Seafloor ecological functioning over two decades of organic enrichment

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Abstract

Climate change and anthropogenic nutrient enrichment are driving rapid increases in ocean deoxygenation, these changes cause biodiversity loss and have severe consequences for marine ecosystem functioning and in turn the delivery of ecosystem services upon which humanity depends (e.g. fisheries). We seek to understand how such changes will impact seafloor functioning using biological traits analysis. Results from a sewage-sludge disposal site in the Firth of Clyde, UK spanning 26 years of monitoring showed that substantial changes in macrobenthic nutrient cycling and the provision of food for predators occurred, with elevated functioning on the margins 1–2 km from the centre of the disposal grounds. Changes in food-web dynamics are expected, that weaken benthic pelagic coupling and lower secondary production (such as fisheries). Generally, functioning was conserved, but declined below a ~6% total organic carbon threshold. Similar to other severely deoxygenated systems, the recovery was slow and hysteresis was apparent.

Keywords: sewage; deoxygenation; hypoxia; ecological functioning; long-term

Introduction

One of the consequences of climatic warming is a decrease in the amount of dissolved oxygen in seawater due to reduced gas solubility, temperature stratification inhibiting vertical mixing, and changes in the delivery of nutrients to the sea. To date most studies of the ecological impacts of climate change have focussed on changes in temperature and the impacts of other significant drivers of ecological change, e.g. ocean deoxygenation and acidification, are less well-known. Ocean deoxygenation, e.g. hypoxia (dissolved oxygen content 1–30% of saturation) or anoxia (no oxygen), is one of the greatest threats to marine ecosystem health and functioning (United Nations, 1992). Over the last 50 years ocean oxygen content has decreased by a mean rate of 0.06–0.43% per year (Stramma et al., 2010) and models predict a continued decline of >7% from present-day levels until 2100 under high CO_2 emissions scenarios (IPCC, 2013). The number of coastal hypoxic zones have also been increasing over the past 50 years and are now documented from >500 systems (Diaz and Rosenberg, 2008) covering ~7% of ocean area. The impacts on ecosystems are complex, being associated with non-linear interactions, thresholds and hysteresis (Cardinale et al., 2012; Stachowicz et al., 2007) and take time to manifest. Thus, we need to study change over timescales that exceed decades. Synergism between ocean deoxygenation and other anthropogenic stressors, e.g. global temperature rise, ocean acidification, marine pollution and fisheries, also make it more challenging to predict and manage the impacts of deoxygenation for ecosystem health (Altieri and Gedan, 2015; Breitburg et al., 2009).

Hypoxia has profound effects on marine organisms and often results in mass mortalities of animals that dwell on the seafloor and in the water column due to low dissolved oxygen, or indirectly due to toxic H_2S (Breitburg et al., 2009; Caddy, 2000; Falkowski et al., 1980). Significant changes may occur in terms of: organism behaviour (Gray et al., 2002; Riedel et al., 2014; Seitz et al., 2003), growth rates and body size (Caswell and Coe, 2013; Cheung et al., 2013), organism health (Keppel et al., 2015), the impairment of reproductive processes, and the contraction of the available area for spawning (Ekau et al., 2009; Nissling and Westin, 1997). These changes have led to 3–5-fold declines in benthic macrofaunal biomass (Hale et al., 2016; Seitz et al., 2009), and declines in secondary productivity of ~10% (Karlson et al., 2002; Sturdivant et al., 2014).

Long-term studies of the ecological impacts of ocean deoxygenation are lacking, but short-term studies (e. g. Breitburg et al., 2018; Cheung et al., 2013; Dauer et al., 1992; Gray et al., 2002; Levin et al., 2009a; Pearson and Rosenberg, 1978) indicate that the consequences of long-term deoxygenation will include decreasing biodiversity and production, changing trophic structure, the decreasing size, health, fitness and reproductive capacity of organisms, and the loss of key habitats. This loss of productivity and biodiversity, and the changes in the biological traits of taxa can profoundly constrain the ways that ecosystems function, and in coastal seas many functions are provided or mediated by benthic communities. For example, by providing food for higher trophic levels (Breitburg et al., 2009; Greenstreet et al., 1997) or stimulating decomposition and nutrient cycling which in turn drives water column primary productivity (Aller and Aller, 1998).

These ecological functions support a number of core regulating and supporting ecosystem services (e.g. nutrient cycling, waste treatment, biodiversity, biological control and habitat provision) which are threatened by deoxygenation, and approximately US$350 billion of services are lost each year to hypoxia (Diaz et al., 2012). This is in addition to other socio-economically important services such as the provision of food and recreational experiences (e. g. Carstensen et al., 2014; Hale et al., 2016). The recovery from short-term severe hypoxia can take years, but for long-term severe hypoxia recovery is hysteretic, associated with thresholds and can exceed decades (Diaz and Rosenberg 2008). Once a system has exceeded the threshold, and turned hypoxic, it may become increasingly susceptible to repeated hypoxia (Conley et al., 2009). The increasing adoption of an ‘ecosystem services’ approach, following the Millennium Ecosystem Assessment (United Nations, 2005) and the increasing availability of tools for mapping ecological functioning to services (Bremner, 2008; Bremner et al., 2003, 2006; Frid et al., 2008) is shifting the basis of environmental management. There is now an explicit recognition of the underpinning of human well-being and economic activity by healthy functioning ecosystems (United Nations, 2005). It is now possible to reinterpret data on the impacts of sewage sludge disposal on sea floor communities and to assess the extent to which the changes in the taxonomic composition of the impacted communities can be used to determine past changes in ecological functioning and hence the delivery of ecosystem services. Investigation of changes in the composition and ecological functioning of natural benthic systems have shown that over decadal scales changes in the faunal composition do not result in shifts in ecosystem functioning, but rather that ecosystem functioning is conserved (Clare et al., 2015; Frid, 2011). Over millennial time scales benthic functioning is also conserved through species turnover, but in periods of rapid and severe environmental change, profound shifts in species composition mean functioning is compromised (Caswell and Frid, 2013, Frid and Caswell, 2015).

In 1986 Pearson and Rosenberg developed a conceptual model of the ecological structure and functioning of benthic systems, which placed the availability of food, in the form of organic matter, at its heart. It was partially developed from a model of the observed impacts of organic pollution on marine benthic communities, in terms of their species richness, abundance and biomass (Pearson and Rosenberg, 1978), and has since been validated in many coastal systems (Diaz and Rosenberg, 1995; Gray et al., 2002), and may also function as a generalised model of disturbance (e.g. Connell and Slatyer, 1977). The core concepts of these ideas have underpinned marine benthic ecology since the 1970s. In this paper we revisit one of the classic benthic macrofaunal data sets from which the Pearson Rosenberg model was derived, the Garroch Head sewage sludge disposal site and ask the questions (i) to what extent do the benthic macrofaunal changes caused by enrichment and deoxygenation drive functional changes, (ii) did the changes in functioning correspond to the change in taxonomic composition, (iii) what was the nature of the recovery, and (iii) what are the consequences of functional changes for the delivery of ecosystem services derived from the benthos?

From 1979 to 1998 Garroch Head in the Firth of Clyde was used as a disposal site for sewage sludge (the solid components of sewage). The site received on average 1.67 ± 0.06 x 10^6 t y^{-1} sludge from primary treatment plants in the Strathclyde region, surrounding the city of Glasgow, Scotland and was deposited on the seafloor for five days each week throughout the year. The sludge contained organic material with elevated heavy metals and organochlorine compounds (e. g. Pearson and Blackstock, 1987), and at this time its disposal at sea was considered an acceptable disposal option because the large dilution reduced chemical contamination and natural processes reduced the biological oxygen demand of the wastes (Frid and Caswell, 2017). Sewage sludge was disposed offshore from a series of sites around the UK and was regulated under the Dumping at Sea Act (1974) and the Food and Environmental Protection Act (1985), which was accompanied by regular sampling at these sites to monitor the environmental impacts. By 1998 the sludge disposal ceased with the adoption of the EU Urban Waste Water Directive (European Economic Community, 1991). Using benthic macrofaunal data from eight sampling stations situated in 3 km radius from the Garroch Head sewage sludge disposal site (plus one reference station at 8.5 km) this study aims to quantify changes in the biological characteristics or 'traits' of macrobenthos along a gradient of organic enrichment and deoxygenation over 26 years. The biological traits of organisms have considerable impacts on the magnitude of ecosystem functioning (Cardinale et al., 2012; Crowe and Frid, 2015), and we anticipate that the considerable ecological changes that occur in enriched and deoxygenated systems will profoundly affect ecosystem functioning and in turn the delivery of ecosystem services. The Garroch Head data are of very high quality, despite some changes in sampling procedures, and are used to explore changes in the ecological functioning of the benthos through time. The wide spatial extent of the Garroch Head sampling programme meant that differing levels of anthropogenic organic enrichment and hypoxia could be explored over the 19 years of sewage sludge disposal and the seven years post-disposal (Pearson and Stanley, 1980; SEAS, 1999). These data represent a unique opportunity to investigate the decade long ecological impacts of varying levels of organic enrichment and hypoxia on benthic ecosystem functioning.

Materials and methods

The Garroch Head dataset

Environmental monitoring at Garroch Head was conducted by the Scottish Association for Marine Sciences (SAMS) and the Scottish Environmental Advisory Services Ltd (SEAS), on behalf of the Department of Agriculture and Fisheries for Scotland, which began in 1979 and sampling occurred every year until disposal ceased in 1998 (Coates and Pearson, 1997, 1999; Pearson, 1981, 1983, 1991, 1992, 1993, 1994; Pearson and Blackstock, 1982, 1983, 1985, 1986, 1987, 1988, 1989; Pearson et al., 1990; Pearson and Coates, 1995, 1998; Pearson et al., 1992; Pearson and Stanley, 1980; SEAS, 1999) when the disposal of sewage sludge at sea was banned. Monitoring at Garroch Head incorporated biological sampling of sediment microbe, benthic macrofauna and demersal fish communities plus a suite of environmental variables and the concentrations of notable pollutants (Supplementary Table S1). In 2000, 2004 and 2005 follow up environmental surveys were conducted by the Scottish Environmental Protection Agency (SEPA) and SAMS in order to assess the recovery of the site after sewage sludge disposal had ceased (Duncan, 2005; Scottish Environment Protection Agency, 2000; Scottish Environmental Protection Agency, 2004).

The centre of the disposal site was located 6 km south of Garroch Head on the Isle of Bute (station P7, Fig. 1), Firth of Clyde, Scotland. This site replaced an earlier sewage sludge disposal site located 4 km to the north that received Strathclyde's sewage from 1904-1974. This site was found to be an accumulating site with low current speeds <10 cm s^{-1} and so in 1974 was relocated 2 km further south (Fig. 1; Dooley, 1979; Midgley et al., 2001).

Samples were collected from 40 different sampling stations throughout the 26 years, and of these eight stations were sampled near annually for biotic and abiotic factors. These eight stations plus the reference station, 8.5 km to the north, were used in this study to achieve the maximum temporal duration and the full spectrum of organic enrichment (Fig. 1). The number of sampling points totalled 184 over the nine stations and 26 years. Macrofaunal data are missing for some stations in some years (Supplementary Table S1), e.g. macrofaunal data from stations P4 and V7 could not be completely extracted from the archived report for 1979; macrofauna were not sampled at Station P8.5 in 1984-1985, nor from stations T7 and V7 in 1985. The sampling procedures were modified at times as licensing laws changed and methodology developed. Consequently some of data sets (e.g. dissolved oxygen and full sediment redox profiles) were only collected from 1985 and not always from every station (for a summary see Supplementary Table S2). No abiotic data were collected in 2000 and 2004 from any of the stations.

Benthic macrofauna were collected using a 0.1 m^2 van Veen grab and samples were sieved using a 1 mm mesh and were preserved in 4% formalin with rose Bengal (Pearson and Stanley, 1980). Taxa were identified to species level where possible and the number of individuals were enumerated. The number of grab samples collected varied from 1-3 per station throughout the 26 years (see supplementary information Table S1). The macrofaunal data were checked for spelling mistakes, the taxonomic designations were reviewed, checked for synonymy and if necessary the nomenclature was updated (for details of the data auditing approach see the supplementary information).

Oxygen saturation was measured in the water immediately overlying the sediment surface at each station, and was determined using the Winkler method. Sediment samples of 40 mm depth were collected by Craib corer at each station for analyses of abiotic variables including: sediment redox potential (Eh; measured using a standard redox electrode) and pH were measured at 10 mm sediment depth intervals from 10 mm above the surface down to 40 mm depth, sediment type, interstitial salinity, sediment total organic carbon (TOC) and nitrogen content (using a Perkin-Elmer elemental analyser), the surface sediment concentrations of six organochlorine compounds (α-HCH, γ-HCH [Hexachlorocyclohexane], o'p-Dichlorodiphenyl dichloroethane, p,p'-Dichlorodiphenyl dichloroethane, Dieldrin and PCB 1260 [aka Aroclor]) and eleven heavy metals (Cu, Ni, Pb, Zn, Mn, Cr, Co, Cd, As, Hg and Fe; Pye SP192 Atomic Absorption Spectrophotometer) were determined (Duncan, 2005; Pearson and Blackstock, 1989; Pearson and Stanley, 1980). Similar to the macrofauna the abiotic sampling replication varied somewhat through time (Supplementary Table S2).

Biological traits analysis

Biological traits analysis (BTA) was used to categorise taxa based on their morphology, biology, and behaviour (e. g. Bremner et al., 2006). Ten categories, or biological traits, were chosen to reflect the range of biological and morphological attributes of the 385 taxa. These traits included four based on taxa morphology: organism maximum body length, maximum body volume, relative two dimensional body shape, and exoskeletal composition (Table 1). Five behavioural traits were used to summarise the organism's ecological role including their life habit, burrow depth, bioturbatory mode, feeding mode and type of larval development. The bioturbatory modes used the classification of Queirós et al. (2013) and predecessors (Gerino et al., 2003; Solan et al., 2004) and splitting taxa into five bioturbatory modes: **(1)** *epifauna* that bioturbate at the sediment-water interface only, **(2)** *surficial modifiers* that disturb only the upper 1–2 cm of sediment. **(3)** *Upward/downward conveyors* which actively transport sediment upwards toward the surface from depth (those oriented head downwards) or downward from the surface (taxa oriented head upwards). **(4)** *Biodiffusors* that cause diffusive sediment transport, with no predominant orientation, over small spatial scales; and, **(5)** *regenerators* that excavate large structures within the sediment such as galleries whereby sediment is moved to the surface during construction and is transported away, and later new sediment accumulates within the structures. Increasing sediment reworking occurs as bioturbatory mode shifts from (1) to (5) (Mermillod-Blondin et al., 2004; Queirós et al., 2013).

The final trait considered was the existence of any adaptations to low oxygen and/or the presence of toxic H_2S (e.g. Gray et al. 2002, Vacquer-Sunyer and Duarte, 2008) that prevail where conditions are enriched. Given our interest in the macrofaunal response to such enrichment, this represents a 'response trait' (*sensu* Lavorel and Garnier, 2002). To avoid circularity we do not infer a hierarchy for these adaptations nor do we map this trait to any of the ecological functions we consider (Table 2). Five modalities were selected to encompass specific adaptations to low oxygen conditions (Table 1) which ranged from *no adaptations*, *metabolic adaptations* (e.g. metabolic depression or a switch to anaerobiosis; (Hochachka and Lutz, 2001)), mechanisms that facilitate the *H_2S and O_2 transport* (e.g. the presence of blood and respiratory pigments that efficiently transport O_2, or remove toxic H_2S; Childress and Siebel, 1998), the presence of *chemosymbionts*, beneficial *body features* (e.g. thin body walls that facilitate gas transfer, or respiratory structures such as gills; Levin, 2003) and *behavioural adaptations* to avoid hypoxic water and/or H_2S (e.g. lifting of appendages (e.g. brittlestars) or the whole body above the redox boundary (Riedel et al., 2014)).

For eight of the ten traits five modalities (subcategories) were chosen for each trait to reflect the range of morphology/behaviour/adaptation expressed by the Garroch Head taxa; and for the exoskeletal composition (D) and adaptations to oxygen stress/H_2S (J) traits six modalities were used (Table 1).

For every taxon each trait (Table 1) was coded using the ‘fuzzy coding’ approach (Chevenet et al., 1994) whereby a score from 0 to 1 was awarded based on the individual taxon's affinity to each modality (where 0 represents no affinity) and sums to a total of one for each trait. For example, the anemone *Cerianthus lloydi* typically reproduces using planktotrophic larval development and so this modality is assigned a large proportion of the score (e.g. 0.8); however, it also, albeit less frequently, reproduces asexually by budding thus the remainder of the score (e.g. 0.2) is attributed to this modality. In this way we incorporated the multiple known behaviours common to each taxon e.g. being capable of both suspension and deposit feeding (Bremner et al., 2006; Paganelli et al., 2012) and the ability to switch throughout ontogeny, or as environmental conditions or food supply changed. This approach also allows the incorporation of a degree of uncertainty for taxa whose behaviour is not well-known.

Information on the biological traits for each taxon present at Garroch Head were obtained from a mixture of sources including: the Marine Life Information Network (Marlin.ac.uk 2014) and the Marine Species Identification Portal (species-identification.org, 2014), summaries of the attributes of North Atlantic species (e. g. Hayward and Ryland, 2017; Queirós et al., 2013), monographs on specific taxonomic groups or papers focussing on specific taxa (Fauchald and Jumars, 1979) or adaptations (Diaz and Rosenberg, 1995; Pearson and Rosenberg, 1978; Rosenberg, 1990; Rosenberg et al., 1991; Rosenberg et al., 1992). Additionally, specimens of relevant benthic taxa collected from Liverpool Bay (between 2006 and 2013) were measured to obtain morphological data. As far as possible taxa were coded using species level data. Where trait information was limited for a species, information for the genus was used and if the required data were still unobtainable coding was done at the family level. For taxa originally *recorded* at the genera level and above a conservative approach was adopted, and traits were coded using only the range of taxa, within each classification, present at Garroch Head during the sampling period. For example, the mean trait scores for all taxa from the family Ampharetidae were used to score a taxon recorded as ‘Ampharetidae’, and thus it assumes there were no additional unidentified members of the family Ampharetidae present. If no other members of the genus were present the type species was used. For taxa recorded at higher taxonomic levels there is obviously greater uncertainty surrounding their traits compared with those identified to species or genera. For each taxon the affinity to each modality was multiplied by the abundance of that taxon in each sample (biomass data being available for sample aggregates only). In this way an abundance by trait matrix was created in which each modality within a community was weighted by its abundance.

Mapping biological traits on to ecological functions

Ecological functioning was indexed by summing the trait modalities contributing to the delivery of each of the five selected functions (Tables 1–2), and these were as follows:

- *Nutrient recycling/regeneration* in shelf seas is strongly coupled with benthic processes that regenerate N from sediments and this can provide 20–100% of the annual N requirements for water column primary production (Aller and Aller, 1998). Nutrient cycling/regeneration results primarily from biogeochemical processes including microbial activity. In shallow and shelf systems macrofauna facilitate the mineralization of sedimentary N and the efflux of the mineralisation products into the water column. These fluxes are four fold higher when macrobenthos are present compared with when they are not (Hansen and Blackburn, 1992; Kristensen and Blackburn, 1987) because macrofauna facilitate nutrient cycling through sediment bioturbation and burrow irrigation. These processes oxygenate the sediment, enhance the vertical transfer of organic matter, establish concentration gradients and influence the composition of meiofaunal and microbial communities (Fenchel and Finlay, 2008; Olafsson, 2003). Taxa that burrow more deeply have greater influence on sediment nutrient recycling/regeneration (Mermillod-Blondin et al., 2004). Thus, these traits were used to index nutrient recycling/regeneration (Table 1) and included organisms with a body volume >5,000 mm^3 that have the potential to overturn larger amounts of sediment than those of smaller body volume (Table 2; Norkko et al., 2013; Thrush et al., 2006). Similarly, organisms that disturb a lot of sediment have greater potential influence upon sediment nutrient recycling/regeneration were included (traits E2–E4 and G3–G5; Tables 1–2).
- The value of the benthos as *food for large mobile predators* was indexed by an intermediate to large body size, without exoskeletons and epifaunal or semi-shallow life habits (Tables 1–2). Organisms with these traits would represent optimal prey species for large (≥50 mm) mobile predators.
- *Inorganic carbon sequestration* refers to the long-term sequestration of inorganic carbon by benthic macrofauna. Calcium carbonate accumulation rates derived from sediment cores show that on the continental shelves (0.11–0.13 Pg C yr^{-1}) it is comparable with that in the open ocean despite its ~25 fold smaller spatial area (Iglesias-Rodriguez et al., 2002). Most of the $CaCO_3$ is produced by benthic macrofauna (Table 2) with highly

calcified skeletons (Lebrato et al., 2010). In the present study inorganic carbon sequestration was linked to benthic taxa with a body size >5,000 mm^3, and $CaCO_3$ skeletal composition (Table 1).

- In marine systems the cycling of organic C or *food-web dynamics* is driven by ingestion, respiration, production and reproductive processes. Within sediments macrofauna may contribute between 11 and 43% of total benthic community respiration with the remainder being from bacteria and microbenthos (van Oevelen et al., 2006). Benthic macrofaunal C-cycling was therefore indexed by all life habits (except borers), suspension/filter feeding modes that capture carbon and transfer it between the benthic and pelagic realms, and dispersive reproductive modes (these traits would move C around the ecosystem more than direct developers where C from the adult is packaged into young and retained locally; Greve et al., 2004).
- *Biogenic habitats* are constructed by organisms that are sessile and have an attached epifaunal life habit with a suspension/filter feeding or chemosymbiotic life mode (e. g. Dame et al., 2001; Table 1-2).

Statistical analyses

Spatial and temporal trends in the data were explored using taxonomic and trait richness (as a measure of diversity) and evenness (Pielou's). A simple two way ANOVA was used to explore differences in taxa and trait richness across time and space. Temporal variability in richness and function delivery was characterised by the coefficient of variation. Changes in function delivery through time were explored using Spearman's rank-order correlation on data standardised to the mean and standard deviation.

Similarities between samples were calculated using the Bray-Curtis index after log (x +1) transformation of taxonomic data and the untransformed traits data. Taxonomic data were transformed to account for rare species. Non-parametric multivariate statistical approaches including non-metric multidimensional scaling (nMDS), analysis of similarity (ANOSIM) and the similarity percentages routine (SIMPER) were used to compare the Bray-Curtis similarity of the taxonomic and trait composition of the benthic assemblages using Primer v. 6 (Primer-e, Plymouth, UK) and are available in the supplementary material. In order to understand the quantitative effect of environmental drivers, associated with shifts in composition, we used an equivalent parametric procedure, Canonical Correspondence Analysis (CCA; Legendre and Legendre, 2012).

The CCA was constrained by ten variables, these were: the distance in km from the centre of the disposal site; the water depth (m) at each station; year of sampling; the dissolved oxygen content of the water just above the surface of the sediment; the redox potential (Eh) at 4 cm sediment depth; the total organic carbon (TOC), Hg, Cu, Mn and PCB content of the sediments at each station. Variables that were strongly spatially auto-correlated (sedimentary concentrations of α-HCH, γ-HCH, op' DDD, pp' DDD, Co, Ni, Zn, Cd, Pb, Cr, As, Fe) were excluded from the ordination: thus Hg, Cu and PCB 1260 were used to represent the full suite of pollutants measured. Autocorrelation between these variables was assessed using variance inflation factors. CCA was performed on the weighted averages of the species or traits scores for taxonomic composition and trait composition separately (data were transformed as for nMDS). Monte Carlo global permutation tests were performed on the complete dataset and the individual CCA axes, relationships with the environmental variables were determined using the bi-plot scores. CCA was completed using the *Vegan* v. 3.3.0 package (Oksanen, 2016) in R (R Foundation), and the *Goeveg* v. 0.3.3 package (Goral and Schellenberg, 2017) was used to produce simplified ordination plots with the ordiselect() and ordipointlabel() functions.

Results

The amount of sludge deposited at Garroch Head varied little, with the total organic carbon content (TOC) at the centre (P7) ranging from 6% to 15% of the surface sediments and did not show an appreciable increase or decrease throughout the disposal years (Fig. 2a). The total area of reducing sediments, at 4 cm depth, was initially high then decreased to a mean of 9.6 ± 5.3 km^2 (<0 mV) and 2.7 ± 2.8 km^2 (<-100 mV) up until 1998. Pearson and Stanley (1979) interpreted redox values of <0 mV to indicate reducing conditions, and -100 to -150 mV to indicate long term heavy organic pollution. Thus, conditions at the central and intermediate NE stations were reducing for more than three-quarters of the disposal years, whereas at the periphery and reference stations conditions were reducing for 2–3 years only (V7, P10, P5) or were never reducing (P4, G1). By 2005 the TOC in the sediments at the centre of the grounds had declined to 5% and remained slightly reducing (<0 mV), with 4 km^2 of reducing and 0.15 km^2 of very reducing sediments overall.

Trait and taxonomic diversity

The benthos from the eight sampling stations comprised a total of 397 taxa (263 species, 68 genera, 31 families and 35 higher level classifications). During the disposal years the taxonomic richness (or taxonomic diversity) and abundance of benthic macrofauna (Fig. 3) showed the classic organic enrichment pattern as described by Pearson and Rosenberg (1978). Taxonomic diversity decreased with increasing enrichment from 30–40 taxa at the periphery to <10 at the centre (Figs 2a–b and 3). Macrofaunal abundance reached a maximum and diversity a minimum (the so-called 'peak of opportunists' mostly *C. capitata, M. fragilis or T. benedii*) close to the centre on the W–E transect and ~1 km north on the N–S transect (Fig. 3; Pearson and Blackstock, 1989). Stations P8.5 and M7 were located between the peak of opportunists and the ecotone point to the north and west, however the pattern to the east is complicated by a second abundance peak at P8.5. Stations P4, V7, P5, T7 and P10 represented the transition zone. The assemblage at reference station G1 usually had 10–20 species less than those in the transition zone.

After sludge disposal ceased the number of taxa at the central and intermediate NE stations varied little until 2004 when diversity doubled, and by 2005 exceeded that at the periphery of the disposal grounds (Fig. 2a–b). Mean trait richness ranged from 38–52 trait modalities across all stations, and overall was 20% higher towards the periphery compared with the centre (Table 3), and after sludge disposal ceased at the centre the trait richness increased 25% (Fig. 2b).

Trait richness varied through time by up to 15% at the centre of the disposal grounds (Table 3) and it became less variable with distance from the centre: with a coefficient of variation of just ~3% CV at stations ≥1.7 km from the centre (Table 3). Pielou's evenness showed that the central and intermediate stations were dominated by a small number of taxa whereas the stations at the periphery were more even (Fig. 2). So, although the central and intermediate stations were often dominated by individual taxa their traits were more evenly represented in the community. In both cases the most dominated communities occurred at the central and intermediate NE stations.

Mean taxa and trait richness both significantly differed, across all years, between the five groups of stations (two-way ANOVA, $p<0.001$); and, there was a significant interaction effect with time ($p<0.001$) showing that the effect of time varied between stations (Table 3). Post-hoc Tukey tests showed that mean taxa richness significantly differed between all groups of stations except: the reference station and the intermediate SW stations ($p>0.05$), and between the intermediate SW stations and those at the periphery ($p>0.05$). Whereas, the mean trait richness differed between the centre and all of the other stations (post hoc Tukey test, $p<0.001$). These changes in benthic diversity show a threshold effect: the assemblages from stations with <6% sediment TOC had >20 taxa, while those from stations with TOC ≥6% contained <10 taxa (Fig. 4a). This effect is less marked for trait richness (Fig. 4b), while most of the stations with high TOC had lower trait richness it was more variable when TOC exceeded 6% compared with the other stations (Fig. 4b). Thus, taxonomic composition and to a lesser extent trait richness showed a tipping point at ~6% TOC, and the greater variability of the latter suggests that traits, and so functioning, was conserved.

Faunal and trait composition

Twenty seven percent of the total inertia in taxonomic composition, 5.74, was explained by constrained analysis with 10 variables (Monte Carlo global permutation test X^2 =1.54, F = 3.07, $p<0.01$, permutations 999, df = 10). CCA axis 1 accounted for most of the variation between stations and consistent with the nMDS showed a clear gradient of change from the centre to the reference station (Figs 2–3, Fig. 5, Table 4). Most of the variation in taxonomic composition was explained by distance from the centre of the disposal grounds, the TOC content and the sedimentary redox conditions (Fig. 5d). As the distance from the centre of the disposal grounds increased, TOC content decreased and sediments became less reducing (Fig. 2e–f). The concentrations of pollutants (represented by four pollutants in the CCA) were auto-correlated with TOC that was higher at the centre where the contaminated sewage sludge was deposited (Fig. 5), and so it is not clear to what extent these pollutants contributed to biological variations. Mn concentration was correlated with redox potential (Fig. 5c) that was probably associated with the links between sedimentary conditions (pH and redox) and Mn precipitation (Pakhomova et al., 2007). CCA axis 2 accounted for the changes in water depth (Fig. 5a and 5d; Supplementary Table S1) and much of the temporal variation. Although the data from Garroch Head are excellent the collection of environmental information varied through time (Supplementary Table S2) a complete environmental data set (e.g. for dissolved oxygen concentration and redox profiles) would probably have produced a stronger correlation.

Sixty-six of the 397 taxa contributed substantially (upper 30%) to the total macrofaunal abundance present at Garroch Head *and* made significant contributions (50%) to the ordination fit for CCA axes 1 and 2 (Fig. 5b–c). During disposal the community in the centre was comprised of between 3–10 taxa (Fig. 2c–d) with *C. capitata* accounted for >50% of the total number of individuals, and the polychaetes *M. fuliginosus*, *C. capitata,* Nematoda, *T. benedii* and *Tubificoides* sp. also made significant contributions (Fig. 5). The taxonomic composition at the centre was very consistent over time during the disposal years (Fig. 3c–f; SIMPER average similarity 73% *cf*. 38–50% at the other stations). The intermediate NE stations were dominated by the same taxa as at the centre, however the polychaetes *Cirriformia tentaculata, Prionospio fallax, Cirratulus cirratus*, *Diastylis lucifera*, *M. fragilis*, *Pholoe inornata* and *Eumida* sp., the anemone *Cerianthus lloydi,* gammarids and decapods were also important components of the assemblage. At the intermediate SW stations there were high abundances of the polychaetes *Eulalia viridis*, *Notomastus* sp., *Melinna palmata*, *Dodecaceria* sp., *Lipobranchius jeffreysii, Terebellides stroemi, Ampharete grubei*, *Prionospio malmgreni,* Ampharetidae and the bivalve *Thyasira flexuosa*.

During the disposal years assemblages from the peripheral stations had high abundances of bivalves (*Corbula gibba*, *Nuculana minuta, Abra nitida* and *Parvicardium scabrum*), the ophiuroids (*Ophiocten affinis*, *Amphiura filiformis* and *Ophiura albida*)*,* polychaetes (*Glycera* sp., *Polycirrus* sp., *Oxydromus flexuosus*, *Ophelina acuminata* and *Prionospio cirrifera*)*,* and the caudofoveate *Chaetoderma nitidulum*. The macrobenthic community at the reference station was distinct from the other stations in having abundant of *Amaeana trilobata*, *Spiophanes kroyeri, Levinsinia gracilis, Ancistrocyllis groenlandica, Kirkegaardia dorsobranchialis, Rhodine loveni, Ampharete lindstroemi*, *Prionopsio multibranchiata, Glyphohesione klatti*, *Nepthys incisa, Nepthys hombergii*, nuculid bivalves, the decapod *Calocaris macandrae* and the ophiuroid *Amphiura chiajei* (Fig. 5c).

Post-disposal the fauna became richer (Fig. 2c) and the samples from the central and intermediate stations became compositionally more similar to the peripheral stations, although they were distinct and highly variable during the first six years after disposal operations ended (Fig. 5a). By 2005 the central and intermediate stations were indistinguishable from those at the periphery, which had very similar composition to the reference station (Fig. 5a). Peripheral communities had between 31 and 57 taxa and were more even than those from the centre of the disposal grounds (Fig. 2a–b, Table 3).

The constrained CCA of biological traits composition explained 62% of the total inertia (Monte Carlo global permutation test $X^2 = 0.380$, F = 13.77, $p<0.001$, 999 permutations, df = 10; Table 4). The ordination differed from that for taxonomic composition, and showed clear differences between the trait composition of the central and intermediate NE stations along CCA axis 1. Variations between the central and intermediate NE stations and most of the others were described by CCA axis 2 (Fig. 6a–c). Sedimentary TOC content and the distance from the centre explained most of the variation on CCA axis 1(Table 4, Fig. 6a–c), the former was positively correlated and the latter was negatively correlated (stations further from the centre had lower TOC; Fig. 6). Most of the variation on CCA axis 2 was explained by water depth and Mn.

Communities at the centre were characterised by taxa with vermiform bodies, of 50–100 mm maximum length and with a volume in the range 5000–99, 999 mm^3 (Fig. 6). When TOC was highest the predominant life habit was epifaunal free-living and in some years when TOC was lower taxa which tend to inhabit maintained burrows and that burrow to shallow (0–10 mm) depths only were more common. At the centre most taxa had traits for surface modifying and upward/downward conveyor style bioturbation, and a higher proportion of grazers and parasites/endosymbionts, and larval development was both planktotrophic and lecithotrophic.

From the centre to the intermediate NE stations (1.2–1.6 km distant) there was a transition towards: a greater number of taxa with long vermiform bodies exceeding 200 mm length that did not have exoskeletons. This fauna inhabited maintained and unmaintained burrows at depths of 10–500 mm, and had deposit feeding and scavenging/predatory habits. These changes in behaviour corresponded with a shift from surface modification and upward/downward conveyor bioturbation at the centre to more biodiffusion at the intermediate NE stations. Whereas the peripheral and reference stations were characterised by taxa with spherical bodies of varying size, diverse exoskeletal composition, attached epifaunal and boring life habits and were suspension feeders.

Taxa at the intermediate NE stations had metabolic adaptations to low O_2/high H_2S, at the intermediate SW they used H_2S and O_2 transport and unique body features such as external gills, and at the periphery taxa with chemosymbionts and/or behavioural adaptations for low oxygen were common (Fig. 6b). The lack of such adaptations at the centre of the disposal grounds was attributable to the high abundance of ‘nematodes’ that were indexed with traits for ‘no adaptations’ (J1).

In the post-disposal years the trait composition at all stations became more similar to the peripheral and reference stations suggesting that functional recovery had begun. However, the recovery of the benthos at the centre of the disposal grounds was protracted; after two years although it had changed from that present during sludge disposal it remained distinct in both taxa and traits from all other stations (Figs 5-6). By 2005, seven years after sludge disposal had ceased, the benthos at the centre of the disposal grounds, intermediate NE stations and T7 were more similar in taxa and traits to the peripheral stations during disposal compared with the reference station.

Overall, the large shifts in taxonomic and trait composition at the centre of the disposal grounds, and at the intermediate NE and SW stations, and the smaller shifts at the periphery (Figs 5–6) after dumping ceased suggested that the benthos at these stations were all affected, to some extent, by the sludge disposal. During the disposal years the peripheral stations seemed to have been stimulated by the enrichment rather than being adversely impacted (Fig. 2a–d and 5), and this also seems to be the case for the central and some intermediate stations after disposal ceased.

Changes in function delivery through time

The high macrofaunal abundances at the central and intermediate NE stations resulted in these stations having the highest magnitude of functioning (Table 5). Function delivery at the centre was up to two orders of magnitude larger than at the peripheral and reference station (Table 5). Overall functioning was >20% more variable at the central stations compared with those on the periphery (Table 5). However, for many of these functions a small individual will deliver less than a large individual, for example the degree of burrow irrigation and hence the degree of sediment oxygenation and the rates of corresponding aerobic microbial processes. Although we include information on the maximum body size in our function estimates (Table 2) we do not know their actual size and so are cautious of over interpreting the changes in absolute values.

Considering the relative changes in functioning over time (Fig. 7), nutrient regeneration, food for predators, biogenic habitat provision, and food-web dynamics all show similar patterns. During the first decade the level of function delivery at any station was stable with the relative variability between years being similar within each groups of stations. From 1989–1998, this broadly stable pattern persisted outside of the central station, although variability was a little higher than during the first decade. At the central station the relative level of functioning increased from ~1993 until 1997 when it subsequently declined, while at the reference station in some cases function delivery tracked the changes near the centre (Fig. 7b-e). The post-disposal (1998-2005) pattern of relative change was similar at all the stations, and although there seemed to be a decrease at the peripheral and reference stations the lack of sampling at these stations in 2000 and 2004 mean their dynamics cannot be fully assessed.

Inorganic carbon sequestration (Fig. 9e) showed a different pattern to the other functions: increasing through time across all the groups of stations (Spearman's correlation, $p<0.05$ in all cases; Table 5). After 1998, as for the four other functions, (Fig. 7a-d) C-sequestration declined to the level of functioning found in 1980 suggesting that there was a regional change in dynamics unconnected with the sewage sludge disposal.

Discussion

At Garroch Head a strong gradient of organic enrichment with associated deoxygenation, and reducing conditions, persisted throughout the 19 disposal years. The present study analysed data from the macrofaunal assemblages covering ~28 km^2 of seafloor (excluding the reference station) of which 30–50% was 'reducing' and 10–20% was 'highly reducing' (at 4 cm sediment depth; SEAS, 1999). Sludge disposal produced considerable changes in the diversity, abundance, taxonomic and biological trait composition of the benthic assemblages. Furthermore, the ecological changes (Gray et al. 2002, Nilsson and Rosenberg 1997, Pearson and Rosenberg 1978), presence of sulphur oxidising bacteria (Pearson and Blackstock, 1989; Pearson et al., 1990; Pearson and Coates, 1995, 1998; Levin et al. 2009a), and proximity of the RPD to the surface in many years suggests that conditions at the centre were near-azoic at times (Table 6). The inclusion of results from the post-disposal years showed that, while the macrofaunal assemblage had begun to recover, 7 years after disposal operations ceased there remained marked differences in the abundance, diversity and composition of the benthos at the centre of the disposal site and proximal stations. The incorporation of information on biological traits has uniquely shown how the changes in the taxa present and composition of the assemblage affected the delivery of benthic ecosystem functions, and that functional change was associated with a threshold.

The ecological changes at Garroch Head conformed to, what we now consider to be, the archetypal pattern of the impacts of organic enrichment on the sea floor (e.g. Fig. 3; Table 6). Along a deoxygenation/enrichment gradient ecological succession proceeds from a diverse and even community (with polychaetes, bivalves and echinoderms in similar proportions) with deep dwelling infauna at the periphery, towards a polychaete-dominated community (Table 6) with many infauna adopting epifaunal habits. Compared with the reference station, during the disposal period, the peripheral assemblages had elevated diversity, total abundance and biomass suggesting that they represented the 'transition zone' (Fig. 3; Table 6) and were 'biostimulated' by the allochthonous food supplied from the excess organic matter (Pearson and Rosenberg, 1978). Macrofaunal taxa with traits for larger body sizes, >200 mm maximum body length, predominated at stations 1.2-1.6km from the centre, and taxa of smaller size (50–100 mm maximum length) characterised the centre of the disposal grounds. This shift in body size is consistent with predictions of the organic enrichment model where towards the transition zone individuals benefit from the enriched food supply (Fig. 2; Pearson and Rosenberg 1978). The distribution of macrofaunal biomass supports this observation (Pearson and Blackstock, 1989). Taxa that used respiratory pigments (e.g. *Abra alba* and *Abra nitida*) and specialised body features (e.g. brachiae) were abundant at the intermediate stations, whereas in the transition zone those that used chemosymbionts (*Thyasira* spp.) and behavioural adaptations (e.g. extension of the body into the water column by ophiuroids) were more common.

Taxa within 1.2 km of the centre were characterised by metabolic adaptations to low O_2 and the presence of toxic H_2S (e.g. nuculid species) and taxa without specific adaptations to oxygen stress (e.g. *Ophelina acuminata*). Although such adaptations provide an advantage in disturbed environments so do the more generic 'opportunistic life history' adaptations that allow taxa to establish as pioneers shortly after aperiodic stress events (Gray et al., 2002). All of the dominant taxa, the scolecid worm *Capitella capitata*, nematode worms, the oligochaete *Tubificoides benedii* and the spionid *Malacoceros fuliginosus*, in the assemblages near the centre of the disposal grounds have been documented in high abundances in other enriched systems (e. g. Diaz and Rosenberg, 1995; Pearson and Rosenberg, 1978) and have physiological or behavioural adaptations to such conditions. For instance, *T. benedii* and *M. fuliginosus* have specific adaptations to oxygen stress and species in the *C. capitata* complex, which dominate the assemblage at the centre in most years, have highly opportunistic life histories (Diaz and Rosenberg, 1995; Giere, 2006). For the BTA nematodes were indexed as having 'no adaptations', as this describes the majority of taxa in the phyla. However, subsequent analyses of individuals from some samples showed that the nematodes were predominantly *Pontonema* (Bett and Moore, 1988; SEAS, 1999) which occur in mass aggregations in fjords at <1 ml l^{-1} O_2 (Lorenzen et al., 1987) and anoxic sediments of the Black Sea, Chilean shelf and Gulf of Mexico (Levin et al., 2009a).

The changes in the macrobenthos at Garroch Head were associated with both deoxygenation and organic enrichment (e. g. Diaz and Rosenberg, 1995; Diaz and Rosenberg, 2008; Gray et al., 2002; Jessen et al., 2017; Pearson and Rosenberg, 1978). In many systems it is often difficult to disentangle the effects of the two, in most systems it seems that enrichment stimulates populations of opportunists whereas deoxygenation impacts community diversity. The CCA showed that both TOC and sediment redox conditions were linked with taxa and trait composition, and that the changes in TOC especially corresponded with the changing abundance of enrichment opportunists. Redox conditions (Eh) also strongly corresponded with the changes in diversity along CCA axis 1 (Fig. 5), and so the patterns were largely consistent with that observed in other studies.

Long-term change and the recovery from enrichment/deoxygenation

Seven years after sludge disposal ceased the conditions in the sediments (to 4 cm depth) within ~1.6 km radius of the centre of the disposal grounds remained reducing, and the organic carbon content was twice the background (Fig. 2e–f). Between the 1998 cessation of disposal and 2000 the taxonomic richness at the centre varied little, from 2000–2004 it doubled, and from 2004–2005 it increased 4-fold. Thus, seven years post disposal the fauna near the centre was very similar in diversity, taxonomic and trait composition to the peripheral stations during, but not post, disposal (Figs 5–6), which is to say they were in a ‘biostimulated’ state due to the legacy enrichment. This suggests that the macrofaunal recovery had begun, and although less distinct the benthos between 1.2 and 1.7 km of the centre showed a similar pattern. Thus, the macrofaunal changes near the centre was consistent with that documented in severely deoxygenated systems whereby the ecological succession during the recovery is not simply the reverse of the initial response to pollution i.e. it shows hysteresis *(sensu* Diaz and Rosenberg, 2008).

At the stations ≥2 km from the centre, where enrichment and deoxygenation were less severe, by 2005 the assemblages were indistinguishable, in terms of macrofaunal abundance, the taxa present and their traits, from the reference station (during the disposal years). Temporal changes at the nearby pre-1974 sludge disposal site (Moore and Rodger, 1991) were similar to the present study: a decade after disposal ceased stations >2 km from the centre of the pre-1974 disposal grounds showed no substantial changes, however the benthos nearer the centre underwent a four-fold increase in taxa richness and an 80% decrease in dominance. Major components of the recovery fauna included *Abra alba, Scalibregma inflatum*, *Mediomastus fragilis, Spiophanes kroyeri* and *Ennucula tenuis* taxa that respond to organic enrichment, and have formed part of the recovery assemblage in other systems (Diaz and Rosenberg, 1995; Gogina et al., 2014; Pearson and Rosenberg, 1978; Rees et al., 2006; Whomersley et al., 2007). The recovery of marine communities from short term severe hypoxia can take several years, and for systems that experience long term severe hypoxia it may take several decades (e. g. Diaz and Rosenberg, 1995; Diaz and Rosenberg, 2008; Rosenberg, 1976). At Garroch Head the recovery of the benthos within a 1.6 km radius from the centre had not occurred within 7 years post-disposal, and further north at the pre-1974 site (after ~70 years of sludge disposal) benthic communities had not recovered after a decade (Moore and Rodger, 1991).

One reason proposed for the slow recovery of deoxygenated zones is the accumulation of carbon in the sediments. For example, at the pre-1974 disposal site 11 years post-disposal large quantities of TOC were retained in sediments concentrated at depths of 20-60 cm (Moore and Rodger, 1991; Rodger et al., 1991). Organic carbon accumulates in deoxygenated regions due to the lack of macrofaunal bioturbation and slower rate of anaerobic degradation, and this condition may persist for long periods even once it has again become oxygenated (Jessen et al., 2017). For instance, at the pre-1974 sewage sludge disposal site legacy TOC remained buried in the sediments, with peak concentrations between 50–70 mm depth, for considerable periods (Moore and Rodger, 1991). Thus, although sediment TOC at the intermediate stations seemed to reflect ambient conditions in 2005 (Fig. 2e) it is likely that carbon remained elevated at depth.

The delivery of seafloor functioning

While the Pearson and Rosenberg (1978) model can now be regarded as the classic description of the impacts of organic enrichment on benthic species it does not make any predictions as to the consequences of these changes for ecological functioning. For instance, do large numbers of opportunist provide as much bioturbation and hence nutrient regeneration as the fewer, larger, organisms in an un-impacted assemblage? How does the value of the benthos as a food resource for fish change across the enrichment gradient? How does deoxygenation change the cycling of carbon through marine foodwebs?

Both the taxonomic and trait composition differed significantly between assemblages at the central, intermediate and peripheral stations, however the magnitude of the differences in biological traits were less than those for taxa. This suggests that in spite of the large changes in the taxa present in the assemblages the biological traits, and hence ecological functioning, were conserved to a large extent. A growing body of data now show that minor or even moderate perturbations in diverse systems can produce species changes that mostly conserve functioning (Cardinale et al., 2012; Ehrlich and Ehrlich, 1981; Micheli and Halpern, 2005; Villnäs et al., 2012; Walker, 1992). This can be achieved by direct species substitutions where a ‘sensitive’ species is replaced by a species with similar traits but greater tolerance to enrichment/deoxygenation. Although, in most species rich systems the patterns are more complex and the distributions of multiple species may change (Cardinale et al., 2006), but the net effect on the assemblage’s ‘trait inventory’ is small and so functioning is conserved. Studies of functioning in communities exposed to hypoxia-anoxia also show that functioning is conserved during intermittent and/or moderate deoxygenation (Caswell and Frid, 2013; Villnäs et al., 2012), but that a ‘tipping point’ is reached where species composition changes markedly, usually manifesting as a collapse in diversity, at which point functioning declines precipitously (Cardinale et al., 2012; Caswell and Frid, 2013; Conley et al., 2009; Ehrlich and Ehrlich, 1981; Villnäs et al., 2012).

The ‘tipping point’ dynamic is a classic case for a system to display hysteresis (Scheffer et al., 2001). The limited (7 year) recovery data presented here show that at the severely impacted stations both changes in functioning and taxonomic composition at Garroch Head were hysteretic. Meta-analyses of species responses to deoxygenation show that tolerance varies widely between taxa, but that at dissolved oxygen thresholds of ~4.6 mg l^{-1} only the most sensitive species in marine communities (top 10%) would be adversely affected (Vaquer-Sunyer and Duarte, 2008) however this is expected to change in the future (Steckbauer et al., 2011). In Corpus Christi Bay, Texas a threshold of 3.5–4.0 mg l^{-1} O_2 was proposed for changes in the diversity, abundance and biomass of benthos (Ritter and Montagna, 1999). Furthermore, thresholds for biomass change have been proposed at ~4.5 mg l^{-1} dissolved oxygen (corresponding to sediment TOC of 2.65–7.83%; Seitz et al., 2009), and models of changes in benthic biomass in the Baltic Sea found increases at ~4 mg l^{-1} O_2 when reoxygenated (Timmermann et al., 2012).

At Garroch Head bottom water dissolved oxygen measurements are incomplete and are not a reliable indicator of the benthic conditions, and being retrospective cannot be resampled. Instead we suggest a threshold for total organic carbon (TOC) triggered changes in benthic macrofaunal taxonomic composition and functioning of ~6% TOC by weight. At Garroch Head hysteresis-like patterns of change occurred at stations that had 4.0–10.5% mean TOC in surficial sediments, whereas the macrofaunal assemblages at the stations with TOC <3% during disposal did not (Table 6). Studies on a fossil fauna from a long-term regional period of deoxygenation during the Jurassic found that the threshold for benthic taxonomic and functional changes occurred around 3.8% TOC, although these measurements may not be equivalent to those in the original environment due to preservational factors (Arthur and Sageman, 1994). Determining the benthic ecosystem thresholds for carbon, when information on dissolved oxygen data are not available, would aid the interpretation of the prevailing conditions during geological periods of ocean deoxygenation (Caswell and Frid, 2016). Thresholds have also been determined for the loss of macrofaunal bioturbation on the Pakistan Margin at an estimated at 0.08–0.14 mg l^{-1} O_2 (corresponding to TOC of 2.64–3.53%; (Levin et al., 2009b)), and in the Baltic Sea at 1.40–2.90 mg l^{-1} O_2 (Josefson et al., 2012). At Garroch Head bioturbation seemed to have ceased at the centre of the disposal grounds in many years (where TOC was 5.60–13.80% and interpreted oxygen concentrations were <1.2 mg l^{-1} (Table 6)).

Biological traits analysis uses the inherent biological, life history and behavioural characteristics of a species to define its ecological role. The focus was therefore on the *known* behaviour of the macroinfauna and not directly observed behaviour, however as noted by Riedel et al. (2014) the behavioural changes that precede a taxon's disappearance from the benthos can contribute to changes in functioning. We recognise that many of the traits vary between individuals i.e. body size, ontogenetic stage, or short term behavioural changes driven by environmental conditions (Cesar and Frid, 2012; Norkko et al. 2013). The indexing of traits using 'fuzzy coding' (Chevenet et al., 1994) seeks to capture these variations, but in doing so it reduces the 'sensitivity of the approach' – everything is coded for the taxon's profile rather than the actual individuals in the sample (Bremner, 2008; Bremner et al., 2006). Thus, the BTA reports *potential* ecological functioning as modelled from the 'fuzzy trait' composition.

The high abundances of opportunists within 1.6 km of the centre of the disposal grounds produces estimates of functioning that are up to two orders of magnitude larger than those at the peripheral and reference stations. It is generally recognised that biomass rather than abundance is a better metric to use in functional models (Bremner, 2008; Bremner et al., 2006). For instance, with all other factors being constant the impact of one individual of a small species of worm on sediment oxygenation is smaller than the absolute level of oxygenation provided by the bioturbation of one large worm (e.g. Karlson et al., 2016; Norkko et al., 2013; Thrush et al., 2006). The selection of traits and their combination/weighting in deriving functioning used in the present study attempts to address this short-coming. However, as most benthic studies report abundance data, or biomasses derived by multipliers of the abundance (rather than actual biomasses for each species in each sample; Eleftheriou and Moore, 2013) this remains a constraint. In the context of the present study the extremely high numerical abundances in the ‘peak of opportunists’ may therefore over-estimate their functional role. Whilst there are no data on the biomass of individual taxa, the total biomass within 1.2 km of the centre was 30–84% higher than at the periphery (Table 6) and so we do expect that the delivery of some functions were enhanced at stations towards the centre of the disposal site. It is also worthy of note that our estimates of functioning are based on macrofauna sampled by grab only, and so they do not include the contribution of mobile epifauna or demersal fish.

Benthic nutrient regeneration

Near the centre of the disposal grounds the dominant living habit traits were for unmaintained burrows that were limited to <10 mm sediment depth, and bioturbation that disturbed the surface or performed upward/downward conveyance of sediment. Similar shifts towards taxa that do not perform any bioturbation, or those that burrow only in the top few centimetres of the sediment are observed in many deoxygenated systems (Gogina et al., 2014; Josefson et al., 2012; Solan et al., 2004; Sturdivant et al., 2014). Our estimates for Garroch Head predicted high relative functioning at the centre of the disposal grounds based on high abundances of intermediate sized taxa and those that typically bioturbated by upward/downward conveyance. However, the core descriptions and RPD profiles showed that the sediments at the centre of the disposal ground were often un-bioturbated although an occasional burrow or vagrant worm was found (Table 6).

Remineralisation rates (Jessen et al., 2017) and nutrient fluxes maybe up to four fold higher when bioturbating macrofauna are present compared with when they are not (Hansen and Blackburn, 1992; Kristensen and Blackburn, 1987), although it varies between taxa, and the deeper they burrow the greater the sediment nutrient recycling/regeneration (Mermillod-Blondin et al., 2004). This bioturbation provides nutrients to primary producers in the water column and thus links benthic and pelagic productivity, and underpins overall foodweb productivity. Of the two dominant taxa at the centre of the disposal grounds one was a surface modifier and the other performed upward conveyance, and so the net transport of sediment from any burrowing in these reducing sediments was from depth towards the surface. Furthermore, on the Pakistan Margin it was shown that below a dissolved oxygen threshold of 0.08–0.11 mg l^{-1} organic matter processing by metazoan macrofauna ceased and benthic foraminifera came to dominate the processing of organic matter (Woulds et al., 2007), however Mojtahid et al. (2008) showed that foraminifera were almost completely absent from the centre of the Garroch Head disposal grounds. Therefore, it seems that at these stations: less oxygenated sediment would have been transported to depth and so the sediments will have remained reducing, the rate of organic matter degradation would have slowed and consequently less nutrients would have been recycled by the benthic macrofauna (Jessen et al., 2017). These changes would not necessarily manifest as a net decrease because the dynamics of the biogeochemical cycling of nitrogen and phosphorous change under hypoxia which can create negative feedbacks that promote further deoxygenation (Carstensen et al., 2014; Childs et al., 2002; Conley et al., 2009; Hale et al., 2016). In the transition zone the diverse biostimulated assemblages, particularly those with high biomasses and plentiful TOC, provided ideal conditions for deep burrowers, as shown by RPD depths of 40–75 mm and sediment profiles with much greater mixing evident (Table 6), which may have processed and regenerated large proportions of carbon and nutrients. However, even in the presence of bioturbation carbon may persist in sediments for decades, in oxic regions of the Black Sea, and perhaps centuries in hypoxic-anoxic areas (as estimated from sedimentation rates) where sediment TOC reaches 6% (Jessen et al., 2017) such as at Garroch Head.

Food for large predators and food web dynamics

Predators typically decline along hypoxia gradients (Breitburg et al., 2009; Wu, 2002), and are usually most abundant on the periphery of deoxygenated zones. This is because deoxygenation-related macrofaunal mortality is higher and prey are more vulnerable, due to depressed escape responses (Riedel et al., 2014) and/or other behavioural shifts e.g. infauna adopting epifaunal habits (Seitz et al., 2003). Thus, hypoxic zones may provide a refuge from mobile predators with high oxygen demands (Altieri, 2008; Gray et al., 2002; Gutiérrez et al., 2000; Pihl et al., 1992). For instance, demersal fish such as cod, whiting and flounder leave the area once bottom water oxygen drops below ~30–40% saturation, and invertebrate predators avoid or migrate away from areas where dissolved oxygen drops below 10–20% (Gray, 1992; Miller et al., 2002). The quantification of benthic food for predators (indexed by larger sized taxa without exoskeletons that typically lived on or near the sediment surface;

Table 2) in the present study was comparable between all stations <1.7 km from the centre, and was far lower at the peripheral stations. Although small-sized benthic prey were more numerous at the centre of the disposal grounds, the deoxygenated conditions probably excluded many benthic-feeding predators. As oxygenation increased between 1.2 km and 1.6 km from the centre the less numerous but larger-sized prey without exoskeletons were probably an important source of food for large mobile predators such as fish, cephalopods and crustacea. Benthic macroinvertebrate scavenger/predator abundance was high in this zone suggesting that prey vulnerability/mortality was higher.

The additional supply of food (organic material) and/or the provision of a refuge from predation can enhance fisheries productivity in moderately or intermittently deoxygenated areas (Altieri, 2008; Elmgren, 1989; Nixon and Buckley, 2002). However, it is more common for deoxygenation to negatively impact fisheries, e.g. in the New York Bight in 1976 a mass mortality event caused a US$60 million loss of shellfish stocks (Falkowski et al., 1980). The benthos are an important food supply for fish but under deoxygenation considerable losses of benthic biomass and productivity occur. For instance, in Chesapeake Bay hypoxia develops across more than half the bay in the summer causing 6–12% decreases in total macrobenthic productivity (Sturdivant et al., 2014), and in the Baltic Sea hypoxic or anoxia develops over one quarter of the seafloor and ~8% of productivity is lost (Karlson et al., 2002). It has been shown in deoxygenated systems, including Chesapeake Bay, the Kattegat, the Black Sea and the Adriatic Sea (Caddy, 2000; de Leiva Moreno et al., 2000; ICES, 2014; Kemp et al., 2005) that when deoxygenation persists there is a shift from largely demersal feeding to planktivorous fish as benthic habitats become inaccessible/azoic. Overall, long-term fisheries production declines and may collapse (e. g. Breitburg et al., 2009; Caddy, 2000; Cheung et al., 2013; Daskalov, 2002; Eby and Crowder, 2002; Jørgensen, 1980; Kemp et al., 2005; Rosenberg and Loo, 1988; Wu, 2002) although it is difficult to extrapolate from local to system-wide changes and compensatory mechanisms may exist (e. g. Breitburg et al., 2009). For example, in hypoxic regions of the Baltic Sea changes in food supply combined with the effects of habitat compression have resulted in the long-term deterioration of cod body condition (Casini et al., 2016; ICES, 2014). Given the degree of enrichment and deoxygenation at Garroch Head (and its long history) retrospective analyses of the variations in the fish catches would probably also exhibit similar trends. However, as with many anthropogenic stressors these changes may be difficult to isolate from synergistic stressors such as the impacts of fisheries exploitation (Daskalov, 2002; Kemp et al., 2005).

Deposit feeding taxa, parasites/commensal feeders were abundant within 1.2 km of the centre of the disposal grounds at Garroch Head, scavengers/predators were common at the intermediate stations, and suspension feeders were abundant at the peripheral and reference stations. The trend for greater deposit feeding at enriched stations is consistent with most other deoxygenated systems that have a high organic carbon supply (Diaz and Rosenberg, 1995; Gogina et al., 2014; Karlson et al., 2002; Levin et al., 2009a; Wu, 2002), and the benthos inhabiting these systems usually experience decreased competition for resources allowing them to establish large populations. Shifts in feeding behaviour such as those found in the present study can have profound effects on the cycling of organic carbon through marine systems. For instance, a shift towards a predominantly deposit feeding mode, as occurs near the centre, can result in 25% less pelagic carbon being processed by the benthos compared with suspension feeder dominated systems such as those nearer the periphery (Pearson and Rosenberg, 1992; Rosenberg, 1977). These changes weaken the coupling of benthic and pelagic productivity (primary and secondary), and overall deposit feeder dominated systems can support less epifaunal and nektonic predators (e.g. Chesapeake Bay and the Baltic Sea; (Pearson and Rosenberg, 1992)). Where defaunation has occurred and bacterial mats predominate the organic carbon is remineralised and reused by the bacteria further weakening benthic-pelagic coupling and shortening food chains (Baird et al. 2004; Rosenberg, 1977). So, the shifts in feeding mode and living habit from an infaunal to an epifaunal benthos, to a seafloor covered in bacterial mats would have represented major shifts in food web dynamics and the associated cycling of C and nutrients. During prolonged deoxygenation eventually energy flow through the macrobenthos and higher predators would cease as it was transferred to predominantly microbial biomass (Baird et al., 2004; Levin et al., 2009a; Pearson and Rosenberg, 1992), and as fish and other predators were excluded by the deoxygenation (Gray, 1992). In many deoxygenated areas the complete loss of benthos during seasonal or permanent deoxygenation (Karlson et al., 2002) can further add to the organic matter load.

Benthic inorganic carbon sequestration and the provision of biogenic habitat

At Garroch Head macrofaunal taxa with calcareous exoskeletons were confined to the outer stations (>2 km from the centre). The exclusion of calcareous taxa from near the centre of the disposal grounds reduced the potential for inorganic carbon sequestration by the benthos, as indexed by large animals and those with calcareous exoskeletons. Of the five functions considered in the present study inorganic carbon sequestration made the smallest contribution to total ecological functioning by benthic macrofauna. However, this is not always the case: at other UK sewage sludge disposal sites such as the Tyne the enhanced productivity stimulated populations of bivalves (Rees et al., 2006). These populations will, in turn, have utilised carbonate in construction of their shells and so may have sequestered more inorganic carbon than at Garroch Head.

Within enriched settings the CO_2 produced during carbon decomposition can create localised acidity (Cai et al., 2011; Melzener et al., 2013), and during the disposal years the sediments closest to the centre of the disposal grounds at Garroch Head were 0.2 pH units lower than at the reference station (Table 6). Subnormal seawater pH values of 7.5–7.8 (*cf*. normal values being 7.8–8.3) that are within the range found at Garroch Head have been shown to cause dissolution and/or inhibit the formation of the calcareous tests of e.g. macrofauna (Findlay et al., 2009) and benthic foraminifera (Boltowsky and Wright, 1976; Le Cadre et al., 2003). Thus, the absence of macroinvertebrate calcifiers near the centre of the disposal grounds may be attributed to localised acidity. The benthic foraminiferal assemblage at Garroch Head was also adversely impacted by low bottom water pH (Mojtahid et al., 2008). Foraminifera were rare at the centre of the Garroch Head disposal grounds, and the intermediate NE stations the assemblage was dominated by foraminifera with agglutinated tests (non-calcareous), and taxa with calcareous tests were only abundant nearer the periphery where sediment surface water pH was higher (Mojtahid et al., 2008; Table 6). It seems therefore that the contribution of benthic foraminifera to benthic inorganic carbon sequestration must also have been reduced at this time. Furthermore, the absence of attached epifauna at Garroch Head provided minimal biogenic habitat, e.g. mussel beds that construct complex habitat that would have supported local biodiversity.

Conclusions

- Comparable ecological changes to those in the benthos at Garroch Head have been recognised from many other deoxygenated systems, particularly those with restricted water circulation, and are as described by the Pearson and Rosenberg (1978) model. The inclusion of information on biological traits allowed us to make predictions about the impact of these changes for ecological functioning. Significant shifts in organism morphology and size, life habit, burrow depth, bioturbatory and feeding mode occurred, and was broadly consistent with expectations from other systems in relation to organic enrichment/deoxygenation. We show that under deoxygenated conditions the biological traits of the taxa present, and hence ecological functioning, were conserved to a large extent suggesting that there was ecological redundancy within the benthic communities.

- Although organism abundance was high near the centre bioturbation was minimal and limited to near the sediment surface. Thus, the macrofaunal processing of the accumulating organic matter (during the disposal years) was probably lower than at the intermediate stations that had high abundances, deeper RPD, and taxa with traits for larger body sizes, deeper burrows and more complex bioturbation. These intermediate communities could have made substantial contributions to processing the excess organic carbon, while at the centre this carbon would have been sequestered into the deeper sediment layers by burial (where it may have remained for decades).
- The food available to large mobile predators was probably greatest on the margins of the deoxygenated zone (intermediate stations), where dissolved oxygen concentrations were >10%, macrofaunal abundance was high, most taxa lacked exoskeletons and body sizes were larger. Switches in the feeding mode of the benthic taxa near the centre of the disposal grounds would have severely impacted food-web dynamics (C-cycling), by weakening benthic-pelagic coupling and shortening food chains. These changes would ultimately have resulted in lower overall benthic productivity and within the macrobenthos, as observed in a number of other long-term deoxygenated systems e.g. the Baltic Sea and Chesapeake Bay where productivity is reduced by ~10% (Karlson et al., 2002; Sturdivant et al., 2014). Thus, the benthos would have supported fewer epifaunal and nektonic predators, which would have impacted productivity at higher trophic levels (including fisheries species). Further, work is needed to establish the extent of these changes higher in the food web.
- Changes in TOC drove changes in populations of opportunists and both TOC/deoxygenation drove changes in assemblage diversity. The apparent threshold for taxonomic and functional change in this study was ~6% TOC, or based on our interpretation of deoxygenation at the centre (Table 6) was at dissolved oxygen concentrations of <1.2 mg l^{-1}.
- We show that the recovery of the assemblage, in terms of both the taxa and their biological traits, was slow and apparently hysteretic in assemblages near the centre. This trend matches observations from other severely deoxygenated systems that experience long-term deoxygenation, and like these systems the recovery was on the order of a decade or more.
- Few studies of deoxygenation span decadal timescales (however, see Borja et al., 2006; Carstensen et al., 2014; Gogina et al., 2014; Hale et al., 2016)), but long timescales are required: for the impacts on some ecosystem functions to manifest, e.g. biogeochemical cycling (Jessen et al., 2017) and effects higher in the food chain (Casini et al., 2016); for benthic communities to recover (Diaz and Rosenberg, 2008); and, the degradation of

excess buried carbon may take several decades (Jessen et al., 2017). If we wish to better understand the impacts of these changes on the delivery of ecosystem services we need to look at change over longer timescales and larger spatial scales/system-wide (Breitburg et al., 2018; Thrush et al., 2013).

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Figure captions

Figure 1. Study area within the context of the UK (a), within southwest Scotland (b), inset box shows area illustrated in (c) and black point shows location of disposal site. The sampling stations within the Firth of Clyde (c) near Garroch Head (GH); eight of the nine stations used in this study are shown and the ninth (P7) is at the centre of the cruciform transect (grey point)(SEAS, 1999). Station G1 is the reference station used to reflect background conditions. The 50 m, 100 m and 150 m bathymetric curves are from (Matthews et al., 1999). The deep region in the southwest is referred to as the Arran trough. For the exact locations and depths of the sampling stations see Supplementary Table S1.

Figure 2. (a) Mean (± SE) taxonomic and (b) trait richness, (c) Pielou's taxonomic and (d) trait evenness, and (e) sedimentary total organic carbon (TOC) content through time at Garroch Head within five groups of stations: the centre (P7), the intermediate NE (M7 and P8.5), intermediate SW (P5 and T7), periphery (P4, P10, V7) and the reference station (G1).

Note: y-axes scales for (c) and (d) differ. (f) The wet weight of sludge disposed, total area of reducing (Eh <0 mV) and very reducing (<-100 mV) sediments (at 4 cm sediment depth) at Garroch Head. Sewage sludge disposal occurred from 1979 until 1998, and during 2000–2005 operation of the Garroch Head disposal site had ended.

Figure 3. Mean (± SE) abundance, taxonomic richness and biomass with distance from the centre of the sewage sludge disposal grounds during the years of operation only (1979‑1998); negative values are the southern and western (except G1 which is NW)), and positive values are the northern and eastern branches of the transect. Station names are shown below the x axis. Approximate positions of the TR = transition zone and E = ecotone point of Pearson and Rosenberg (1978) based on Pearson (Pearson and Blackstock, 1989; SEAS, 1999). Biomass peaks occurred at the centre on the W–E transect and 1 km north (near M7) on the N–S, transect with secondary peaks ~2‑3 km east (Pearson and Blackstock, 1989).

Figure 4. (a) Number of taxa and (b) number of biological traits within macrofaunal assembalges in each area of the disposal grounds by the sedimentary total organic carbon content (TOC; measured at 4 cm sediment depth). Data for regions with >1 station are means (intermediate NE, intermediate SW and periphery).

Figure 5. Canonical correspondence analysis (CCA) ordination of benthic macrofaunal taxonomic composition for two axes. (a) Samples from the eight stations sampled at Garroch Head between 1979 and 2005; samples from 2000-2005 are indicated. (b) All taxa present in the benthic communities. (c) The most frequent species (upper 30% of total abundance) that contributed 50% of the fit to CCA axes 1 and 2 (crosses correspond to physical position of each four-letter taxa within the ordination). (d) Vectors for 10 environmental variables (secondary horizontal and vertical axes correspond to the biplot scores for these vectors). Taxonomic names in (c) and (d) are abbreviated to the first 2-4 letters of the taxa name if family/class/order, and for species are the first two letters of the genus and species name (see Supplementary Table S3 for abbreviations). Abbreviations: Distance from centre of the disposal grounds, Depth = water depth, Eh = Eh at 4 cm sediment depth, PCB = polychlorinated biphenols, TOC = total organic carbon; Cu, Hg and Mn are elemental abundances bound to sediments.

Figure 6. Canonical correspondence analysis (CCA) ordination of macrofaunal biological trait composition with two CCA axes. (a) Samples from the eight Garroch Head stations sampled from 1979-2005; with location of 2000–2005 samples indicated. (b) Biological traits of the benthic communities (crosses correspond to physical position of each trait within the ordination). For trait codes see Supplementary Table S1. Note the scale of the primary axes differ from (a) and (c). (c) Vectors for 10 variables (secondary horizontal and vertical axes correspond to the biplot scores for these vectors) including: the distance from centre of the disposal grounds, the water depth, the redox potential (Eh) at 4 cm sediment depth, concentrations of polychlorinated biphenols (PCBs), sedimentary total organic carbon (TOC) content, the dissolved oxygen (DO) concentration of the overlying water; and, the sedimentary concentrations of Cu, Hg and Mn. (The three metals and the PCBs are used to reference the full suite of pollutants all of which were strongly autocorrelated).

Figure 7. Changes in the amount of function delivery (standardised by each station mean and standard deviation) during 19 years of sewage sludge disposal and the seven years post-dumping. Data are the mean function delivery for the central (P7), intermediate SW (P8.5 and M7), intermediate NE (P5 and T7) and peripheral stations (P4, P10 and V7). (a) Nutrient regeneration, (b) food for predators, (c) biogenic habitat provision, (d) food-web dynamics, (e) carbon-sequestration. For details on the indexing of each function see Tables 1–2, and for non-standardised values see Table 5.

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Table 1

Table 1. Summary of the six biological traits and modalities chosen to represent the morphological and biological diversity of the benthic macro invertebrate communities found at the Garroch Head sewage sludge dumping ground between 1979 and 2005.

Traits	**Modalities**
A. Max. body length (mm)	**(1)** <10, **(2)** 10–50, **(3)** 50–100, **(4)** 100–200, **(5)** >200
B. Max. body volume (mm^3)*	**(1)** <100, **(2)** 100–4,999, **(3)** 5,000–99,999, **(4)** 100,000–999,000, **(5)** >1,000,000
C. Relative 2d body shape† (length: width)	**(1)** <0.8, **(2)** 0.8–1.2, **(3)** 1.25–5, **(4)** 5.01–10, **(5)** >10.01
D. Exoskeletal Composition	**(1)** None, **(2)** Chitin/collagen, **(3)** Aragonite, **(4)** Low Mg calcite, **(5)** High Mg calcite, **(6)** Calcium phosphate
E. Living habit	**(1)** Tube/borer, **(2)** Maintained burrow, **(3)** Unmaintained burrow, **(4)** Epifaunal attached, **(5)** Epifaunal free living
F. Burrow depth (mm)	**(1)** 0–10, **(2)** 10–50, **(3)** 50–150, **(4)** 150–250, **(5)** 250–500
G. Bioturbatory mode	**(1)** Epifauna, **(2)** surface modifier, **(3)** upward/downward conveyor, **(4)** biodiffusors, **(5)** regenerators
H. Feeding Mode	**(1)** Deposit, **(2)** suspension/filter, **(3)** scavenger/predator, **(4)** grazer, **(5)** parasite/endosymbiont
I. Larval Development	**(1)** Planktotrophic, **(2)** lecithotrophic, **(3)** brood to larva, **(4)** brood to juvenile, **(5)** asexual budding
J. Adaptations to low oxygen/H_2S	**(1)** None, **(2)** metabolic, **(3)** H_2S and O_2 transport, **(4)** chemosymbiont, **(5)** body feature, **(6)** behavioural

*Body volume assumed bivalves and crustacea were cuboids, starfish were composed of one large central cylinder and five smaller cylinders, gastropods and scaphopods were cones, annelids and holothurians were single cylinders.

†Relative body length approximates; C1 circular, C2 subrectangular, C3 short vermiform, and C4 long vermiform, C5 filiform.

Table 2

Table 2: Ecological functions delivered by recorded taxa, and the modalities (Table 1) used to index them. For ecological basis of assignment see SI methods.

Function	**Contributory trait modalities**
Nutrient regeneration	Body vol. ≥5000mm³ (**A3-A5)** + all living habits except attached epifauna and tubes/borer **(E2–E4**) + those bioturbators which overturn significant quantities of sediment from depth (**G3–G5).**
Food for predators	Body vol. ≥5000mm³ (**A3–A5**) + those with exoskeletons **(D2–D6)** + epifaunal habit **(E4, E5) +** shallow burrowers **(F1–F2)**.
Biogenic habitat	Body vol. ≥5000 mm³ **(A3–A5) +** attached epifaunal habit (**E4) +** suspension/ filter feeding **(H3)** + chemosymbiont **(H4)**.
Food web dynamics	All body vol. (**A1–A5**) + all living habits **(E1–E5)** + suspension/filter feeding **(H2) +** all reproductive modes except direct developers **(I1–I2, I5).**
C-sequestration	Body vol. ≥5000 mm³ (**A3–A5**), $CaCO_3$ skeleton **(D3–D5)**

Table 3

Table 3. Mean (± SD) trait and taxonomic richness, coefficient of variation (%) for richness, Pielou's evenness for each of the eight stations during the disposal years 1979-1998; and, the numerically dominant taxa (accounting for >50% of the total abundance) in the central, intermediate NE, intermediate SW and peripheral stations. For locations of stations see Fig. 1.

Stn	Mean richness		CV richness		Mean Pielou's evenness	
	Taxa*	Traits†	Taxa	Trait	Taxa	Trait
Central stations						
P7	6.6 ± 2.3	37.9 ± 5.6	35.2	14.7	0.48 ± 0.12	0.83 ± 0.04
Intermediate NE stations						
P8.5	24.7 ± 11.2	48.4 ± 2.8	45.7	5.7	0.41 ± 0.09	0.82 ±0.04
M7	23.2 ± 9.2	48.4 ± 2.5	39.6	5.2	0.36 ± 0.18	0.79 ± 0.05
Intermediate SW stations						
P5	53.3 ± 13.6	50.6 ± 1.5	25.6	3.0	0.61 ± 0.12	0.85 ± 0.04
T7	35.7 ± 11.6	50.0 ± 1.2	32.5	2.5	0.44 ± 0.11	0.83 ± 0.03
Peripheral stations						
V7	43.6 ± 11.8	50.1 ± 1.5	27.1	3.0	0.76 ± 0.06	0.86 ± 0.02
P4	42.7 ± 9.8	49.6 ± 1.6	23.0	3.3	0.70 ± 0.12	0.86 ± 0.03
P10	46.0 ± 11.3	50.0 ± 1.4	24.7	2.9	0.78 ± 0.08	0.87 ± 0.02
Reference station						
G1	33.6 ± 12.4	49.4 ± 1.5	8.6	3.0	0.79 ± 0.06	0.87 ± 0.02

*Two-way ANOVA (sqrt transformed) between location (central, intermediate NE, intermediate SW, and periphery) and time (1979-1989, 1990-1998 and recovery years) had an interaction effect (F=6.93, p<0.001), location had a main effect (F=25.1, p<0.001), but time did not (F=0.63, p>0.05). †Two-way ANOVA (sqrt transformed) between locations and time (using the same groups as for taxa) showed an interaction effect (F=3.4, p<0.001), location had a main effect (F=37.2, p<0.001) but time did not (F=0.002, p>0.05). NB residuals for traits were not normally distributed.

Table 4

Table 4. Results from the constrained CCA for the first three axes for the taxonomic and trait composition at the eight Garroch Head sampling stations from 1979 to 2005. The total inertia for taxonomic composition was 5.74 and for traits was 0.61.

Axis	**Eigenvalue**	**Proportion explained**	**Permutation test**		
			X^2	**F**	**p**
Taxonomic composition					
CCA1	0.493	0.321	0.493	9.84	<0.001
CCA2	0.260	0.176	0.271	5.40	<0.001
CCA3	0.138	0.167	0.257	5.13	<0.001
Trait composition					
CCA1	0.235	0.620	0.235	85.32	<0.001
CCA2	0.116	0.310	0.120	42.11	<0.001
CCA3	0.021	0.055	0.021	7.56	<0.01

Table 5

Table 5. Functioning through time for five functions (mean, standard deviation and coefficient of variation (%)) for each of the eight stations. Spearman's rank order correlation for functioning (standardised values; Fig. 7) at the five groups of stations through time (during the disposal years only); *indicates significant at p<0.05, †indicates significant at p<0.01. For relative positions of stations see Fig. 1.

	Nutrient regeneration			Food for predators			Biogenic habitat			Food-web dynamics			Inorganic C-sequestration		
	Mean	**CV**	**r**	**Mean**	**CV**	**r**	**Mean**	**CV**	**r**	**Mean**	**CV**	**r**	**Mean**	**CV**	**r**
Central stations															
P7	12,882	95%	0.56^{*}	13,471	88%	0.52^{*}	8,599	87%	0.52^{*}	17,075	89%	0.54^{*}	5,757	88%	0.52^{*}
Intermediate NE stations															
P8.5	20,866	80%	-	11,612	79%	-	10,658	81%	-	17,562	78%	-	7,038	83%	$0.78^{\dagger}$
M7	21,830	78%		10,993	74%		11,120	78%		16,961	73%		7,380	79%	
Intermediate SW Stations															
T7	8,108	71%	$0.62^{\dagger}$	5,364	67%	$0.62^{\dagger}$	4020	73%	$0.66^{\dagger}$	8,376	63%	$0.62^{\dagger}$	2,443	88%	$0.63^{\dagger}$
P5	1,064	87%		875	74%		459	96%		1677	71%		275	78%	
Peripheral stations															
V7	522	72%	-	585	55%	0.46^{*}	192	60%	-	1,083	66%	-	237	65%	0.55^{*}
P4	443	50%		452	62%		159	55%		880	56%		186	90%	
P10	364	60%		456	60%		149	63%		736	55%		230	64%	
Reference station															
G1	111	37%	-	119	30%		46	41%	-	225	35%	-	47	38%	0.60^{*}

Table 6

Table 6. Summary of the macrobenthic community (mean taxonomic diversity, abundance (0.1 m^{-2}), biomass (g m^{-2}), Pielou's evenness (J), diagnostic taxa/phyla (%), RPD depth, mean total organic carbon content of the sediment (TOC; wt. %)), and mean sediment surface pH from the different sampling areas and stations during the disposal years (Coates and Pearson, 1997, 1999; Pearson, 1981, 1983, 1991, 1992, 1993, 1994; Pearson and Blackstock, 1982, 1983, 1985, 1986, 1987, 1988, 1989; Pearson et al., 1990; Pearson and Coates, 1995, 1998; Pearson et al., 1992; Pearson and Stanley, 1980; SEAS, 1999). The interpretation of benthic state and dissolved oxygen concentrations (at bottom water temperatures of 9°C) in relation to the species richness-abundance-biomass (SAB; Fig. 3) model of Pearson and Rosenberg (1978), successional stages and benthic habitat quality Nilsson and Rosenberg (1997), and faunal composition (Gray, 1992). Echin. = echinoderms.

Parameter	**Centre**	**Intermediate NE**		**Intermediate SW**		**Periphery**	**Reference**
	P7	P8.5	M7	T7	P5	P4, P10, V7	G1
Taxa	7	25	23	36	53	43–46	34
Abundance	6550	8382	8673	4077	809	265–370	78
Biomass	38.6	28.0	32.3	33.5	29.2	18.7–26.2	6.8
Evenness	0.48	0.36	0.41	0.44	0.61	0.70–0.78	0.80
C. capitata	55%	13%	24%	28%	1%	0–1%	2%
Nematodes	37%	4%	6%	5%	1%	<1%	3%
***Thyasira* sp.**	0%	≤0.1%	≤0.1%	1%	>1%	1–2%	≤0.1
Bivalves	0%	0%	<1%	4%	13%	18-19%	16%
Echin.	0%	0%	<1%	<1%	1%	2–8%	2%
RPD[*] (range)	<4[†]	<4[‡]	<4[◊]	>4[#]	>4[§]	>7.5[§]	>10[‡]
Mean TOC	10.50	4.10	4.70	4.00	3.10	2.60	2.70
Mean pH	7.66	7.72	7.79	7.86	7.82	7.72-7.78	7.89

Interpretation of benthic conditions and successional stages

SAB	*Azoic–Peak of Opportunists*	*Peak of Opportunists*	*Ecotone?*	*Transition Zone*		*Climax Community*
Stage	0–1	1–2	2	2–3	3	4–5?
BHQ	<2	2–4	4–10	4–10	>10	10–15
Assemblage attributes	• *Capitella* & nematode dominated (RPD ≤1) • Azoic & sulphur bacteria (RPD ≈0)	• *Capitella* dominated • Decline of bivalves & echin. • RPD ≤4	• *Capitella* dominated • *Thyasira* increase • RPD ≤4	• Diverse with bivalves, echin. & crustacea • *Thyasira* increase • RPD ≥4		• Diverse with bivalves, echin. & crustacea • RPD ≥ 10
O_2 (%)	0–10	5–10	10-25	10–25	25–40	>80?
O_2 mg l^{-1}	0.0–1.2	0.6–1.2	1.2–2.9	1.2–2.9	2.9–4.6	>9.3

[*] From 1979-1985 redox was only measured at 4 cm sediment depth and so can only be concluded to be more or less than 40 mm, from 1985 redox was measured at the surface, 1 cm and 4 cm depth and so data are more accurate. [†] At P7 in 5 years the RPD was at the surface, and during a further 7 years it was <1.5 cm, and another 7 years was <4 cm. In [‡]18, [§]15, [◊]13 and [#]11 disposal years this was the case. Mats, up to 30 cm thick, of the sulphur oxidising bacteria *Beggiatoa* were present in 1989-90, 1995, and 1997

Figure 1

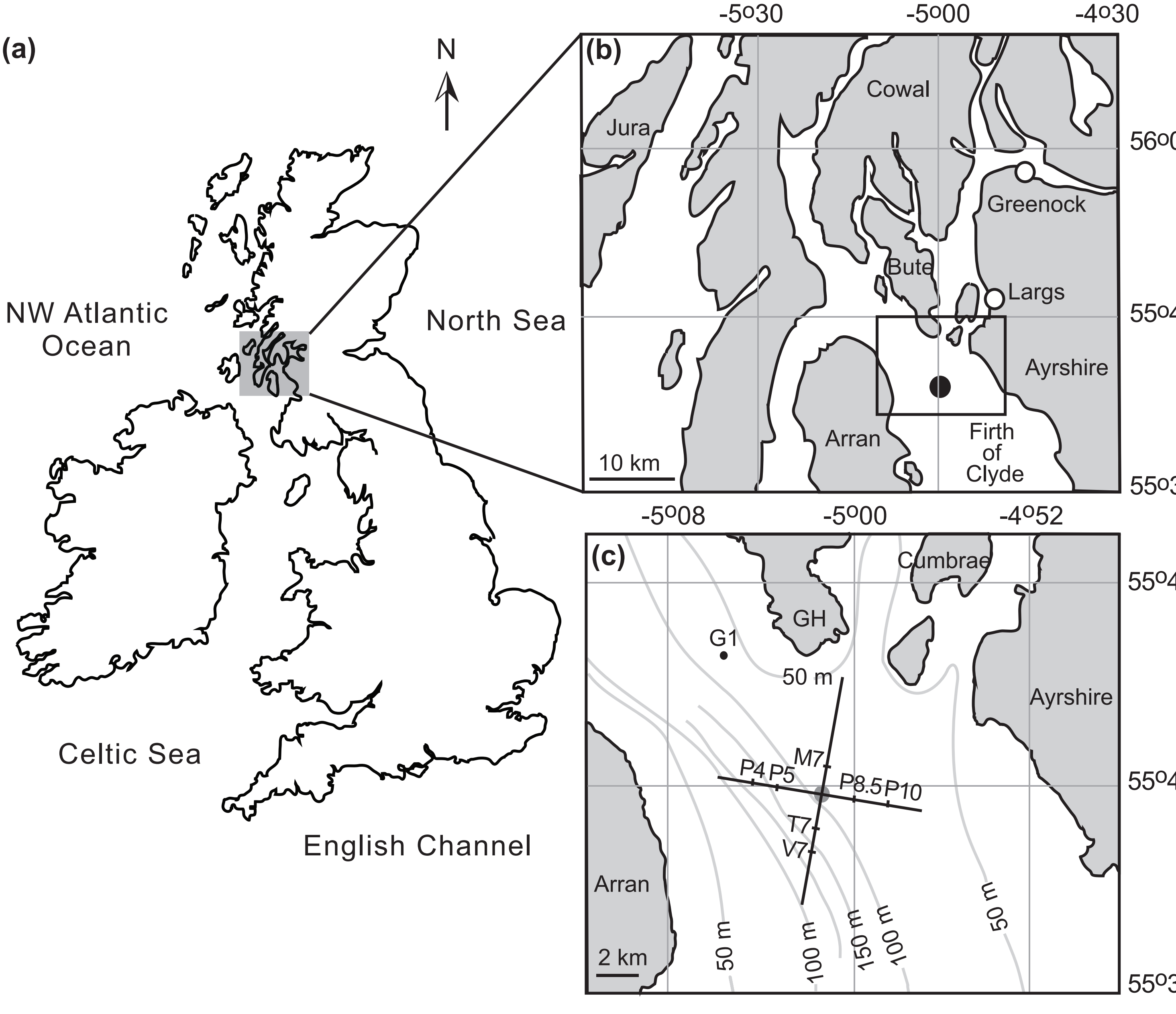

Fig. 1 Caswell and Frid 2018

Figure 2

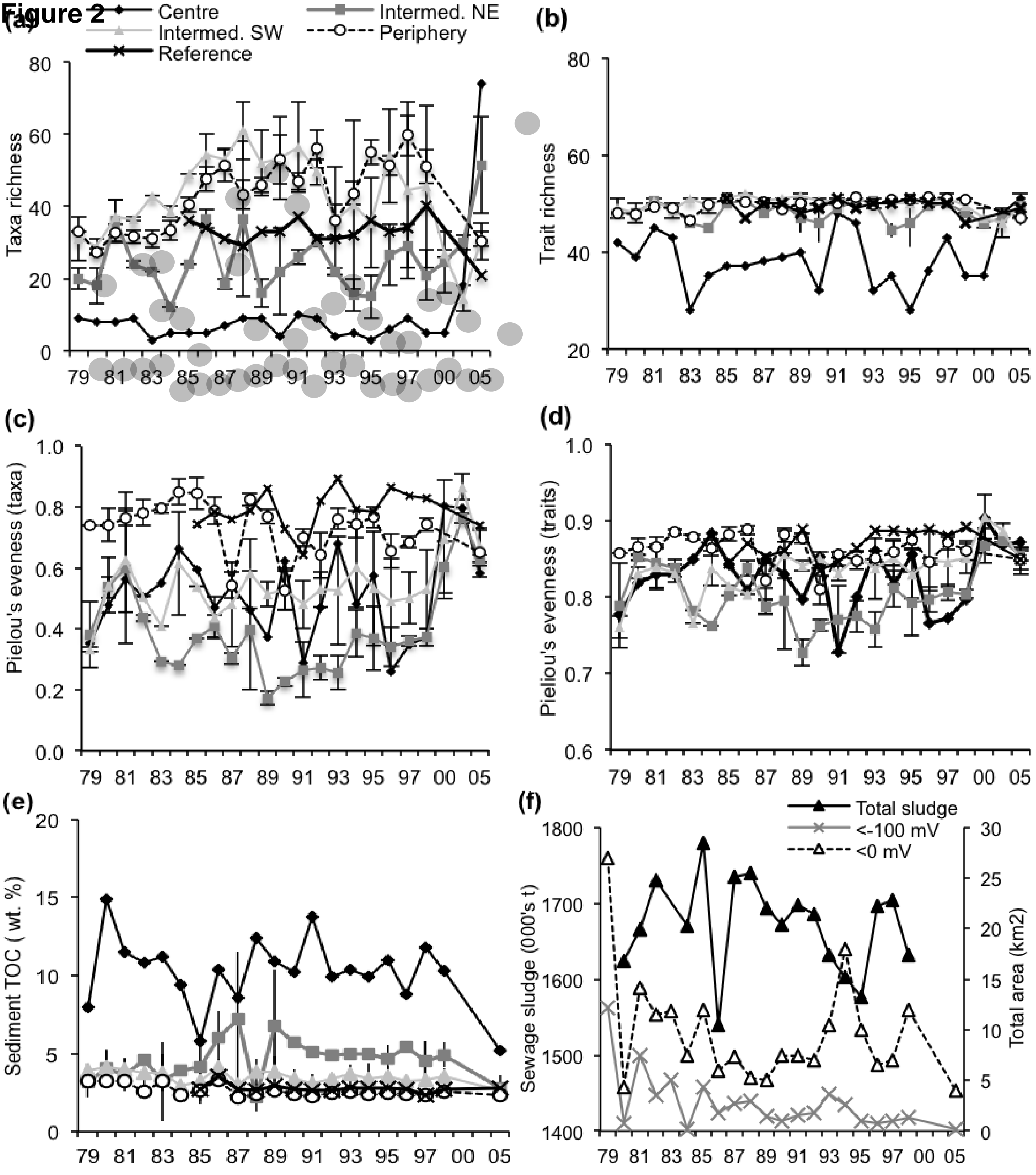

Fig. 2 Caswell and Frid 2018

Figure 3

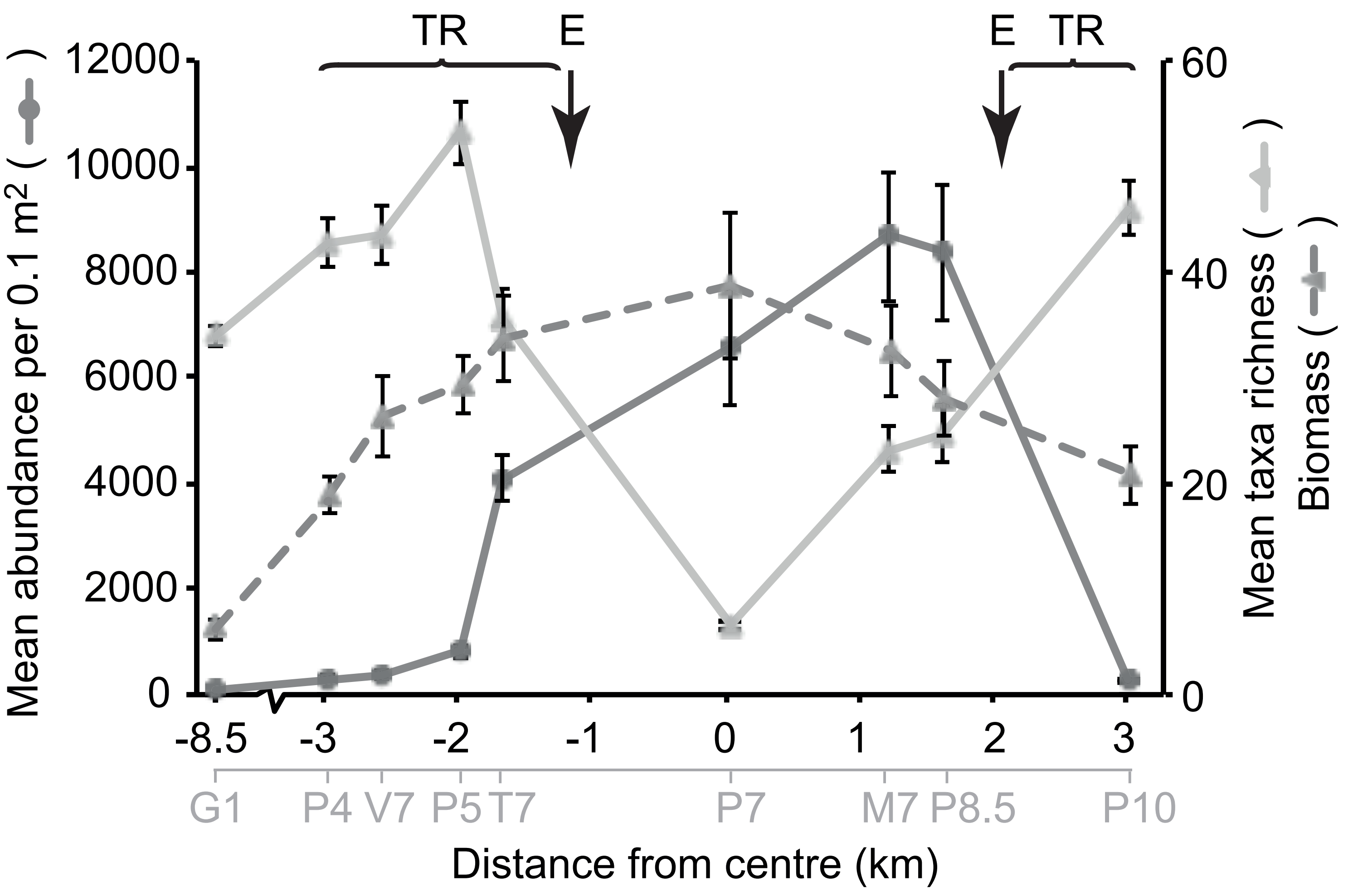

Figure. 3 Caswell and Frid 2018

Figure 4

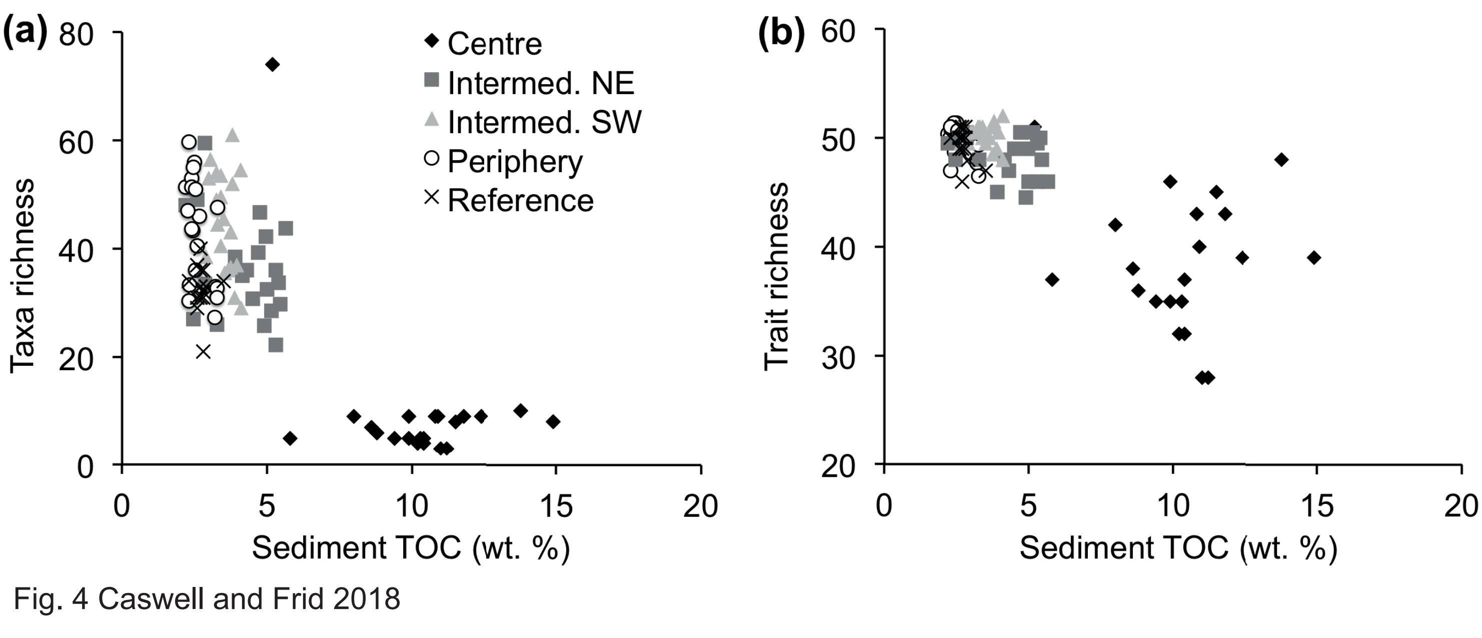

Fig. 4 Caswell and Frid 2018

Figure 5

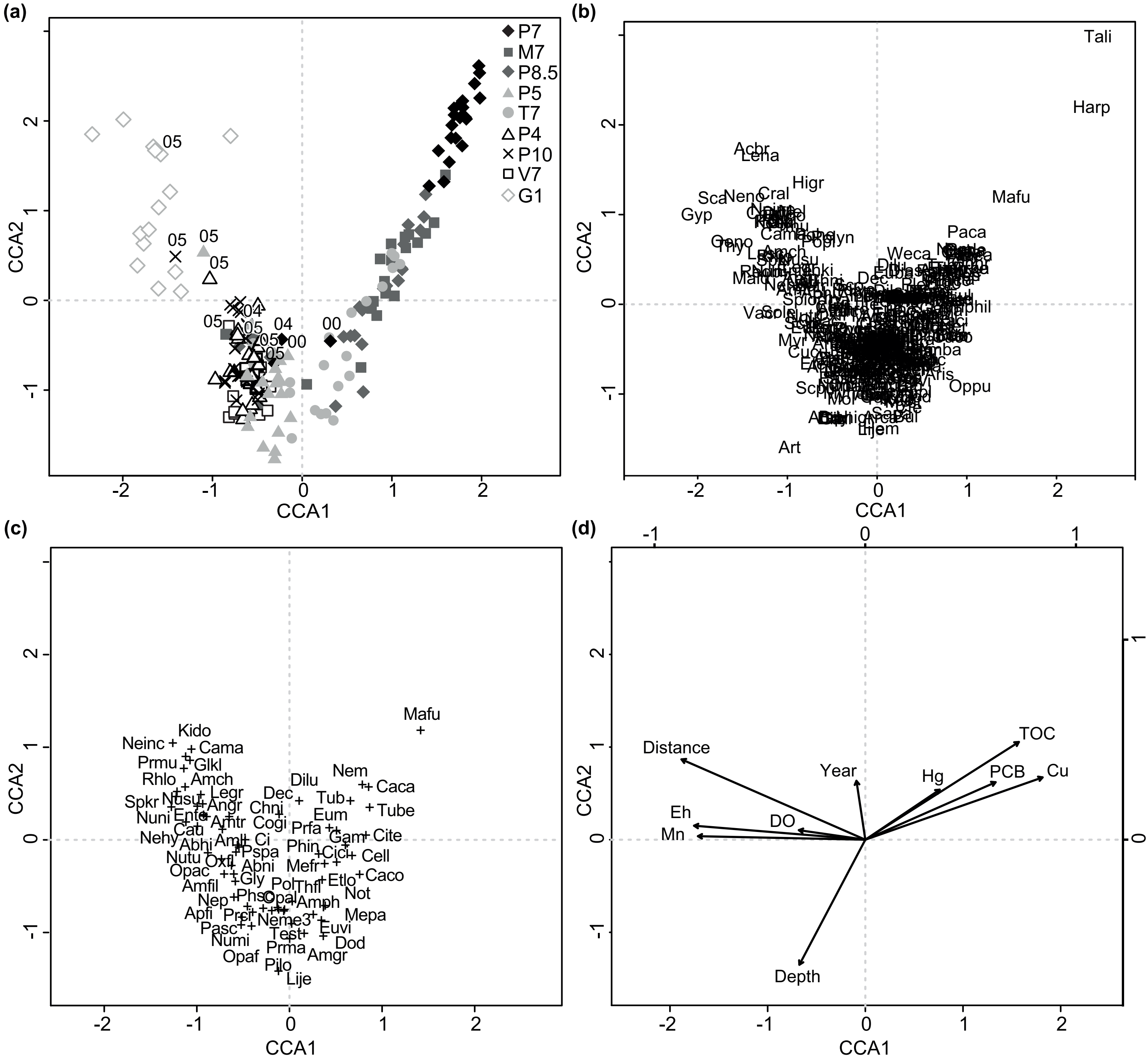

Figure 5 Caswell and Frid 2018

Figure 6

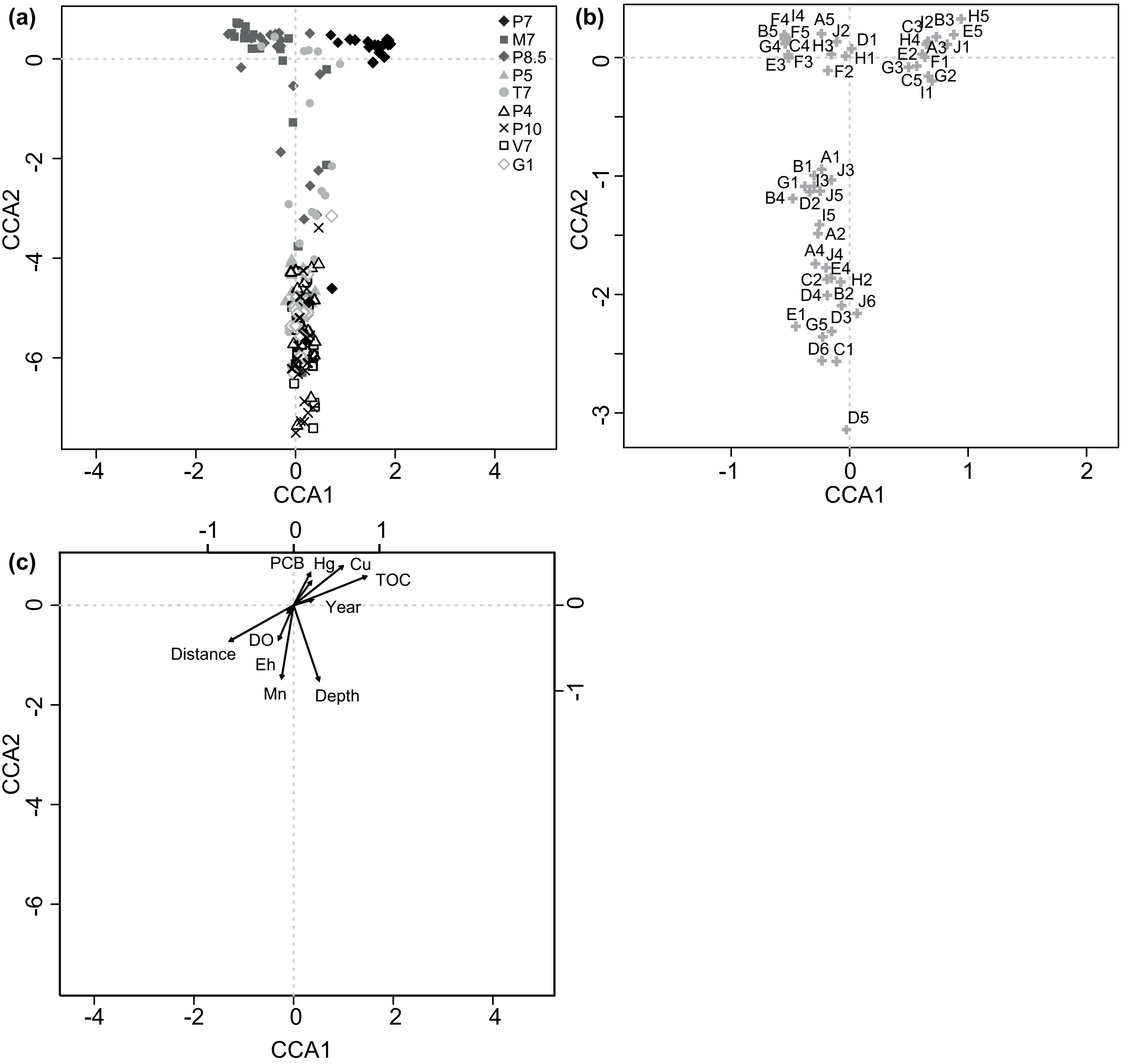

Figure 6 Caswell and Frid 2018

Figure 7

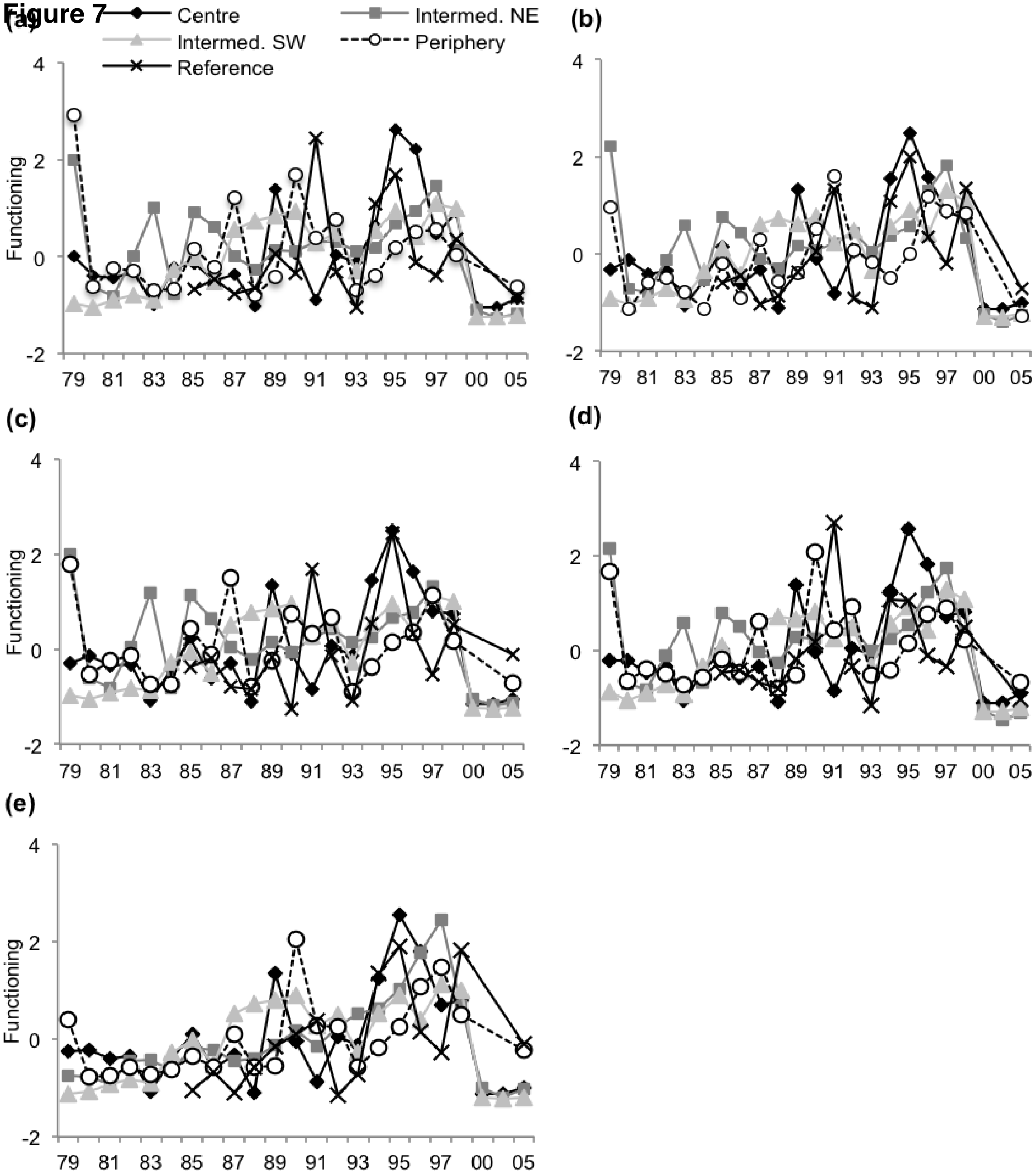

Figure 7 Caswell and Frid 2018

Supplementary Data