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Research Article

RESEARCH ARTICLE

Sediment microbial assemblage structure is modified by marine polychaete gut passage

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One sentence summary: Digestive tracts of the marine deposit feeder *Hediste diversicolor* contain distinct transitory sediment microbial assemblages.

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ABSTRACT

Invertebrate activities in sediments, predominantly the redistribution of particles and porewater, are well-known to regulate the structure of associated microbial assemblages; however, relatively little attention has been given to the effects of sediment ingestion, gut passage and excretion by deposit-feeding invertebrates. Here, we use high-throughput sequencing and quantitative PCR to examine how passage through the gut of the marine polychaete *Hediste diversicolor* affects the structure of bacterial and archaeal assemblages and the abundance of nitrogen cycling taxa. We show that the digestive tract of *H. diversicolor* contains unique transitory microbial assemblages that, during gut passage, become more like the surrounding sediment assemblages. Enrichment of similar microbial taxa in both the hindgut and the burrow wall suggest that these transitory gut assemblages may influence the composition of the local sediment community. The hindgut of *H. diversicolor* also forms a reservoir for unique ammonia-oxidising archaeal taxa. Furthermore, distinct microbial assemblages on external polychaete surfaces suggest that deposit-feeding invertebrates act as vectors that transport microbes between sediment patches. Collectively, these findings suggest that the passage of sediment and associated microbial assemblages through the gut of deposit feeding invertebrates is likely to play a significant role in regulating sediment microbial assemblages and biogeochemical functioning.

Keywords: invertebrate–microbe interactions; ecosystem functioning; functional traits; nitrogen cycling; microbiome

INTRODUCTION

Coastal sediment environments are globally important sites for organic matter decomposition and remineralisation (Middelburg, Soetaert and Herman 1997). The biological complexity associated with sediment ecosystems remains, however, insufficiently constrained in biogeochemical models to generate accurate projections (Snelgrove *et al.* 2018). In particular, the representation of microbial processes underpinning biogeochemical transformations requires more thorough consideration because sediment-dwelling invertebrates directly and indirectly alter the structure and activity of microbial communities (Mermillod-Blondin, Francois-Carcaillet and Rosenberg 2005; Gilbertson, Solan and Prosser 2012).

Invertebrate burrows are sites of steep chemical gradients, high levels of organic matter cycling and increased oxygen penetration (Kristensen 2000; Nielsen *et al.* 2004; Jovanovic *et al.* 2014)

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that vary in relation to the feeding, burying and ventilatory activity of the invertebrate inhabitant. Consequently, burrow walls support distinct microbial communities that display both spatial and inter-specific variation (Bertics and Ziebis 2009; Laverock *et al.* 2010; Pischedda *et al.* 2011; Taylor and Cunliffe 2015). Whilst it is known that changes in the structure and diversity of microbial assemblages related to invertebrate activities (e.g. mucopolysaccharide production) can have substantive effects on biogeochemical cycles (Satoh, Nakamura and Okabe 2007; Foshtomi *et al.* 2015; Dale *et al.* 2018; Foshtomi *et al.* 2018), studies to date have largely focused on the effects of particle and fluid displacement by infauna and do not consider the roles of other significant animal–environment–microbial interactions.

As marine sediment ecosystems are dominated by deposit-feeding invertebrates, transit of sediment through the digestive tract of these communities is likely to be particularly important in determining the benthic contribution to biogeochemical cycling (Thorsen 1998; Biron *et al.* 2014; Troussellier *et al.* 2017). In terrestrial systems, the specific organic matter, pH and redox conditions of the earthworm (*Lumbricus rubellus*) gut means that ingestion of soil significantly alters the abundance of certain microbial taxa in the transitory substrate assemblage (Furlong *et al.* 2002; Pass *et al.* 2015). In marine deposit-feeders, abundance-based techniques have demonstrated a general loss of bacteria in the foregut, followed by regrowth towards the hindgut (Plante, Jumars and Baross 1989; Hymel and Plante 2000). Bacteriolytic activity and digestion are centred in the stomach and decline towards the gut posterior (Plante and Mayer 1994; Mayer *et al.* 1997), where bacterial growth can be stimulated in the absence of competitors and the presence of elevated levels of organic matter (Andresen and Kristensen 2002). As the digestion and subsequent regrowth of bacterial assemblages can be species-specific and vary between individuals within an invertebrate population (Plante, Jumars and Baross 1989; Plante and Mayer 1994; Mayer *et al.* 1997), it follows that the nature of invertebrate–microbial gut interactions within a community will be of functional importance to ecosystem processes within the sediment profile. Clone library studies have also shown that gut passage can alter assemblage diversity and the ratio of aerobic to anaerobic taxa (Lau, Jumars and Ambrust 2002; Li *et al.* 2009), but the extent to which the surrounding sediment assemblages are influenced by these changes remains unclear (King 2018).

Invertebrate guts may introduce functionally important taxa into the surrounding sediment or they may act as a vector, which transports a subset of microbes between sediment patches (Troussellier *et al.* 2017). Earthworm guts have been shown to contain active nitrate-reducing populations (Furlong *et al.* 2002; Wust, Horn and Drake 2011) at abundances that are orders of magnitude higher than the surrounding substrate (Karsten and Drake 1997). The presence of these ingested denitrifying taxa means that earthworm guts are sources of nitrous oxide (N_2O) (Horn, Drake and Schramm 2006a; Horn *et al.* 2006b). In marine sediments, gut emissions from deposit feeding invertebrates also form a significant contribution to N_2O fluxes because of incomplete denitrification occurring in the gut (Heisterkamp *et al.* 2010). The significance of these emissions is constrained by a lack of mechanistic understanding of microbe–invertebrate interactions during gut passage, including consideration of whether gut conditions encourage the growth of microbial functional groups that contribute to sediment nitrogen cycling.

Here, we assess the variation between bacterial and archaeal assemblage structure in the surrounding sediment environment, the external body surface and the internal gut of the sediment-dwelling polychaete *Hediste diversicolor*, using both 16S rRNA gene amplicon sequencing and quantitative PCR (Q-PCR). Our aim was to determine whether the transitory microbial assemblages within the gut or on the external surfaces of the polychaete are distinct from the assemblages located in the local burrow wall or surrounding peripheral sediment, and to establish the extent to which these assemblages contribute to the wider sediment microbial community and potential nitrogen cycling.

METHODS

Sample collection and processing

Samples were collected from three mud flat sites (~15 m apart) at St Johns Lake, Cornwall, UK (50°21'51' N, 004°14'08' W), in September 2017 (Fig. 1a). A previous survey at this location showed that the sediment in the area is predominantly silt (16–63 μm) with an organic carbon content of 6.9% (Ecospan, 2010). For each site, three surficial sediment samples ('surface', upper 0.5 cm) were obtained using a sterile syringe and six burrows occupied by *H. diversicolor* were identified (Fig. 1b and c). Individual *H. diversicolor* were removed from each burrow, swabbed (Fisherbrand swabs, Fisher Scientific (Loughborough, UK)) and then anaesthetised in 40 ml of $MgCl_2$ in seawater (2.5% $MgCl_2$) to prevent gut evacuation (Rouse 2004). Sediment from the burrow wall ('burrow', 3 cm depth from the sediment-water interface, 0.5 cm of burrow wall thickness sampled) and from the surrounding, non-bioturbated area ('deep', 3 cm depth from the sediment–water interface, 4 cm from the burrow) was obtained with a sterile syringe. All sediment samples and swabs were snap frozen using a liquid N_2 dry shipper and stored at -80°C. Individual *H. diversicolor* were washed in distilled water, measured (10 ± 4 cm, n = 18) and dissected on the day of collection. An incision was made after the foregut apparatus (foregut, ~31 ± 6.9% body length, n = 13) and a separate incision towards the end of the hindgut (hindgut, ~79 ± 5.6% body length, n = 16) to facilitate the removal of two 1 cm sections of gut contents using sterile tweezers. Dissection tools were washed with ethanol between each incision. All samples were stored at -80°C.

DNA extraction and Q-PCR

DNA was extracted (sediment samples, 0.25 g wet weight; swab samples, whole swab; gut samples, available sediment content) from 11 burrow systems (Site 1, n = 4; Site 2, n = 3; Site 3, n = 4) using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA). DNA yield was quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific) and the extracted DNA stored at −20°C.

Q-PCR was used to assess the abundance of 16S rRNA genes, *amo*A genes and the bacterial *nir*S gene in four of the burrow systems (full protocol, Text S1; reaction efficiencies, Table S1 (Supporting Information)). A total of 10 μL reactions contained 5 μL 2x SensiFast SYBR No-ROX master mix (Bioline, UK), 0.1 μL 10 pM forward and reverse primers, 1 μL template DNA and 3.8 μL molecular grade H_2O and were run in a Rotor-Gene 6000 (Corbett Life Science, Australia), with duplicate technical replicates for each sample. Swab samples were excluded from Q-PCR analyses because of difficulties in obtaining accurate sample weights. Results were converted from ng μl^{-1} to copy number

Figure 1. Sample site at St John's Lake, Cornwall **(a),** with a vertical cross-section of the sediment profile displaying visible *H. diversicolor* burrow systems with oxidised interfaces **(b)** and an individual worm within burrow **(c)**.

mgww.sediment^{-1}. Data are reported according to MIQE guidelines (Bustin *et al.* 2009).

16S rRNA gene sequencing and analysis

Bacterial and archaeal 16S rRNA gene PCR was used to establish sequencing viability (see Supplementary Methods for full protocol). Based on these results, the swab samples from site 2 (n = 3) were excluded from both bacterial and archaeal sequencing analyses, with additional samples excluded from the archaeal sequencing analysis (Table S2, Supporting Information). 16S rRNA gene sequencing was performed on the Illumina MiSeq platform using V6-V8 primer sets (Comeau, Douglas and Langille 2017). Poor quality sequencing runs excluded 4 archaeal samples from further analysis (Table S2, Supporting Information).

Sequences were analysed using the R package DADA2 (Callahan *et al.* 2016). Based on the forward and reverse read error profiles, reads were truncated at position 100 to account for the poor read quality of some of the gut and swab samples. The resulting read length was not enough for acceptable overlap between the forward and reverse reads; therefore, only the forward reads were used for the remainder of the analysis. Forward primer sequences were removed, and then reads were filtered to allow a maximum of four errors per read to obtain the best fit between the expected and estimated error rates. The sequence data were dereplicated to remove redundancy, sequence variance was inferred and a sequence table produced. Taxonomy was assigned to operational taxonomic units (OTUs) using the SILVA database (Quast *et al.* 2013) and loaded into the phyloseq package (McMurdie and Holmes 2013). Chloroplast plastid and other eukaryotic sequences were removed from the dataset before further analysis. Sequence data have been deposited in the European Nucleotide Archive (accession code PRJEB29031).

Statistical analysis

The single archaeal foregut sample was excluded from the statistical analyses because it was not replicated. Diversity was calculated with the phyloseq package using the Chao 1 diversity index and ANOVA to determine statistical significance. Variations in assemblage composition between environmental DNA sources (surface, burrow, deep, swab, foregut and hindgut) and sample sites (1, 2 and 3) were calculated from weighted Bray–Curtis dissimilarity matrices rarefied to the minimum number of reads using PERMANOVA (999 permutation), and visualised using Non-metric Multidimensional Scaling (NMDS) (vegan; v2.4–6; Oksanen *et al.* 2016) and Minimum Spanning Trees (phyloseqGraphTest). As sample site had no significant effect on assemblage structure, the three sites were pooled and site effects were disregarded. Pairwise PERMANOVA analyses (999 permutations) were also carried out between each sample type. Relative abundance plots were produced for the abundant bacterial orders and archaeal classes (>5% relative abundance). This high cut-off value was selected to minimise any potential bias introduced by the low read numbers obtained for some samples. Differential abundances of taxa (Log2-FoldChange) between pairs of sample types relevant to the aims of this study were calculated for the bacterial assemblages in the DESEQ2 package (Love, Huber and Anders 2014) using the Wald parametric test ($P < 0.05$). Changes in the abundance of nitrifying taxa were assessed using ANOVA once the key taxa were identified from the sequence table and sequence identity confirmed using online BLAST searches. Differences in bacterial and archaeal 16S rRNA genes, bacterial and archaeal *amoA* genes, the ratio of bacterial and archaeal *amoA* (AOB:AOA) genes and bacterial *nirS* genes between sample types were calculated using ANOVA,

and multiple comparisons to identify the source of any significant differences were performed using Tukey's post hoc tests. Bacterial and archaeal 16S rRNA, archaeal amoA and AOB:AOA were log transformed before analysis. For ecological clarity, we present untransformed data in the figures. All statistical analyses were performed with R (version, 3.2.2; R Core Team 2015)

RESULTS

For the bacterial community, sequencing analysis generated a total of 1 847 603 reads, which, after filtering, grouped into 4917 unique OTUs. Read depth was slightly higher and less variable in the sediment samples (burrow, 33 726 ± 5238; deep, 41 375 ± 6020; surface, 34 189 ± 4928) relative to the worm-associated samples (foregut, 9602 ± 10,144; hindgut, 27 687 ± 25 380; swab, 37 952 ± 22 679) (Table S3, Supporting Information). For the archaeal community, sequencing analysis generated a substantially lower total of 307 073 reads, which, after filtering, grouped into 268 unique OTUs. This low read depth, along with a more uneven distribution of reads between samples (burrow, 8924 ± 5187; deep, 13 269 ± 4303; surface, 7246 ± 3647; hindgut,105 ± 129; swab, 1035 ± 397) (Table S4, Supporting Information), means that the archaeal community cannot be assessed with the same degree of certainty but is included here to support the trends observed within the bacterial community.

Microbial abundance and alpha diversity

Bacterial abundance, determined by 16S rRNA gene QPCR, was dependent on the sample type (Log_{10}, $F_{4, 14} = 3.7$, $P = 0.0295$), with a greater abundance of bacteria present in the hindgut (1 192 363 ± 293 596 copies mgww.sediment^{-1}) compared to the foregut (87 145 ± 61 045 copies mgww.sediment^{-1}) (Log_{10}, Tukey's, $P = 0.039$; Table S7, Supporting Information) (Fig. 2a). Archaeal abundance also differed between sample types (Log_{10}, $F_{4, 14} = 59.82$, $P < 0.001$), with the greatest mean abundance in the burrow and deep sediment assemblages (burrow, 55 671 ± 29 315 copies mgww.sediment^{-1}; deep, 116 472 ± 51 386 copies mgww.sediment^{-1}; $Log_{10,}$ Tukey's, $P < 0.001$ (Table S8, Supporting Information)), and the lowest mean abundance in the foregut assemblage (203 ± 94 copies mgww.sediment^{-1}; Log_{10}, Tukey's, $P < 0.05$ (Table S8, Supporting Information)) (Fig. 2b). Overall, archaeal abundance was lower than bacterial abundance, but followed a generally similar trend despite the low abundance in the hindgut.

Bacterial diversity, determined using the Chao1 index, was also dependent on sample type ($F_{5, 55} = 17.79$, $P < 0.001$), with greater diversity in the sediment and swab assemblages (surface, 590 ± 55; burrow, 620 ± 50; deep, 674 ± 72; swab, 657 ± 372) than the gut assemblages (foregut, 144 ± 97; hindgut, 286± 206) (Fig. 3a). Archaeal diversity was generally lower, but was still dependent on sample type ($F_{5, 35} = 15.59$, $P < 0.001$) and showed greater diversity in the sediment assemblages (surface, 68 ± 24; burrow, 65 ± 24; deep, 80 ± 15) than in the worm-associated (swab, 26 ± 24; hindgut, 6 ± 3) assemblages (Fig. 3b).

Microbial assemblage structure

Bacterial assemblage structure, determined by 16S rRNA sequencing, was dependent on sample type ($F_{5, 55} = 10.27$, $P = 0.001$), with all sources being distinguishable from one another (Supplementary Table S5, Supporting Information). Overall, the sediment samples clustered separately from the gut samples. Within the sediment cluster, the burrow samples formed an intermediate cluster between the surface and deep sediment assemblage clusters (Fig. 4a and b), although the majority of the burrow samples were more affiliated to the surface assemblages (Fig. 4b). The gut assemblages clustered together, though in more variable and less distinct groups, with hindgut and sediment assemblages more closely affiliated with one another than the foregut and sediment assemblages (Fig. 4a and b). The external swab samples formed a distinct bacterial group between the sediment and gut clusters, being most closely affiliated to the surface and hindgut assemblages (Fig. 4a and b).

Sample type also affected archaeal assemblage structure ($F_{4, 35} = 9.62$, $P = 0.001$), with each source forming an independent cluster (Supplementary Table S6, Supporting Information). As with the bacterial assemblages, sediment samples clustered together with the burrow samples forming an intermediate cluster between the deep and surface assemblages (Fig. 4c). Unlike the bacterial assemblages, however, the archaeal burrow assemblages appeared to be more closely affiliated with the deep sediment assemblages (Fig. 4d). Similarly, distinct hindgut assemblages showed greatest affiliation with the external swab assemblages, which formed an intermediate group between the gut and sediment assemblages (Fig. 4c and d).

Summary of abundant microbial taxa

The abundant bacterial assemblage (>5% relative abundance), determined by 16S rRNA gene sequencing, was made up of 18 orders that accounted for 54.6 ± 12.9% of the total OTU abundance (Fig. 5a). This abundant subset differed between the sediment (surface, burrow and deep) and gut environments (foregut and hindgut), with the sediment assemblages dominated by the orders Desulfobacterales, Flavobacterales and Xanthomonadales. Comparing the surface and deep sediment assemblages, the surface samples had greater abundances of Cytophagales and Rhodobacterales, whilst the deep samples had greater abundances of Desulfobacterales and Myxococcales. The burrow assemblages were a combination of both the surface and deep assemblages, with abundant populations of Desulfobacterales, Myxococcales and Rhodobacterales. In the gut, the majority of the samples were dominated by Flavobacterales and Rhodobacterales. Additionally, as observed previously in the community structure analyses (Fig. 4), there seems to be greater variation in the overall assemblage composition between the individual *H. diversicolor* gut tracts than between the individual burrow systems. The external swab assemblages were also more varied in composition relative to the burrow systems, with some individuals showing high abundances of Xanthomonadales, as observed in the sediment assemblages, whilst others showed high abundances of Rhodobacterales, similar to the gut assemblages.

The abundant archaeal assemblage (>5% relative abundance) was made up of five classes that accounted for 94.6 ± 3.5% of the total OTU abundance (Fig. 5b). This abundance assemblage also differed between the sediment and gut environments, with the external swab and sediment assemblages dominated by Group C3 and Thermoplasmata (Fig. 5b). Methanomicrobia was also abundant in the surface and swab assemblages, with some surface sediment assemblages also enriched in Marine Group I. As with bacteria, the burrow archaea assemblage was a combination of the surface and deep assemblages, which were dominated by Group C3 and Thermoplasmata, with high abundances of Marine Group I and Methanomicrobia in some individual burrows. In contrast, the gut assemblages were dominated by the Soil Crenarchaeotic Group, with Methanomicrobia only present in high abundances in the hindgut assemblages.

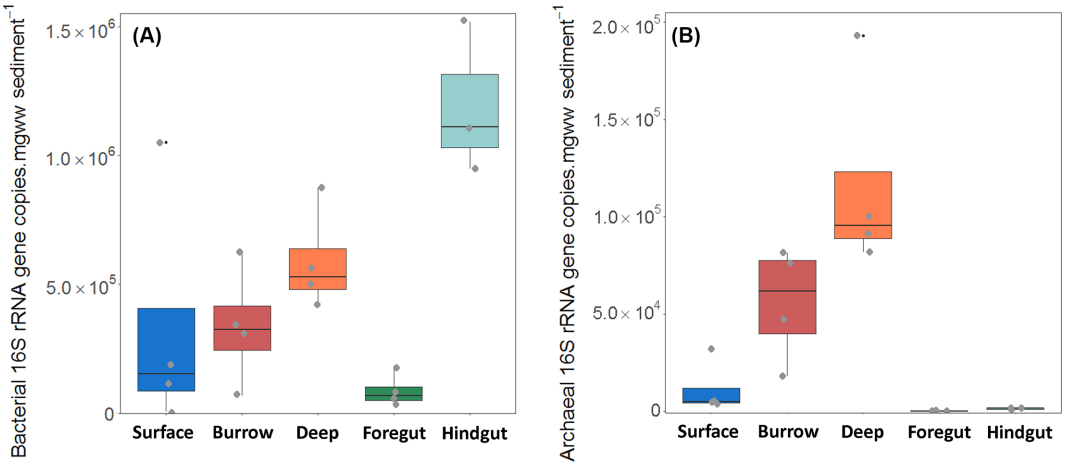

Figure 2. Variations in gene abundance between environmental sources. Bacterial **(A)** and archaeal **(B)** 16S rRNA genes. Boxplot indicates median, 25% and 75% quartiles, and 95% of the data spread (n = 4). Untransformed data presented.

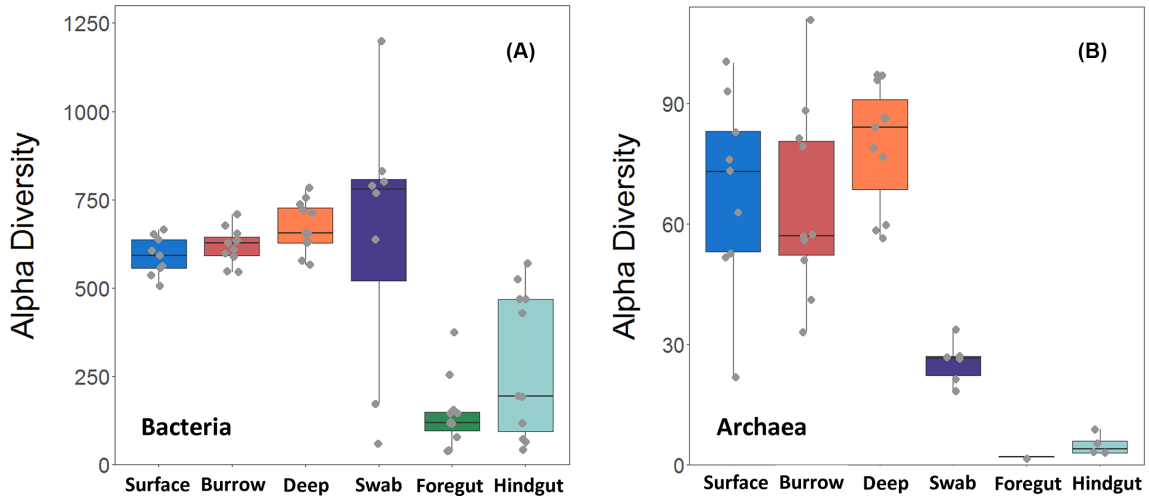

Figure 3. Variations in bacterial **(A)** and archaeal **(B)** alpha diversity between each of the environmental sources. Boxplot indicates median, 25% and 75% quartiles, and 95% of the data spread (Bacteria: n = 11, except surface n = 9; Archaea: surface n = 9, burrow n = 10, deep n = 11, swab n = 6, foregut n = 1, hindgut n = 4).

Taxonomic variations between sample type assemblages

Pairwise comparisons of the abundance of taxonomic groups between sample types (plotted as log2 fold changes) revealed that bacterial burrow assemblages were significantly enriched in Desulfobacterales and Myxococcales relative to surface assemblages, which were enriched in Flavobacteriales and Rhodobacterales (Fig. 6a). In contrast, comparison between burrow and deep sediment assemblages revealed the reverse trend (Fig. 6b), which indicates that burrow assemblages were enriched in both surface and deep sediment taxa. Generally, fewer taxa varied between the foregut and hindgut, but greater abundances of Cytophagales, Flavobacteriales and Myxococcales were present in the hindgut relative to the foregut (Fig. 6c). Between the hindgut and the burrow assemblages, these three orders were generally enriched in the burrow environment, although some representatives were more abundant in the hindgut (e.g. *Ekhidna sp.*, *Actibacter sp.*) (Fig. 6d).

Between the swab and surface assemblages, only one Cyanobacteria representative differed between the two sample types (Supplementary Figure S1, Supporting Information). Swab assemblages were enriched in some Desulfobacterales taxa relative to burrow and hindgut assemblages and in Flavobacterales when compared to the hindgut assemblage alone (Fig. 6e and f). Bacterial orders enriched during gut passage (i.e. Cytophagales, Flavobacteriales and Myxococcales) were also more abundant in the external swab assemblage relative to the deep anoxic sediment (Supplementary figure S2, Supporting Information).

Abundance of nitrogen cycling taxa

Abundance of ammonia oxidising bacteria (AOB) was dependent on sample type ($F_{4, 14}$ = 5.935, P = 0.005), although the significance of this term was driven by a greater abundance in the burrow and deep sediment assemblages (burrow, 629 ± 210 copies mgww.sediment^{-1}; deep, 789 ± 365 copies mgww.sediment^{-1}) relative to the foregut assemblage (69 ± 32 copies mgww.sediment^{-1}; Tukey's, $P < 0.05$ (Table S9, Supporting Information)) (Fig. 7a). Concomitantly, the sequencing analysis showed that the majority of ammonia oxidising and nitrite oxidising (NOB) bacterial taxa (*Nitrosomonadaceae, Nitrosococcus, Nitrobacter and Nitrospina*) were either absent or did not differ with sample type. *Nitrospira* (NOB) was the exception ($F_{5, 55}$ =

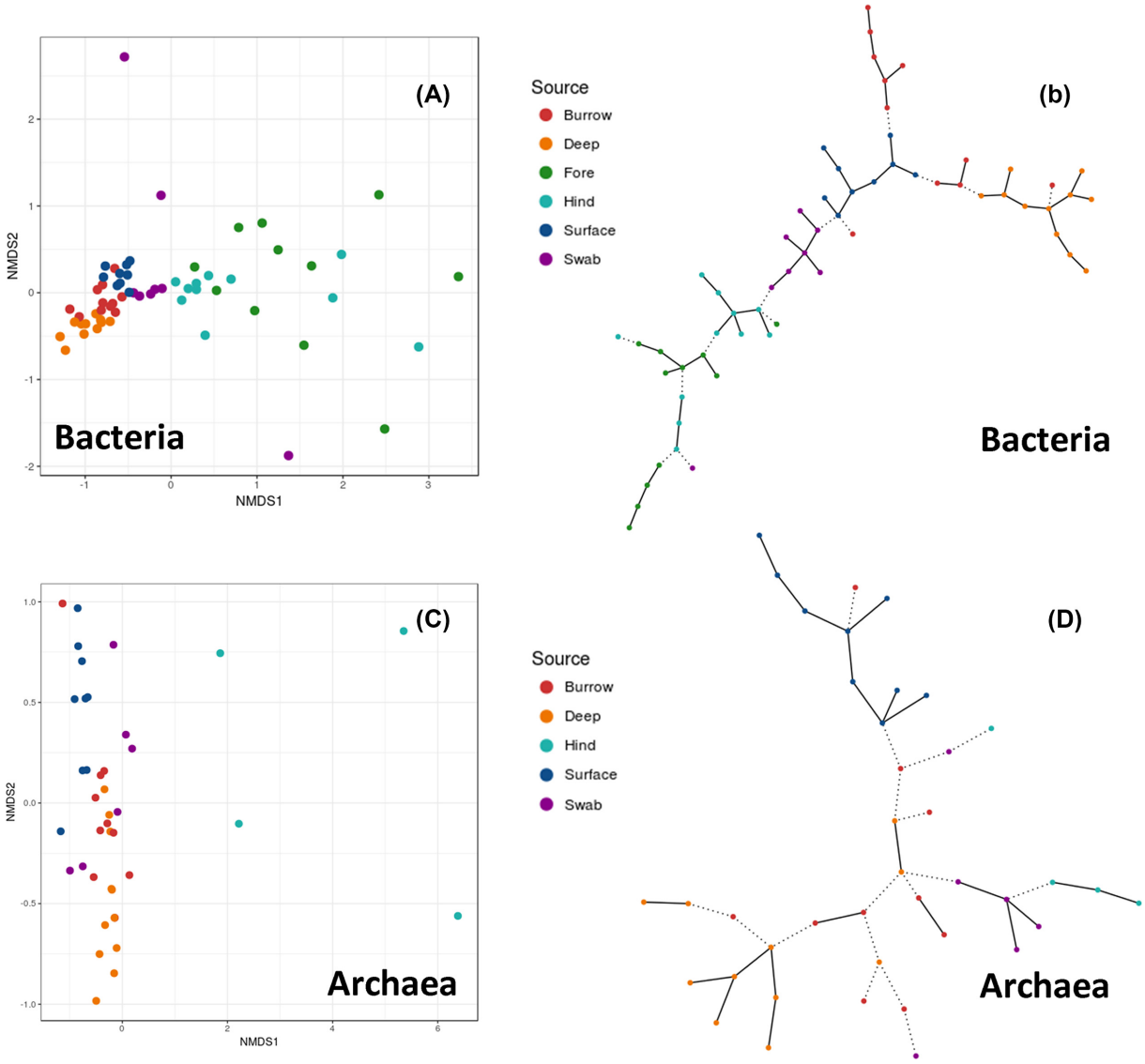

Figure 4. NMDS (Bray–Curtis) and network analysis (Minimum Spanning Tree, Bray–Curtis, $P = 0.002$) plots of bacteria **(A, B)** and archaeal **(C, D)** community structure.

2.97, $P = 0.02$) and had a lower relative abundance in the hindgut (0.02 ± 0.03% relative abundance) compared to the deep sediment assemblage (0.15 ± 0.10% relative abundance; Tukey's, $P < 0.05$).

AOA abundance was dependent on sample types (Log_{10}, $F_{4, 14} = 10.65$, $P < 0.001$), with greater abundances in sediment and hindgut assemblages (burrow, 363 ± 66 copies mgww.sediment^{-1}; deep, 517 ± 121 copies mgww.sediment^{-1}; hindgut, 1951 ± 1129 copies mgww.sediment^{-1}) relative to the foregut assemblage (90 ± 148 copies mgww.sediment^{-1}; Log_{10}, Tukey's, $P < 0.05$ (Table S10, Supporting Information) (Fig. 7b). The ratio of AOB to AOA abundance (AOB:AOA) was also influenced by sample type (Log_{10}, $F_{4, 14} = 4.08$, $P = 0.021$), with the lowest mean ratio present in the hindgut (0.267 ± 0.323), intermediate ratios in the sediment (surface, 1.11 ± 0.630; burrow, 1.70 ± 0.291; deep, 1.48 ± 0.406), and the highest mean ratio in the foregut (4.43 ± 2.86; Log_{10}, Tukey's, $P < 0.05$ (Table S11, Supporting Information) (Fig. 7c). A ratio less than one indicates an AOA dominated community, whilst a ratio greater than one indicates an AOB dominated community. Interestingly, the dominant AOA in most of the hindgut samples (*Nitrosocosmicus*) was from the Soil Crenarchaeotic Group, whilst the dominant group in the sediment samples (*Nitrosopumilus*) was from Marine Group I, which was only observed in some individual surface and burrow samples.

Bacterial *nirS* copy numbers, calculated as a proxy for the denitrifying bacterial assemblage, differed in abundance with sample type ($F_{4, 14} = 10.26$, $P < 0.001$). Denitrifying bacterial abundance was generally lower in the gut assemblages (foregut, 671 ± 484 copies mgww.sediment^{-1}; hindgut, 27 442 ± 7546 copies mgww.sediment^{-1}) relative to the sediment assemblages (surface, 82 577 ± 40 786 copies mgww.sediment^{-1}, 112 541 ± 37 716 copies mgww.sediment^{-1}; deep, 106 859 ± 34 420 copies mgww.sediment^{-1}; Tukey's, $P < 0.05$ (Table S12, Supporting Information)), with the exception of the hindgut and surface sediment assemblage (Fig. 7d). All Q-PCR abundance data are provided in supplementary material (Table S13, Supporting Information).

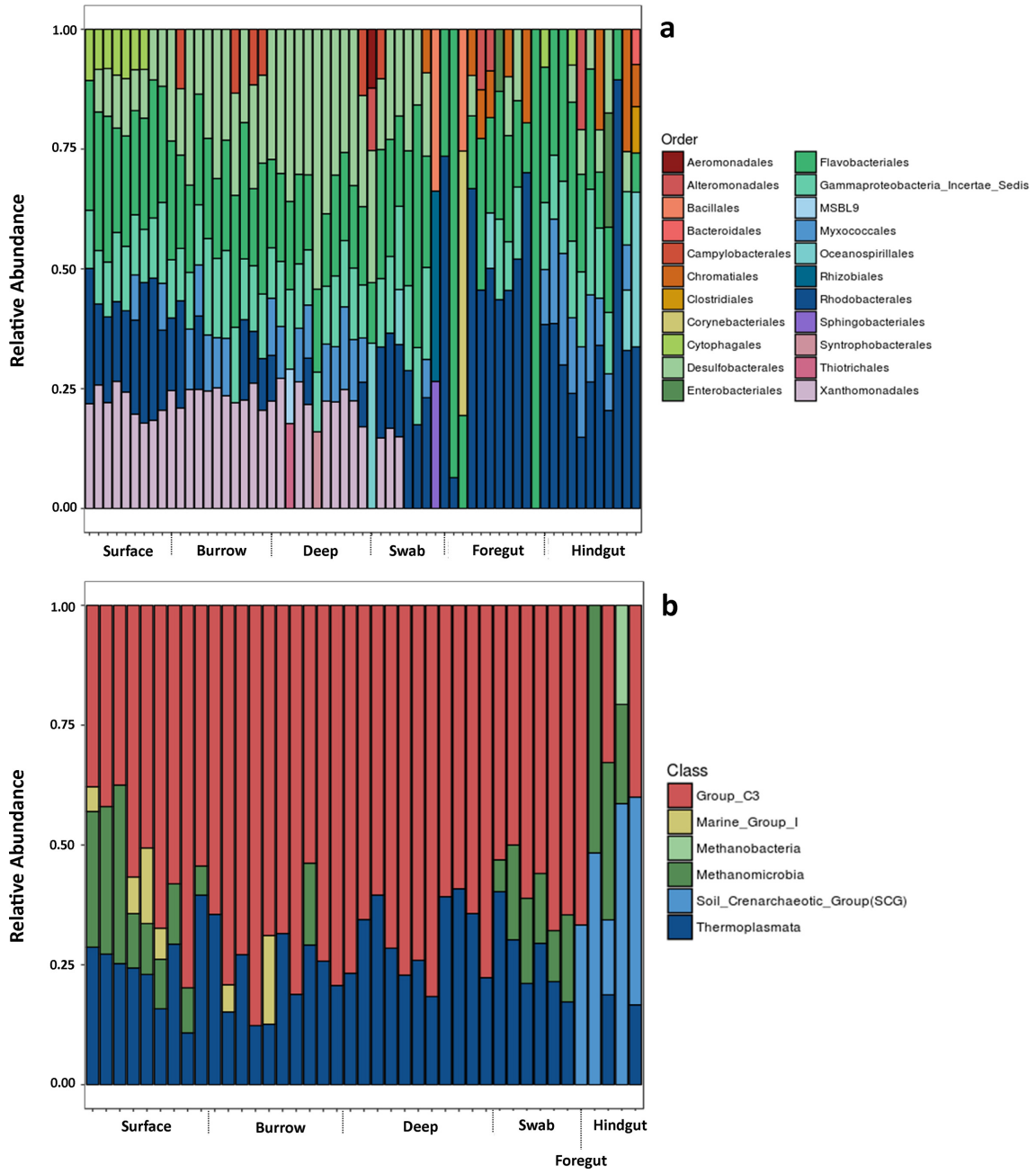

Figure 5. Relative abundance of bacterial orders **(a)** and archaeal classes **(b)** (>5% relative abundance) between each of the samples for each site.

DISCUSSION

The passage of sediment through the gut of an invertebrate has the potential to be a key mechanistic ecological process that helps explain the influence of deposit feeding invertebrates on sediment microbial assemblages and subsequent biogeochemical cycling. Using high-throughput sequencing, we demonstrate that a common sediment-dwelling invertebrate (*H. diversicolor*) has a distinct transitory gut assemblage, with regrowth of both bacterial and archaeal taxa at the posterior end of the digestive tract. Hindguts of *H. diversicolor* also appear to be 'incubators' for distinct ammonia-oxidising archaeal assemblages. This specific transitory assemblage, and the distinct assemblage on the external surface of the polychaete, has the potential to introduce higher abundances of specific taxa to the surrounding sediment, and therefore modify the sediment assemblage structure and facilitate the transport of microbial taxa between sediment patches (Godbold, Bulling and Solan 2011).

Bacterial burrow assemblages have been shown to be similar to both surface (Laverock *et al.* 2010; Pischedda *et al.* 2011) and deep sediment assemblages (Papaspyrou *et al.* 2005; Papaspyrou *et al.* 2006). Here, we show that bacterial and archaeal abundances were generally similar between all surface, burrow and surrounding sediment, and abundances observed were consistent with those in other bioturbated sediments (Laverock *et al.* 2013). Assemblage composition analysis, however, indicated that the burrow assemblages were a combination of both

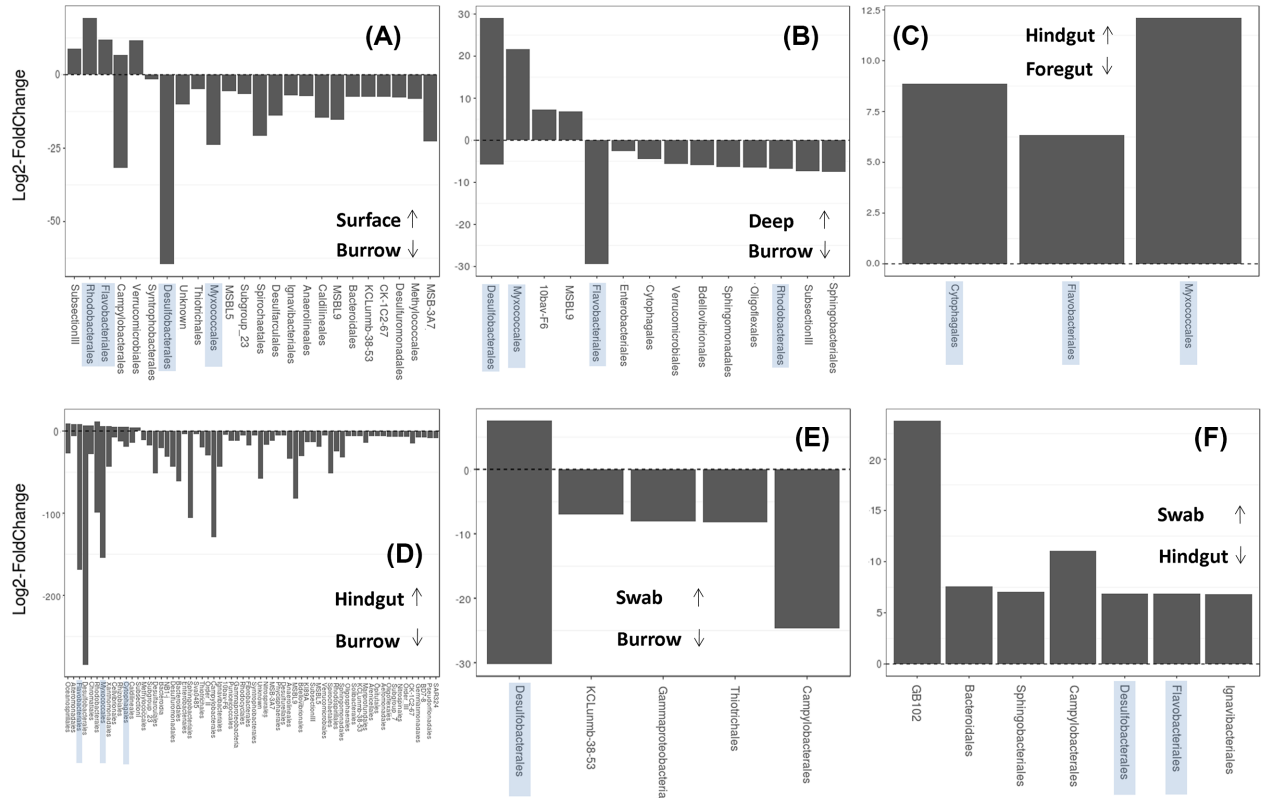

Figure 6. Change in abundance of taxa (Log2 fold; $P < 0.05$) between ecologically relevant combinations of environmental sources. (a—Burrow vs Surface; b—Burrow vs Deep; c—Foregut vs Hindgut; d—Burrow vs Hindgut; e—Burrow vs Swab; f—Hindgut vs Swab). For each combination, taxa below 0 are more abundant in first stated sample and taxa above 0 are more abundant in second stated sample. Key taxa are highlighted.

the surface and deep sediment assemblages, with the bacterial assemblage most similar to the surface sediment assemblage and the archaeal assemblage seemingly most similar to the deep sediment assemblage. Overall, this fits with the paradigm that intermittent irrigation of burrows by invertebrates creates an oscillating oxic-anoxic environment (Volkenborn *et al.* 2012), which switches the habitat between 'surface-like' and 'deep-like' available O_2 conditions and allows the coexistence of aerobic and anaerobic microbial taxa in the burrow. Other invertebrate activities occurring within burrows, such as mucopolysaccharide production (Dale *et al.* 2018) or the secretion of biocides (King 1988), are also likely to contribute to burrow assemblage structure.

The gut tracts of *H. diversicolor* contained distinct bacterial and archaeal assemblages that were less diverse than the surrounding sediment (King 2018) and included taxa, such as Thermoplasmatales and Methanomicrobiales, which have been previously observed in polychaete digestive tracts (Li *et al.* 2009). The transitory assemblage was most distinct from the surrounding sediment after passage through the foregut, where both bacterial and archaeal abundance and alpha diversity declined. Within the hindgut, the assemblages remained distinct but seemed to become more similar to the sediment and abundance increased, though this was most likely not due to the addition of taxa as there was no significant increase in bacterial or archaeal diversity. These observations follow the abundance patterns observed in other marine deposit feeders, where bacteriolytic activity is highest in the fore and midgut (Plante, Jumars and Baross 1989; Plante and Mayer 1994; Mayer *et al.* 1997), but are not consistent with previous studies of *H. diversicolor* where abundance-based techniques using epifluorescence microscopy have shown higher lytic activity and limited regrowth in the hindgut (Lucas and Bertru 1997; Lucas, Bertru and Hofle 2003). Here, the use of molecular tools with increased resolution has shown that Cytophagales, Flavobacteriales and Myxococcales increase in abundance during *H. diversicolor* gut transit. Both Cytophagales and Flavobacteriales have previously been observed in the guts of deposit-feeding shrimp (Lau, Jumars and Ambrust 2002) and are known to degrade complex macromolecules (Reichenbach and Dworkin 1981; McBride 2014) that are likely to be abundant in hindgut environments. As these taxa are also present in the surrounding sediment bacterial assemblages, gut conditions appear to affect assemblage composition by altering the abundance of existing transitory sediment taxa (Furlong *et al.* 2002).

As both bacterial and archaeal assemblages increased in abundance in the hindgut, it is possible that this microbial 'incubator' could contribute to the wider sediment assemblage once excreted. Because the distinct hindgut assemblage was more similar to the surrounding sediment than the foregut assemblage was, and greater abundances of the hindgut-enriched taxa were observed in the burrow environment relative to the surrounding deep anoxic sediment, it is possible that there was some introduction of hindgut enriched taxa to the sediment. Organic enrichment within the burrow environment, however, could have encouraged the proliferation of Cytophagales and Flavobacterales (Aller and Aller 1986; Papaspyrou *et al.* 2006), whilst Flavobacterales may have been translocated from surficial sediments. Overall, the majority of taxa were more abundant in the sediment than the hindgut, but the hindgut may act as a reservoir for at least some representatives of specific taxa

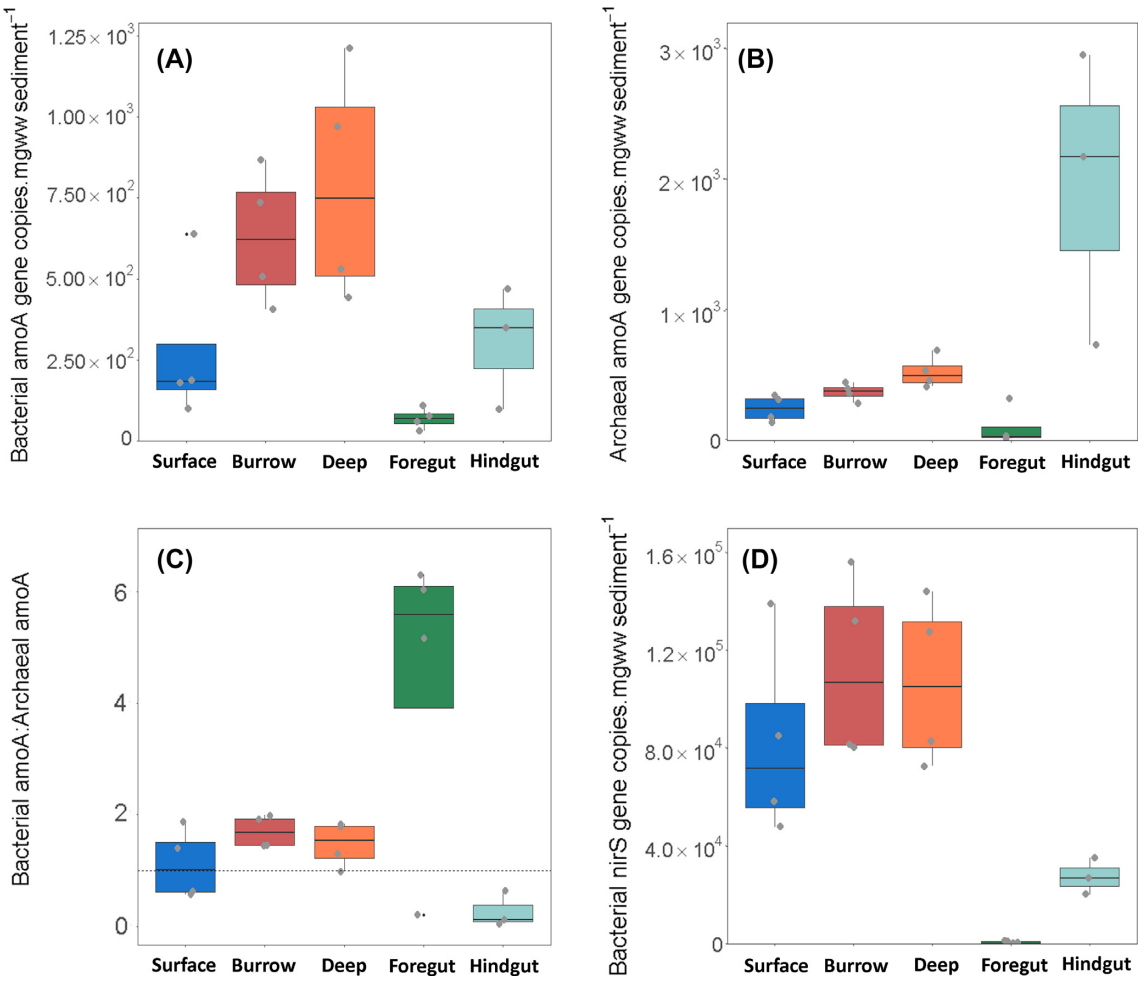

Figure 7. Variations in gene abundance between sample types. Bacterial **(A)** and archaeal **(B)** *amoA*, bacterial:archaeal *amoA* copy ratio **(C)**, and bacterial *nirS* **(D)**. Boxplot indicates median, 25% and 75% quartiles, and 95% of the data spread (n = 4). Untransformed data presented.

(King 2018). Hence, to fully characterise how sediment functioning may be affected by gut passage and the introduction of specific taxa, it will be beneficial to consider the activity levels of microbial functional groups in both gut, faecal cast and sediment environments.

Invertebrates have been suggested as potential transporters of microbial groups within sediment (Troussellier *et al.* 2017), but the significance of such transport has not been assessed. In this study, the composition of the external microbial assemblages of *H. diversicolor* reflected a combination of both hindgut and sediment assemblages, particularly the surface sediment assemblage. This observation supports the view that a proportion of the hindgut assemblage may be excreted into the sediment. The close relationship with the surficial sediment also suggests that either *H. diversicolor* spends a portion of time at the surface (e.g. foraging, Vedel and Andersen 1994), or that the very inner burrow sediment immediately in contact with the invertebrate is more closely related to the surface sediment than the other burrow wall assemblages (Bertics and Ziebis 2009). As the *H. diversicolor* external assemblages had greater abundances of some Desulfobacterales taxa compared to both the burrow and hindgut assemblages, specific taxa may be concentrated on the external surfaces of individuals and therefore be redistributed within the burrow system. Additionally, the taxonomic groups that increased in abundance during gut passage were more abundant on the external surfaces of the polychaete than they were in the deep sediment, and so *H. diversicolor* individuals could transport taxa into anoxic sediment during burrow construction and extension (Davey 1994).

Nitrification is a significant process in benthic nitrogen cycling as it converts NH_4^+ released from organic matter back into NO_3^- (Herbert 1999) that can then support primary productivity in the overlying water column (Boynton and Kemp 1985). Although the abundances of AOB and archaea (AOA) in the sediment were consistent with other sediment environments (Laverock *et al.* 2013; Bowen *et al.* 2014), they were also consistent between the surface, burrow and surrounding sediment, which suggests that these burrow systems were not hotspots for nitrifying taxa. In the foregut of *H. diversicolor*, there seemed to be an overall reduction of both AOB and AOA in the transitory assemblage, though the high AOB:AOA ratio suggests that AOA taxa were generally more susceptible to potential digestive foregut loss processes.

Conversely, passage through the hindgut supported a substantial increase of AOA to abundances comparable with the surrounding sediment so that AOA dominated the nitrifying community within the hindgut environment. This suggests that the organic content differed between the surrounding sediment and the hindgut, most likely because of the digestion of organic matter in the foregut and the release of NH_4^+ in the hindgut,

and indicates that hindguts have the potential to contribute AOA to the surrounding sediment through excretion. Sequencing analysis, however, demonstrated that the dominant AOA taxon in the *H. diversicolor* hindguts was *Nitrosocosmicus sp.*, which has a higher tolerance for NH_4^+ and NO_2^- than other AOA (Lehtovirta-Morley *et al.* 2016), whilst the dominant AOA in the sediment samples was instead *Nitrosopumilus sp.* (Reyes *et al.* 2017), which tends to dominate under low NH_4^+ conditions (Martens-Habbena *et al.* 2009). It is therefore possible that the high organic matter content in deposit feeder gut passages supports a unique AOA assemblage, which is then outcompeted when introduced to sediment. Whether the AOA are actively undertaking ammonia-oxidation in these low O_2 environments (Plante and Jumars 1992) to the extent that they significantly contribute to sediment nitrogen cycling remains to be determined.

Earthworm gut passages and faecal casts can contain larger and more active populations of denitrifying microbial groups than the surrounding soil (Karsten and Drake 1997; Furlong *et al.* 2002). Here, denitrifying bacteria were reduced during foregut passage and, although certain nitrite reducing taxa did form a significant portion of the transitory assemblage, there was no significant regrowth in the hindgut. *Hediste* diversicolor guts are, therefore, unlikely to contribute denitrifiers to sediment assemblages, but the presence of these taxa will still have functional value. Complete and incomplete denitrification by ingested soil taxa means that earthworms are sources of both N_2 and N_2O (Horn, Drake and Schramm 2006a; Horn *et al.* 2006b). Release of N_2O from deposit feeder guts have been shown to contribute to the overall flux from sediment systems (Stief *et al.* 2009; Heisterkamp *et al.* 2010), though this study also indicated that *H. diversicolor* has a slightly lower N_2O release rate than other deposit feeders (Heisterkamp *et al.* 2010). This may be due to a generally lower abundance of denitrifiers in *H. diversicolor* guts, though this would need to be confirmed by comparisons with other deposit-feeding invertebrate taxa. It also cannot be ruled out that complete denitrification (i.e. N_2 release instead of N_2O accumulation) may occur within *H. diversicolor* guts, but that remains to be determined via either direct activity measurements or identification of genes responsible for the final denitrification step (i.e. *nosZ*). Understanding how denitrifying assemblages differ between deposit feeding taxa, and whether this is related to subsequent variations in N_2O and N_2 release, will be beneficial to efforts seeking to improve current estimates of sediment N budgets.

Collectively, our findings indicate that the internal and external transport of microbial assemblages by deposit feeders has the potential to regulate sediment microbial assemblages. By accumulating both sediment and gut-associated taxa on external surfaces, burrowing invertebrates may alter local sediment microbial distributions. The transitory sediment assemblage in gut passages also has the potential to contribute to sediment nitrogen cycling, either by introducing key microbial functional groups or by supporting these taxa within the gut and excreting products. *Hediste diversicolor* often dominates the biomass of intertidal mudflats and is known to reach densities of 3700 ind. m^{-2} (Scaps 2002). As related polychaete populations have been estimated to ingest 5 kg (dry weight) sediment m^{-2} $year^{-1}$ (Cammen 1980), sediment microbial assemblages will be regularly and consistently exposed to gut conditions. Yet, the wider ecological consequences of this process are understudied, especially in the context of expanding polychaete fisheries (Watson *et al.* 2017; Cole, Chick and Hutchings 2018). Future effort should assess transitory taxa activity and establish whether the effects of gut passage vary between alternative invertebrate groups. Additionally, based on previous assessments of *H. diversicolor* emissions (Heisterkamp *et al.* 2010), these populations have the potential to release 8.8 μmol N_2O m^{-2} day^{-1} with significant implications for sediment denitrification and nitrogen fluxes. Further examination of gut passage and external transport as invertebrate functional traits will improve our understanding of invertebrate–microbe interactions, and the role this ecological process plays in regulating sediment ecosystem functioning.

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