RESEARCH PAPER

Metagenetic analysis of patterns of distribution and diversity of marine meiobenthic eukaryotes

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ABSTRACT

Aim Meiofaunal communities that inhabit the marine benthos offer unique opportunities to simultaneously study the macroecology of numerous phyla that exhibit different life-history strategies. Here, we ask: (1) if the macroecology of meiobenthic communities is explained mainly by dispersal constraints or by environmental conditions; and (2) if levels of meiofaunal diversity surpass existing estimates based on morphological taxonomy.

Location UK and mainland European coast.

Methods Next-generation sequencing techniques (NGS; Roche 454 FLX platform) using 18S nuclear small subunit ribosomal DNA (rDNA) gene. Pyrosequences were analysed using AmpliconNoise followed by chimera removal using Perseus.

Results Rarefaction curves revealed that sampling saturation was only reached at 15% of sites, highlighting that the bulk of meiofaunal diversity is yet to be discovered. Overall, 1353 OTUs were recovered and assigned to 23 different phyla. The majority of sampled sites had *c.* 60–70 unique operational taxonomic units (OTUs) per site, indicating high levels of beta diversity. The environmental parameters that best explained community structure were seawater temperature, geographical distance and sediment size, but most of the variability ($R^2 = 70\%$–80%) remains unexplained.

Main conclusions High percentages of endemic OTUs suggest that meiobenthic community composition is partly niche-driven, as observed in larger organisms, but also shares macroecological features of microorganisms by showing high levels of cosmopolitanism (albeit on a much smaller scale). Meiobenthic communities exhibited patterns of isolation by distance as well as associations between niche, latitude and temperature, indicating that meiobenthic communities result from a combination of niche assembly and dispersal processes. Conversely, isolation-by-distance patterns were not identified in the featured protists, suggesting that animals and protists adhere to radically different macroecological processes, linked to life-history strategies.

Keywords
454 Roche pyrosequencing, environmental metagenetics, macroecology, marine eukaryotes, meiofauna, metabarcoding, microscopic biodiversity, next-generation sequencing.

INTRODUCTION

Biological diversity is unevenly distributed over the surface of the planet, and several global patterns of spatial variation in biodiversity have been explored. Such patterns can vary from hotspots to coldspots (highs and lows) of diversity, change with spatial scale and along gradients across space or environmental conditions (Gaston, 2009). There is still no consensus that explains all the diversity patterns found in nature, with assumptions ranging from niche theory (Tilman, 1982) to neutral theory (Hubbell, 2001). Distribution patterns are understood relatively well in macroscopic organisms such as plants and animals, but are hotly debated in small-bodied taxa (Lambshead & Boucher, 2003), including eukaryotic meiobenthic fauna. For example, the wide or even cosmopolitan distribution of marine meiofauna has previously been considered a paradox, because meiofaunal organisms do not typically have a planktonic larval stage (Giere, 2009). Importantly for the consideration of macroecological patterns and processes, meiofaunal communities are characterized by a breadth of phyla, differing in body size, morphology and life-history strategies, which is predicted to strongly affect patterns of alpha and beta diversity (Giere, 2009), especially in the marine environment where the opportunities for gene flow are high (Palumbi, 1994). Consequently, in-depth analysis of communities that share similar habitats but exhibit high taxonomic and biological diversity provides an insightful opportunity to characterize and interpret distribution trends in microbial taxa, regardless of phylogenetic or taxonomic constraints. Here, we exploit such an opportunity to decipher the biodiversity and distribution patterns of intertidal microbial marine benthos using next-generation sequencing (NGS) technologies.

The application of NGS sequencing platforms for large-scale environmental studies focusing on homologous taxonomic markers, termed 'metagenetics' (Creer *et al.*, 2010; Fonseca *et al.*, 2010), has driven a paradigm shift in environmental microbiology (Zinger *et al.*, 2011) that has been recently extended to eukaryotes (Fonseca *et al.*, 2010; Stoeck *et al.*, 2010; Bik *et al.*, 2012; Logares *et al.*, 2014) and the expanding field of environmental DNA analysis (Hajibabaei *et al.*, 2011; Taberlet *et al.*, 2012; Thomsen *et al.*, 2012; Ji *et al.*, 2013). Many marine habitats remain poorly sampled, and several species-rich taxonomic groups, especially smaller organisms, are under-studied (Costello *et al.*, 2006), making it impossible to infer the drivers of diversity and structuring in these groups. Previously, we revealed a novel perspective of meiobenthic diversity at a microgeographical scale (Fonseca *et al.*, 2010), and here we aim to elucidate the structure and drivers of marine eukaryote community across macroecological scales, using the 18S rDNA marker gene. We sampled the marine benthos intensively around the UK, extending to France, Spain and Portugal, and with an outgroup reference site in Africa. Collectively, the data provide unique and important insights into the distribution and diversity of marine meiobenthic eukaryotes that inhabit the spatially constrained but globally important marine benthic biosphere.

MATERIAL AND METHODS

Sample collection

Sixty-six marine benthic samples were collected from the low-tide mark using a standard corer methodology (Platt & Warwick, 1988) at 23 sampling stations of sandy sediments around the UK, France, Spain, Portugal and Gambia (Appendices S1 & S2 in Supporting Information) during the summers of 2007 and 2008: 16 sampling sites in the UK; two sites each in France, Spain and Portugal; and one sampling site from Gambia representing a geographical outgroup. Three biological samples, each composed of a single 44 mm diameter × 100 mm benthic core, were taken approximately 10 m apart at each sampling site. An additional core was taken for sediment analysis, performed using a Malvern Mastersizer 2000 as in Fonseca *et al.* (2010). Seawater salinity and seawater surface temperature (SST) values were obtained using the data archives of DEFRA (http://chartingprogress.defra.gov.uk/) and NOAA (http://www.ncdc.noaa.gov/), respectively.

All sediment samples were immediately preserved in 500-mL storage pots containing 300 mL of DESS (20% DMSO and 0.25 M disodium EDTA, saturated with NaCl, pH 8.0) (Yoder *et al.*, 2006) and subsequent meiofaunal size fraction and DNA extraction were performed according to Fonseca *et al.* 2010, 2011) and Creer *et al.* (2010), using the whole core from each sample site.

Primer design and PCR strategy

The primers SSU_FO4 (5′-GCTTGTCTCAAAGATTAAGCC-3′) and SSU_R22 (5′- GCCTGCTGCCTTCCTTGGA-3′) were used to amplify approximately 450 bp of the V1–V2 regions of the nuclear small subunit rDNA (18S rDNA). These primers anneal to regions of 18S rDNA that are highly conserved in meiofauna, and flank a highly divergent region that is used to discriminate operational taxonomic units (OTUs) (Creer *et al.*, 2010). Fusion primers, PCR amplification and 454 sequencing were then executed following Creer *et al.* (2010) and Fonseca *et al.* (2010).

Data analysis and generation of OTUs

Amplicons were generated from 23 triplicate benthic sampling sites from the low-tide zone of marine sandy beaches around the UK, France, Spain, Portugal and Gambia. Sequences generated from four half-plates of 454 Roche GSFLX pyrosequencing were analysed using AmpliconNoise, which denoises pyrosequencing data, reducing per-base sequencing errors, through filtering, flowgram and sequence-clustering steps (Quince *et al.*, 2011). Singletons and sequences shorter than 199 bp were discarded; the average resulting sequence size was 200–220 bp. Chimeras are known to inflate diversity levels (Fonseca *et al.*, 2012) and were therefore identified and removed using Perseus (Quince *et al.*, 2011). Denoised and chimera-removed sequences were then used to identify operational taxonomic

units (OTUs). OTUs were calculated using a complete linkage-clustering algorithm, measuring the distance between the most dissimilar members in each cluster, at 96% and 99% identity cut-offs. Given the nature of 18S rDNA intraspecific genetic diversity, potential undetected molecular biological errors and the nature of OTU clustering, 99% OTUs are used here to investigate the distribution of genotypic diversity. Conversely, the AmpliconNoise analysis of a reference nematode community (Porazinska *et al.*, 2009) across a range of similarity cut-offs showed that a 96% similarity algorithm most closely emulated taxonomic species richness. We therefore refer throughout to 99% clustering as the distribution metric and 96% clustering as the richness metric. Taxonomic assignment was performed using megablast against the downloaded GenBank/EMBL/DDBJ nucleotide database, and OTU annotation was restricted to matches of 90% and higher, using the octupus annotation and parsing toolkit (Fonseca *et al.*, 2010). Sequence data have been deposited in the GenBank/EMBL/DDBJ short-read archive as submission PRJNA185478.

Diversity and community analysis

For direct ecological comparisons of sample OTU richness, the original data set was standardized using 9490 (the lowest coverage achieved in any one sample) randomly picked sequences (over 200 bp in length; $n = 218{,}276$) from each sample, prior to denoising and OTU clustering. Sample-specific rarefaction curves were performed using the DiversityEstimates software package via AmpliconNoise and phylum-specific rarefaction curves were generated using EstimateS 8.2.0(Colwell, 2013) using a range of richness estimators (*ACE*, *Chao1*, *Jackknife1* and *Bootstrap*) that yielded very similar results; the *Chao1* richness estimator was chosen because it is relatively independent of sample size and it is particularly informative for data sets that are skewed towards the low-abundance classes (Chao *et al.*, 1992), as is the case for reads derived from a heterogeneous range of microorganisms. Sørensen's similarity coefficient among samples was computed based on a presence/absence similarity matrix and was used to create cluster dendrograms and multidimensional scaling (MDS) with 50 random starts, using primer 6 (Clarke & Gorley, 2006). Using the same software, a similarity profile ('SIMPROF') permutation test, was performed on group-average cluster analysis to test whether meiobenthic samples differ from each other. In order to further test for significant differences in community assemblage among sampling sites, a permutational multivariate analysis of variance ('PERMANOVA') was performed. Analyses were based on Sørensen's similarity coefficient on untransformed data of an OTU presence/absence matrix over the 22 sampled sites, with 1000 permutations. To test if there was an association between geographical distance and phylum community composition, a non-parametric Mantel-type test ('RELATE') based on distance matrices derived from geographical distance (calculated as the minimum coastal dispersal distance between sampling sites) and community composition (presence–absence data) was performed using primer. To further examine phylum community

composition in relation to potential drivers, environmental variables such as seawater salinity, seawater surface temperature (SST) and sediment grain size were examined using the RELATE test for each environmental matrix, calculated based on Euclidean distances. Sequential Bonferroni corrections, which are more sensitive to false positives than the standard Bonferroni technique (Rice, 1988), were applied where appropriate. Additionally, in order to find the combination of parameters (environmental and/or geographical) that best explained the patterns within each phylum, a partition multivariate analysis of variance was performed using the *adonis* function in VeganR (Oksanen *et al.*, 2010). This partitions dissimilarities into sources of variation, and uses permutation tests to inspect the significances of those partitions. Community-composition dissimilarities were calculated as Bray–Curtis distances for each phylum against the several parameters (sea water temperature, salinity, sediment grain size and latitude).

RESULTS

Sequence data and sampling efficiency

The total number of reads derived from the Roche 454 FLX platform for all sampled sites was 877,423; this was reduced to 694,802 sequences after denoising and chimera removal. Sampling saturation profiles varied between sample sites, showing that sequencing effort was sufficient for some samples, but far from complete for most samples (Appendix S3).

Community diversity (α and β), composition and richness

Comparing the frequency of shared versus unique OTUs showed that the majority of sampled sites had 25%–40% unique OTUs, corresponding on average to *c.* 60–70 unique OTUs per site within the UK and all sampling sites. Preliminary analyses also indicated that unique OTUs were uniformly distributed between taxa. Samples from Gambia (Africa), Cap Ferret (France) and Sheerness (UK) all exhibited high percentages of endemism, with 74%, 60% and 53% unique OTUs, respectively (Fig. 1). Additional scrutiny of the 26% shared OTUs in the Gambia samples revealed, according to blast matches with 99% identity to accessions in public databases, that the most ubiquitous meiobenthic species within the sampled areas were likely to be conspecific with, or close relatives of, the nematodes *Daptonema setosum* and *Viscosia* sp., *Vannuccia* platyhelminths and *Paramenophia* copepods. Moreover, the percentages of shared and unique OTUs in all samples were highest in the Nematoda and the Platyhelminthes, with the remaining meiofauna OTUs representatives being equally distributed among phyla (Fig. 2).

The taxonomic distribution and richness of the main meiofauna representatives was explored (Fig. 3, Appendix S4). In total, across all sampled sites at a 96%-similarity cut-off, 1353 OTUs could be assigned to 23 different phyla, including 14 corresponding to marine meiofauna. Throughout the phylogenetic breadth of sampling, 12 phyla were represented by
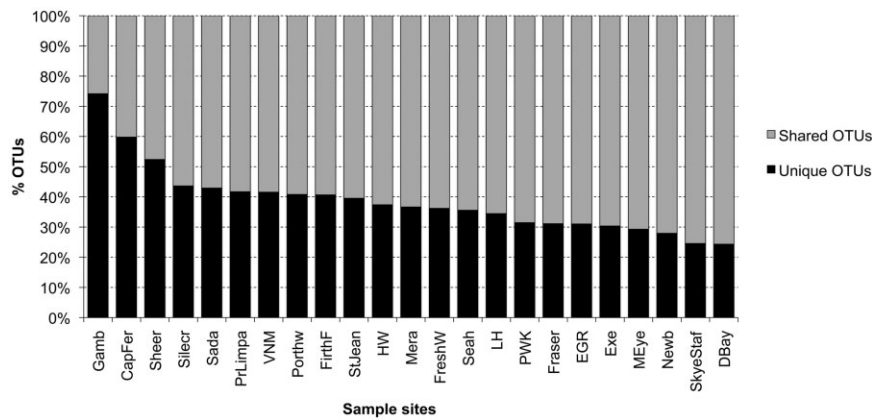
Figure 1 Relative percentages of shared and unique OTUs for each sample site from the standardized data (see Appendix S1 in Supporting Information for site abbreviations). Meiobenthic communities are niche driven but also show high levels of cosmopolitanism.
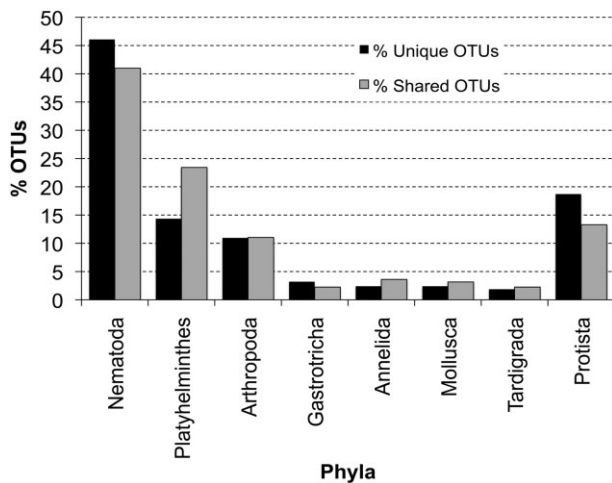

Figure 2 Relative proportions of shared and unique OTUs between all sampled sites for the main meiofauna representatives and protists, showing higher levels of shared OTUs in the most abundant phyla. The data set was normalized to the same number of reads per ecological sample; clustered OTUs that were only found in a single sampling location (i.e. among three ecological replicates per site) represent the 'unique OTUs', and OTUs common to all sampled sites are denominated as 'shared OTUs'.

no more than six OTUs (Cnidaria, Rotifera, Bryozoa, Brachiopoda, Nemertea, Apusozoa, Kinorhyncha, Orthonectida, Tunicata, Gnathostomulida, Porifera and Excavata). There were clear qualitative differences in community composition between geographical regions. Samples from the UK, for example, appeared to have a more homogeneous composition of phyla, whereas those from continental Europe proved to be more heterogeneous (Fig. 3). There was a significant positive association between the Nematoda and Platyhelminthes OTU richness across all samples (sequentially Bonferroni-corrected Spearman's correlation $\rho = 0.0025$; $P < 0.05$) but no other relationships were uncovered between the meiobenthic phyla (Fig. 3). By far the most abundant phylum throughout all sample sites was the Nematoda, which typically represented up to 40% of all OTUs within the main meiobenthic phyla retrieved. Platyhelminthes was the second richest, followed by the Arthropoda (mainly

represented by Copepoda), with 20% and 10% representation in general, respectively. Overall, there is a clear community-richness ranking hierarchy among the dominant phyla (Nematoda > Platyhelminthes > Arthropoda), followed by more random ordering across the remaining meiofaunal phyla (Fig. 4), although exceptions existed in some continental European samples where Platyhelminthes or even Arthropoda were ranked as the richest phylum. No correlations were observed between OTU richness and any of the environmental variables ($P > 0.05$), with the exception of a negative correlation between mollusc richness and latitude ($\rho = -0.658$; $P = 0.0006$). Further Mantel-based tests (RELATE) revealed significant associations between phylum community composition and geographical distance, together with finer sediment grain size, seawater surface temperature and latitude, in most meiofaunal taxa ($P < 0.05$), but not for the protist phyla (Appendix S5). The variance decomposition pattern of each phylum into environmental and geographical parameters showed that seawater temperature and latitude accounted for much of the variance in the community and were significantly correlated with community structure for the main meiobenthic phyla ($P < 0.001$) (Table 1). The combination of environmental and geographical factors that best explained community structure were, in decreasing order, latitude, seawater temperature, sediment and salinity. Latitude and seawater temperature are highly correlated and so their influence could not be estimated simultaneously in the partition analysis, but they exhibited the same level of significance and variance (R^2). Much of the variation in the communities was not explained by the parameters analysed (residual $R^2 > 0.70$; Table 1).

Clustering analysis revealed that the majority of independent samples from within each sampling station generally clustered together (Fig. 5). Of these, community composition was generally significantly different between the majority of sites and displayed clear groupings, with some geographical coherence throughout (PERMANOVA, $P < 0.001$). Communities that were geographically further apart tended to be less similar, although samples from Spain, Portugal and France shared similarities with Littlehampton, Porthtowan and Sheerness from the UK. Unsurprisingly, samples from Gambia represented a completely disparate group of samples in terms of community composition (Fig. 5).

Figure 3 Taxonomic distribution of OTUs assigned to the main representatives of meiofauna phyla (with more than four OTUs) found in the 23 sampled sites, showing the highly conserved meiobenthic community structure. (See Appendix S1 in Supporting Information for the geographical distribution and abbreviations of the sample sites.)
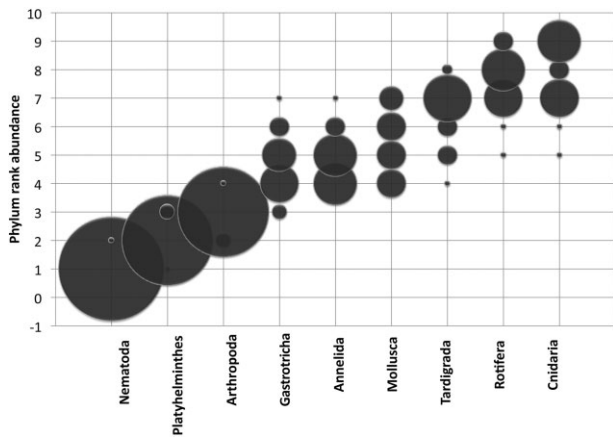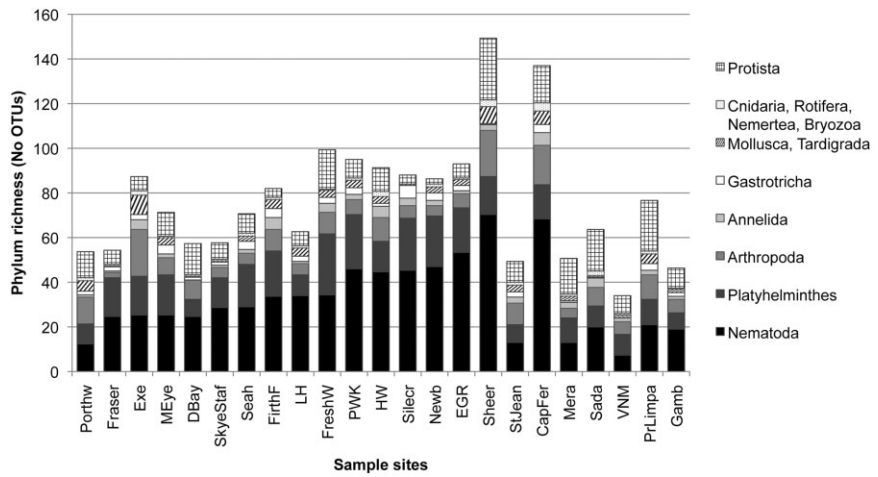

Figure 4 OTU rank abundance for the main phyla recovered from the European sites (after data standardization), providing evidence that the marine benthos is not structured neutrally. The frequency of ranking (out of nine samples) is represented by the diameter of the symbol at each rank and the multiple symbols highlight variance in phylum rank order throughout the samples.

Table 1 Environmental and geographical factors that best explain meiobenthic community structure shown by variance-partitioning analysis. Values represent the variance partitioned by each factor (R^2). Latitude and seawater temperature (SST), followed by sediment grain size (S01) and salinity, represent the factors that best account for the variation in meiobenthic community structure. Much of the variation remains to be explained (residual R^2).

Phylum	Latitude/SST	S01	Salinity	Residual
Nematoda	0.173***	0.051	0.051	0.725
Platyhelminthes	0.103***	0.058	0.048	0.791
Copepoda	0.127**	0.061	0.039	0.772
Gastrotricha	0.130**	0.046	0.127*	0.699
Annelida	0.099*	0.074	0.055	0.772
Mollusca	0.096**	0.069	0.028	0.806
Tardigrada	0.061	0.054	0.086	0.798

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

DISCUSSION

The prevailing paradigm in microbial macroecology is that, in general, the most abundant and dominant species are predicted to have higher dispersal rates and levels of ubiquity (Finlay, 2002). Interestingly, our study supports this idea, as the shared OTUs were not proportional in abundance amongst the different phyla, with higher levels of shared OTUs in the most abundant phyla (Nematoda and Platyhelminthes). Such an observation corroborates the accepted paradigm of how species' ecology affects dispersal and suggests that the level of dispersal differs in relation to relative abundance, where more abundant taxa exhibit higher chances of dispersal. The Nematoda nearly always dominated the marine sediments, generally followed by decreasing numbers of Platyhelminthes and Arthropoda, accompanied by more random assemblages of Gastrotricha, Annelida, Mollusca, and so on. Such concordant geographical patterns support a previous smaller-scale study of alpha diversity (Fonseca et al., 2010) and indicate that the marine benthos
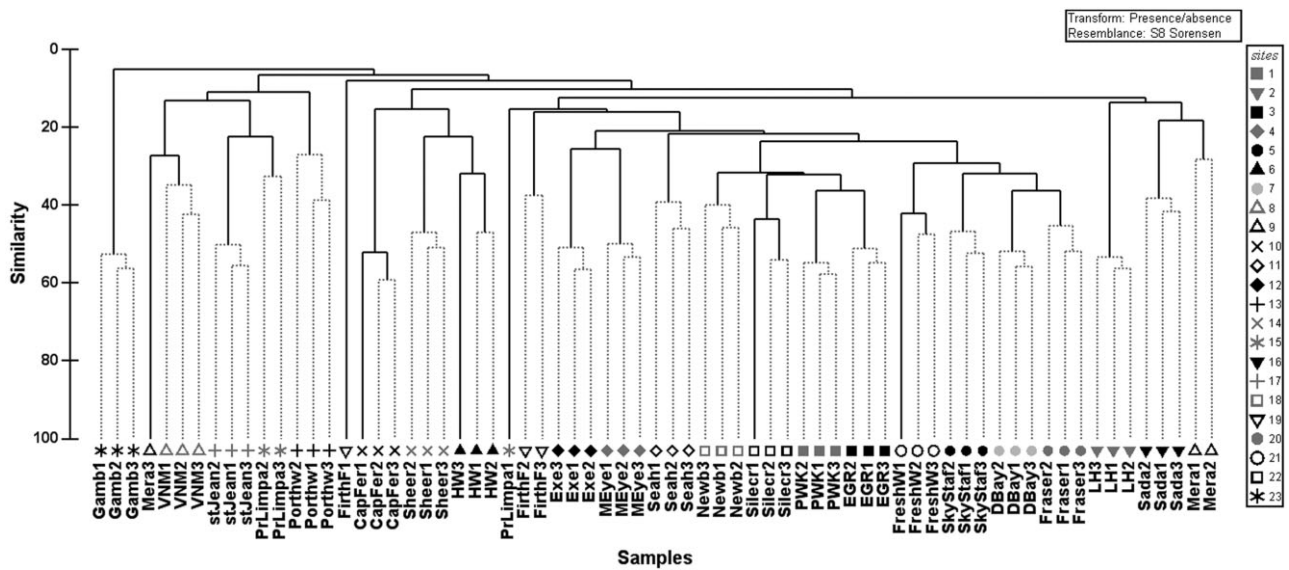
The slope of OTU rarefaction curves at the 96% cut-off for the main meiofauna phyla did not approach an asymptote and displayed a typically linear positive trend throughout the Nematoda, Platyhelminthes, Arthropoda, Annelida and Gastrotricha, suggesting an undersampling of these phyla (Fig. 6). Throughout the meiofauna, richness estimates were highest for Nematoda and lowest for the Annelida, with 827 and 71 OTUs, respectively. Almost 5% of the recovered OTUs gave no significant match to known ribosomal databases [< 90% identity; not assigned (NA)] and are likely to represent unsampled genetic diversity that is not available in public databases. Further manual analysis of these OTUs showed that the majority were placed within the Nematoda (43% OTUs, 5168 sequences) and around 14% corresponded to taxa that are annotated simply as 'environmental samples'. Of these, manual annotations of the 454 Roche reads showed that many were again likely to be derived from nematode worms.

Figure 5 Clustering dendrogram for the sampled sites based on Sørensen's similarity coefficient on a presence/absence similarity matrix (standardized data). Plotted are sixteen samples around the United Kingdom, two samples from each of France, Spain and Portugal, and one from Gambia (replicates are marked 1, 2 and 3; see Appendix S1 in Supporting Information for site abbreviations).
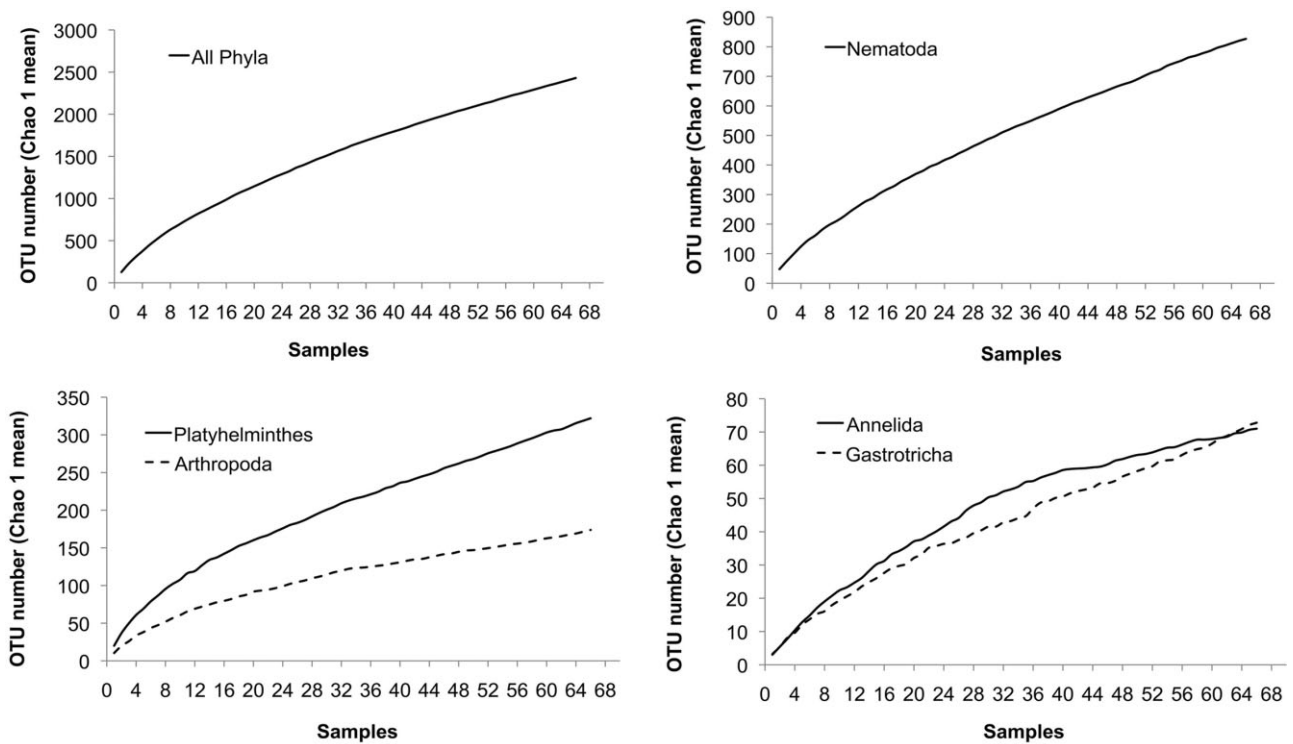

Figure 6 Rarefaction curves showing mean expected OTU numbers (*Chao1*) as a function of sample size, confirming that the bulk of meiofaunal diversity is yet to be discovered. Plots are shown for (a) all phyla, (b) Nematoda, (c) Platyhelminthes (solid line) and Arthropoda (dashed line), (d) Annelida (solid line) and Gastrotricha (dashed line) at 96% identity OTU cut-off from all European ecological samples, excluding Gambia. Curves were estimated from 100 randomizations without replacement using ESTIMATES 8.2.0.

is not structured neutrally, at least amongst the dominant phyla. Further to this, the observed significant association between Nematoda and Platyhelminthes richness suggests that at a local scale, the most abundant vermiform phyla may compete for niche space, with their corresponding impacts on meiobenthic community composition. Some members of the community might serve as 'keystone' or 'driver' species (Walker, 1992), with a crucial role in regulating community function. Thus, because

nematodes are the most abundant metazoans in marine sediments (Lambshead, 2004; Bhadury *et al.*, 2006), they are likely to directly affect community dynamics, assemblage patterns and ecological function (Danovaro *et al.*, 2008). Ecological grouping is often related in nematodes to substrate type (mud or sand) and feeding mode (e.g. microvores, predators) (Giere, 2009). Previous studies have corroborated associations between sediment granulometry at a local scale (Fonseca *et al.*, 2010; Pitcher *et al.*, 2012). This study has shown that at a macrogeographical scale meiobenthic community composition, and in particular that of Nematoda and Platyhelminthes, was associated with sediment granulometry, with fine silt in particular affecting composition. This observation suggests that, at larger scales, it may be possible to identify specific phylum requirements and preferences for sediment texture and size, and maybe to further characterize community functional groups.

Regional differences in the composition of metazoan meiofauna along continental margins have generally been hard to detect, partly because of technical/taxonomic challenges related to the small size of specimens, but also because of pronounced local variations in the biotic and abiotic factors that influence community assemblages (Soltwedel, 2000). Here, we found extremely low levels of within-site variation and identified highly conserved communities representative of the biogeographical areas sampled. Nevertheless, certain samples shared OTUs between geographically disparate locations across the UK and France/Spain/Portugal that may reflect conspecific organisms with shared ecology, or different species sharing closely related 18S rDNA genotypes. The decrease in community similarity with geographical distance is a universal biogeographical pattern observed in communities from all domains of life (Green *et al.*, 2004; Pitcher *et al.*, 2012) and is a predominant feature of the current data derived from 'animals', rather than protists. More specifically, the Gambian samples exhibited high beta diversity but the lowest estimated OTU richness, which may be the result of skewed evenness levels both at the organism and genomic levels. Alternatively, communities showing high levels of both alpha and beta diversity, such as Cap Ferret, Sheerness and Harwich, suggest high species turnover on this scale of sampling, which is probably attributable to microscopic changes in spatial/trophic niche dynamics.

Apart from a clear association between geographical distance/ latitude and community similarity for eukaryotic marine meiofauna, dispersal cannot be considered a limiting factor for all species because a high proportion of shared OTUs was found in all phyla at all sites. Here, we provide evidence of a heterogeneous meiobenthic local distribution where *c.* 40% of the taxa identified were endemic, similar to the 30% previously reported for protists (Foissner, 2006), clearly reflecting high species turnover among sites (β-diversity) and thus high regional diversity. Our data reinforce the overall consensus view that some meiofauna are highly cosmopolitan (Giere, 2009), but they are also in alignment with the existence of metapopulations that are proposed to be characteristic of many coastal invertebrates (Harrison & Hastings, 1996), because a high level of endemism occurred among sites. A recent high-throughput study of meiofauna revealed that deep-sea nematodes are mainly site-restricted, despite showing some degree of cosmopolitanism (Bik *et al.*, 2012). In our study, the existence of unique OTUs with narrow range sizes independent of sample size, suggests that, in addition to spatially driven factors such as dispersal and isolation, biotic interactions and local adaptation also determine local patterns of structure and diversity of the meiobenthos.

An intensively debated issue in macroecology is whether distribution patterns are caused by spatially limited dispersal or by niche-related factors. This issue has recently been challenged by a growing body of evidence showing that many microorganisms have restricted distributions with well-structured spatial patterns of assemblage composition (Green *et al.*, 2004; Foissner, 2006; Ghiglione *et al.*, 2012). Our analyses contribute to our understanding of the mechanisms underpinning the widespread distribution of meiobenthic species when compared to even smaller microorganisms such as protists. The evident selection for habitat found in meiobenthic communities, and thus high endemism, contrasts with the patterns found generally in protists, because most 'microorganisms can be found everywhere' (Fenchel & Finlay, 2004). Of particular relevance to the microbial macroecology debate is that our data set did not show a community isolation-by-distance relationship among the featured protist phyla. In addition, and at a much larger scale, Ghiglione *et al.* (2012) recently found that *c.* 80% of bacteria OTUs in the Arctic and Antarctic were unique to each pole. Such patterns of strong geographical isolation and low levels of dispersal highlight the importance of abiotic barriers to gene flow in relation to divergent life-history characteristics. In fact, we found that niche features (habitat) probably have the same impact as dispersal limitation in shaping meiobenthic communities, because the variation found in community structure was mainly determined by seawater temperature and geographical distance. Chust *et al.* (2013) observed similar patterns in phytoplankton communities and thus, clearly, the 'environment selects' meiofaunal communities (Baas Becking, 1934). On the other hand, much of the variation in meiobenthic community structure remained to be explained by the parameters analysed, which is often seen in biogeographical studies with dispersal limitation (Nekola & White, 1999). Thus, apart from high levels of cosmopolitanism, abiotic and biotic factors, together with dispersal constraints at local scales, can determine the variation in meiobenthic community composition, i.e. the driving factors that contribute to beta diversity.

A fundamental problem in quantifying biodiversity in many taxa is that more species will be recorded as more individuals are sampled (Bunge & Fitzpatrick, 1993), which is probably also the case for small marine organisms such as meiofauna. Nonetheless, with the advance of high-throughput techniques, it is now possible to enhance the accuracy of global marine biodiversity estimates, which are essential for an understanding of ecosystem ecology. More specifically, the present study indicates that meiobenthic diversity is likely to exceed contemporary conservative estimates, because our rarefaction curves were far from saturation. In fact, in the majority of samples, site-specific eukaryotic diversity was characterized incompletely, indicating

that a 'hidden' component of community composition probably remained to be sampled. Taxonomic sequence saturation for 18S rDNA metagenetic data sets is likely to be a result of the dynamic interplay between species richness, evenness and the number of intragenomic repeats/variants present in each sample. The dominant species may also mask the detection of low-abundance OTUs in ultrasequencing data sets. Further to this, phylum-specific rarefaction estimators of richness suggest the existence of 2500 OTUs of meiobenthic eukaryotes and more than 830 OTUs just for nematodes from a single habitat (i.e. a narrow marine littoral range) along the UK coast, augmented by limited sampling from France, Spain and Portugal. Along the UK coast, we found an average of *c.* 60 unique OTUs per site within a minimum distance between sites of 20 km, yielding a gross richness estimate of around one million new marine meiofauna species that remain to be identified along the 356,000 km of the world's coastline. Remarkably, these numbers apply to a very conservative 96% cut-off on a restricted intertidal marine benthos habitat and suggest that the recently predicted 2.21 million eukaryote marine species may indeed be a gross underestimate (Mora *et al.*, 2011). For marine Nematoda, there are *c.* 450 species estimated around the British Isles Giere, (2009) and Costello *et al.* (2006) estimates 1837 species for the Northern European marine nematodes. Lambshead & Boucher (2003) also report that 30%–40% of free-living Nematoda identified in field surveys of European seas are new to science. Despite the controversy surrounding marine species richness, it is clear that richness is currently underestimated, and it is likely that microorganisms in less-explored areas such as the deep sea and soil make up the bulk of diversity yet to be discovered (Mora *et al.*, 2011).

Our study yields insights into the biodiversity of marine meiobenthic eukaryotes in terms of magnitude, novelty and organization. More specifically, it has revealed that some meiobenthic species are likely to be highly ubiquitous, whereas others exhibit local segregation in marine coastal environments along a discrete littoral habitat across Europe. Local drivers of global environmental biodiversity will be the result of the abiotic factors, in addition to other microclimatic properties such as sediment chemical properties interacting with organism life-history strategies in benthic environments. Acknowledging the environmental challenges that are likely to be imposed on all ecosystems in the current century, it is increasingly important to understand the macroecology and concomitant levels of local adaptation of functionally important microbial communities. Specifically, further refinements will be required to fully synthesize the extent of community change, including the analysis of seasonal variation, direct analysis of relative abundance, additional metadata and clarifying the taxonomic significance of molecular genetic OTUs.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix S1 Coordinates of the 23 sampled sites.
Appendix S2 Map of the 23 sampling stations of sandy sediments around the UK and France, Spain, Portugal and Gambia.
Appendix S3 Operational taxonomic unit saturation profiles.

Appendix S4 Number of total OTUs for all metazoan phyla found in the 23 sampled sites.
Appendix S5 Spearman's correlation (ρ) and significance test (*P*) between community similarity and grain size, surface seawater temperature, seawater salinity, geographical distance and latitude, derived from a Mantel test, for the main meiofauna and protist phyla.

BIOSKETCH

The research team comprise molecular ecologists, taxonomists, marine, evolutionary, genome, functional and computational biologists who share interests in defining and understanding biodiversity patterns and processes in the marine biosphere.

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