Human access impacts biodiversity of microscopic animals in sandy beaches

Alejandro Martínez 1,10, Ester M. Eckert 1,10, Tom Artois 2, Giovanni Careddu 3, Marco Casu 4, Marco Curini-Galletti 4, Vittorio Gazale 3, Stefan Gobert 2, Viatcheslav N. Ivanenko 5, Ulf Jondelius 6, Marinella Marzano 7, Graziano Pesole 7,8, Aldo Zanello 3, M. Antonio Todaro 9 & Diego Fontaneto 1

Whereas most work to understand impacts of humans on biodiversity on coastal areas has focused on large, conspicuous organisms, we highlight effects of tourist access on the diversity of microscopic marine animals (meiofauna). We used a DNA metabarcoding approach with an iterative and phylogeny-based approach for the taxonomic assignment of meiofauna and relate diversity patterns to the numbers of tourists accessing sandy beaches on an otherwise un-impacted island National Park. Tourist frequentation, independently of differences in sediment granulometry, beach length, and other potential confounding factors, affected meiofaunal diversity in the shallow “swash” zone right at the mean water mark; the impacts declined with water depth (up to 2 m). The indicated negative effect on meiofauna may have a consequence on all the biota including the higher trophic levels. Thus, we claim that it is important to consider restricting access to beaches in touristic areas, in order to preserve biodiversity.
Coastal areas and sandy beaches in particular are popular locations for recreational activities and holiday destinations, and are therefore subjected to intense stressors as a result of increasing urbanization and coastal infrastructure. Marine protected areas have been created as a tool to protect biodiversity of marine and coastal environments from these alterations. The creation of marine protected areas aims also at developing sustainable eco-tourism, which should have a positive effect on biodiversity and landscape, maintaining ecosystem services. Even if direct alterations of the ecosystem are reduced in such projects, tourism might nevertheless impact marine biota. Activities such as fishing or diving have conspicuous effects on the animal communities and have been widely investigated and regulated by environmental authorities. Other apparently harmless human activities, such as the simple presence of people, could already affect animal communities, e.g. through the physical effect of trampling on the sand; the microbiological interference of human-related bacteria discharged in water; and the chemical release of pollutants from sunscreen creams.

Most of the studies addressing the impact of human presence on beaches were performed in highly urbanized and tourist-rich areas, where other stressors inherent to coastal development are present (e.g. pollution, coastal infrastructure, beach nourishment) potentially masking the effect of human presence on beach communities. Moreover, they were performed on large invertebrates, which often present hard shells and cuticles and can dig into the sediment layers beyond the one that is directly affected by tourists. In contrast, microscopic animals, collectively called meiofauna, are rich in species consisting of members of almost all animal phyla with a wide range of ecological features, differentially responding to human stresses, with a short generation time, allowing for a rapid detection of different types of impacts at different time scales. Although these features make meiofauna a candidate to test the impact of human-driven changes in marine coastal areas, very few studies on the effect of tourists on meiofauna of sandy beaches are available, mostly because it is difficult to obtain reliable estimates of diversity on meiofauna dealing with morphological approaches in species identification. The main problems are due to the vast diversity of meiofaunal organisms, with different extraction methods for each group to obtain animals for morphological identification, and to the lack of taxonomic expertise for several taxa making it almost impossible to identify meiofauna in biodiversity inventories.

We here investigate the effect of tourist presence on sandy beach ecosystems to provide quantitative estimates with the final goal of helping biological conservation of coastal areas, especially in countries that are highly impacted by tourism. Two problems have to be bypassed: on the one hand the presence of potential confounding factors other than human frequentation in the analysed area (e.g. pollution, coastal infrastructure, beach nourishment), and on the other hand the difficulties in using and identifying meiofauna. To minimize the effect of confounding factors, we selected the Asinara National Park in Sardinia, Italy as a study area, one of the least impacted localities with sandy beaches in the North-Western Mediterranean Sea. The area has no local inhabitants and has controlled access of tourists, present mostly during the summer season; far from cities and large harbours, the only human-induced impact on the beaches of the island is therefore due to tourists; moreover, activities other than walking on the beach and swimming in water, e.g. access to motorized vehicles, camping, beach grooming, are forbidden in the Park. To apply meiofauna as a metric for biological diversity, we used a DNA metabarcoding approach from high-throughput sequencing of the V1–V2 region of the 18S rDNA with an iterative and phylogenetically informed taxonomic assignment for the identification of the different taxa, paired with the identification of taxa also with a morphological approach for some selected groups of meiofauna.

Our hypothesis is that any effect of human presence on meiofauna living in sandy beaches will be detected more intensely where tourists have higher densities and can walk on the sand, namely in sand at the waterline in the swash level in the beach face (0 m depth), or in sand in shallow waters just below the low-tide shoreface (0.3 m depth), compared to sand in deeper waters (2 m depth), where people cannot walk but only swim.

Results

ZOTU and species diversity. All 11 beaches of Asinara longer than 10 m (Fig. 1a, b) were sampled at three different depths (Supplementary Data 1), here called swash (on the beach face, at 0 cm water depth), shallow (below the low-tide shoreline, at 30 cm water depth), and deep (in the open water, at 2 m water depth, within a few metres offshore). A total of more than 460,000 high-quality merged reads clustered into 1069 ZOTUs (zero-radius operational taxonomic units); of these, 416 belonged to unicellular eukaryotes, 13 to non-miofaunal metazoans (e.g. Asciidae, Porifera, Phoronida), and 640 to meiofauna (Supplementary Table 1). The 640 meiofaunal ZOTUs accounted for 60% of the ZOTUs and for 99% of the reads: almost all the reads in the dataset were indeed from meiofauna. Meiofaunal ZOTUs, assigned to taxonomic groups with an iterative approach, corresponded mostly to Nematoda (32.2% of the meiofaunal ZOTUs) and Copepoda (19.7%), followed by interstitial Annelida (12.3%), free-living Platyhelminthes (10.3%), Acoela (8.6%), and Gastropod (6.8%). These six groups (Fig. 1), representing 90% of the meiofaunal ZOTUs, were used also separately as major groups in subsequent analyses. The other groups, Acari, Gnaurostromulida, Mollusca, Nemertea, Ostracoda, Rotifera, and Tardigrada were represented by 3–14 ZOTUs, Kinorhyncha by a single ZOTU (Supplementary Table 1).

Richness. ZOTU richness per sample ranged from 24 to 180 (Supplementary Table 1). The overall number of meiofaunal metazoan ZOTUs was significantly different at different depths (generalized linear mixed effect model, GLMEM; depth: LR chi-squared = 25.4, p < 0.0001), with more ZOTUs in sand in deep water (Fig. 2), and at different beaches (likelihood ratio chi-squared, LR = 31.4, p < 0.0001), without being affected by the potential bias due to number of reads per sample (LR = 0.8, p = 0.380). Analysing each major meiofaunal group separately, results were congruent with the overall analysis on all ZOTUs, except for Gastropod and Copepoda, for which richness was not affected by differences between beaches, and Acoela, whose richness was not affected by depth and was marginally related to the number of reads (Supplementary Table 2), suggesting a complex scenario of potential drivers of ZOTU richness in different beaches at different water depths for the different taxa.

Thus, to be able to understand the actual patterns of diversity, we build a second set of models in which we analysed the effect of tourists against the total number of ZOTUs separately for each depth level (swash, shallow, and deep), including the length of the beach and the type of sediment granulometry as confounding factors. We found a negative relationship between the total number of ZOTUs and the number of tourists at swash and shallow levels, although the relationship was significant only at the swash level (generalized linear model, GLM: LR = 4.8, p = 0.028) (Table 1, Fig. 2). Significant negative correlations were found between number of ZOTUs and number of tourists for Copepoda at the swash (LR = 4.6, p = 0.031) and for Acoela at the shallow level (LR = 5.9, p = 0.015), whereas no clear
information was found for the other taxa (Supplementary Table 3).

In contrast, the analyses using the morphological dataset at each level did not show clear results, with no significant relationships recovered between the total number of species and the number of tourists at any water depth (Supplementary Table 4). Only the richness of Annelida was positively correlated with number of tourists at the swash level.

Community composition. Mirroring the results obtained for the meiofaunal richness analyses, our analyses also suggest complex scenario of potential drivers of the meiofaunal community composition at different beaches with different number of tourists interacting at different depths. As expected, changes in the overall ZOTU composition of the meiofaunal communities, analysed with presence/absence data, were better explained by the inherent differences between beaches (PERMANOVA: $R^2 = 0.381$, $p = 0.0038$), followed by differences in sediment granulometry ($R^2 = 0.263$, $p = 0.002$). We detected a significant effect of the number of tourists, even if it explained a smaller proportion of the variability in community composition ($R^2 = 0.058$, $p = 0.0021$) (Table 2).

A second set of models testing the effect of the number of tourists at each level and including sediment granulometry as a confounding factor revealed that the explanatory power of number of tourists was higher in the swash ($R^2 = 0.184$, $p = 0.204$) and the shallow ($R^2 = 0.171$, $p = 0.241$) levels than in the deep one ($R^2 = 0.111$, $p = 0.204$), even if not statistically significant. Such scenario was consistent across all meiofaunal groups (Supplementary Tables 5 and 6).

Phylogenetic diversity. No clear effect of the explanatory variables was found on phylogenetic diversity and mean phylogenetic distance, except for the number of ZOTUs significantly affecting phylogenetic diversity of all the groups, and sediment granularity affecting phylogenetic diversity of Annelida (GLM: LR = 36.1, $p < 0.0001$) and Nematoda (LR = 19.8, $p < 0.0001$) (Supplementary Table 7).
Discussion
Our results suggest that the presence of tourist on beaches may have an impact on the communities of microscopic animals: a negative correlation between the number of meiofaunal ZOTUs and the number of tourists on beaches could be seen for the swash (slightly significant) and the shallow level (even if not significant). We thus found a stronger effect of tourists at the swash level, but we cannot disentangle whether this could be due only to a higher presence of tourists in the swash level than in the other two water levels or if this habitat is indeed more sensitive to human presence. Changes in the taxonomic composition of meiofauna, as expected from previous studies\textsuperscript{15,17}, were due mostly to differences between beaches and sediment granulometry, but also to the presence of tourists and not so much to the differences between depths. We suggest that the effect of presence of people could be assigned mostly to trampling, since walking on the beach at the swash level is the major disturbance activity of humans in the sampled areas, where other more impacting recreational activities (e.g. access to motorized vehicles, camping, beach grooming, etc.) are forbidden. Other potential effect could be indirectly related to human presence, such as to the amount of sunscreen cream and of faecal-related bacteria that enter the water, which are likely proportional to the number of tourists. We acknowledge that sample size for the most densely frequented beaches in Asinara is low (Fig. 2); yet, the statistical approach is robust and accounts for potential confounding factors. In any case, the negative effect of tourists was always present, more significantly at the swash level, where people indeed walk on the beach, and only marginally present in shallow waters but completely absent in deep waters. Such effect was visible even for the low density of tourists that are present in the near-pristine habitats of the Asinara National Park. A maximum of 300 people per day walking every 10 m\textsuperscript{2} means that during all the day less than 30

| Predictor | $R^2$ | $P$ |
|-----------|-------|-----|
| All water depths | | |
| Tourists | 0.058 | 0.021 |
| Granulometry | 0.263 | 0.002 |
| Depth | 0.090 | 0.003 |
| Beach | 0.381 | 0.004 |
| Residual | 0.202 | |
| Swash | | |
| Tourists | 0.184 | 0.204 |
| Granulometry | NA | NA |
| Residual | 0.816 | |
| Shallow | | |
| Tourists | 0.171 | 0.241 |
| Granulometry | 0.678 | 0.200 |
| Residual | 0.151 | |
| Deep | | |
| Tourists | 0.111 | 0.204 |
| Granulometry | 0.404 | 0.366 |
| Residual | 0.486 | |

Results are reported from permutational multivariate analyses of variance (PERMANOVA) on the effect of different sets of explanatory variables on community composition calculated as pairwise Jaccard dissimilarities of the ZOTU occurrence. Analyses are performed for all the dataset, as well as the swash, shallow, and deep level. Differences in granulometry were not analysed at the swash level because only one granulometry type was present for the level. $R^2$ and $P$ values are reported. $P$ values for significant predictors are marked in bold.

Table 1 Effect of the number of tourists together with the potential confounding factor of type of sediment granulometry and beach length on the richness of meiofaunal ZOTUs (zero-radius operational taxonomic units) at the three water depths, according to a type II ANOVA output of generalized linear models.

| Predictor | LR Chisq | Estimate ± s.e. | df | $P$ |
|-----------|----------|-----------------|----|-----|
| Swash | | | | |
| Tourists | 4.786 | $-0.170 ± 0.078$ | 1 | 0.028 |
| Granulometry | NA | NA | NA | NA |
| Length | 0.003 | $-0.004 ± 0.072$ | 1 | 0.956 |
| Shallow | | | | |
| Tourists | 0.000 | $-0.005 ± 0.300$ | 1 | 0.987 |
| Granulometry | 53.428 | NA | 4 | <0.0001 |
| Length | 0.003 | $-0.017 ± 0.298$ | 1 | 0.957 |
| Deep | | | | |
| Tourists | 2.388 | $0.070 ± 0.045$ | 1 | 0.122 |
| Granulometry | 101.570 | NA | 4 | <0.0001 |
| Length | 0.060 | $0.011 ± 0.043$ | 1 | 0.807 |

$LR$ Chisq likelihood ratio chi-square values, $df$ degrees of freedom, $P$ chi-square goodness of fit, s.e. standard error. Differences in granulometry were not analysed at the swash level because only one granulometry type was present for the level. $P$ values for significant predictors are marked in bold.

Fig. 2 Number of meiofaunal ZOTUs (on a logarithmic scale) in relationship to the maximum number of tourists per day every 10 m\textsuperscript{2}. The black dashed line represents the (non-significant) regression line for the entire dataset. Points and lines represent the relationship for each water depth: swash (black dots), shallow (red squares), and deep (blue triangles). The significant regression line for swash water level is reported with 95% confidence interval.
people passed on every square metre at the peak of the tourist season.

Previous studies on meiofauna or on larger invertebrates were not always consistent in their results, and provided evidence of human disturbance only at very high levels of frequentation by tourists\textsuperscript{6-9}. Moreover, they were performed in urbanized areas where other stressors continuously affect beach ecosystems throughout the year potentially masking the effect of people during the touristic season\textsuperscript{13}. This is not the case in the Asinara National Park, never populated or subjected to intensive touristic exploitation. The use of the metabarcoding approach with an iterative and phylogeny-informed taxonomic assignment allowed us to overcome the impediment to describe meiofaunal communities using morphological approaches, in contrast with other studies that had to focus on certain groups only, often copepods or nematodes, which are easier to extract and to preserve\textsuperscript{13}, neglecting the most sensitive soft-bodied taxa\textsuperscript{5,e}, e.g. flatworms, gastrotrichs, acocels, gnathostomulids, rotifers, for which sampling and preservation for morphological analyses is more problematic\textsuperscript{18}. Metabarcoding from meiofauna is now a common approach in biodiversity studies\textsuperscript{21,25} and our results demonstrate that we can strengthen the support for the use of metabarcoding of meiofauna in routine environmental monitoring, potentially not only for sandy beaches\textsuperscript{26,27}. We found an indication of the extent of human presence notwithstanding the use of one single marker and with a certain proportion of samples that did not work, potentially because of non-optimal processing and storage before DNA extraction. Using appropriate storage and a multi-marker approach\textsuperscript{28,29} may work even better. The metabarcoding protocol can be considered more efficient than the approach based on morphological identification of species: especially for meiofauna, metabarcoding is faster, less subjective, and mostly cheaper than a morphological approach\textsuperscript{25}. We managed to handle all meiofaunal groups for all beaches for metabarcoding, whereas the same team of taxonomic experts who worked for this study could handle only few groups and for only a selection of the beaches (Supplementary Data 2). As a negative side, the problem of metabarcoding of meiofauna is that no abundance data can be considered reliable, and because of occurrence-only data, biological monitoring through metabarcoding is still under discussion for freshwater and marine habitats\textsuperscript{30}, but will surely become a reality in the near future\textsuperscript{31}. In our case, with at least 88 of the 196 morphologically identified species being potentially new for science (Supplementary Data 2), the process of morphological identification for biological monitoring is massively slowed down. Due to such high number of yet unknown species with no DNA sequence data, the taxonomic assignment of metabarcoding data may be flawed. What is known from previous studies is that metabarcoding and morphology may provide different results for the assessment of biological diversity\textsuperscript{19,21,23}. We confirm such indication, showing that several genera and families have been found uniquely with metabarcoding or with a morphological approach for the six taxonomic groups on which both approaches were applied (Fig. 3). In addition, it is known that the 18S rDNA marker we selected may underestimate diversity for some taxa\textsuperscript{32}, potentially explaining the low number of ZOTUs for some groups such as Rotifera and Kinorhyncha. Regardless of potential problems and differences between different approaches, one of the main messages of our study is that we confirm the use of metabarcoding on meiofauna as a tool for biodiversity monitoring\textsuperscript{19,21,22,25}.

The other main message of our results is that if human access may impact biodiversity beaches with restricted access to tourists should be considered when planning marine reserves, in order to preserve biodiversity, especially in an impacted but highly species-diverse area such as the Mediterranean Sea\textsuperscript{33}. Since many meiofaunal taxa are restricted to sandy beaches and may be sensitive to trampling and other indirect influences from the mere presence of people, our results highlight the necessity of implementing management strategies including integral protection for specific sandy beaches for conservation purposes. Protection can consist either of including certain beaches into areas of integral protection within marine protected areas, as is the case in Asinara, or alternatively by defining specific zones within beaches in which tourist access is forbidden. This last approach has already been implemented in areas with extensive coastal sandy areas with positive effects on coastal marine communities\textsuperscript{14}. However,
in areas in which most sandy beaches are short, such as the Mediterranean, efficient subdivision of beaches might not be possible. In such areas, integral protection within marine protected areas seems the only solution, but it needs to be carefully planned and supported by campaigns of social awareness on the importance of such a measure34, potentially using metaborcoding as a tool to show the effect of human presence.

From an ecological perspective, the negative effects on meiofauna that we highlighted may not be strictly limited to these microscopic animals: any negative impact on them will have a general effect on the ecosystem functioning35,36. Meiofaunal organisms play a key role in production, consumption, decomposition, nutrient regeneration, and energy transfer, but also in preventing sediment erosion in sandy beaches37. It is true that we highlighted a negative effect on meiofauna only at the swash zone, thus, not allowing any supported inference on the effects on ecosystem functioning of the beach ecosystem.

In conclusion our results have two important implications: (1) The use of biological monitoring through DNA metaborcoding in aquatic habitats should be pursued as an efficient and reliable future research methodology to identify ecosystems38 and inform environmental stakeholders and politicians in their decision-making process. (2) Even low intensity of presence of tourists might produce an overall loss of biodiversity, especially in the sensitive groups of microscopic animals; it is possible that such effect could not be demonstrated any more in heavily disturbed coastal areas, where most beaches are frequently trampled and the most sensitive organisms would have disappeared already, but our sampling in Asinara allowed us to identify such effects, even if we acknowledge that the results are only slightly significant and limited to the swash zone.

### Methods

#### Sampling design

The Asinara National Park (http://www.parks.it/parco-nazionale-asinara/Iindex.php), located at the island of Asinara in the North-Western tip of Sardinia, Italy, covers a Mediterranean Protected Area of about 1,100 km². Before a National Park was established in 1997, the island hosted a hospital in the nineteenth century and then a prisoner camp and a maximum-security prison39, nobody lives permanently on the island, and the only people who can impact marine biodiversity are tourists. The National Park receives an average of 1,989 tourists every day and all tourists from October to March; the total number of tourists is anyway limited, providing one of the best examples of potentially sustainable tourism along the coastline of the Mediterranean Sea39. Meiofauna on the island, even if disturbed by tourists, may have the possibility to recover during the tourist-free period from October to March. Asinara island is sinuous shaped with four mountainous sections linked by a narrow, flat coastal belt (Fig. 1b). The tidal range in the area is just a few centimetres, making the physical impact of tides on meiofauna very limited. The west side of the island is rocky and steep, while the east side has flat areas occupied by coves and beaches. Most of the 11 beaches longer than 10 m are open to tourists, except for two within the area of total protection, corresponding to “Cala Sant’Andrea and Cala di Scombro di Dentro” at the centre and “Cala Arena and Punta dello Scorno” at the northern tip of the island (Fig. 1b).

We sampled all 11 beaches longer than 10 m present in the park (Fig. 1b). The beaches are mostly pocket beaches, between 20 m and 400 m along the shoreline, relatively homogeneous in their physical, ecological, and geographic conditions (Fig. 1d): they are within a maximum distance of 15.5 km, which minimizes spatial and biogeographic confounding factors; they are on the more protected Eastern coast of the island, which minimizes ecological factors of physical exposition to waves; and they are all sandy beaches, which minimizes ecological differences due to sediment granulometry. The major difference between the beaches is the number of tourists received during the summer months (Supplementary Data 1). The daily affluence of tourists ranged from beaches with no tourists to beaches with peaks of 300 tourists per day every 10 m², even if such relatively high numbers were reached for only one or a few days in the season. Two major beaches (Cala Sant’Andrea and Cala d’Arena) within the areas of integral protection are restricted to the public (Supplementary Data 1). The number of tourists per beach was estimated by the authorities of the park by direct observations and the data are stored in their unpublished archives. The samples for the extraction of meiofauna were collected at the end of the tourist season between 22 September and 1 October 2014. Sediment samples were collected manually from four replicates of 1 liter of sediments from the upper 5 cm of sand collected over a homogenous area of 1 m² by scooping the top layer of sand with a jar. Immediately after collection, samples were taken to the laboratory on the island. All samples were processed within few hours after collection. Total meiofauna for each replicate with high-throughput sequencing (HtS) was extracted from two replicates using the MgCl₂ decantation technique through a mesh size of 63 µm and immediately preserved in ethanol at −20 °C. The other two replicates were used one for the analysis of sediment granulometry and one for morphological identification of meiofauna (see below).

#### Metaborcoding

Overall, 11 beaches were samples, with three levels (here called swash, shallow, and deep). Each of the 33 samples was sequenced twice for a total of 66 sequencing reads (the replicates with the highest number of reads for each of the 33 samples was used for metaborcoding. Eight of the total 33 samples were discarded (Supplementary Data 1), due to the low quality of the DNA in both replicates. Sequence reads are publicly accessible at NCBI (GenBank) with accession number PRJNA369046. Index, adapter, and primers were removed with cutadapt (ref. 39). The USEARCH pipeline was used for alignment, quality control; USEARCH for the clustering of operational taxonomic units (OTUs)40. The pipeline was essentially used following the author’s online tutorial with the following settings: when merging sequences the maximum number of nucleotides that were allowed to be different in the overlap (max-diff) was set to 10 and the merged sequences had to have a minimum length of 300 bp. ZOTUs were calculated using the UNOISE algorithm, which attempts to identify all correct biological sequences in the reads (high-quality requirements and more than eight reads in the dataset) and cluster the other sequences around them, resulting in presumed 100% identity termed ZOTUs for zero-radius cluster. To reduce rarefaction of the dataset, raw data was used to maintain all the sequences we obtained41; yet, we explicitly tested for the potential confounding effect of number of reads in the statistical tests (see below).

We used an iterative and phylogenetically informed approach for the taxonomic assignment of ZOTUs. Non-metazoan meiofaunal sequences, identified through Blast against the whole Genbank database42, were amplified using the MgCl₂ decantation technique but also by siphoning off the water just above the sediment surface18. Live material was studied using dissecting and compound light microscopes. The major difference between the beaches is the number of tourists received during the summer months (Supplementary Data 1). The daily affluence of tourists ranged from beaches with no tourists to beaches with peaks of 300 tourists per day every 10 m², even if such relatively high numbers were reached for only one or a few days in the season. Two major beaches (Cala Sant’Andrea and Cala di Scombro di Dentro) at the centre and “Cala Arena and Punta dello Scorno” at the northern tip of the island (Fig. 1b).

We sampled all 11 beaches longer than 10 m present in the park (Fig. 1b). The beaches are mostly pocket beaches, between 20 m and 400 m along the shoreline, relatively homogeneous in their physical, ecological, and geographic conditions (Fig. 1d): they are within a maximum distance of 15.5 km, which minimizes spatial and biogeographic confounding factors; they are on the more protected Eastern coast of the island, which minimizes ecological factors of physical exposition to waves; and they are all sandy beaches, which minimizes ecological differences due to sediment granulometry. The major difference between the beaches is the number of tourists received during the summer months (Supplementary Data 1). The daily affluence of tourists ranged from beaches with no tourists to beaches with peaks of 300 tourists per day every 10 m², even if such relatively high numbers were reached for only one or a few days in the season. Two major beaches (Cala Sant’Andrea and Cala d’Arena) within the areas of integral protection are restricted to the public (Supplementary Data 1). The number of tourists per beach was estimated by the authorities of the park by direct observations and the data are stored in their unpublished archives. The samples for the extraction of meiofauna were collected at the end of the tourist season between 22 September and 1 October 2014. Sediment samples were collected manually from four replicates of 1 liter of sediments from the upper 5 cm of sand collected over a homogenous area of 1 m² by scooping the top layer of sand with a jar. Immediately after collection, samples were taken to the laboratory on the island. All samples were processed within few hours after collection. Total meiofauna for each replicate with high-throughput sequencing (HtS) was extracted from two replicates using the MgCl₂ decantation technique through a mesh size of 63 µm and immediately preserved in ethanol at −20 °C. The other two replicates were used one for the analysis of sediment granulometry and one for morphological identification of meiofauna (see below).

#### Phylogenetic diversity

Phylogenetic diversity was assessed from ultrametric trees obtained by BEAST package v2.4.8 (ref. 45). Blast taxonomic assignment to confirmed metazoan taxa27,28. The high-throughput sequencing reads were used for each taxonomic group, adding all the overlapping sequences available in GenBank for each group at the date of March 2019 (Supplementary Figs. 2–20). Such additional analyses allowed us to be more confident about the taxonomic assignment of each ZOTU to each group at the desired taxonomic level (phylum, class, or order), regardless of the species or genus assignment, due to the potentially high number of unknown species in meiofauna19. Sequences were downloaded from GenBank, added to our dataset and handled for the analyses using the R packages rentrez 0.4.1 (ref. 46) and ape 3.2 (ref. 47). Only the ZOTUs that were eventually unambiguously nested within their target groups including identified Genbank sequences were retained, excluding those to the 99.9% of the sequences of non-strictly meiofaunal groups, e.g. Cnidaria, were removed (Supplementary Table 1).

#### Morphological analyses

One of the four replicates collected for each sample was used to perform a parallel analysis on the effect of human frequeanation on species identified using morphological criteria. These samples were processed mostly using the MgCl₂ decantation technique but also by siphoning off the water just above the sediment surface and using small volumes in the method. According to the focal taxon of study, as in previous studies covering different meiofaunal groups from the same samples48. Live material was studied using dissecting and compound light microscopes. Additional material for identification and/or descriptive purposes was preserved using methods appropriate for the respective taxon49.

#### Explanatory variables

As a proxy to account for the effect of human frequeanation we used the maximum number of tourists for each 10 m² of the analysed beaches, as measured by the records of the surveillance personnel of the park. Water depth was considered as a categorical explanatory variable (three fixed levels:
0 m, called swash; 0.3 m, called shallow; 2 m, called deep). Other potentially confounding factors that we included in our statistical models were: number of reads, intrinsic differences between beaches, and interactions between these factors, in addition to beach length and differences in sediment granulometry. Granulometry was assessed by passing 150 g of dry sediment through six sieves with mesh sizes corresponding to a range from 1 mm to 50 µm, shaking, fractioning, and weighing to obtain mean grain size, sorting coefficient, kurtosis, and skewness58,59. (Supplementary Data 1). From such measurements, we grouped sediments defined by granulometry by a k-means analysis to find the optimal number of groups, selected using Bayesian Information Criterion (BIC) for expectation–maximization algorithm initialized by hierarchical clustering for parameterized Gaussian mixture models51 using the mclust v5.3 R package52. All values were scaled before performing the analysis53. The highest BIC was obtained for 7 groups (BIC = -106.6, EEV ellipsoidal, equal volume, and equal shape multivariate mixture model). We accounted also for the effects of different length of each beach on the meiofaunal composition and richness by including such measure in the models.

Response variables. The effect of tourists was evaluated on three different types of community descriptors, included as response variables in the different sets of models: richness, community composition, and phylogenetic diversity. Richness was calculated as the number of ZOTUs (or morphological species) for the total meiofauna and for each major group defined as representing at least 5% of the total ZOTUs. Community differences between samples were measured using the Jaccard dissimilarity index from binary presence/absence data calculated with the R package betapart v. 1.5.1 (ref. 54). Phylogenetic diversity was measured as diversity and standardized effect size of the mean phylogenetic diversity (MPD), equivalent to 1-branched branch length for one or multiple samples) and as phylogenetic clustering (standardized effect size of the mean phylogenetic diversity (MPD), equivalent to 1-Nearest Relative Index, NRI55), with the R package picante v. 1.6-2 (ref. 56).

Statistical models. We developed statistical models to test the effect of tourists on meiofauna richness, community composition, and phylogenetic diversity. In order to mirror the complex structure of biological reality, our models included additional explanatory variables that could affect the response variables. To be able to account for a combination of such accounted and unaccounted effects in the models we used GLMEMs56,57, designed exactly for these kinds of analyses, with violations of the assumption that data are independent58. Thus, in the first set of GLMEMs we used number of tourists per 10 m², depth (three levels: swash, shallow, deep), sediment granulometry (7 levels), and beach length, as explicit explanatory variables; the identity of the 11 beaches was included as a random effect to account for a combination of such accounted and unaccounted effects in the analyses on ZOTU richness. All analyses were performed in R 3.6.3 (ref. 60).

All PCR products were made up of the following reagents: template DNA (40 ng), 1× Buffer HF, dNTPs (0.5 µM of each primer, and 1 U of Phusion High-Fidelity DNA polymerase in a final volume of 50 µl). The cycling parameters for PCR were standardized as follows: initial denaturation 98 °C for 30 s, followed by 12 cycles of 98 °C for 10 s, 50 °C for 30 s, 72 °C for 15 s, and subsequently 18 cycles of 98 °C for 10 s, 62 °C for 30 s, 72 °C for 15 s, with a final extension step of 7 min at 72 °C. All PCRs were performed in triplicate and in the presence of a negative control (Molecular Biology Grade Water, RNase/DNase-free water). The PCR products were purified on a 1.2% agarose gel and purified using the AMPure XP Beads (Agencourt Bioscience Corp., Beverly, MA, USA), at a concentration of 1.2 x vol/ vol, according to the manufacturer’s instructions. The purified amplicons were used as templates in the second PCR round, which was performed with the Nextera indices priming sequences as required by the dual index approach reported in the Nextera Sample Preparation Guide (Illumina). The DNA extraction and sequencing

References
1. Cheong, S.-M. et al. Coastal adaptation with ecological engineering. *New. Clim. Change* 3, 787 (2013).
2. Agardy, T. S. *Marine Protected Areas and Ocean Conservation* (Academic Press, 1997).
3. Badalamenti, F. et al. Cultural and socio-economic impacts of Mediterranean marine protected areas. *Environ. Conserv.* 27, 110–125 (2000).
4. Deleo, O. et al. Threats to sandy beach ecosystems: a review. *Estuar. Coast. Shelf Sci.* 81, 1–12 (2009).
5. Gherskier, T., Vincx, M., Weslawski, J. M., Scapini, F. & Degraer, S. Meiofauna as descriptor of tourism-induced changes at sandy beaches. *Mar. Environ. Res.* 60, 245–265 (2005).
6. Jaramillo, E., Contreras, H. & Quijon, P. Macronfauna and human disturbance in a sandy beach of south-central Chile. *Rev. Civil. de Historia Nat.* 69, 655–663 (1996).
7. Moffett, M., McLachlan, A., Winter, P. & De Ruyster, A. Impact of trampling on sandy beach macrofauna. *J. Coast. Conserv.* 4, 87–90 (1998).
Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s42003-020-0912-6.

Correspondence and requests for materials should be addressed to D.F.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.