

Macrobenthic community structure and influence on denitrification capacity in soft sediments (Mobile Bay, Alabama, USA)

M. P. Jacquot^{1,2,3}, K. M. Dorgan^{1,4,*}, B. Mortazavi^{1,5}, A. A. Kleinhuizen^{1,5}, W. C. Clemo^{1,4}

¹Dauphin Island Sea Lab, 101 Bienville Blvd., Dauphin Island, AL 36528, USA

²Université de Bretagne Occidentale, Laboratoire des Sciences de l'Environnement Marin (LEMAR, UMR 6539, UBO/CNRS/IRD/Ifremer), Institut Universitaire Européen de la Mer (IUEM), 29280 Plouzané, France

³Environmental and Marine Biology, Åbo Akademi University, BioCity, Artillerigatan 6, 20520 Åbo, Finland

⁴Department of Marine Sciences, University of South Alabama, Mobile, AL 36688, USA

⁵Department of Biological Sciences, University of Alabama, Tuscaloosa, AL 35487, USA

ABSTRACT: Benthic macrofauna play important roles in coastal ecosystems through their impact on nutrient cycling and by serving as an important trophic link. This study investigated the influence of environmental stressors on macrofaunal community abundance, biomass and diversity and evaluated impacts of those benthic organisms on nutrient fluxes and denitrification capacity in Mobile Bay, a shallow subtropical estuary in the northern Gulf of Mexico. Between January and May 2016, 22 stations in the lower bay were sampled, and the distance from the sea and sediment characteristics were identified as the principal constraints for macrofaunal distribution and taxonomic composition. Higher diversity of macrofauna was found near the mouth of the bay, likely due to hydrological characteristics of the bay, in addition to distance from anthropogenic disturbances near coasts. Sediments comprised of 60–80 % sand showed higher abundances, biomass and diversity compared to muddier or sandier sediments, potentially because these sediments were more stable. Although macrofaunal abundance, biomass and diversity differed across stations in the bay, we did not find a relationship between macrofaunal abundances or biomass and denitrification capacity. Both abundance and biomass of infauna were low in Mobile Bay (<4000 ind. m⁻² and <5 g DW m⁻² at most sites) compared to those in previous studies which showed a positive relationship between macrofaunal abundances and denitrification. The lack of the relation between macrofauna and denitrification suggests that a threshold of abundance, biomass and/or burrowing activity is necessary to increase bioturbation or irrigation to sufficient levels to affect denitrification.

KEY WORDS: Benthic macrofauna · Denitrification · Nitrogen flux · Sediments · Community diversity · Environmental stressors · Mobile Bay

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Estuaries are among the most productive ecosystems and provide habitats for many commercially and recreationally important species (e.g. oysters, crabs and shrimp). They are also active sites of sediment organic matter decomposition and associated nutrient regeneration. Benthic macrofauna, especially

deposit-feeders, play an important role in linking organic material with higher trophic levels and alter biogeochemical cycling in sediments through bioturbation and bioirrigation. Due to their restricted mobility, benthic macrofauna have limited ability to avoid environmental and anthropogenic stressors (Pearson & Rosenberg 1978, Dauer 1993), including eutrophication, hypoxia, salinity fluctuations and sedi-

ment resuspension resulting from storms and tides. As a result, infauna can be particularly impacted by stressors that occur in estuarine ecosystems. Shallow areas subject to episodic storm disturbance are often dominated by opportunistic early colonizers (McCall 1978). Additionally, exposure to hypoxia (oxygen levels below 2 mg l^{-1}) can result in decreased functioning or mortality of benthic macrofauna and alteration in community structure, e.g. increased abundances of opportunists, shifts in species composition and trophic structure (Middelburg & Levin 2009).

Shallow coastal sediments can act as either a source or sink for nutrients. Nutrients regenerated in sediments can be a substantial component of those required for primary production in the water column (Nixon 1981, Fisher et al. 1982, Zimmerman & Benner 1994, Cowan et al. 1996, Fulweiler et al. 2010). Through their activities of feeding, burrowing, irrigation of burrows, excretion of wastes and locomotion, macrofauna can enhance the exchange of materials between the sediment and overlying water column and modify sediment redox conditions (Aller 1982, 1994, Aller & Yingst 1985). Burrow irrigation aerates sediments and increases the area of the oxic–anoxic interface and the transport of ions through the sediment, enhancing nutrient cycling rates (Kristensen et al. 1991, Gilbert et al. 1995). Natural and/or anthropogenic disturbances may influence nutrient fluxes by either disrupting macrofaunal activity or by directly affecting chemical processes (Middelburg & Levin 2009).

Due to their bioturbation, bioirrigation and feeding activities, the presence of macrofauna has been shown to enhance denitrification rates in laboratory experiments. Denitrification, the microbially driven stepwise reduction of nitrate (NO_3^-) to dinitrogen gas (N_2), represents an important nitrogen sink in estuarine systems (Seitzinger 1988) that is subject to complex interactions between organic matter supply, water column nitrate concentrations, sediment properties and bioturbation (Christensen et al. 1990, Risgaard-Petersen et al. 1994, Rysgaard et al. 1994). Increases in coupled nitrification–denitrification (Dn; 3-fold) and water column nitrate-driven denitrification (Dw; 5-fold) were measured in the presence of high densities of the amphipod *Corophium volutator* (19800 ind. m^{-2}) (Pelegri et al. 1994). Similarly, the presence of the amphipod *Monoporeia affinis* (1500 ind. m^{-2}) increased Dn in sediments 1.5-fold (Tuominen et al. 1999), and the polychaete *Nereis* spp. increased Dw 3-fold in estuarine sediments (Nizzoli et al. 2007). This enhancement of denitrification rates can be explained by the increased surface area of the oxic–anoxic transition where coupled ni-

trification–denitrification occurs (Gilbert et al. 1995) and the enhanced supply of water column nitrate into the anoxic sediments (Pelegri et al. 1994, Nizzoli et al. 2007).

Impacts of macrofauna on water–sediment exchanges, and therefore on denitrification rates, vary considerably among taxa, depending on species-specific feeding, ventilation and irrigation modes. Bonaglia et al. (2013) found that oxygenation of anoxic sediment increased denitrification rates, but addition of the deep-burrowing, irrigating spionid polychaete, *Marenzelleria* spp., reduced the enhancement of denitrification (Bonaglia et al. 2013). This reduction in denitrification in the presence of *Marenzelleria* spp. is likely due to enhanced bacterial reduction of sulfate to sulfide in the burrow walls, which can have a toxic effect on nitrifying bacteria, suppressing Dn (Bonaglia et al. 2013). The presence of the polychaete, *Nereis* sp., leads to higher aerobic respiration, Dw, and Dn than bivalves do (*Mya arenaria* and *Cerastoderma* sp.) (Pelegri & Blackburn 1995). This suggests that the impact of macrofauna on denitrification in sediments may depend strongly on the community composition (and traits) and sediment geochemistry, and that disturbances that impact macrofaunal species composition could consequently alter denitrification rates.

As a shallow estuarine ecosystem experiencing high anthropogenic nitrogen loads (Lehrter 2008), Mobile Bay, Alabama, is an ideal system in which to study the macrofaunal influence on sediment denitrification rates. Cowan et al. (1996) showed considerable intra-annual variability in nutrient fluxes and sediment oxygen consumption at a site in Mobile Bay, with variability that was best explained by bottom water dissolved oxygen (DO) concentrations and temperature. Cowan et al. (1996) did not examine macrofaunal communities in their study, and suggested that macrofauna were unlikely to be important because of their small size and therefore low biomass. Species-specific effects on benthic nutrient fluxes and denitrification rates (Pelegri et al. 1994, Tuominen et al. 1999, Nizzoli et al. 2007) have clearly demonstrated direct impacts of benthic organisms on sediment biogeochemistry. However, organisms selected for targeted studies tend to be larger-bodied and motile, i.e. the most likely taxa to impact nutrient fluxes. Moreover, given the dynamic feedbacks between diverse macrofaunal communities and sediment biogeochemistry in the natural environment, it is difficult to extrapolate these results to an ecosystem level such as natural infaunal communities within an estuary (Ferguson & Eyre 2013).

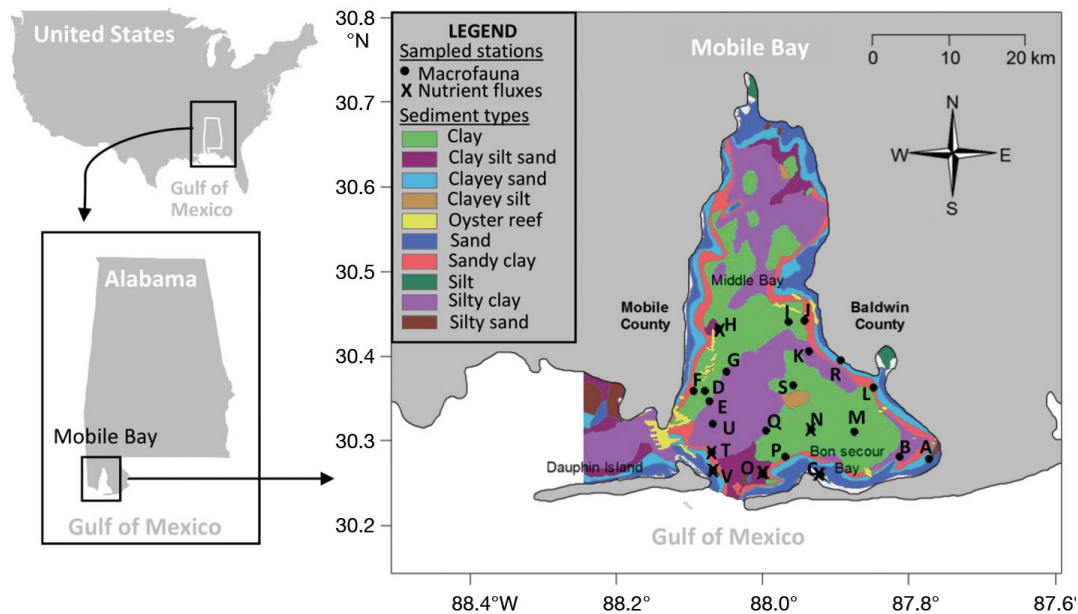


Fig. 1. Location of sampling stations in Mobile Bay covering different sediment types (sediment map based on Esri, HERE, De-Lorm, MapmyIndia, OpenStreetMap contributors and the GIS user community)

Since macrofaunal diversity of soft-sediment habitats within the Mobile Bay is not well studied, with the last survey conducted in 1988 (Dardeau 1988, Clarke & Miller-Way 1992, Stout et al. 1998), the first step of this study was to determine the distribution of macrofauna in the bay. Within this estuary, natural fluctuations in the physical environment play an important role in structuring the soft-sediment habitats (Stout et al. 1998).

This study aimed to determine the impact of location in the bay, sediment type, and potential stressors (bottom water salinity, DO levels) on the abundance, biomass and taxonomic diversity of infaunal communities in the lower region of Mobile Bay. Based on these results, we next evaluated impacts of natural macrofaunal communities with varying abundance and diversity on benthic nutrient fluxes and denitrification. This study therefore aimed to link potential stressors on macrofaunal communities to sediment denitrification rates in a shallow estuarine environment.

MATERIALS AND METHODS

Study area: Mobile Bay, Alabama

Mobile Bay is a shallow estuarine embayment located in the northern Gulf of Mexico (Fig. 1) with an average depth of 3 m and a 14 m deep shipping channel running north–south. The lower part of the bay opens to the Gulf of Mexico and the Mississippi

Sound. Because of the shallow water and variable freshwater inputs, salinity fluctuates dramatically, with strong vertical stratification under moderate to high river discharge and weak winds (Park et al. 2007). The tidal influence in Mobile Bay is small, with an average range of 0 to 20 cm. Mobile Bay experiences hypoxic and anoxic conditions, mostly during the summer months (Park et al. 2007). This study was conducted during predominately normoxic conditions (between January and May 2016).

To characterize variability in the macrofaunal community in Mobile Bay and determine the relationship between community structure and environmental parameters, 22 randomly selected stations were sampled across a range of sediment types in the southern part of Mobile Bay between January and March 2016 (Fig. 1). At each station, temperature, salinity and DO of bottom water were measured with a YSI Multi-parameter probe, and turbidity was measured with a Secchi disk. In the second phase of the study (April–May 2016), based on the initial survey of sediment type and macrofaunal abundance, biomass and diversity of 6 of the initial 22 stations were sampled for nutrient fluxes (Fig. 1).

Sediment analysis

For the initial sampling, bulk sediment samples were collected with a 0.1 m² Petersen grab and haphazardly subsampled for grain size, porosity and car-

bon to nitrogen (C:N) ratio. In the follow-up sampling for nutrient fluxes, sediment cores (95 mm i.d.) were collected and the upper 10 cm was homogenized and analyzed for grain-size, porosity and C:N content. The top 1 cm was sampled for chlorophyll *a* (chl *a*) analysis and frozen for later analysis.

Sediments were treated with a 30% hydrogen peroxide solution to oxidize organic matter prior to grain size analysis. Grain-size analysis followed the pipette method adapted from Folk (1974). Proportions of sand, clay and silt were plotted in a Shepard diagram to characterize the sediment type for each site. Sediment porosity was calculated from the percentage of water in the sediment sampled at each station by weighing sediment before and after drying at 60°C for 24 h. Care was taken to collect sediment that had been brought up in a cohesive clump from the middle of the Peterson grab rather than that near the edges that was often partially fluidized, but porosity measurements from those samples were likely less accurate than those from cores collected by divers during the 2nd sampling phase because of lower disturbance to the sediment during collection. Carbon and nitrogen content of the sediment (% wt) were measured after homogenizing the dried sediment on a CHNSO elemental combustion system analyser (mod. ECS 4010; Costech Analytical Technologies). The molar C:N ratio (mol:mol) was used as a proxy for sediment organic matter quality (Blackburn & Henriksen 1983). Sediment chl *a* content was determined fluorometrically after cold extraction in 90% acetone in triplicate (Welschmeyer 1994).

Macrofaunal sampling

Macrofaunal sampling strategy and laboratory procedures

Benthic infauna were collected using the same Petersen grab used for bulk sediment collection. Three grab samples were taken at each station. Each replicate was treated separately to assess intra-station variability. Sediments were washed through a 500 µm sieve, and retained material was preserved in 95% ethanol and stained with 1% Rose Bengal.

Macrobenthic organisms were removed from all debris, identified and counted. Taxonomic identification was carried out at family level. The wet weight of each taxon for each sample was measured to 0.001 g after blot-drying on tissue paper. Taxa-specific conversion factors provided by Brey (2001) were used to convert wet weight to dry weight.

Macrofaunal community analysis

Macrofauna diversity was assessed using the following primary community variables and diversity indices (Gray & Elliott 2009): abundance, biomass, taxonomic richness (*S*), Shannon's diversity index (*H'*), Pielou's evenness (*J'*) and Simpson's diversity index (1-*D*, hereafter *D*). Before performing statistical tests on these univariate characteristics of macrofaunal α -diversity, all faunal and environmental variables were checked for normality (graphically and using Agostino test) and fourth-root, log or arcsine transformed if necessary. If faunal data did not meet the assumptions for 1-way ANOVA, the Kruskal-Wallis 1-way ANOVA by ranks was used. If significant differences were detected by the test ($p < 0.05$), post-hoc tests for pairwise multiple comparisons (Conover's test) were used to identify differences between pairs of stations.

Hierarchical cluster analysis based on Bray-Curtis distances was applied to abundance and biomass data in order to identify similarities among sites. The same analysis was also applied to diversity metrics *S*, *H'*, *J'* and *D*, but these data were transformed based on Euclidean distances, which is more appropriate for index numbers. An agglomerative procedure was used beginning with discontinuous collection of objects, which were successively grouped into larger and larger clusters until a single, all-encompassing cluster was obtained (Legendre & Legendre 1998). The flexible clustering method proposed by Lance & Williams (1966) was used.

Distance-based redundancy analysis (db-RDA) provides a robust approach to identify the relative influence of different ecological factors in driving community assembly. This method is used for carrying out constrained ordinations on data using Bray-Curtis distances (Legendre & Gallagher 2001). The usual methods for constrained ordinations (canonical correspondence analysis [CCA], RDA) use Euclidean measures, but this distance measure has been found to be inappropriate for some types of data such as contingency tables gathering the species abundances as a function of sites (Legendre & Anderson 1999). db-RDA synthesizes the complete dataset of taxa abundances at different sites with a triplot projection (sites, stations and explaining factors) on 2 axes. This analysis assesses the influence of the following variables on taxa abundances across stations: sand content in sediment, mud composition (clay/clay+silt), sediment C:N ratio, total carbon content, porosity, temperature, salinity, turbidity, depth, dissolved oxygen, distance from the bay mouth, distance from the shore, distance from the channel, distance from fresh-

water input source and sampling date. To determine the significance of the analysis, an ANOVA was conducted (Legendre & Anderson 1999). Permutation tests were then conducted to assess significance of each of the constraint variables (Legendre & Anderson 1999). This last step returns a formula containing only variables with a significant influence on the community structure, independent from each other.

The rarefaction method allows for comparison of the number of species found in different regions when the sampling effort differed. Replication was not sufficient to plot rarefaction curves for individual stations, but rarefaction curves were plotted for aggregated samples from station clusters identified by the db-RDA results. All analyses were performed using R Statistical Software (R Core Team 2013) using the Vegan (Oksanen et al. 2011) and PCNM (Legendre et al. 2013) packages.

Nutrient fluxes and denitrification rates

Sampling strategy

Sampling sites were chosen based on the results obtained in the first part of the study (see Fig. 1, Table 1, Table S1 and Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m605p017_suppl.pdf). To span the range of variability in Mobile Bay, sites with relatively high, low and intermediate values of abundance, biomass and diversity (*S*) were selected for both mud and sand. Samples were collected over 2 d, with muddy sites on one day and sandy sites on the other. At each of the 6 sites, triplicate sediment cores were collected by divers (18 cores in total) in 95 mm i.d. × 270 mm long clear Plexiglas tubes, retaining approximately 190 mm sediment and 50 mm overlying water. Care was taken to ensure that no air bubbles remained on the inside of the Plexiglas tubes before coring and to select only cores with an undisturbed sediment surface. An additional diver-collected core was processed for sediment analysis. A

150 l sample of bottom water was also collected from each site and used to replenish the overlying water in the core throughout the duration of the laboratory experiment.

Benthic *ex situ* incubation set-up

Cores were transported within 8 h of collection to the laboratory, where they were placed uncapped in buckets with aerated water from the corresponding site in an environmental chamber maintained at the average bottom temperature of the 3 sites. Cores were capped underwater, ensuring that no air bubbles were trapped, and continuous flow of water collected from the site through the cores was established. Overlying water in the core was gently stirred with a magnetic stir bar. Water collected from each site ('inflow') was filtered (0.7 µm), enriched to ~100 µM Na¹⁵NO₃⁻ (99 atom%) and pumped into the overlying water of each core at a flow rate of 1.2 ml min⁻¹ with a multi-channel proportioning pump. The positive displacement of the overlying water forced water through the outflow tubing, which, following a 24 h pre-sampling incubation period, was collected for analyses ('outflow'). Incubations were carried out in the dark to prevent both interference by photosynthetic algae (An & Joye 2001) and air bubble formation, which alters gas concentrations in water (Reeburgh 1969).

Nutrient fluxes

After a 24 h pre-sampling incubation period (Eyre et al. 2002), triplicate water samples for nutrient analysis were collected from the outflow and inflow lines from each core, GF/F filtered and immediately frozen. Nutrient concentrations were analyzed for dissolved inorganic nitrogen (DIN) (NO₂⁻, NO₃⁻, NH₄⁺) and PO₄³⁻ on a Skalar autoanalyzer. Benthic nutrient fluxes (*F*) were calculated using the standard flux equation (Miller-Way & Twilley 1996):

Table 1. Characteristics of selected sites (see Fig. 1) according to sediment type, showing macrofaunal abundance, biomass and taxonomic richness. Data are classified as low, medium and high, and values are mean ± SE

Sediment type	Site	Abundance (ind. m ⁻²)	Biomass (gDW m ⁻²)	Taxonomic richness (no. taxa)
Mud	N	Low (721.98 ± 74.66)	Medium (1.50 ± 0.17)	Medium (11.67 ± 2.08)
	H	Medium (897.99 ± 213.99)	Low (0.27 ± 0.02)	Low (10.00 ± 1.00)
	T	High (1429.60 ± 224.32)	High (2.88 ± 1.32)	High (19.67 ± 1.52)
Sand	C	High (3444.68 ± 568.81)	Medium (3.32 ± 0.44)	Medium (17.00 ± 3.00)
	O	High (2816.09 ± 541.09)	High (16.13 ± 0.96)	High (31.33 ± 4.04)
	V	Low (790.23 ± 12.44)	Low (1.58 ± 0.53)	Medium (18.67 ± 1.53)

$$F = \frac{(C_e - C_i) \times V}{A} \quad (1)$$

where C_e is the outflow concentration (μM), C_i is the inflow concentration (μM), V the flow rate (l h^{-1}) and A the surface area of the sediment (m^2). Nutrient fluxes were measured after $^{15}\text{NO}_3^-$ was added, and this increased nitrate in the water column may have affected fluxes. We opted to measure both fluxes and denitrification simultaneously to minimize the time between collection of sediments and measurement of denitrification rates.

Oxygen concentrations in inflow and outflow water were measured with calibrated microelectrodes and a Unisense multimeter and used to calculate the sediment oxygen demand (SOD). Outflow water overflowed exetainers by $\sim 2.5\times$ the volume immediately before oxygen was measured.

Denitrification

Triplicate samples from the inflow and outflow lines were collected in 12 ml exetainers by overflowing the volume of the vial twice and preserving them with 250 μl of 50 % (w/v) ZnCl_2 . Samples were capped and stored underwater in the environmental chamber until dissolved gas analysis on a membrane inlet mass spectrometer (MIMS). The MIMS was equipped with a copper reduction column set at 600°C to remove oxygen upstream (O_2) (Eyre et al. 2002). Rates of denitrification in the cores were determined following the isotope pairing technique (IPT) (Nielsen 1992) with standard gas concentrations determined by Hamme & Emerson (2004). Denitrification is explicitly calculated from the $^{29}\text{N}_2$ and $^{30}\text{N}_2$ fluxes calculated from dissolved $^{29}\text{N}_2/^{28}\text{N}_2$ and $^{30}\text{N}_2/^{28}\text{N}_2$ measured with the MIMS (Kunu et al. 1998). Denitrification from added $^{15}\text{NO}_3^-$ (D_{15}) was calculated directly:

$$D_{15} = F^{29} + 2 F^{30} \quad (2)$$

where F^{29} and F^{30} represent the steady-state fractions of dissolved $^{29}\text{N}_2$ and $^{30}\text{N}_2$ gas fluxes relative to that of $^{28}\text{N}_2$. *In situ* denitrification from water column $^{14}\text{NO}_3^-$ and $^{14}\text{NO}_3^-$ produced from nitrification (D_{14}) was calculated (to exclude the contribution from added $^{15}\text{NO}_3^-$) according to Nielsen (1992):

$$D_{14} = \frac{F^{29}}{2 F^{30}} D_{15} \quad (3)$$

Total denitrification, henceforth referred to as denitrification capacity, was then calculated as the sum of D_{14} and D_{15} .

Denitrification and benthic fluxes ($\mu\text{mol m}^{-2} \text{h}^{-1}$) were determined from the inflow and outflow concentrations, flow rate, and the surface area of the sediment according to Eq. (1). All rates and fluxes pertaining to N species are expressed on a N atom basis. A positive flux indicates release from the sediments to the water column, and a negative flux indicates uptake by the sediment.

Macrofauna extraction and quantification

At the end of the incubation, the sediment cores were sectioned by depth (0–2 cm, 2–5 cm, 5–10 cm, >10 cm). Each segment was sieved through a 500 μm sieve for macrofauna. Macrofaunal samples were then processed following methods for field samples (see 'Macrofaunal sampling strategy and laboratory procedures'). Additionally, to relate the potential activity of the community to the impact on nutrients, the bioturbation potential index (BP_i) for each taxon and the summed community bioturbation potential index (BP_c) were calculated taking into account the population biomass of each species based on the macrofauna samples and the species' impact on sediment bioturbation through its specific mobility and sediment reworking traits (Solan et al. 2004, Queirós et al. 2013).

Statistical analysis

Pearson product-moment correlation analyses were conducted to determine correlations among parameters. When correlations were significant, linear regressions were plotted. All analysis were performed using R Statistical Software (R Core Team 2013).

RESULTS

Identification of factors structuring benthic infaunal communities

Sediment characteristics in Mobile Bay

Sediment composition in Mobile Bay ranges from sandy in nearshore to muddy throughout much of the bay (Fig. 1, Fig. S1 and Table S1 in the Supplement). Our results (Fig. S1, Table S1) followed published data fairly well (Fig. 1). Muddy sites were more clayey than silty, and sites tended to be mixtures of sand and clay (Fig. S1, Table S1). Organic matter quantity var-

ied from 0.0048 mmol C at the sandiest site near the mouth of the bay (V) to ~0.04 mmol C at silty clay sites (P,Q) in the middle of the bay (Table S1). Organic matter quality (C:N) was fairly consistent (9.6–11.4), except for one sandy site (O) near the mouth of the bay, with C:N = 19.6 (Table S1).

Macrobenthic taxonomic composition

A total of 65 different families of infauna were identified in Mobile Bay, a third of which were Annelida (Table S2). Annelids dominated the abundance and biomass of the macrobenthic community (Fig. 2, Tables S2 & S3), except at Stns O and T, located near the mouth of the bay, where other taxa such as Mollusca and Arthropoda were more abundant. The high biomass of Echinodermata in Stn O was due to a few individual brittle stars (Amphiuridae) and the high biomass of other taxa (others) in Stn L was mostly attributed to Platyhelminthes (Fig. 2b, Table S3).

Differences in abundance (Kruskal-Wallis = 57.253, $p < 0.01$) and in biomass (Kruskal-Wallis = 57.197, $p < 0.01$) were significant among stations (Fig. 2). Macrofaunal abundance ranged from 474 to 3444 ind. m^{-2} (1192 ± 814 ; mean \pm SE) and biomass from 0.27 to 16.13 gDW m^{-2} (2.16 ± 3.28 ; mean \pm SE) (Fig. 2). Significantly higher abundances (Conover's post-hoc, $p < 0.05$) were found in stations composed of 60–80 % sand, and higher biomass (Conover's post-hoc, $p < 0.05$) at Stn O, which had a few large brittle stars (Fig. 2, Tables S2 & S3).

Hierarchical clustering analysis conducted on abundance data grouped sites with similar abundances and species compositions (Fig. 3a). The resulting clusters reflected both sediment characteristics of sites and their locations in the bay. Muddy sites, excluding Stn T at the mouth of the bay, grouped together and formed 2 clusters split by location in the bay (Fig. 3a). Another cluster included coastal sites with 60–80 % sand, and Stn O was distinct from other sites with the highest abundance (Fig. 3a). Hierarchical cluster analysis conducted on biomass data did not show strong

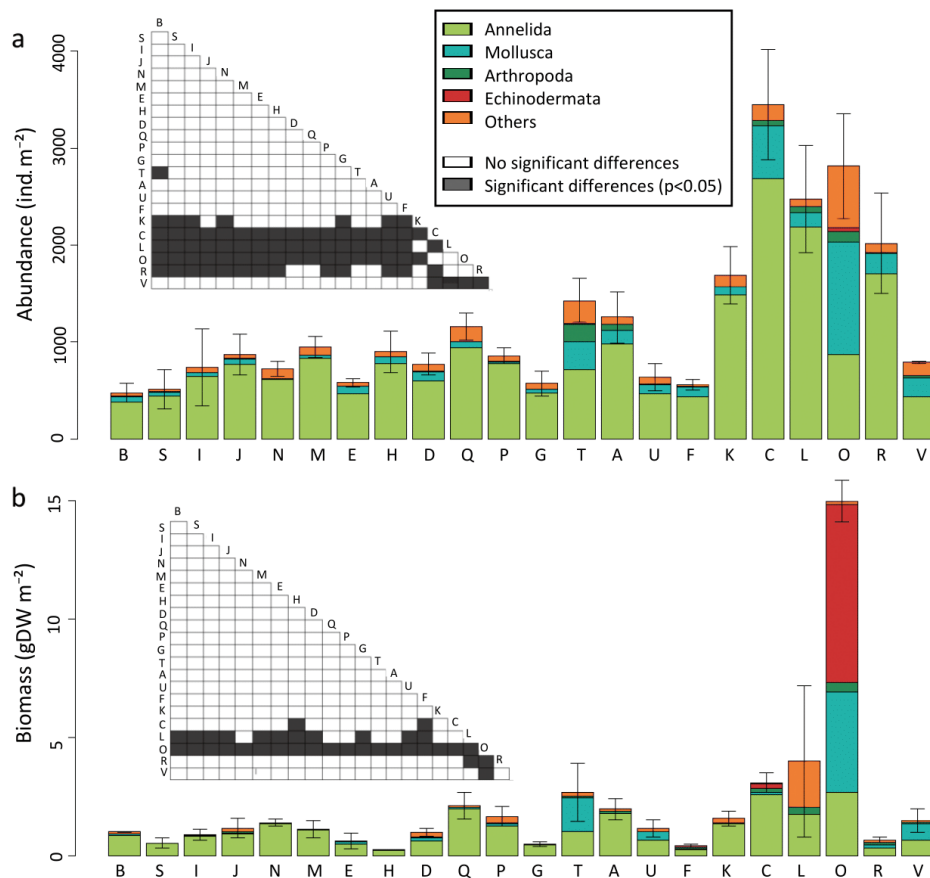


Fig. 2. Mean \pm SE ($n = 3$) (a) abundance and (b) biomass of macrofaunal taxa in Mobile Bay. Other taxa (others) include Cnidaria, Hemichordata, Nemertea, Oligochaeta and Platyhelminthes. Stations are ordered by increasing sand content. The matrix insets show significant differences ($p < 0.05$) between stations, based on Kruskal-Wallis tests with Conover's post-hoc tests

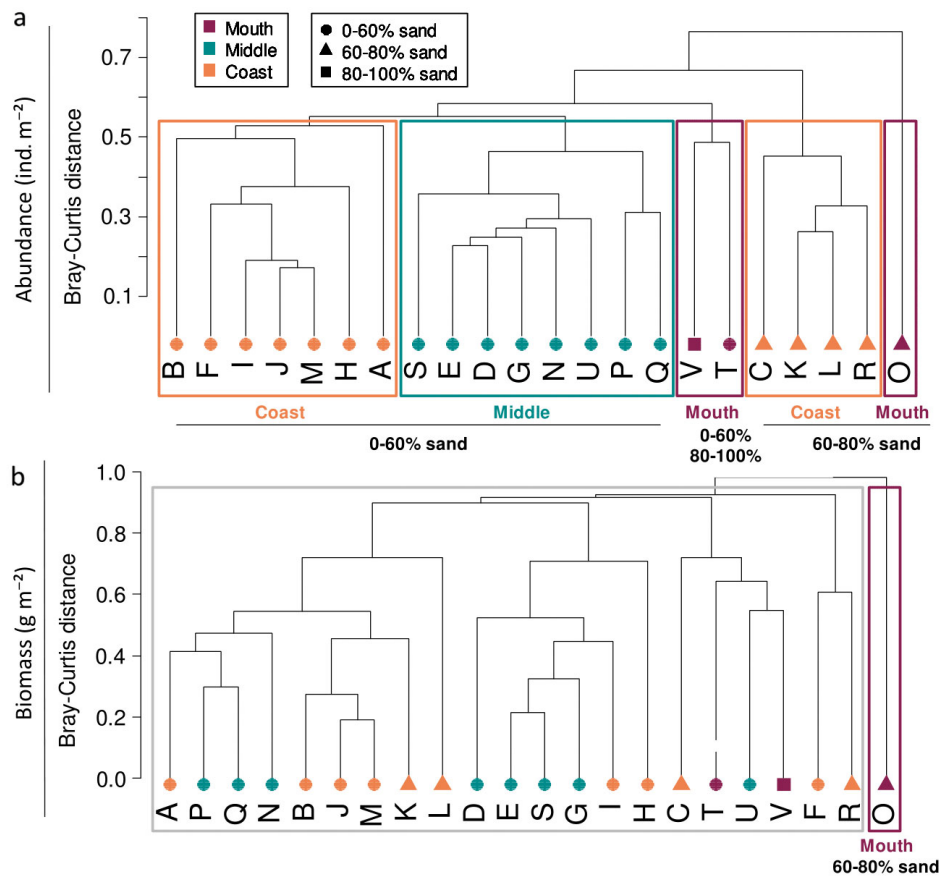


Fig. 3. Dendrogram from group-averaged hierarchical cluster analysis based on Bray-Curtis distances, produced using transformed macrofaunal (a) abundances and (b) biomass (data at 22 sites in Mobile Bay, grouped according to location in the bay (coast, middle, mouth) and sand content). Clusters grouped in the gray box show stations that cannot be explained by sediment characteristics or location in the bay

clustering based on sediment and location, but Stn O was, again, distinct from all other stations (Fig. 3b).

Macrofaunal diversity

The sand content and location in the bay both influenced macrofaunal abundance, biomass and diversity metrics (S , H' , D and J') (Fig. 4, Table S4). There was considerable variability in abundance and biomass within sediment types and locations in the bay (Fig. 4ab). Taxonomic richness ranged from 9 to 35 families (13.76 ± 4.94 ; mean \pm SE) and was higher in stations close to the mouth of the bay (Fig. 4c). Of note is the high taxonomic richness at Stn O (total of 35 families) with 60–80 % sand and near the mouth of the bay (Table S2). H' varied from 1.32 to 2.95 (2.06 ± 0.35 ; mean \pm SE) (Fig. 4d), D varied from 0.52 to 0.92 (0.81 ± 0.09 ; mean \pm SE) (Fig. 4f) and J' varied from 0.52 to 0.94 (0.80 ± 0.10 ; mean \pm SE) (Fig. 4e). Diversity increased from the coastline to the mouth (S , H' ,

D : Coast < Middle < mouth; $p < 0.01$) (Fig. 4c,d,f, Table S4). Lower values of evenness were found in sediments containing 60–80 % sand than in 0–60 % sand (Fig. 4e, Table S4). Despite a differential sampling effort, macrofaunal communities from stations close to the mouth of the bay and containing 60–80 % sand showed a higher taxonomic richness on rarefaction curves (not shown). Diversity metrics were not higher for sites with 60–80 % sand (Fig. 4), so the lack of difference in diversity among sediment types likely resulted from there being more sites with 60–80 % sand near the coast (with lower diversity) than near the mouth (with higher diversity); a more even sampling effort may have shown differences.

Influence of environmental factors on macrobenthic community structure

The db-RDA (Fig. 5) highlights the influence of the sediment characteristics, notably the sand content

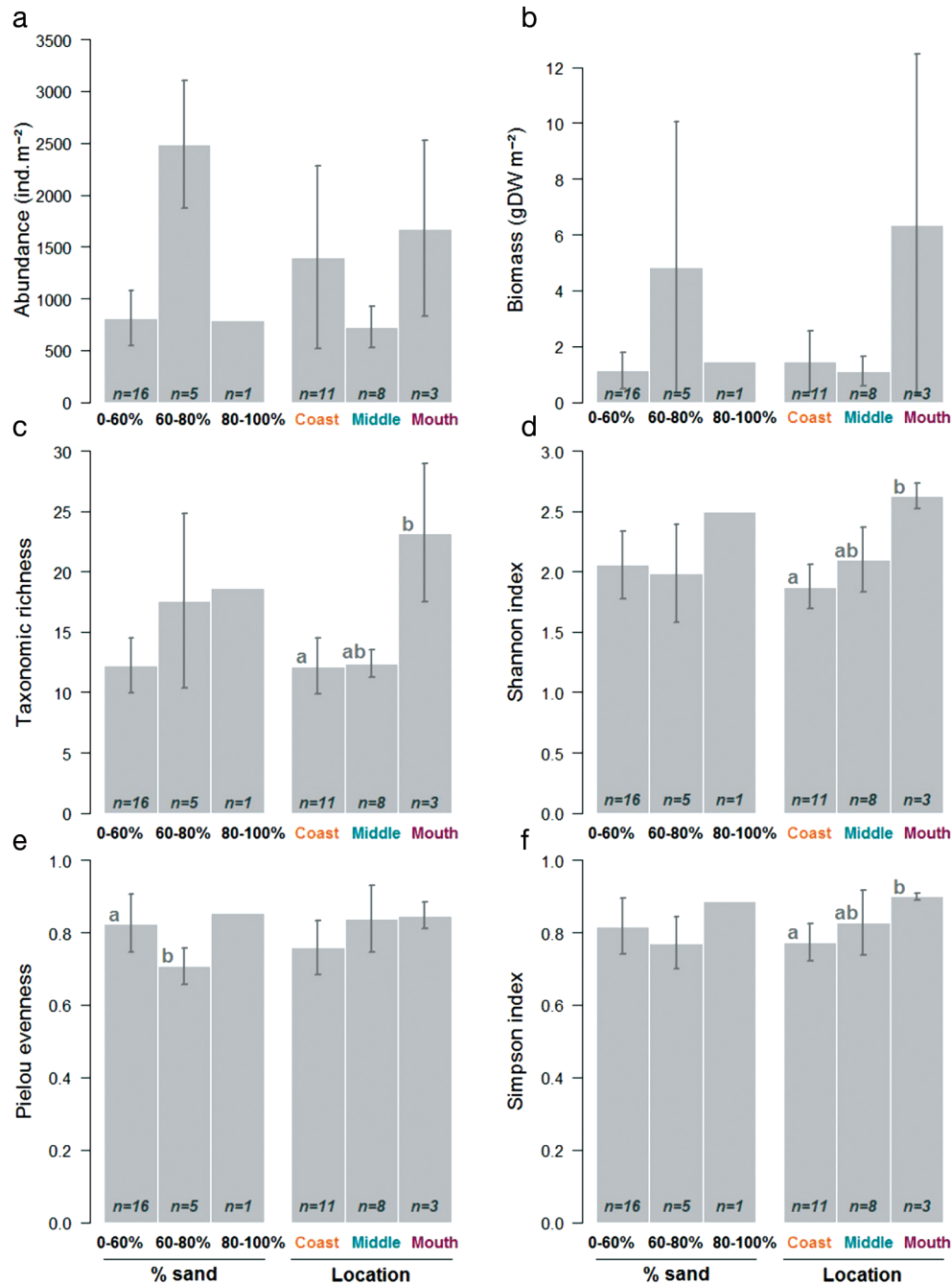


Fig. 4. Mean \pm SE (a) total abundance, (b) total biomass, (c) taxonomic richness, (d) Shannon's diversity, (e) Pielou's evenness, (f) Simpson's diversity index of macrofauna sampled at 22 sites classified depending on their sand content (<60 %, 60–80 %, >80 %) and their location (stations near the coast in orange, stations in the middle of the bay in blue and stations near the mouth of the bay in violet). Different lowercase letters above the bars show significant differences ($p < 0.05$) (Kruskal-Wallis tests with Conover's post-hoc tests). Replicates are pooled together for each station, and numbers of stations (n) are indicated at the bottom of the bars

(Fig. 5a,c) and location in the bay (Fig. 6a,d), on macrobenthic community structure. The ANOVA performed on the db-RDA analysis showed the significance of the results ($p = 0.001$). Permutation tests were conducted to assess the significance of constraints with

fauna abundances as a function of environmental parameters (% sand, distance from the sea, total carbon content, C:N ratio, distance from the channel, salinity, temperature, turbidity, porosity, clay/clay + silt). This analysis showed that the combination of these envi-

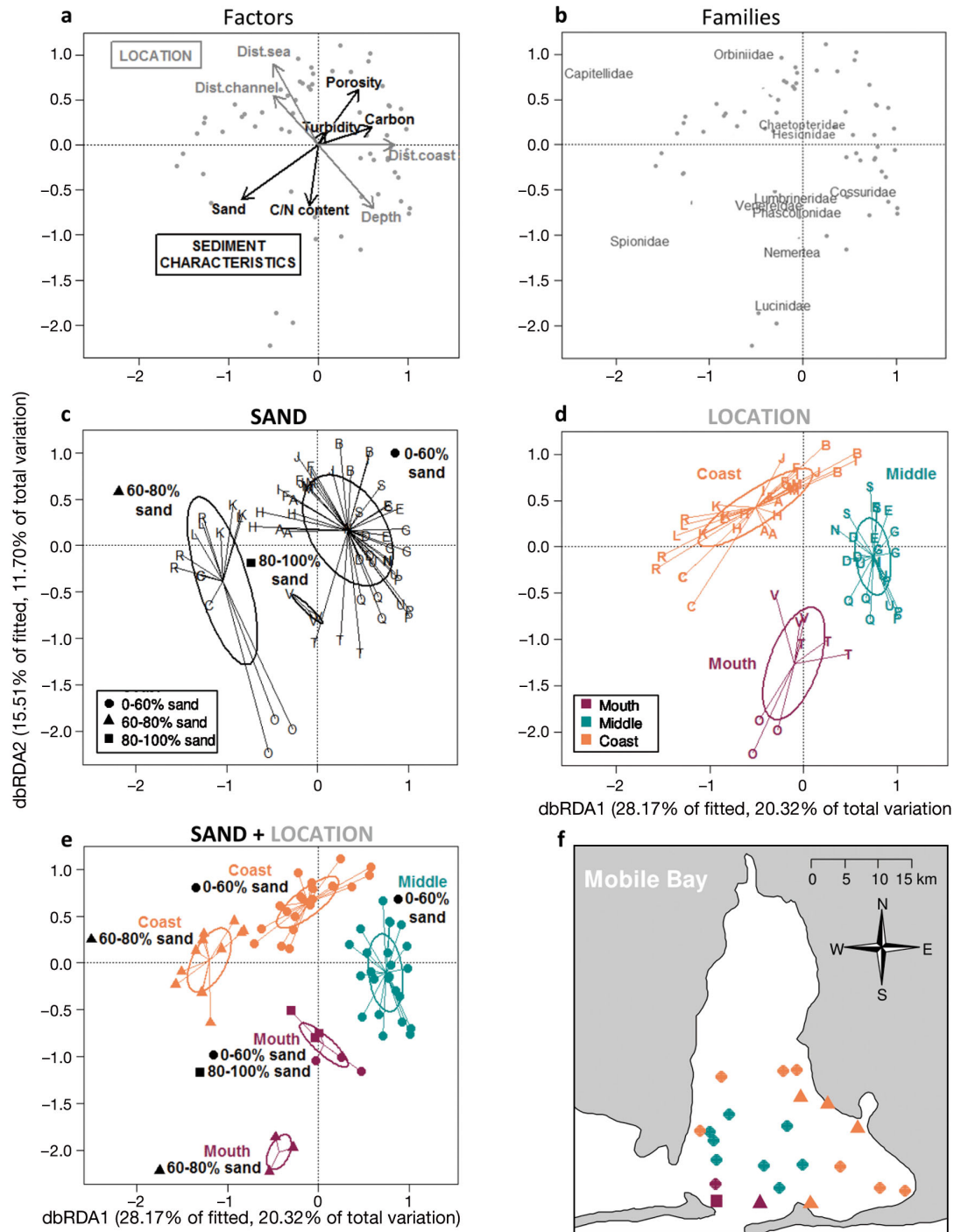


Fig. 5. Distance-based redundancy analysis (db-RDA) resulting from macrobenthos data based on Bray-Curtis similarities. This analysis has been divided into 5 graphs for clarity. (a) Environmental variables with a significant influence on the distribution of sites: distance from the sea (Dist.sea, m), distance from the channel (Dist.channel, m), distance from the coast (Dist.coast, m), water column depth (Depth, m), sand content (Sand, %), C:N content (C/N content, mol mol⁻¹), turbidity (Turbidity, m), porosity (Porosity) and carbon content (Carbon, mol). See Table S1 in the Supplement for additional information on environmental variables. (b) Macrofaunal families present in the different sites and showing a significant influence on the distribution of those sites in the analysis. Possible clusters have been highlighted depending on (c) sand content, (d) location in the bay and (e) the combination of both % sand and location in the bay, revealed by hierarchical clustering analysis based on transformed abundances data (Bray-Curtis). (f) Map showing stations' characteristics in % sand and location in the bay. 43.68% of fitted and 32.02% of the total information is summarized with the first 2 axes (dbRDA1 and dbRDA2) (Table S5). This analysis is significant (ANOVA, $p = 0.001$)

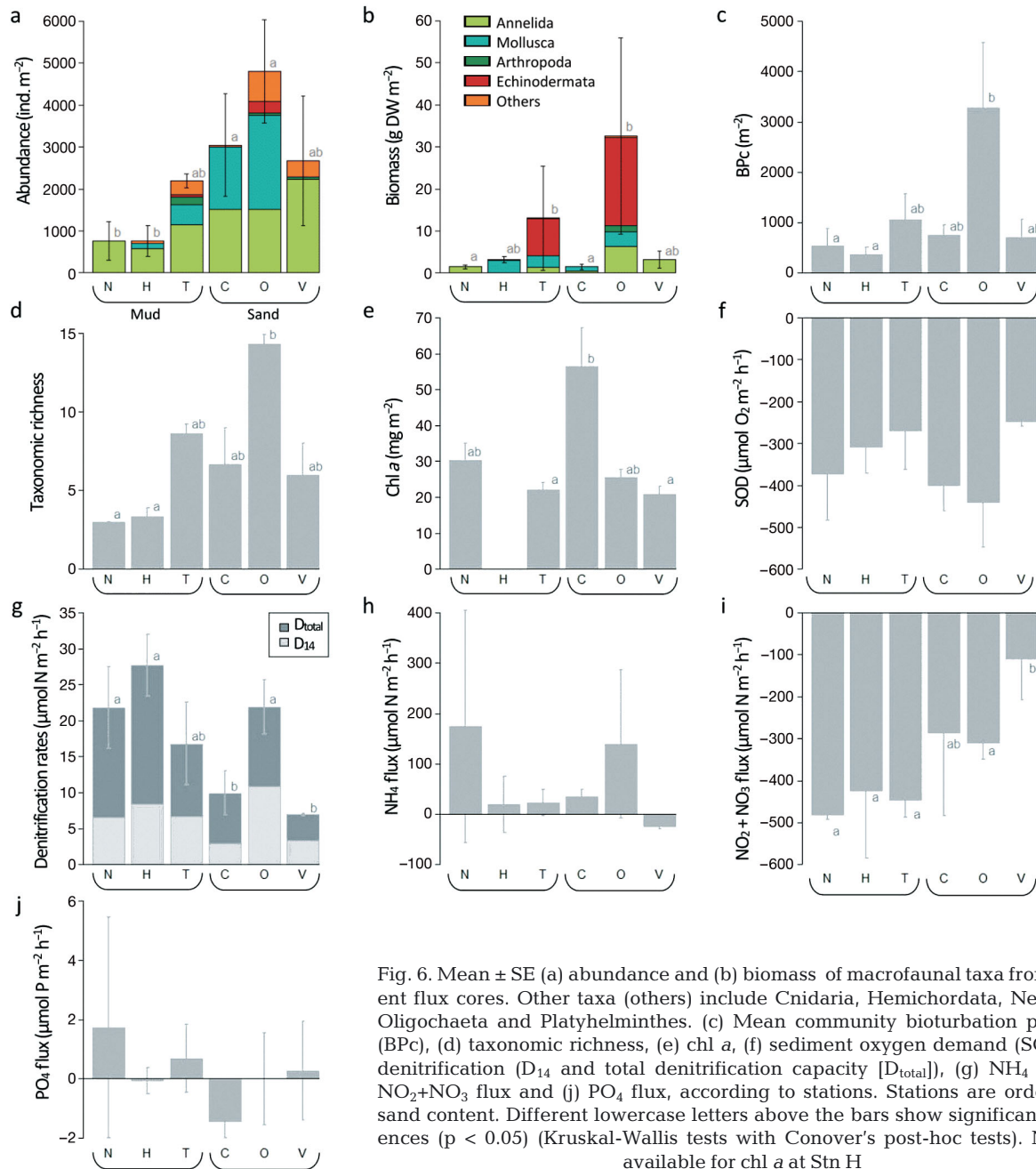


Fig. 6. Mean \pm SE (a) abundance and (b) biomass of macrofaunal taxa from nutrient flux cores. Other taxa (others) include Cnidaria, Hemichordata, Nemertea, Oligochaeta and Platyhelminthes. (c) Mean community bioturbation potential (BPC), (d) taxonomic richness, (e) chl *a*, (f) sediment oxygen demand (SOD), (g) denitrification (D₁₄ and total denitrification capacity [D_{total}]), (g) NH₄ flux, (i) NO₂+NO₃ flux and (j) PO₄ flux, according to stations. Stations are ordered by sand content. Different lowercase letters above the bars show significant differences (p < 0.05) (Kruskal-Wallis tests with Conover's post-hoc tests). No data available for chl *a* at Stn H

ronmental variables explained 62.1 % of the variability in community structure (Table S5). Specifically, community structure (Table S2) was significantly influenced by sediment characteristics (Table S1) (sand content: 12.5 %, p < 0.01; total carbon content: 6.4 %, p < 0.01; C:N ratio: 5.0 %, p < 0.01; turbidity: 3.8 %, p < 0.05; porosity: 3.4 %, p < 0.01; clay/clay+silt: 2.9 %, p < 0.01), the location in the bay (distance from the sea: 14.2 %, p < 0.01; distance from the channel: 4.5 %, p < 0.01), salinity (5.5 %, p < 0.01) and temperature (3.8 %, p < 0.01) (Table S5).

Higher abundances of Spionidae were found in stations with 60–100 % sand, whereas Chaetopteriidae and Hesioniidae were more abundant in stations with 0–60 % sand (Fig. 5b,c). Capitellidae and Orbiniidae were particularly abundant in stations located along the coastline, whereas stations close to the mouth of the bay were characterized by the presence of 3 families of annelids (Lumbrineridae, Cossuridae and Phascolionidae [Sipuncula]), a bivalve (Lucinidae) and nemerteans (Nemertea) (Fig. 5b,d). Clusters identified with the hierarchical

cluster analysis were also clearly identified in the db-RDA (Fig. 5e), supporting the combined effects of sediment characteristics and location in the bay explaining the similarities in macrofaunal community structure among sites (Fig. 5f).

Influence of macrofaunal community structure on nutrient fluxes

Patterns of macrofaunal abundance (Fig. 6a) and biomass (Fig. 6b) in cores collected for nutrient fluxes followed those of the earlier sampling (Fig. 2) with the same significant differences among sites (Figs. 6a,b & 2). Absolute values for both abundance and biomass were slightly higher, although the sampling was done later in the spring and sample size was much smaller—a 0.007 m² core rather than the 0.1 m² Petersen grab. Macrofaunal biomass at Stns O and T were largely driven by a few brittle stars (Amphiuridae) (Table S6).

BPC (Fig. 6c) showed a similar pattern to biomass, as expected from the calculation. Both BPC (Fig. 6c) and *S* (Fig. 6d) were higher in Stn O (*RS* = 15, BPC = 3291 ± 1273 m⁻²) than in N (*RS* = 3, BPC = 537 ± 348 m⁻²) and H (*RS* = 3, BPC = 379 ± 132 m⁻²) (Table S7). Highest benthic chl *a* concentrations were found in Stn C and averaged 56.4 ± 10.9 (SE) mg m⁻² (Fig. 6e, Table S7). Due to a problem during sample processing, no chl *a* data for Stn H are available. Higher denitrification rates were measured at the muddy stations, N and H, and the sandy station, O (Fig. 6g). Nitrate (plus nitrite) fluxes were higher in the muddy sites than the sandiest site (V), although these fluxes were likely enhanced by the added nitrate for denitrification measurements (Fig. 6i). Sediment oxygen consumption (Fig. 6f), NH₄⁺ fluxes (Fig. 6h), or PO₄³⁻ fluxes (Fig. 6j) did not differ among sites.

Denitrification capacity (*D*_{total}) and *in situ* denitrification (*D*₁₄) increased with increasing sediment oxygen demand (*r*² = 0.40 and 0.44, respectively, *p* < 0.01) (Fig. S2a,e, Table 2). Neither macrofaunal abundance, biomass, nor BPC, however, were correlated with either *in situ* or total denitrification (Fig. S2b–d, f–h, Table 2) or correlated with any nutrient fluxes (all *p* > 0.05) (Table 2, Fig. S2).

Table 2. Pearson product-moment correlation matrix relating station characteristics to biogeochemical process rates. Correlations in **bold** are significant. Positive values show a positive relationship and negative values a negative relationship. SOD: sediment oxygen demand; BPC: community bioturbation potential. **p* < 0.05, ***p* < 0.01, ****p* < 0.001

	SOD	Fluxes					
		NH ₄ ⁺	NO ₃ ⁻	NO ₂ ⁻	D ₁₄	D _{total}	PO ₄ ⁻
SOD	1.00						
Fluxes							
NH ₄ ⁺	-0.108	1.00					
NO ₃ ⁻	0.270	-0.274	1.00				
NO ₂ ⁻	-0.369	-0.023	-0.514*	1.00			
D ₁₄	-0.660**	0.084	-0.396	0.345	1.00		
D _{total}	-0.631**	0.077	-0.635**	0.416	0.878***	1.00	
PO ₄ ⁻	-0.204	0.091	-0.084	0.309	0.211	0.247	1.00
Bottom water							
Salinity	0.254	0.390	-0.813	0.813	0.252	0.675	0.844
Temp. (°C)	0.300	0.291	-0.841	0.890*	0.315	0.755	0.764
DO (mg l ⁻¹)	-0.159	-0.529	0.623	-0.517	-0.161	-0.458	-0.928
Sediment:							
C:N	-0.620	0.528	-0.024	-0.282	0.807	0.371	-0.028
Chl <i>a</i> (mg m ⁻²)	-0.514	0.007	-0.009	0.031	-0.393	-0.393	-0.695
Macrofauna							
Abundance (ind. m ⁻²)	-0.259	-0.066	0.245	-0.277	0.118	-0.179	-0.200
Biomass (gDW m ⁻²)	-0.038	0.058	-0.036	-0.024	0.410	0.121	-0.136
BPC (m ⁻²)	-0.163	0.084	0.027	-0.083	0.404	0.107	-0.135

DISCUSSION

Macrobenthic community structure depends on sediment characteristics and location in Mobile Bay

Influence of location in the bay on macrofaunal diversity

Macrofaunal abundance and biomass were comparable to samples collected from Mobile Bay in August 1988 (Clarke & Miller-Way 1992). Although abundance and biomass from several of our sites exceeded their ranges of 443–1410 ind. m⁻² and 0.07–0.95 gWW m⁻², respectively, those sites tended to be near coasts or the mouth of the bay, whereas sampling by Clarke & Miller-Way (1992) followed transects across the middle of the bay (cf. Fig. 1 in Clarke & Miller-Way 1992). *S*, *H'* and *J'* were higher but fairly comparable to values determined by Clarke & Miller-Way (1992) (*S* = 7–20, *H'* = 1.11–2.21 and *J'* = 0.39–0.73). These differences could also be due to differences in sampling locations or to lower oxic and thermal stress during the spring in our sampling, but could also represent temporal or inter-annual variability. Our study found high abundances of similar taxa found in the 1988 survey (Clarke & Miller-Way 1992), including polychaetes (Capitellidae, Goniadi-

dae, Pilargidae and Spionidae) and bivalves (Tellinidae) (Table S2).

The lower region of the bay close to the mouth is characterized by the highest diversity, similar to previous sampling (Dardeau 1988, Clarke & Miller-Way 1992). Macrofaunal diversity in the bay is mostly dependent on the distance from the sea, with the discrimination of 3 different zones: along the coastline, in the middle of the bay, and close to the mouth of the bay. Stations along the coastline are characterized by lower diversity and biomass, with communities dominated by a few, small and opportunistic (r-selected) taxa such as capitellids (Tsutsumi 1987) and tolerant orbinids (Kruse et al. 2004) (Fig. 5).

Communities inhabiting more variable environments (e.g. in salinity and temperature) tend to be dominated by high densities of relatively smaller-sized, r-selected species (Flint & Kalke 1985). Even though our time-point measurements of the salinity at our sites were not always higher near the mouth (Table S1), time-averaged salinity shows higher salinities at the mouth of the bay, although with considerable variability depending on rainfall. This salinity gradient has been previously identified as a prevailing structuring factor for benthic communities in Mobile Bay (Clarke & Miller-Way 1992). Salinity has also been shown to affect benthic community structure in Nueces Bay, Texas, USA, with higher biomass and diversity in the higher-salinity region of the bay (Mannino & Montagna 1997). In addition, on sunny days, bottom temperature can increase rapidly in shallow environments, and high bottom temperature can have a negative impact on macrofauna. Located close to freshwater inputs, the shallow-water stations therefore may experience higher variability in salinity and temperature (Flint & Kalke 1985) and therefore higher stress.

Natural stressors associated with the shallow coastal parts of Mobile Bay in which diversity was low may be exacerbated by anthropogenic factors. Coastal development and shoreline modification in Mobile Bay have led to the transformation of 38 % of the bay's shoreline into engineered structures (vertical walls and revetments) (Jones et al. 2009). Approximately 93 % of the shoreline is experiencing erosion (Jones et al. 2009), and a loss of at least 5 to 10 ha of intertidal habitat has been estimated in this microtidal bay (Douglass & Pickel 1999). Moreover, as a transitional area between land and sea, estuaries receive large amounts of pollutants derived from urban, agricultural, industrial and domestic waste effluents (Lillebø et al. 2005, Paerl 2006). Coastal habitat alteration, release of pollutants from coastal

development, and low salinity and high temperatures with high variability in both salinity and temperature may all contribute to stressful living conditions for macrofaunal communities.

The extent to which a marine species is able to penetrate into an estuary depends primarily on the amount and variability of the freshwater discharge relative to the tidal inflow of seawater (Ysebaert et al. 1993). The combination of small tidal ranges and long tidal periods that characterize Mobile Bay is expected to produce weak tidal currents at the estuary mouth (Stigebrandt 1977, Lee et al. 2013). In addition, the shape of the mouth of Mobile Bay has the potential to support gyre formation on both sides (Lee et al. 2013) that can oxygenate and keep higher salinity levels in the area close to the mouth as well as transport and retain larvae from outside of the bay. Larval transport patterns and reduced variability in oxygen, salinity and temperature are potential factors explaining the higher diversity of taxa near the mouth, including the polychaetes Lumbrineridae and Phascolionidae, ophiuroids, and bivalves (e.g. Lucinidae, Pandoridae).

Influence of sediment characteristics on macrofaunal abundance, diversity and taxonomic composition

Whereas diversity showed a clear geographic pattern within Mobile Bay, macrofaunal abundances and taxonomic composition appear to depend more on sediment characteristics, including sand content as well as quantity and quality of the organic matter, porosity and turbidity. Benthic soft-sediment organisms generally show strong relationships with the grain-size characteristics of the sediments they inhabit (Gray 1974). Sediment characteristics are a significant explanatory factor in the taxonomic composition of northern Gulf of Mexico infaunal communities (Coblentz et al. 2015). Highest macrofaunal abundances are found in 60–80 % sand, which are likely more stable environments than fine, easily resuspended muds. As sandier sediments are more highly oxygenated environments, they may be favored by macrofauna over organic-rich muds when hypoxic events occur with some frequency (Flint & Kalke 1985, Wilson et al. 2008). Interestingly, the site with highest sand content had lower abundances and diversity, suggesting a non-linear relationship between community structure and sediment grain size, with a peak at intermediate sand content. More sites with higher sand contents would be needed to determine

the relationship between sediment type and community structure, but this pattern is consistent with previous studies (Van Hoey et al. 2004, Anderson 2008). In the North Sea, macrofauna show maximum abundances at 5.8% mud and decrease with increasing median grain size and decreasing mud content (Van Hoey et al. 2004). Optimum percentage mud for most abundant macrofaunal taxa is estimated to $17.3 \pm 11.8\%$ in Auckland (New Zealand) which is equivalent to a range from 70.9 to 94.6% sand (Anderson 2008). In Neuces Bay, abundance and diversity were higher in >50% sand than in muds but did not, however, differ between 50–75% and 75–100% sands (Mannino & Montagna 1997). This may reflect sediment stability resulting from a combination of cohesion by muds and compaction by heavier sand grains or a trade-off between oxygenated sands and higher food content of organic muds.

Macrofaunal abundance and biomass do not significantly impact nutrient fluxes in Mobile Bay

We hypothesized that the differences in macrofaunal abundance, biomass and diversity observed in Mobile Bay would result in differences in nutrient fluxes and denitrification. Although macrofauna have been shown to affect denitrification in laboratory experiments (Svensson et al. 2001, Bonaglia et al. 2013), the present study did not show a relationship between natural macrofaunal abundances or biomass and nutrient fluxes in Mobile Bay.

There was no detectable impact of the macrofauna on nutrient and oxygen fluxes. Fluxes of ammonium, phosphate and SOD were within ranges of reported values for Mobile Bay (Cowan et al. 1996), Weeks Bay, a sub-estuary of Mobile Bay (Mortazavi et al. 2012), and other Gulf of Mexico estuaries (Twilley et al. 1999). SOD is low in these systems compared to many other estuaries, with the majority of organic matter processing occurring in the water column (Mortazavi et al. 2012). However, because the water column was enriched with $^{15}\text{NO}_3^-$ ($\sim 100\mu\text{M}$), nitrate + nitrite fluxes were much higher than previously measured fluxes in Mobile Bay (-14 to $67\mu\text{mol N m}^{-2} \text{ h}^{-1}$) or in Weeks Bay ($-30\mu\text{mol N m}^{-2} \text{ h}^{-1}$). Interestingly, only a fraction of the nitrate + nitrite uptake by the sediments was denitrified, suggesting that most of the N uptake by the sediments is retained within the sediments either through dissimilatory nitrate reduction to ammonium (DNRA) or assimilation by microorganisms. While we do not have the data to address the role of DNRA in this system, these results

are consistent with the findings of Hou et al. (2012), who used a mass balance for sediments in a Texas estuary to show that 50–70% of the sediment uptake of $^{15}\text{NO}_3^-$ was assimilated by microorganisms.

Numerous laboratory studies have shown species-specific effects on benthic fluxes by comparing inhabited sediments with a control of defaunated cores (Pelegrini et al. 1994, Tuominen et al. 1999, Karlson et al. 2005, Nizzoli et al. 2007, Bonaglia et al. 2013) (Table 3). This approach helps to understand direct impacts of benthic organisms on processes by quantifying the impacts of an active burrower or irrigator such as nereid polychaetes (Henriksen et al. 1980, Sayama & Kurihara 1983, Kristensen & Blackburn 1987, Kristensen et al. 1991, Gilbert et al. 1995, 1998) and corophium amphipods (Henriksen et al. 1980, Pelegrini et al. 1994, Rysgaard et al. 1995, Gilbert et al. 1998) but differences do not necessarily represent fluxes driven by natural assemblages. Several studies, however, have also found enhancement of denitrification and nutrient fluxes from sediments with higher densities of infauna compared to lower-density sediments (Table 3). Communities studied by Engelsen et al. (2008) and Van Colen et al. (2012), enhanced nutrient fluxes at high densities, but at abundances that were much higher than those in Mobile Bay ($>35\,000$ versus $<4800 \text{ ind. m}^{-2}$) (Table 3). Macrofaunal abundances in the present study were similar to those measured in Mobile Bay (Dardeau 1988, Clarke & Miller-Way 1992), as well as in Perdido Bay, a nearby estuary (Flemer et al. 1998), and the coastal northern Gulf of Mexico (Baustian & Rabalais 2009, Engle et al. 2009). These low macrofaunal abundances in Mobile Bay and nearby regions that also experience fluctuating oxygen and salinity may be insufficient to substantially influence nutrient fluxes. The idea that there may be a threshold in macrofaunal abundance above which denitrification and nutrient fluxes are enhanced but below which the impact is low is supported by results from a controlled experimental and modeling study by Gilbert et al. (2003). Their aim was to determine the influence of macrofaunal density on sedimentary denitrification by simulating different distances between oxygenated burrow centers using sediment plugs of different thicknesses immersed in aerated seawater reservoirs (Gilbert et al. 2003). Highest denitrification/nitrification occurred for the intermediate plug thickness representing burrow spacing of 5000 ind. m^{-2} of large animals (burrow radius of 5 mm), likely because this density allows both O_2 flux from burrows and NH_4^+ flux from anoxic zones between burrows to fuel nitrification. Thicker plugs correspon-

Table 3. Summary of macrofaunal impact on nutrient fluxes in different experimental conditions and with variation in abundance, biomass and community bioturbation potential (BPc). Y: yes; N: no; blank: absence of information. I: impact on denitrification rate but calculation of the stimulation rate not possible with available data; F: denitrification rate not measured but impact on other nutrient fluxes shown; X: no impact on denitrification rate or other nutrient fluxes shown. See Gilbert et al. (1998) and Karlson et al. (2005) for additional information on denitrification stimulation rate

Bioturbating organism	Abundance (ind. m ⁻²) Min. Max.	Biomass (gWW m ⁻²) Min. Max.	BPc (m ⁻²) Min. Max.	Control used (defaunated sediment)	Denitrification stimulation rate (%)	Source
Laboratory						
<i>Oligochaeta</i>	0 12000			Y	180	Chatarpaul et al. (1980)
<i>Nereis virens</i>	0 2000			Y	171	Henriksen et al. (1980)
<i>Nereis virens</i>	0 1810			Y	240	Kristensen & Blackburn (1987)
<i>Nereis virens</i>	0 875			Y	300	Kristensen et al. (1991)
<i>Nereis diversicolor</i>	0 1430			Y	114	Gilbert et al. (1995)
<i>Nereis</i> sp.	0 2000			Y	500	Pelegrí & Blackburn (1995)
<i>Hediste japonica</i>	0 1000			Y	300	Sayama & Kurihara (1983)
<i>Corophium volutator</i>	0 6000			Y	161	Henriksen et al. (1980)
<i>Corophium volutator</i>	0 19800			Y	300 to 500	Pelegrí et al. (1994)
<i>Monoporeia affinis</i>	0 1500			Y	866	Tuominen et al. (1999)
<i>Monoporeia affinis</i>	0 5000			Y	141	Karlson et al. (2005)
<i>Mya arenaria</i>	0 1100			Y	163	Pelegrí & Blackburn (1995)
<i>Cerastoderma</i> sp.	0 1100			Y	254	Pelegrí & Blackburn (1995)
<i>Macoma baltica</i>	0 1200			Y	253	Karlson et al. (2005)
Field						
<i>Corophium</i> spp.	300 9000			N	950	Rysgaard et al. (1995)
Assemblage	0 3160			Y	160 to 529	Gilbert et al. (1998)
Assemblage (high nereids abundances)	0 1130			N	I	Ferguson & Eyre (2013)
Assemblage	0 70000			N	I	Van Colen et al. (2012)
Assemblage	1800 35350	5 180		N	F	Engelsen et al. (2008)
Assemblage (high maldanids abundances)	500 7000		100 6000	N	I	McTigue et al. (2016)
Assemblage	527 4600	17 412	100 5500	N	I	Braeckman et al. (2014)
Assemblage	430 4800	7 107	379 3291	N	X	Present study

ding to lower densities of 1600 and 400 ind. m⁻² did not differ in denitrification, likely because both were limited by NO₃⁻, which was formed closer to the sediment–water interface, rather than at the oxic–anoxic boundary around burrow walls, and thus more likely to diffuse to the water column. These results are consistent with a minimum density, in this case between 1600 and 5000 large burrows, being necessary to substantially enhance denitrification rates.

Macrofaunal abundance is not the only factor that needs to be taken into account. The burrow spacing predicted by Gilbert et al. (1998) to enhance denitrification near the maximum of the range of abundances found in Mobile Bay, and other studies have shown an influence of macrofauna on nutrient fluxes at abundances comparable to this study (Ferguson & Eyre 2013, Braeckman et al. 2014). Differences in biomass and taxonomic composition are likely important as well. However, we found no relationship between denitrification and nutrient fluxes and either biomass or BPc. BPc depends heavily on biomass (and therefore, unsurprisingly, shows a similar trend in our study) but includes species mobility and sediment reworking traits (Solan et al. 2004, Queirós et al. 2013) and therefore would be more likely to correlate with nutrient fluxes. High macrofaunal biomass at a couple of sites in Mobile Bay was driven by a few individual brittle stars (Table S6). Brittle stars have been shown to increase the sediment release of nitrite and nitrate with higher densities (Wood et al. 2009). All other individuals, however, are small, limiting their bioturbation impact.

At abundances similar to those found in Mobile Bay, an active burrower such as *Corophium* spp. enhances denitrification rates, in contrast to our findings for the macrofaunal community of Mobile Bay (cf. Fig. 7 of Rysgaard et al. 1995). A macrofaunal community including high abundances of taxa that irrigate deep burrows or actively bioturbate sediments may have a greater impact on denitrification. Potentially, the fewer bioturba-

tors in the community, the greater the abundance that would be needed to have an impact. Functionally contrasting species of macrofauna stimulate microbial nitrogen transformations to different extents or can even decrease denitrification (Bonaglia et al. 2014): for instance, impacts on oxygen and nutrient fluxes by shallow biodiffusers (*Cerastoderma edule*) are much less than those by deeper gallery-diffusers (*Corophium volutator* and *Nereis diversicolor*) (Mermillod-Blondin et al. 2004).

Microbial processing should be higher in muddy sediments with higher organic content than in sand, potentially explaining the higher levels of denitrification in muds in this study (Figs. 6g & S2a–h) (Deutsch et al. 2010, Braeckman et al. 2014). The one sandy site with higher denitrification rates, O (Fig. 6g), also had much higher biomass than the other 2 sandy sites (Fig. 6b). Higher macrofaunal biomass could potentially explain the high denitrification, however; if biomass were driving denitrification, we would expect to also see higher SOD at that site (Fig. 6f). SOD is often used as a proxy for the quality of the organic matter that is being remineralized (Eyre et al. 2013a). It is somewhat surprising that SOD did not differ between muddy and sandy sites (Fig. 6f), although the organic matter quantity and quality did not differ substantially between the sandy and muddy sites (Table S1). The higher denitrification rates at sites with higher SOD, but a lack of correlation between denitrification and the macrofauna abundance or biomass, is consistent with prior investigations suggesting that the quality of the organic matter is a major driver of denitrification rates (Eyre et al. 2013b, Fulweiler et al. 2013).

One of the sandy sites near the bay mouth (Stn O), however, appears to be an outlier in the bay with higher macrofaunal abundance, biomass, diversity and denitrification rates. All replicates have fairly high denitrification rates (Fig. S2). The core that had the highest denitrification rate had the lowest abundance at this site but contained a large tube-building worm (Polychaeta: Maldanidae) (Table S6). Not only do maldanids irrigate their burrows, but *in situ* tracer experiments revealed that maldanids can rapidly subduct freshly deposited algal carbon and inorganic materials to at least 10 cm below the surface (Levin et al. 1997). As a result of this transport, relatively fresh organic matter becomes available to deep-dwelling microbes rapidly after deposition. Both aerobic and anaerobic processes may be enhanced by maldanid activity (Levin et al. 1997), which therefore has the potential to impact nutrient fluxes. *Maldane sarsi* at high densities (5057 ± 484 ind. m^{-2}) and high BPc

in shallow Arctic shelf sediments contribute to enhanced denitrification (McTigue et al. 2016). However, based on the results of the first part of the present study, this family of polychaetes appears to be rare in the bay (Table S2).

This study shows that, given unfavorable abundance, biomass and/or bioturbation or irrigation activity, an enhancement of fluxes by benthic macrofauna does not become measurable or statistically detectable. In this study, it seems that small numbers of burrows associated with low biomass result in macrofauna not being a predominant factor to explain variability of nutrient fluxes and denitrification rates in the bay. Our community analysis suggests that sandy-mud sites and those near the mouth of the bay with higher macrofaunal abundance and diversity are the most promising regions for future study to determine whether seasonal or environmental variables interact with macrofaunal community structure to impact nutrient cycling. However, on a bay-wide scale, our study indicates rather that physical processes and microbial communities drive nutrient cycling in Mobile Bay.

Acknowledgements. Research reported in this manuscript was supported by an Early-Career Research Fellowship to K.M.D from the Gulf Research Program of the National Academies of Sciences, Engineering, and Medicine. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Gulf Research Program of the National Academies of Sciences, Engineering, and Medicine. Thanks to the support of the international mobility of the LabexMER, a cluster of Excellence funded by the French 'Investment for the future' program, supported by the French Ministry of Research and Education (reference ANR-10-LabX-19-01). We are very grateful to Grant Lockridge, Sarah Frankenberg, Ryan Parker and Alex Leynse for their help in the field. We also thank Steve Dykstra for help with Fig. 1.

LITERATURE CITED

- Aller RC (1982) The effects of macrobenthos on chemical properties of marine sediment and overlying water. In: McCall PL, Tevesz MJ (eds) Animal-sediment relations. Plenum Press, New York, NY, p 53–102
- ✦ Aller RC (1994) Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation. *Chem Geol* 114:331–345
- ✦ Aller RC, Yingst JY (1985) Effects of the marine deposit-feeders *Heteromastus filiformis* (Polychaeta), *Macoma balthica* (Bivalvia), and *Tellina texana* (Bivalvia) on averaged sedimentary solute transport, reaction rates, and microbial distributions. *J Mar Res* 43:615–645
- ✦ An S, Joye SB (2001) Enhancement of coupled nitrification-denitrification by benthic photosynthesis in shallow estuarine sediments. *Limnol Oceanogr* 46:62–74
- ✦ Anderson MJ (2008) Animal-sediment relationships revisited: characterising species' distributions along an en-

- vironmental gradient using canonical analysis and quantile regression splines. *J Exp Mar Biol Ecol* 366:16–27
- ✦ Baustian MM, Rabalais NN (2009) Seasonal composition of benthic macroinfauna exposed to hypoxia in the northern Gulf of Mexico. *Estuaries Coasts* 32:975–983
- ✦ Blackburn TH, Henriksen K (1983) Nitrogen cycling in different types of sediments from Danish waters. *Limnol Oceanogr* 28:477–493
- ✦ Bonaglia S, Bartoli M, Gunnarsson JS, Rahm L and others (2013) Effect of reoxygenation and *Marenzelleria* spp. bioturbation on Baltic Sea sediment metabolism. *Mar Ecol Prog Ser* 482:43–55
- ✦ Bonaglia S, Nascimento FJA, Bartoli M, Klawonn I, Brüchert V (2014) Meiofauna increases bacterial denitrification in marine sediments. *Nat Commun* 5:5133
- Braeckman U, Foshtomi MY, Van Gansbeke D, Meysman F, Soetaert K, Vincx M, Vanaverbeke J (2014) Variable importance of macrofaunal functional biodiversity for biogeochemical cycling in temperate coastal sediments. *Ecosystems* 17:720–737
- ✦ Brey T (2001) Population dynamics in benthic invertebrates. A virtual handbook. Version 01.2. <http://www.thomas-brey.de/science/virtualhandbook>
- ✦ Chatarpaul L, Robinson JB, Kaushik NK (1980) Effects of tubificid worms on denitrification and nitrification in stream sediment. *Can J Fish Aquat Sci* 37:656–663
- ✦ Christensen PB, Nielsen LP, Sørensen J, Revsbech NP (1990) Denitrification in nitrate-rich streams: diurnal and seasonal variation related to benthic oxygen metabolism. *Limnol Oceanogr* 35:640–651
- Clarke D, Miller-Way T (1992) An environmental assessment of the effects of open-water disposal of maintenance dredged material on benthic resources in Mobile Bay, Alabama. US Army Engineer Waterways Experiment Station, Vicksburg, MS
- ✦ Coblenz KE, Henkel JR, Sigel BJ, Taylor CM (2015) Influence of sediment characteristics on the composition of soft-sediment intertidal communities in the northern Gulf of Mexico. *PeerJ* 3:e1014
- ✦ Cowan JLW, Pennock JR, Boynton WR (1996) Seasonal and interannual patterns of sediment-water nutrient and oxygen fluxes in Mobile Bay, Alabama (USA): regulating factors and ecological significance. *Mar Ecol Prog Ser* 141:229–245
- Dardeau MR (1988) Mobile Bay trend station infaunal macroinvertebrates (winter 1987; spring 1988): life history and environmental requirements. Technical report 88-004. Dauphin Island Sea Lab, Dauphin Island, AL
- ✦ Dauer DM (1993) Biological criteria, environmental health and estuarine macrobenthic community structure. *Mar Pollut Bull* 26:249–257
- ✦ Deutsch B, Forster S, Wilhelm M, Dippner JW, Voss M (2010) Denitrification in sediments as a major nitrogen sink in the Baltic Sea: an extrapolation using sediment characteristics. *Biogeosciences* 7:3259–3271
- Douglass SL, Pickel BH (1999) The tide doesn't go out anymore—the effect of bulkheads on urban shorelines. *Shore Beach* 67:19–25
- ✦ Engelsens A, Hulth S, Pihl L, Sundbäck K (2008) Benthic trophic status and nutrient fluxes in shallow-water sediments. *Estuar Coast Shelf Sci* 78:783–795
- ✦ Engle VD, Hyland JL, Cooksey C (2009) Effects of Hurricane Katrina on benthic macroinvertebrate communities along the northern Gulf of Mexico coast. *Environ Monit Assess* 150:193–209
- ✦ Eyre BD, Rysgaard S, Dalsgaard T, Christensen PB (2002) Comparison of isotope pairing and N₂:Ar methods for measuring sediment denitrification—assumption, modifications, and implications. *Estuaries* 25:1077–1087
- ✦ Eyre BD, Maher DT, Squire P (2013a) Quantity and quality of organic matter (detritus) drives N₂ effluxes (net denitrification) across seasons, benthic habitats, and estuaries. *Global Biogeochem Cycles* 27:1083–1095
- ✦ Eyre BD, Santos IR, Maher DT (2013b) Seasonal, daily and diel N₂ effluxes in permeable carbonate sediments. *Biogeosciences* 10:2601–2615
- ✦ Ferguson A, Eyre B (2013) Interaction of benthic microalgae and macrofauna in the control of benthic metabolism, nutrient fluxes and denitrification in a shallow subtropical coastal embayment (western Moreton Bay, Australia). *Biogeochemistry* 112:423–440
- ✦ Fisher TR, Carlson PR, Barber RT (1982) Sediment nutrient regeneration in three North Carolina estuaries. *Estuar Coast Shelf Sci* 14:101–116
- ✦ Flemer DA, Kruczynski WL, Ruth BF, Bundrick CM (1998) The relative influence of hypoxia, anoxia, and associated environmental factors as determinants of macrobenthic community structure in a northern Gulf of Mexico estuary. *J Aquat Ecosyst Stress Recovery* 6:311–327
- Flint RW, Kalke RD (1985) Benthos structure and function in a south Texas estuary. *Contrib Mar Sci* 2:33–53
- Folk RL (1974) Petrology of sedimentary rocks. Hemphill Publishing Company, Austin, TX
- ✦ Fulweiler RW, Nixon SW, Buckley BA (2010) Spatial and temporal variability of benthic oxygen demand and nutrient regeneration in an anthropogenically impacted New England estuary. *Estuaries Coasts* 33:1377–1390
- ✦ Fulweiler R, Brown S, Nixon S, Jenkins B (2013) Evidence and a conceptual model for the co-occurrence of nitrogen fixation and denitrification in heterotrophic marine sediments. *Mar Ecol Prog Ser* 482:57–68
- ✦ Gilbert F, Bonin P, Stora G (1995) Effect of bioturbation on denitrification in a marine sediment from the West Mediterranean littoral. *Hydrobiologia* 304:49–58
- ✦ Gilbert F, Stora G, Bonin P (1998) Influence of bioturbation on denitrification activity in Mediterranean coastal sediments: an *in situ* experimental approach. *Mar Ecol Prog Ser* 163:99–107
- ✦ Gilbert F, Aller RC, Hulth S (2003) The influence of macrofaunal burrow spacing and diffusive scaling on sedimentary nitrification and denitrification: an experimental simulation and model approach. *J Mar Res* 61:101–125
- Gray JS (1974) Animal–sediment relationships. *Oceanogr Mar Biol Annu Rev* 12:223–261
- Gray JS, Elliott M (2009) Ecology of marine sediments from science to management, 2nd edn. Oxford University Press, Oxford
- ✦ Hamme RC, Emerson SR (2004) Measurement of dissolved neon by isotope dilution using a quadrupole mass spectrometer. *Mar Chem* 91:53–64
- Henriksen K, Hansen JL, Blackburn TH (1980) The influence of benthic infauna on exchanges rates of inorganic nitrogen between sediment and water. *Ophelia* 1:249–256
- ✦ Hou L, Liu M, Carini SA, Gardner WS (2012) Transformation and fate of nitrate near the sediment–water interface of Copano Bay. *Cont Shelf Res* 35:86–94
- Jones SC, Tidwell DK, Darby SB (2009) Comprehensive shoreline mapping, Baldwin and Mobile Counties, Alabama: Phase I. Open-file report 09210. Geological Survey of Alabama, Tuscaloosa, AL

- ✦ Karlson K, Hulth S, Ringdahl K, Rosenberg R (2005) Experimental recolonisation of Baltic Sea reduced sediments: survival of benthic macrofauna and effects on nutrient cycling. *Mar Ecol Prog Ser* 294:35–49
- ✦ Kristensen E, Blackburn TH (1987) The fate of organic carbon and nitrogen in experimental marine sediment systems: influence of bioturbation and anoxia. *J Mar Res* 45:231–257
- ✦ Kristensen E, Hjorth Jensen M, Aller RC (1991) Direct measurement of dissolved inorganic nitrogen exchange and denitrification in individual polychaete (*Nereis virens*) burrows. *J Mar Res* 49:355–377
- ✦ Kruse I, Strasser M, Thiermann F (2004) The role of ecological divergence in speciation between intertidal and subtidal *Scoloplos armiger* (Polychaeta, Orbiniidae). *J Sea Res* 51:53–62
- ✦ Kunu TM, Sullivan MB, Cornwell JC, Groxzkowski KM (1998) Denitrification in estuarine sediments determined by membrane inlet mass spectrometry. *Limnol Oceanogr* 43:334–339
- ✦ Lance GN, Williams WT (1966) A generalized sorting strategy for computer classifications. *Nature* 212:218
- ✦ Lee J, Webb BM, Dzwonkowski B, Park K, Valle-Levinson A (2013) Bathymetric influences on tidal currents at the entrance to a highly stratified, shallow estuary. *Cont Shelf Res* 58:1–11
- ✦ Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: testing multispecies responses in multifactorial ecological experiments. *Ecol Monogr* 69:1–24
- ✦ Legendre P, Gallagher ED (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia* 129:271–280
- Legendre P, Legendre L (1998) Numerical ecology, 2nd English edition. Elsevier, Amsterdam
- ✦ Legendre P, Borcard D, Blanchet G, Dray S (2013) PCNM: MEM spatial eigen function and principal coordinate analyses. R package version 2.1-2/r109. <http://R-Forge.R-project.org/projects/sedar/>
- ✦ Lehrter JC (2008) Regulation of eutrophication susceptibility in oligohaline regions of a northern Gulf of Mexico estuary, Mobile Bay, Alabama. *Mar Pollut Bull* 56:1446–1460
- ✦ Levin L, Blair N, DeMaster D, Plaia G, Fornes W, Martin C, Thomas C (1997) Rapid subduction of organic matter by maldivid polychaetes on the North Carolina slope. *J Mar Res* 55:595–611
- ✦ Lillebø AI, Neto JM, Martins I, Verdelhos T and others (2005) Management of a shallow temperate estuary to control eutrophication: the effect of hydrodynamics on the system's nutrient loading. *Estuar Coast Shelf Sci* 65:697–707
- ✦ Mannino A, Montagna PA (1997) Small-scale spatial variation of macrobenthic community structure. *Estuaries* 20:159–173
- McCall PL (1978) Spatial-temporal distributions of Long-Island Sound infauna: the role of bottom disturbance in a nearshore marine habitat. In: Wiley ML (ed) *Estuarine interactions*. Academic Press, New York, NY, p 191–219
- ✦ McTigue ND, Gardner WS, Dunton KH, Hardison AK (2016) Biotic and abiotic controls on co-occurring nitrogen cycling processes in shallow Arctic shelf sediments. *Nat Commun* 7:13145
- ✦ Mermillod-Blondin F, Rosenberg R, François-Carcaillet F, Norling K, Maclaure L (2004) Influence of bioturbation by three benthic infaunal species on microbial communities and biogeochemical processes in marine sediment. *Aquat Microb Ecol* 36:271–284
- ✦ Middelburg JJ, Levin LA (2009) Coastal hypoxia and sediment biogeochemistry. *Biogeosciences* 6:1273–1293
- ✦ Miller-Way T, Twilley RR (1996) Theory and operation of continuous flow systems for the study of benthic-pelagic coupling. *Mar Ecol Prog Ser* 140:257–269
- ✦ Mortazavi B, Riggs AA, Caffrey JM, Genet H, Phipps SW (2012) The contribution of benthic nutrient regeneration to primary production in a shallow eutrophic estuary, Weeks Bay, Alabama. *Estuaries Coasts* 35:862–877
- ✦ Nielsen LP (1992) Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiol Lett* 86:357–362
- Nixon SW (1981) Remineralization and nutrient cycling in coastal marine ecosystems. In: Neilson BJ, Cronin LE (eds) *Estuaries and nutrients*. Springer, New York, NY, p 111–138
- ✦ Nizzoli D, Bartoli M, Cooper M, Welsh DT, Underwood GJC, Viaroli P (2007) Implications for oxygen, nutrient fluxes and denitrification rates during the early stage of sediment colonisation by the polychaete *Nereis* spp. in four estuaries. *Estuar Coast Shelf Sci* 75:125–134
- ✦ Oksanen J, Blanchet GF, Kindt R, Legendre P, O'Hara RB (2011) Vegan: community ecology package. <https://cran.r-project.org/web/packages/vegan/index.html>
- ✦ Paerl HW (2006) Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: interactive effects of human and climatic perturbations. *Ecol Eng* 26:40–54
- ✦ Park K, Kim CK, Schroeder WW (2007) Temporal variability in summertime bottom hypoxia in shallow areas of Mobile Bay, Alabama. *Estuaries Coasts* 30:54–65
- Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr Mar Biol Annu Rev* 16:229–311
- ✦ Pelegri SP, Blackburn TH (1995) Effect of bioturbation by *Nereis* sp., *Mya Arenaria* and *Cerastoderma* sp. on nitrification and denitrification in estuarine sediments. *Opheelia* 42:289–299
- ✦ Pelegri SP, Nielsen LP, Blackburn TH (1994) Denitrification in estuarine sediment stimulated by the irrigation activity of the amphipod *Corophium volutator*. *Mar Ecol Prog Ser* 105:285–290
- ✦ Queirós AM, Birchenough SNR, Bremner J, Godbold JA and others (2013) A bioturbation classification of European marine infaunal invertebrates. *Ecol Evol* 3:3958–3985
- R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- ✦ Reeburgh WS (1969) Observations of gases in Chesapeake Bay sediments. *Limnol Oceanogr* 14:368–375
- ✦ Risgaard-Petersen N, Rysgaard S, Nielsen LP, Revsbech NP (1994) Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnol Oceanogr* 39:573–579
- ✦ Rysgaard S, Risgaard-Petersen N, Sloth NP, Jensen K, Nielsen LP (1994) Oxygen regulation of nitrification and denitrification in sediments. *Limnol Oceanogr* 39:1643–1652
- ✦ Rysgaard S, Christensen PB, Nielsen LP (1995) Seasonal variation in nitrification and denitrification in estuarine sediment colonized by benthic microalgae and bioturbating infauna. *Mar Ecol Prog Ser* 126:111–121
- ✦ Sayama M, Kurihara Y (1983) Relationship between burrowing activity of the polychaetous annelid, *Neanthes japon-*

- ica (Izuka) and nitrification-denitrification processes in the sediments. *J Exp Mar Biol Ecol* 72:233–241
- Seitzinger SP (1988) Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnol Oceanogr* 33:702–724
- ✦ Solan M, Cardinale BJ, Downing AL, Engelhardt KAM, Ruesink JL, Srivastava DS (2004) Extinction and ecosystem function in the marine benthos. *Science* 306:1177–1180
- ✦ Stigebrandt A (1977) On the effect of barotropic current fluctuations on the two-layer transport capacity of a constriction. *J Phys Oceanogr* 7:118–122
- Stout JP, Heck KL Jr, Valentine JF, Dunn SJ, Spitzer PM (1998) Preliminary characterization of habitat loss: Mobile Bay national estuary program. Dauphin Island Sea Lab, Dauphin Island, AL
- ✦ Svensson JM, Enrich-Prast A, Leonardson L (2001) Nitrification and denitrification in a eutrophic lake sediment bioturbated by oligochaetes. *Aquat Microb Ecol* 23:177–186
- ✦ Tsutsumi H (1987) Population dynamics of *Capitella capitata* (Polychaeta; Capitellidae) in an organically polluted cove. *Mar Ecol Prog Ser* 36:139–149
- ✦ Tuominen L, Mäkelä K, Lehtonen KK, Haahti H, Hietanen S, Kuparinen J (1999) Nutrient fluxes, porewater profiles and denitrification in sediment influenced by algal sedimentation and bioturbation by *Monoporeia affinis*. *Estuar Coast Shelf Sci* 49:83–97
- Twilley RR, Cowan J, Miller-Way T, Montagna P, Mortazavi B (1999) Benthic nutrient fluxes in selected estuaries in the Gulf of Mexico. In: Bianchi TS, Pennock JR, Twilley RR (eds) *Biogeochemistry of Gulf of Mexico estuaries*, John Wiley & Sons, New York, NY, p 163–209
- ✦ Van Colen C, Rossi F, Montserrat F, Andersson MGI and others (2012) Organism–sediment interactions govern post-hypoxia recovery of ecosystem functioning. *PLOS ONE* 7:e49795
- ✦ Van Hoey G, Degraer S, Vincx M (2004) Macrobenthic community structure of soft-bottom sediments at the Belgian Continental Shelf. *Estuar Coast Shelf Sci* 59:599–613
- ✦ Welschmeyer NA (1994) Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. *Limnol Oceanogr* 39:1985–1992
- ✦ Wilson AM, Huettel M, Klein S (2008) Grain size and depositional environment as predictors of permeability in coastal marine sands. *Estuar Coast Shelf Sci* 80:193–199
- ✦ Wood HL, Widdicombe S, Spicer JJ (2009) The influence of hypercapnia and the infaunal brittlestar *Amphiura filiformis* on sediment nutrient flux—Will ocean acidification affect nutrient exchange? *Biogeosciences* 6: 2015–2024
- ✦ Ysebaert T, Meire P, Maes D, Buijs J (1993) The benthic macrofauna along the estuarine gradient of the Schelde estuary. *Neth J Aquat Ecol* 27:327–341
- ✦ Zimmerman AR, Benner R (1994) Denitrification, nutrient regeneration and carbon mineralization in sediments of Galveston Bay, Texas, USA. *Mar Ecol Prog Ser* 114: 275–288

Editorial responsibility: Erik Kristensen,
Odense, Denmark

Submitted: November 26, 2017; Accepted: September 17, 2018
Proofs received from author(s): October 19, 2018