component of the Butterknife macrofaunal community (20.4 % of total abundance). Peracarida is a superorder of malacostracan crustaceans, and representative orders in the pre-disturbed Butterknife community include Amphipoda, Isopoda, Cumacea and Tanaidacea. Peracarids are mainly defined by the presence of a brood pouch, in which eggs are carried until they are released as post-larval juveniles. Because peracarids lack a swimming phase in their development, their ability to disperse is significantly reduced, which may be responsible for high levels of endemism observed in isopods (Svavarsson et al. 1993, Brandt et al. 2007) and genetic divergence in tanaids (Larsen et al. 2014). Mobility differs between peracarid orders with some isopods and amphipods able to swim, while tanaids and cumaceans (despite vertically migrating) are mainly bottom-dwellers (Brandt et al. 2012). Due to their reproductive strategy and limited dispersal, peracarid recovery from seabed mining impacts may be hampered more than taxa with planktonic larval stages. However, direct development and extended maternal care of offspring may allow peracarids to recolonise local patches of seabed relatively successfully (Thiel 2003), but when sediments are completely defaunated and patch sizes increase, larval dispersal becomes an increasingly important factor for colonisation success (Smith and Brumsickle 1989). For the Butterknife peracarid fauna, the

abundance to biomass ratio before disturbance was 2.6:1 (abundance: biomass (mg)). After disturbance, this ratio was 12.47:1 and suggests that larger peracarid fauna were more vulnerable to the SCIP disturbance than small peracarid fauna, which is the opposite response observed in polychaetes and the wider macrofaunal community. This vulnerability, however, did not reduce the ability for peracarids to recover; after one year, an abundance:biomass ratio of 2.71:1 suggests that the average size of peracarids reflected their pre-disturbed state. That the peracarid fauna recovered well from the SCIP disturbance is likely a consequence of a patchy disturbance regime that left undisturbed patches in close proximity to SCIP tracks and allowed for relatively fast peracarid colonisation by lateral migration of adults. Where complete seabed defaunation occurs, particularly over a wide area, the ability for peracarids to recolonise may be severely reduced.

Differences in life history strategies are important to consider when attempting to understand how communities may recover from disturbance. The theory of *r/K* selection was proposed as a way of explaining colonisation success for different species based on trade-offs between the quantity and quality of offspring (MacArthur and Wilson 1967). Species with fast growth rates, high fecundity, early maturation, and the ability to produce widely-dispersing offspring are considered *r*-selected. Conversely, *K*-selected species are typically slow-growing, mature later, produce fewer offspring, and provide more parental care. After disturbance, *r*-selected species are expected to rapidly colonise the disturbed patch, and over time, will slowly be outcompeted by *K*-selected species, which will eventually dominate. Applying *r/K* selection theory at the Butterknife is complicated by the fact that polychaetes exhibit more *r*-selected traits (e.g., larval dispersal) than peracarids (parental care), yet were numerically dominant in the community before disturbance. The *r/K* selection theory is normally applied at species level, and given that individuals at the Butterknife were identified to order level at most (except *Taiaroa tauhou*), a more suitable approach is to consider how the different life history strategies of polychaetes and peracarids, and the ecological interactions between them, influence their colonisation/recovery success differently under different disturbance regimes. For disturbances that create mosaics of undisturbed and disturbed patches, lateral migration and extended parental care may favour peracarids as early colonisers. However, in large defaunated patches, the ability for polychaetes to colonise is enhanced through planktonic

larval dispersal. At the Butterknife, polychaetes had not recovered to their pre-disturbed level of abundance but peracarids had. This finding is possibly explained by the patchy mosaic disturbance caused by SCIP, which favoured early colonisation by peracarids. When the dominant taxon in a community has very consistent abundances in replicate samples before disturbance, it suggests that the community has been undisturbed for a long time. At the Butterknife, polychaetes displayed this pattern (Table 5 in Appendix C: Sim/SD = 9.25) indicating that the Butterknife macrofaunal community was in a mature state prior to the SCIP disturbance, and on this premise, polychaetes were the superior competitors in this community. Provided the seabed is left undisturbed for an extended period of time, polychaete abundance and dominance will likely be restored through larval recruitment and competitive interactions with other taxa. On the other hand, if frequent and prolonged disturbance occurs, polychaete abundance is less likely to recover, which could lead to a long-term change in community composition and uncertainty about the impacts on the more opportunistic taxa under these circumstances.

For abundance-based community structure, log C:N molar ratio was found to be an important sediment predictor variable in DistLMs that investigated relationships between the environment and macrofauna following the impact of the SCIP disturbance (pre- to after disturbance) and recovery from that disturbance (after- to one year after disturbance). The C:N molar ratio was derived from the proportions of organic carbon and organic nitrogen in each sediment sample. The marine environment is generally nitrogen-limited (Rabalais 2002); thus, when nitrogen becomes available, benthic macrofauna consume it more rapidly than carbon (Kristensen and Blackburn 1987). The C:N molar ratio acts as a proxy for food quality, and as its value increases, the organic material is considered refractory (older and more decomposed). Through the deposition of fresh (labile) organic matter, surface sediments tend to have lower C:N molar ratio values compared to subsurface sediments, especially in areas where macrofauna are more abundant (Heilskov and Holmer 2001). At the Butterknife, the C:N molar ratio was much higher after the SCIP disturbance in both disturbed and undisturbed areas at the 0-1 and 1-5 cm depths compared to the other sampling periods (Fig. 12). This pattern was mostly driven by a decrease in % PN rather than any change in % POC. Interestingly, the other measure of food quality, chl*a*:phaeo, did not change for the same sampling period. It is possible that the SCIP disturbance resuspended

refractory organic matter buried in the sediment, which subsequently settled around the wider Butterknife area; however, under this scenario, high C:N molar values would likely only be observed in the top layer of sediments (0-1 cm depth) in undisturbed areas, but high C:N molar values were observed in subsurface sediments (1-5 cm depth) also. Under the premise that the SCIP disturbance resuspended refractory organic matter, it is expected that C:N molar ratios would be high in subsurface sediments prior to disturbance; however, C:N molar ratios were relatively low and did not increase with depth before disturbance. But, given that the penetration depth of SCIP was set at 15 cm (10 cm deeper than the sediment variables were measured), it is possible that refractory material was indeed buried deeper in the sediment and was resuspended upon disturbance. Bioturbation activity by macrofauna is a more important factor in stimulating the mineralization of refractory organic material than for labile organic material (Andersen and Kristensen 1992, Kristensen et al. 1992). Although labile organic material is a higher-quality food source than refractory material, the latter will still be consumed by benthic fauna if it becomes available (Kristensen et al. 1992, Aspetsberger et al. 2007). The SCIP disturbance occurred on a much larger scale than macrofaunal bioturbation and caused a lot more refractory organic material to become available which may have previously been inaccessible due to burial in anoxic sediments (Hulthe et al. 1998). The increase in the C:N molar ratio after disturbance in both disturbed and undisturbed areas aligns very closely to the observed increase in SCOC rates, which responded similarly in both disturbed and undisturbed areas. The availability of refractory organic material may have stimulated a metabolic response in the benthic community across the Butterknife that was captured in the SCOC measurements. Finding a significant correlation between SCOC rates and macrofaunal/bacterial abundance or biomass is made more difficult when, after a disturbance, the rate at which these groups consume oxygen increases, meaning that SCOC rates would not necessarily depend on a level of abundance or biomass, but rather, on increased metabolic activity. It is not known whether changes in the C:N molar ratio caused macrofaunal community structure to change at the Butterknife, or if both responded independently to the SCIP disturbance. Even so, there is ample evidence that food quality can alter macrofauna community structure more than food quantity (Wieking and Kröncke 2005, Campanyà-Llovet et al. 2017, Zaborska et al. 2018, Włodarska-Kowalczuk et al. 2019), and at the Butterknife, where C:N molar ratios increased

and decreased over time while community structure changed and then recovered, the influence of C:N molar ratios on community structure cannot be ruled out.

For the DistLM that examined the recovery period (after- and one year after disturbance), depth was also found to be a significant predictor variable that explained an additional 10 % of variation in abundance-based community structure after the C:N molar ratio. At the Butterknife, depth ranged between 444 and 465 m, so there was little variation in depth or other variables that may covary with depth over larger scales, such as hydrostatic pressure (Leroy and Parthiot 1998). The correlation between depth and community structure is potentially related to some emergent or depressed topographic features of the seafloor that may influence currents that, in turn, can affect food supply and larval settlement (Butman 1987, McClain and Barry 2010). A previous study on the Chatham Rise found that macrofaunal diversity was correlated most strongly with seafloor areas of variable slope compared to flat areas (Leduc et al. 2015), and a seafloor environment containing small-scale topographic features is likely to be permanently altered by seafloor mining operations. The depth range across the Butterknife may incorporate areas of both variable slope and flat seafloor, and may have contributed to the structure of the macrofaunal community; however, small-scale seafloor topography at each multicore sampling point was not recorded.

Biomass-based community structure was different one year after disturbance compared to pre- and after disturbance periods only in disturbed areas. Biomass patterns did not particularly align with the impact that was expected from the SCIP disturbance, unlike the clearer pattern observed in the multivariate abundance response. The difference in biomass one year after disturbance compared to other sampling periods was mostly due to changes in polychaetes and ophiuroids which were the biomass-dominant taxa. Polychaete biomass was lower one year after disturbance compared to pre-disturbance, but because abundance was recovering at a faster rate, polychaete body size was smaller on average. It is not unexpected that abundance will recover at a faster rate than biomass as organisms can quickly colonise but require time to grow to maturity, or smaller bodied species colonise before larger bodied species. Previous simulated mining experiments have found that small-bodied taxa more readily colonise disturbed patches and that biomass recovery lags behind

most other community variables (Gollner et al. 2017, Jones et al. 2017). Around the Butterknife, it is not known how long is required for macrofaunal biomass to return to pre-disturbed levels.

For biomass-based community structure, % medium silt and % fine silt were found to be significant predictor variables in the DistLM that investigated relationships between the environment and macrofauna immediately after disturbance and one year later. As biomass-based community structure did not vary in accordance with what was expected from the SCIP disturbance, these correlations are difficult to explain and may constitute a spurious relationship, particularly because no sediment variables were found to correlate with multivariate macrofaunal biomass between sampling periods pre- and one year after disturbance.

4.3 SCOC analyses

SCOC rates in undisturbed areas responded almost identically to the disturbed areas at each sampling period; SCOC gave a more far-reaching indication of the impact of the disturbance than the community analysis revealed. SCOC is potentially a more sensitive measure of the impact of the disturbance, as macrofaunal community structure in the undisturbed area immediately after disturbance was relatively unaffected, but functioned differently as indicated by the SCOC PERMANOVA results. Because disturbed and undisturbed areas did not differ in SCOC rates immediately after disturbance, and macrofaunal abundance decreased more in disturbed than undisturbed areas, it may be assumed that macrofauna were respiring at a faster rate in disturbed areas. However, the SCOC data I used for my PERMANOVA tests were derived from core incubations of seabed sediment, and therefore, any changes in SCOC could only be indirectly related to macrofauna. The regression results show that SCOC and macrofaunal abundance, biomass, and diversity were not related, suggesting that SCOC rates at the Butterknife are either controlled by other factors, or that the biological groups present are respiring at a faster rate and SCOC rates are not as closely tied to their abundances or biomasses, but rather, are nonetheless affected by disturbance. A study of the factors driving SCOC at a regional scale across the Chatham Rise revealed that, in austral summer (December), variability in SCOC rates were accounted for by a

combination of macrofaunal and environmental variables, but in winter (when the ROBES samples were collected), variability in SCOC rates were mostly accounted for by environmental variables alone; thus, the interaction between the factors driving SCOC make it difficult to predict how benthic ecosystem function may be affected under different conditions (Leduc et al. 2020). The samples analysed in this and many other studies were collected at regional scales from undisturbed sites, but the ROBES study site is at a relatively small spatial scale with limited environmental variability and a portion of core samples being disturbed prior to incubation; thus, the environmental factors correlated with SCOC in previous studies may not correlate with samples collected during the ROBES experiment.

It is difficult to predict how SCOC may be affected by disturbance as few studies have assessed the effect of disturbance/sedimentation on benthic ecosystem function in the deep sea, but those that have found contrasting results. For example, in an experimental study, Mevenkamp et al. (2017) observed reduced rates of SCOC after the deposition of 0.1 cm of mine tailings (low total organic carbon) on an incubated benthic community, but increased rates of SCOC after the deposition of natural sediments (relatively high total organic carbon); experimental physical disturbance has also been observed to have no effect on SCOC (Leduc and Pilditch 2013). In the Mevenkamp (2017) study, increased oxygen consumption was observed in sediments with higher organic carbon content and this may help explain the SCOC results from the ROBES experiment. Immediately after the SCIP disturbance, both SCOC and C:N molar ratios increased (albeit statistically nonsignificant) in both disturbed and undisturbed areas. Bacteria have been found to account for 60-90 % of benthic metabolism (Pfannkuche 1993, Hubas et al. 2006), and they play a key role in carbon cycling after pulses of particulate organic carbon (Sweetman et al. 2019). Bacterial metabolism may have been stimulated by the SCIP disturbance, as in the DISCOL experiment (Vonnahme et al. 2020), however, when examining the relationship between bacterial abundance and SCOC, I found no significant correlation.

A few recent studies that focus on the DISCOL disturbance site give an indication as to how benthic ecosystem function may be affected by a more intense simulated mining disturbance. Processing rates of phytodetritus by benthic communities were still reduced in disturbed areas from the DISCOL experiment after 26 years (Stratmann et al. 2018b). For the

same recovery period, carbon-based food web models estimated ecosystem function in disturbed areas to be only 56 % of the value in undisturbed areas (Stratmann et al. 2018a). However, the undisturbed areas sampled in this study were still within the wider experimental disturbance area, and based on the response of SCOC in ROBES, it is possible that ecosystem function may also be affected by sedimentation in undisturbed areas; thus, it is necessary to measure function in a separate reference area unlikely to be affected by the disturbance. In another study of the DISCOL site, in situ measurements of ecosystem function were conducted in disturbed, undisturbed and reference areas 26 years after disturbance (Vonnahme et al. 2020). Oxygen consumption was significantly reduced in both disturbed and undisturbed areas compared to the reference sites, suggesting that sedimentation alone can negatively affect oxygen consumption rates. The majority of oxygen consumption was attributed to respiration by microbial communities as opposed to larger meio- and macrofauna, and the authors estimate that microbially-mediated functions will require more than 50 years to return to pre-disturbed levels. Although SCOC rates at the Butterknife increased immediately after disturbance, they fell below pre-disturbed levels after one year (though these differences are not statistically significant), and may indicate that ecosystem function can recover faster on the Chatham Rise than in deeper study sites. The results of ROBES and other simulated mining experiments are important to consider regarding the proposed mining plan of CRP (see Fig. 4) in which mining in a 'spiralling out' pattern leaves a central unmined area of seabed that may still be subjected to some level of sedimentation. Even if macrofaunal community structure is unimpacted in this area, it is possible that ecosystem function may be affected.

4.4 Benthic community impacts at larger scales

The difference in the benthic macrofauna community response in the ROBES and DISCOL experiments provides evidence that the negative impact expected from mining-related disturbance can vary considerably. The macrofaunal community around the Butterknife had almost fully recovered after one year, whereas the macrofaunal and bacterial communities around the DISCOL experimental site had still not recovered structurally or functionally after 26 years. There are a number of factors that may have caused this difference. The two sites differ in depth considerably (ROBES = 450 m, DISCOL = 4150 m) and there is a general trend

that metabolism decreases with depth (Childress 1995, Drazen and Seibel 2007), which may be related to decreased organic flux to deeper sites from surface waters that affect benthic communities (Wedding et al. 2013). Net primary production (NPP) in surface waters between these two sites also differs; NPP over the Chatham Rise is estimated at 300–600 g C m^{-2} y^{-1} but may only be 100-200 g C m^{-2} y^{-1} at the DISCOL site (Field et al. 1998). Bottom current flows are also much faster across the crest of the Chatham Rise (20-30 cm s^{-1}; Heath (1983), Chiswell (1994)) compared to the DISCOL site (mostly 1-3 cm s^{-1} but may reach up to 10 cm s^{-1} (Klein 1993)). As the Chatham Rise is a more dynamic environment with potentially higher levels of natural disturbance, the biological communities there may be adapted accordingly, allowing them to recover more quickly from seabed disturbances than biological communities at the DISCOL site. However, given that commercial-scale mines will be operating at much larger spatio-temporal scales than both ROBES and DISCOL (which was only slightly more intense than ROBES, relatively speaking), the impact on benthic communities is likely to be much more severe and longer-lasting than indicated by these mining-simulation experiments. For example, the impact on ecosystem function could be far-reaching, potentially being significantly reduced in unmined areas that are subjected to sedimentation, and it is possible that such function could take decades or more to recover (Stratmann et al. 2018a, Stratmann et al. 2018b, Vonnahme et al. 2020). It is also unknown how the services provided by deep-sea soft-sediment ecosystems will be affected under deep-sea mining scenarios (Orcutt et al. 2020).

6. Conclusions and future research directions

The macrofaunal community at the Butterknife was negatively impacted by the SCIP disturbance in both a uni- and multivariate sense. In areas that were physically run over by SCIP, macrofaunal community structure significantly changed from the pre-disturbed structure; however, a level of resilience was displayed, as evidenced by most community parameters returning to baseline levels after one year. Polychaetes were recovering, though more slowly than peracarid fauna, likely due to a combination of differences in reproductive strategies, and a patchy disturbance regime that did not completely defaunate large areas of seabed. In undisturbed areas that were subject to sedimentation alone, community structure had changed after disturbance, and this was especially noticeable for cumaceans and ostracods which were both shown to be sensitive to disturbance and may be early indicators of a community-wide impact that may occur under increasing levels of sedimentation. Cumaceans and ostracods, despite their sensitivity, also exhibited a good ability to recover from disturbance which is consistent with previous studies. Across both disturbed and undisturbed areas, the macrofaunal community showed an ability to recover from the direct and sedimentation impacts of the SCIP disturbance over a short to medium timeframe (one year). The structure of the macrofaunal community in disturbed areas correlated with an increase in refractory organic material and potentially small-scale topographic features of the seafloor that may indirectly affect benthic communities. Ecosystem functioning, as measured by SCOC, increased similarly in all areas of the Butterknife after disturbance, even in areas where community structure was much less affected. Increased SCOC rates may have been a response to the increase of refractory organic matter that was made available after the SCIP disturbance.

Based on the results of this study and others, it appears that the ability for a macrofaunal community to recover from disturbance is largely improved under a patchy disturbance regime that does not completely defaunate large areas of seabed. The vast difference in macrofaunal community structure between disturbed and undisturbed areas of the Butterknife indicate that undisturbed areas, despite being subjected to some level of sedimentation, may act as a source of colonising fauna to aid the recovery of more impacted communities in disturbed areas. This finding has implications for the deep-sea

mining industry, where the chosen mining pattern could significantly affect the ability for benthic communities to recover. Mining patterns that leave some areas undisturbed, such as that proposed by CRP, will allow benthic communities to recover more quickly, but this also depends on the spatial scales of disturbed and undisturbed areas and the dispersal distance and recolonisation potential of particular taxa. Unmined blocks will not be completely unaffected by sedimentation as the more sensitive fauna can be negatively affected and ecosystem function, it would appear, can respond somewhat independently from macrofaunal community structure. Therefore, having structurally intact communities adjacent to areas where community structure has been significantly negatively affected will facilitate recovery upon cessation of mining.

Future macrofaunal community studies should aim to record small-scale topographic features of the seafloor to more accurately determine their relationship with distributional patterns of the fauna and how these relationships may be potentially altered by seabed disturbance. Research should also involve controlled experiments where recolonisation of soft-sediment communities are assessed after being subjected to different disturbance regimes, such as variations in the size, patchiness and penetration depth of disturbed areas. This research may reveal whether different disturbance scenarios favour recolonisation of some fauna over others, potentially resulting from different behaviours or life-history strategies, and this could subsequently affect the faunal composition of recovering communities. Finally, future studies should also aim to partition the contributions of macrofauna, meiofauna and bacteria to SCOC rates under both undisturbed and disturbed conditions, with increasing levels of disturbance and organic matter input, in order to determine what may be driving increased SCOC rates. This approach may shed light on how structure and function are linked under different disturbance conditions for each biological community. When the responses of each community are understood, changes in SCOC rates may be better understood at an ecosystem level where all communities are present, and this will help explain the effects of sedimentation on ecosystem functioning.

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Appendices

Appendix A – Univariate figures with REF samples included

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d
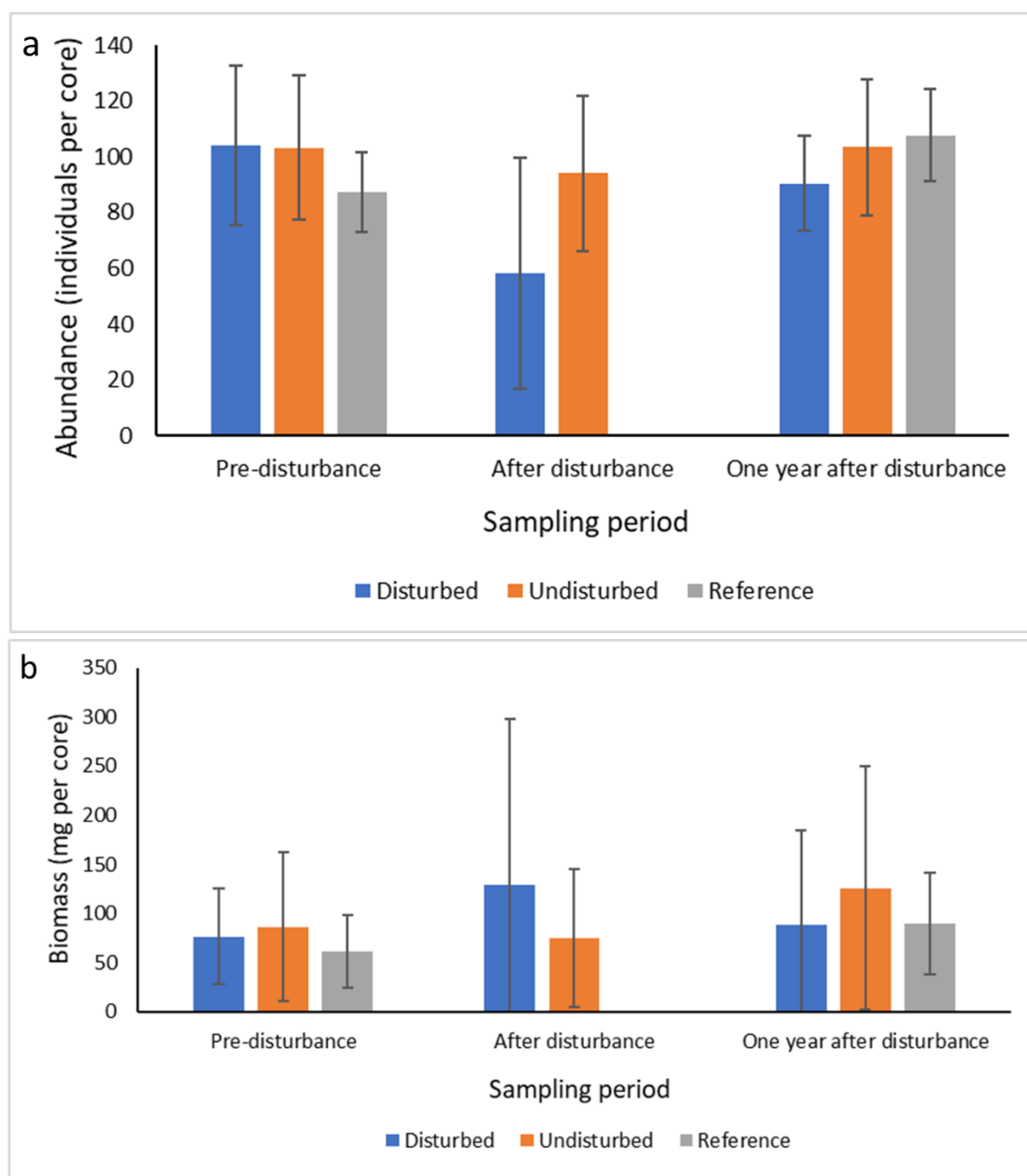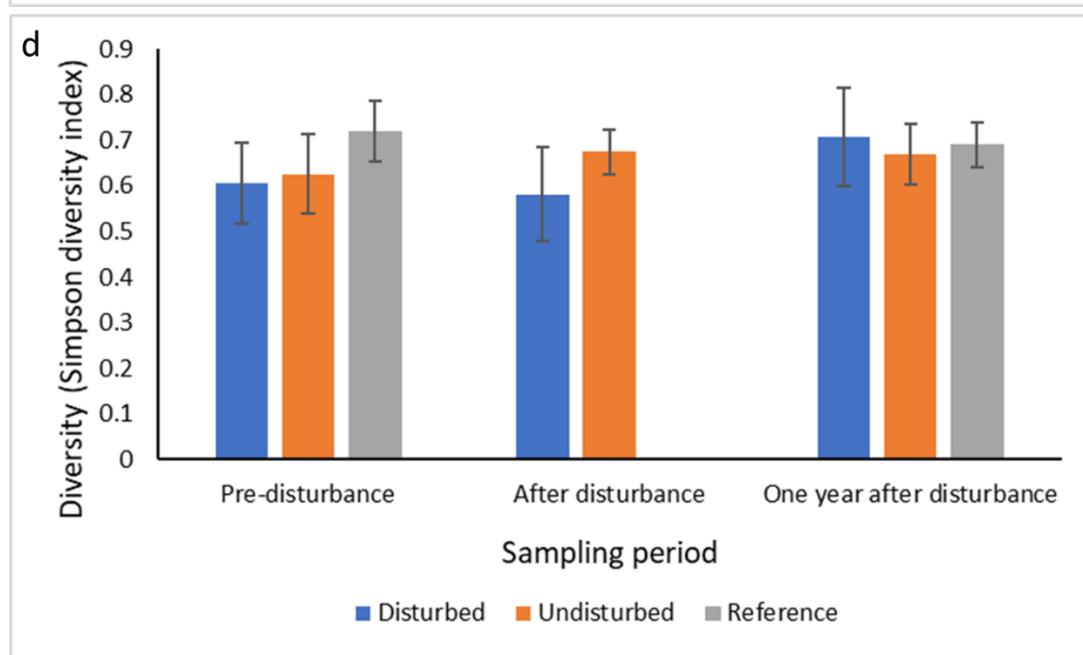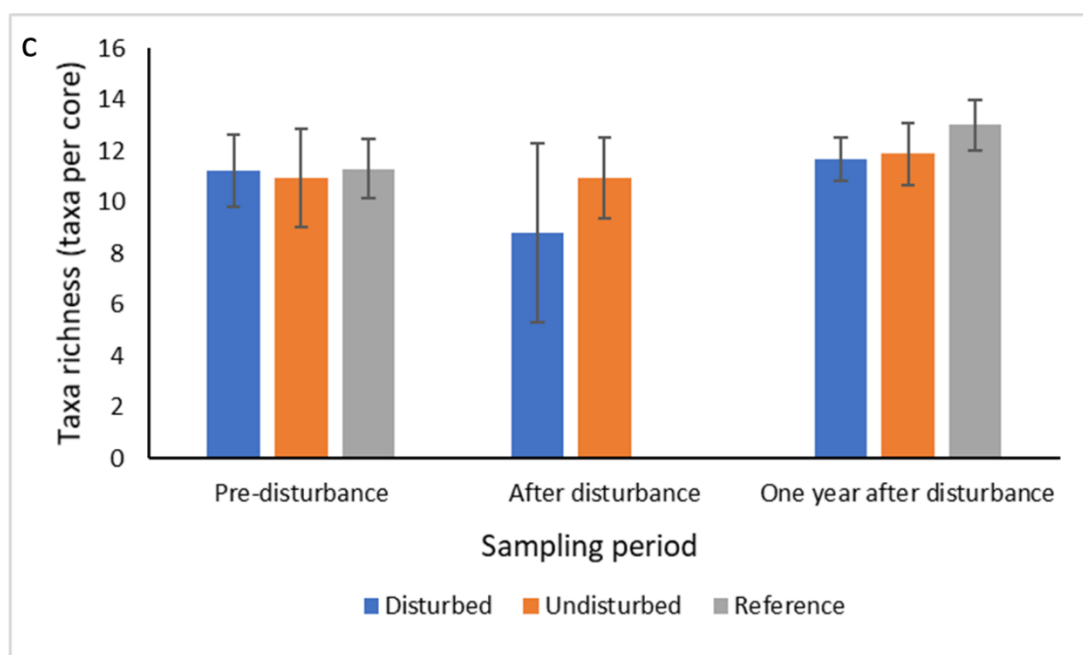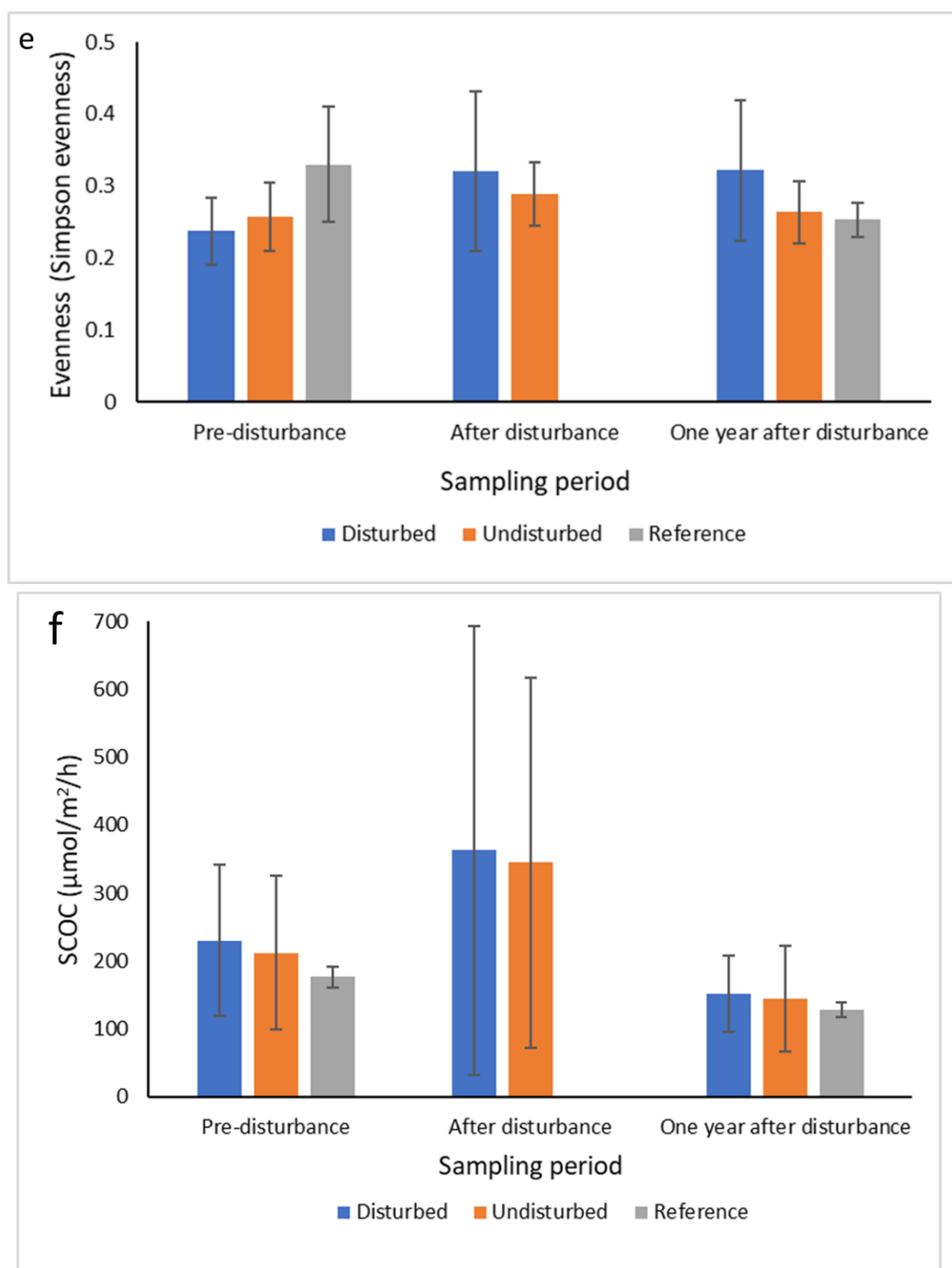
Figure 1. Univariate bar charts with Butterknife and REF samples included, (a) abundance, (b) biomass, (c) taxa richness, (d) diversity, (e) evenness, and (f) SCOC.

Appendix B – Non-metric multidimensional scaling plots with REF samples included
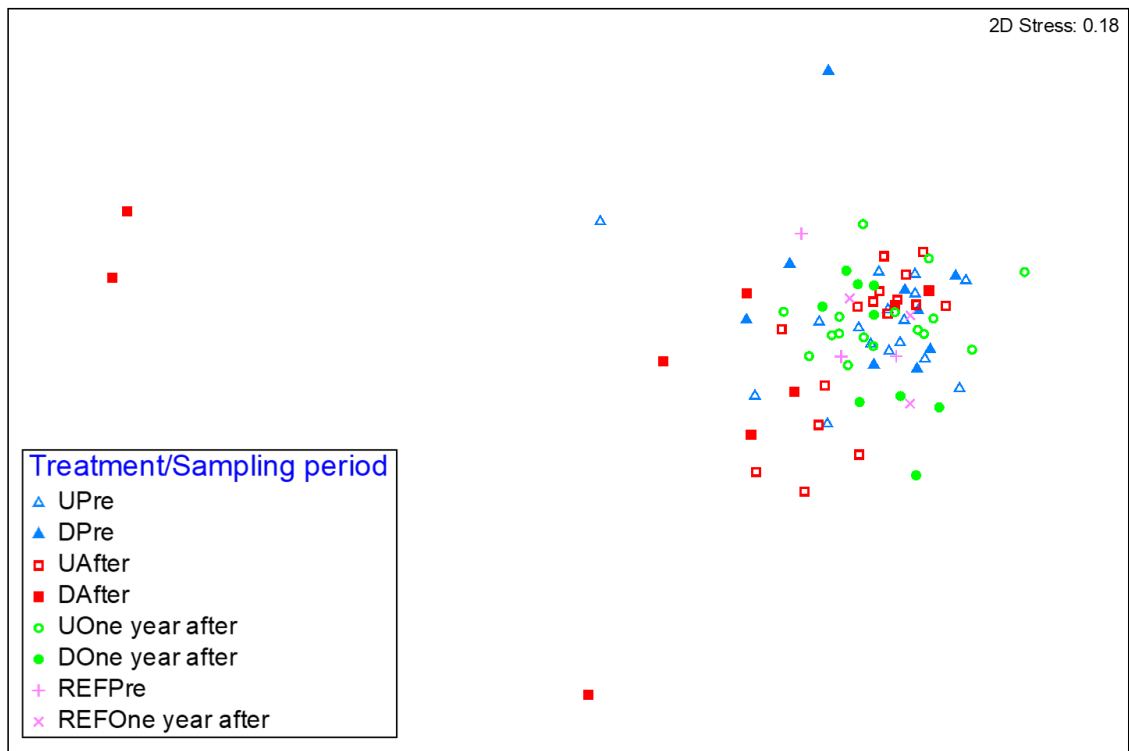
Figure 2. nMDS for multivariate abundance including REF samples. Symbols represent treatments (U or D) and sampling periods (Pre, After, One year after).
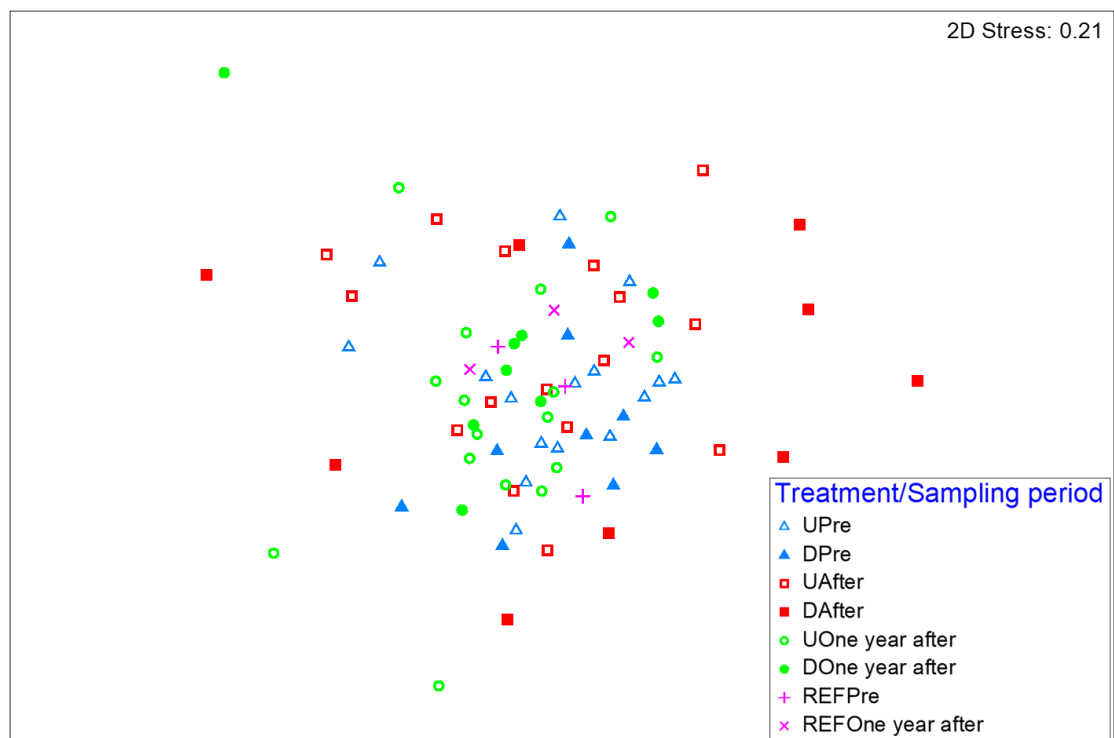
Figure 3. nMDS for multivariate biomass including REF samples. Symbols represent treatments (U or D) and sampling periods (Pre, After, One year after).

Appendix C – SIMPER results without cut-off values

Table 1. Full SIMPER abundance results for treatments U and D at sampling period After.

Groups U & D
Average dissimilarity = 35.93

Taxon	Group U Av.Abund	Group D Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	7.08	5.64	5.14	1.34	14.32	14.32
Ostracoda	2.49	1.18	3.83	1.51	10.65	24.97
Isopoda	2.55	1.58	3.38	1.1	9.41	34.37
Tanaidacea	2.45	1.7	3.32	1.24	9.23	43.6
Bivalvia	1.94	1.36	2.82	1.43	7.84	51.44
Amphipoda	2.16	1.57	2.69	1.35	7.48	58.92
Nemertea	1.62	1.22	2.34	1.19	6.5	65.43
Sipuncula	0.92	0.67	2.23	1.13	6.22	71.65
Aplacophora	1.76	1.12	2.2	1.05	6.12	77.77
Ophiuroidea	0.87	0.46	1.91	1.25	5.32	83.09
Actiniaria	0.73	0.56	1.42	1.04	3.96	87.05
Scaphopoda	0.51	0.38	1.32	0.94	3.67	90.72
Cumacea	0.24	0.33	1.05	0.78	2.93	93.65
Gastropoda	0.3	0.19	0.87	0.66	2.43	96.08
Leptostraca	0.06	0.11	0.34	0.43	0.94	97.02
Taiaroa tauhou	0.13	0	0.3	0.36	0.83	97.85
Pycnogonida	0.13	0	0.27	0.37	0.75	98.6
Asteroidea	0.06	0	0.18	0.25	0.49	99.09
Hydrozoa	0.06	0	0.16	0.25	0.46	99.54
Echinoidea	0.06	0	0.16	0.25	0.46	100
Platyhelminthes	0	0	0	-	0	100
Mysidacea	0	0	0	-	0	100
Holothuroidea	0	0	0	-	0	100
Ascidiacea	0	0	0	-	0	100

Table 2. Full SIMPER abundance results for sampling periods Pre and After for treatment U.

Groups Pre & After
Average dissimilarity = 25.12

Taxon	Group Pre Av.Abund	Group After Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	7.71	7.08	2.57	1.29	10.24	10.24
Bivalvia	1.69	1.94	2.23	1.26	8.89	19.13
Sipuncula	1.14	0.92	1.97	1.26	7.86	26.99
Tanaidacea	2.33	2.45	1.9	1.19	7.55	34.54
Amphipoda	2.66	2.16	1.88	1.16	7.5	42.05
Nemertea	1.61	1.62	1.8	1.25	7.17	49.22
Cumacea	1	0.24	1.74	1.41	6.94	56.16
Isopoda	2.51	2.55	1.72	0.98	6.84	63
Ostracoda	2.23	2.49	1.57	1.32	6.25	69.25
Aplacophora	1.54	1.76	1.54	1.24	6.13	75.38
Scaphopoda	0.86	0.51	1.41	1.18	5.61	81
Ophiuroidea	0.68	0.87	1.29	1.17	5.13	86.12
Actiniaria	0.28	0.73	1.29	1.17	5.12	91.24
Gastropoda	0.24	0.3	0.79	0.72	3.16	94.4
Echinoidea	0.13	0.06	0.35	0.45	1.38	95.78
Taiaroa tauhou	0	0.13	0.24	0.37	0.96	96.74
Pycnogonida	0	0.13	0.22	0.37	0.88	97.62
Asteroidea	0	0.06	0.14	0.26	0.55	98.17
Hydrozoa	0	0.06	0.13	0.26	0.52	98.69
Platyhelminthes	0.06	0	0.11	0.26	0.46	99.15
Leptostraca	0	0.06	0.11	0.26	0.43	99.57
Holothuroidea	0.06	0	0.11	0.26	0.43	100
Mysidacea	0	0	0	-	0	100
Ascidiacea	0	0	0	-	0	100

Table 3. Full SIMPER abundance results for sampling periods (a) Pre and After, (b) After and One year after (OYA), and (c) Pre and One year after for treatment D.

(a) *Groups Pre & After*

Average dissimilarity = 37.66

Taxon	Group Pre Av.Abund	Group After Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	7.82	5.64	6.24	1.41	16.56	16.56
Ostracoda	2.41	1.18	3.59	1.49	9.53	26.09
Tanaidacea	2.51	1.7	3.44	1.36	9.13	35.22
Amphipoda	2.62	1.57	3.09	1.36	8.2	43.42
Isopoda	1.66	1.58	2.83	1.23	7.51	50.93
Sipuncula	1.5	0.67	2.78	1.48	7.38	58.3
Aplacophora	1.61	1.12	2.68	1.29	7.13	65.43
Nemertea	1.44	1.22	2.4	1.24	6.38	71.81
Bivalvia	1.97	1.36	2.05	1.11	5.43	77.24
Ophiuroidea	0.6	0.46	1.53	1.09	4.07	81.31
Scaphopoda	0.47	0.38	1.42	0.77	3.76	85.07
Cumacea	0.6	0.33	1.28	1.02	3.4	88.47
Actiniaria	0.49	0.56	1.28	0.99	3.39	91.86
Gastropoda	0.33	0.19	0.97	0.78	2.57	94.43
Echinoidea	0.22	0	0.58	0.52	1.55	95.98
Taiaroa tauhou	0.22	0	0.51	0.52	1.35	97.33
Platyhelminthes	0.11	0	0.32	0.34	0.84	98.16
Mysidacea	0.11	0	0.26	0.34	0.68	98.84
Leptostraca	0	0.11	0.23	0.35	0.61	99.46
Pycnogonida	0.11	0	0.2	0.35	0.54	100
Hydrozoa	0	0	0	-	0	100
Asteroidea	0	0	0	-	0	100
Holothuroidea	0	0	0	-	0	100
Ascidiacea	0	0	0	-	0	100

(b) *Groups After & OYA*
Average dissimilarity = 36.76

Taxon	Group After Av.Abund	Group One year after Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	5.64	6.74	4.83	1.28	13.14	13.14
Ostracoda	1.18	2.49	3.79	1.49	10.32	23.46
Amphipoda	1.57	2.75	3.22	1.44	8.75	32.21
Tanaidacea	1.7	2.27	3.12	1.21	8.49	40.7
Isopoda	1.58	2.34	2.91	1.18	7.92	48.62
Nemertea	1.22	2.04	2.52	1.24	6.84	55.46
Aplacophora	1.12	1.58	2.28	1.12	6.2	61.66
Bivalvia	1.36	2	2.08	1.1	5.65	67.32
Ophiuroidea	0.46	0.83	1.84	1.19	5.01	72.32
Cumacea	0.33	0.9	1.84	1.1	5	77.32
Sipuncula	0.67	0.49	1.67	1	4.54	81.86
Scaphopoda	0.38	0.61	1.54	0.93	4.18	86.04
Gastropoda	0.19	0.49	1.27	0.93	3.44	89.48
Actiniaria	0.56	0.11	1.12	1.05	3.05	92.53
Taiaroa tauhou	0	0.38	0.92	0.66	2.49	95.03
Hydrozoa	0	0.27	0.63	0.51	1.72	96.75
Mysidacea	0	0.22	0.5	0.52	1.37	98.11
Leptostraca	0.11	0.11	0.43	0.49	1.18	99.29
Ascidiacea	0	0.11	0.26	0.34	0.71	100
Platyhelminthes	0	0	0	-	0	100
Pycnogonida	0	0	0	-	0	100
Asteroidea	0	0	0	-	0	100
Echinoidea	0	0	0	-	0	100
Holothuroidea	0	0	0	-	0	100

(c) *Groups Pre & OYA*
Average dissimilarity = 26.24

Taxon	Group Pre Av.Abund	Group One year after Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	7.82	6.74	2.59	1.28	9.86	9.86
Nemertea	1.44	2.04	2.19	1.51	8.33	18.19
Isopoda	1.66	2.35	2.1	1.2	8.02	26.21
Sipuncula	1.5	0.49	2.1	1.55	7.99	34.2
Aplacophora	1.61	1.58	2.06	1.32	7.85	42.06
Tanaidacea	2.51	2.27	1.68	1.42	6.4	48.45
Ostracoda	2.41	2.49	1.53	1.21	5.82	54.27
Amphipoda	2.62	2.75	1.45	1.4	5.51	59.79
Scaphopoda	0.47	0.61	1.43	0.98	5.45	65.24
Cumacea	0.6	0.9	1.38	1.13	5.25	70.49
Bivalvia	1.97	2	1.36	1.35	5.16	75.65
Ophiuroidea	0.6	0.83	1.27	1.13	4.85	80.5
Gastropoda	0.33	0.49	0.99	0.98	3.78	84.28
Actiniaria	0.49	0.11	0.92	0.88	3.51	87.79
Taiaroa tauhou	0.22	0.38	0.87	0.82	3.31	91.1
Mysidacea	0.11	0.22	0.53	0.62	2.01	93.11
Hydrozoa	0	0.27	0.51	0.52	1.95	95.07
Echinoidea	0.22	0	0.46	0.53	1.75	96.82
Platyhelminthes	0.11	0	0.24	0.35	0.92	97.74
Ascidiacea	0	0.11	0.21	0.35	0.81	98.55
Leptostraca	0	0.11	0.21	0.35	0.79	99.34
Pycnogonida	0.11	0	0.17	0.35	0.66	100
Asteroidea	0	0	0	-	0	100
Holothuroidea	0	0	0	-	0	100

Table 4. Full SIMPER biomass results for (a) sampling periods Pre and One year After (OYA) and (b) After and One year after for treatment D.

(a) *Groups Pre & OYA*

Average dissimilarity = 43.63

Taxon	Group Pre Av.Abund	Group One year after Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	0.22	0.18	6.83	1.47	15.64	15.64
Ophiuroidea	0.02	0.08	5.63	1.05	12.89	28.54
Nemertea	0.02	0.05	3.81	1.42	8.72	37.26
Amphipoda	0.06	0.06	3.68	1.15	8.43	45.69
Bivalvia	0.06	0.03	3.64	0.88	8.34	54.03
Ascidiacea	0	0.05	3.16	0.35	7.24	61.27
Sipuncula	0.02	0.01	2.06	1.28	4.72	65.99
Echinoidea	0.02	0	1.87	0.42	4.3	70.28
Isopoda	0.01	0.03	1.84	1.09	4.22	74.5
Cumacea	0.01	0.02	1.66	0.93	3.81	78.31
Scaphopoda	0.01	0.01	1.47	0.8	3.38	81.69
Aplacophora	0.02	0.02	1.39	1.25	3.18	84.87
Actiniaria	0.02	0	1.32	0.39	3.02	87.89
Ostracoda	0.03	0.03	1.27	1.24	2.92	90.81
Gastropoda	0.01	0.01	1.15	1.01	2.63	93.44
Mysidacea	0	0.01	1.01	0.37	2.31	95.75
Taiaroa tauhou	0	0.01	0.74	0.82	1.7	97.46
Tanaidacea	0.01	0.01	0.58	1.33	1.34	98.8
Platyhelminthes	0	0	0.26	0.35	0.6	99.39
Pycnogonida	0	0	0.11	0.35	0.24	99.64
Hydrozoa	0	0	0.09	0.4	0.21	99.85
Leptostraca	0	0	0.07	0.35	0.15	100
Asteroidea	0	0	0	-	0	100
Holothuroidea	0	0	0	-	0	100

(b) *Groups After & OYA*
Average dissimilarity = 56.13

Taxon	Group After Av.Abund	Group One year after Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Polychaeta	0.19	0.18	10.29	1.25	18.33	18.33
Ophiuroidea	0.04	0.08	7.22	1.14	12.87	31.2
Actiniaria	0.12	0	7	0.56	12.46	43.66
Amphipoda	0.02	0.06	4.55	1.55	8.11	51.77
Nemertea	0.02	0.05	4.22	1.3	7.52	59.29
Bivalvia	0.04	0.03	4.14	1	7.37	66.66
Ascidiacea	0	0.05	3.34	0.34	5.96	72.62
Ostracoda	0.01	0.03	2.13	1.21	3.79	76.41
Isopoda	0.01	0.03	2.11	1	3.77	80.18
Aplacophora	0.02	0.02	1.87	1.07	3.34	83.51
Cumacea	0	0.02	1.87	0.8	3.33	86.84
Gastropoda	0.01	0.01	1.65	0.89	2.95	89.78
Scaphopoda	0	0.01	1.36	0.69	2.43	92.21
Sipuncula	0.01	0.01	1.28	0.86	2.28	94.49
Mysidacea	0	0.01	1.1	0.36	1.97	96.46
Tanaidacea	0	0.01	0.7	1.41	1.24	97.7
Taiaroa tauhou	0	0.01	0.67	0.65	1.19	98.89
Leptostraca	0.01	0	0.52	0.4	0.93	99.82
Hydrozoa	0	0	0.1	0.4	0.18	100
Platyhelminthes	0	0	0	-	0	100
Pycnogonida	0	0	0	-	0	100
Asteroidea	0	0	0	-	0	100
Echinoidea	0	0	0	-	0	100
Holothuroidea	0	0	0	-	0	100

Table 5. Full SIMPER abundance result for all Butterknife cores at sampling period Pre. All Butterknife samples at this sampling period were considered undisturbed in order to increase the sample size for this analysis.

Group U
Average similarity: 75.02

Taxon	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Polychaeta	7.75	27.22	9.25	36.28	36.28
Amphipoda	2.64	8.31	3.41	11.07	47.35
Ostracoda	2.29	6.87	3.48	9.16	56.51
Tanaidacea	2.39	6.85	2.57	9.13	65.64
Isopoda	2.2	6.01	1.65	8.01	73.65
Bivalvia	1.79	4.91	1.85	6.54	80.2
Nemertea	1.55	3.81	1.36	5.08	85.28
Aplacophora	1.57	3.67	1.47	4.9	90.18
Sipuncula	1.27	2.67	1.05	3.56	93.74
Cumacea	0.85	1.77	0.87	2.35	96.09
Scaphopoda	0.72	1.18	0.64	1.57	97.66
Ophiuroidea	0.65	1.14	0.64	1.52	99.18
Actiniaria	0.35	0.32	0.32	0.43	99.61
Gastropoda	0.27	0.18	0.23	0.24	99.85
Echinoidea	0.16	0.09	0.14	0.12	99.97
Platyhelminthes	0.08	0.01	0.06	0.02	99.98
Taiaroa tauhou	0.08	0.01	0.06	0.02	100
Hydrozoa	0	0	-	0	100
Mysidacea	0.04	0	-	0	100
Leptostraca	0	0	-	0	100
Pycnogonida	0.04	0	-	0	100
Asteroidea	0	0	-	0	100
Holothuroidea	0.04	0	-	0	100
Ascidiacea	0	0	-	0	100