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RESEARCH PAPER

Species richness, species turnover and functional diversity in nematodes of the deep Mediterranean Sea: searching for drivers at different spatial scales

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ABSTRACT

Aim Understanding how biodiversity varies at different spatial scales and the drivers behind these patterns is a crucial issue in ecology. This is evident for the deep sea, the largest biome of the biosphere, where there is little information available on the spatial and temporal variability of biodiversity. Here, we investigated the variability and potential drivers of species richness, turnover and functional biodiversity of deep-sea nematodes at a depth of 3000 m across different spatial scales and in two periods of contrasting pelagic productivity.

Location The Mediterranean Sea.

Methods We used for the first time a hierarchical sampling strategy that includes different spatial scales, from tens of metres (small scale) to hundreds of kilometres (macroscale).

Results We show that the variability in biodiversity is greatest at the macroscale, although the rate of variability is about two- to three-fold lower than observed for nematode abundance. Also, turnover diversity is highest at the macroscale (and uncoupled from species richness), and significantly decreases to the meso- and local scales. Functional diversity is positively related to species richness and its variability is associated with the change in richness of predators. The drivers of spatial variability of biodiversity are different at different spatial scales. Our data identify the pivotal role of food quantity in the control of variability in biodiversity attributes at the macroscale, while the quality and bioavailability of food sources have a key role in driving beta diversity and biodiversity attributes at small spatial scales. Also, the largest variations in biodiversity attributes at both macroscales and mesoscales are related to periods of high food input from the euphotic zone.

Main conclusions We conclude that changes in food availability, which can also be expected as a consequence of climate change, have a significant impact in setting biodiversity attributes at different spatial scales in the deep sea.

Keywords

Beta diversity, deep sea, functional diversity, Mediterranean Sea, spatial scales, species richness.

INTRODUCTION

Deep-sea ecosystems cover approximately 65% of the world's surface and have a pivotal role in global biogeochemical cycles; however, they are certainly the least explored of the environments on Earth (Costello *et al.*, 2010). Deep-sea benthic biodiversity is closely linked to ecosystem functioning (Danovaro *et al.*, 2008a), such that its conservation is crucial for sustainability of the ecosystem services provided. Understanding the processes that control deep-sea biodiversity patterns at different

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spatial scales is a central issue in ecology, and determining the ecological impact due to climate change is a priority (Smith *et al.*, 2009; Tittensor *et al.*, 2010, 2011). Since the pioneering work of Jumars (1976), several studies have been carried out on this topic, and the discovery of longitudinal, latitudinal and bathymetric gradients of species richness in the deep sea has revealed conflicting results when different benthic components (e.g. prokaryotes, meio-, macro- and megafauna) and oceanic regions are considered (Rex & Etter, 2010, and references therein). Deep-sea species diversity and composition have been shown to vary globally, regionally and locally, which suggests that changes in environmental factors will influence the community structure at all spatial scales (Stuart *et al.*, 2003; Rex & Etter, 2010). Several studies have investigated the processes that support high levels of biodiversity under natural conditions (Gaston, 2000) and the drivers that lead to changes in species diversity over time or along ecological gradients (Cottingham *et al.*, 2001; Levin *et al.*, 2001; Rex & Etter, 2010). From these studies, it also appears that the same process/driver can have different effects on biodiversity at different spatial scales. For instance, at the local scale, species and energy relationships are generally described according to a hump-shaped curve, in which the number of species increases up to some energy value, above which a further increase of energy promotes decreased diversity; at the regional scale, this relationship might become positive linear (Chase & Leibold, 2002). This not only reinforces the view that there is not a unique spatial scale at which ecological phenomena occur, but also emphasizes the inherent difficulty in linking patterns and processes across different spatial scales (Levin *et al.*, 2001).

For a long time, the deep sea has been considered to be extremely stable in terms of physical and chemical conditions. However, recent evidence has suggested that this ecosystem is much more temporally and spatially variable than previously thought, with potentially important implications for biodiversity patterns (Lampitt *et al.*, 2010; Pusceddu *et al.*, 2010, 2013; Rex & Etter, 2010). Several mechanisms have been invoked to explain the spatial patterns of biodiversity: (1) sediment grain size and substrate/ habitat heterogeneity (Etter & Grassle, 1992; Danovaro *et al.*, 2009, 2010; Bongiorni *et al.*, 2010; McClain & Barry, 2010; Vanreusel *et al.*, 2010; Zeppilli *et al.*, 2012); (2) productivity (Smith *et al.*, 2008; Lampitt *et al.*, 2010; Tittensor *et al.*, 2011; McClain *et al.*, 2012); (3) food resources (Danovaro *et al.*, 2008b; Gambi *et al.*, 2010); (4) oxygen availability (Diaz & Rosemberg, 1995); (5) water currents (Lambshead *et al.*, 2001); and (6) occasional catastrophic disturbances (Levin *et al.*, 2001; Pusceddu *et al.*, 2010, 2013). Nonetheless, all of these factors are subjected to strong scientific debate because they are often site-specific and constrained by local (or regional) conditions (Levin *et al.*, 2001). The recent literature reveals the pivotal role of productivity (in the form of particulate organic carbon input) in shaping deep-sea biodiversity (both as species richness and turnover) along spatial patterns that are related especially to bathymetric gradients (Wei *et al.*, 2010; Tittensor *et al.*, 2011; McClain *et al.*, 2012; Brault *et al.*, 2013a,b). However, the role of the nutritional quality and the diversification of food sources available in deep-sea sediments in influencing the spatial patterns of the different attributes of biodiversity have been little investigated to date (Danovaro *et al.*, 2008b).

Marine ecosystems generally cover very large spatial scales, although they can also have high heterogeneity at small spatial scales (Fraschetti *et al.*, 2008; McClain & Barry, 2010; Vanreusel *et al.*, 2010). Unfortunately, the limited knowledge about the scale of variability in the attributes of the different benthic components in the deep sea does not allow the definition of a general relationship that can describe the spatial patterns of benthic biodiversity and their regulating factors, nor does it allow us to understand whether different attributes of biodiversity show similar patterns at the same spatial scale(s). To fill in these gaps of knowledge, hierarchical sampling designs and multivariate analyses of variance have been used extensively to quantify spatial and temporal variation in species assemblages, and particularly in marine environments (Fraschetti *et al.*, 2005). A variety of regression-based methods have extended this approach to enable the specification of covariates (as potential drivers of biodiversity) and to allow for spatial (or temporal) autocorrelation of the residuals (Beale *et al.*, 2010). The ability to model spatial autocorrelation has provided additional opportunities to distinguish between extrinsic (unmeasured environmental variables) and intrinsic (aggregation and dispersal) causes of variation in diversity distribution (Legendre, 1993; Lichstein *et al.*, 2002). Lichstein *et al.* (2002) used an interesting approach to unravel spatial dependency between predictor and ecological response variables, which was based on the outcomes of models that accounted for spatial variation at different scales. The procedure required the evaluation of how the apparent importance of environmental predictors changed from models that ignored both large-scale and fine-scale spatial variation (ordinary least squares, OLS) to models that account for large-scale spatial trends (trend surface analysis) and finally to models that also account for fine-scale autocorrelation (conditional autoregressive models). Using this approach, Lichstein *et al.* (2002) successfully distinguished between local-scale and landscape-scale habitat variables that can account for the breeding–habitat relationships of migrant songbirds.

Here, we investigated the patterns of distribution of deep-sea nematode biodiversity from the macroscale (thousands of kilometres) to the small scale (tens of metres). We used the biodiversity of deep-sea nematodes as a model, as they account for three-fifths of metazoans on Earth and 80–90% of the total abundance in the deep sea. Moreover, although comparative studies are rare, deep-sea nematode diversity appears to be related to that of other benthic components, including Foraminifera (Gooday *et al.*, 1998), macrofauna (Levin *et al.*, 2001) and the richness of higher meiofaunal taxa (Danovaro *et al.*, 2008a). Nematodes are also characterized by a very high species number (more than a million species; Lambshead, 2004) and the morphological features of their buccal apparatus allow the identification of their feeding habits, which thus provides an important insight into their functional diversity (Soetaert & Heip, 1995; Danovaro *et al.*, 2008a).

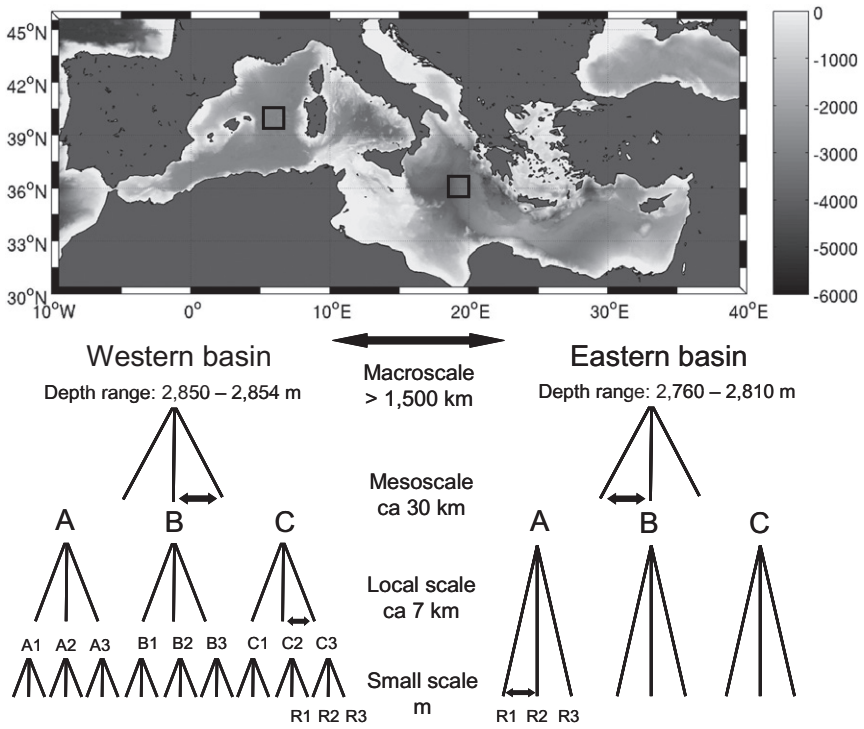

Figure 1 Sampling areas and schematic representation of the hierarchical sampling strategy in the deep Mediterranean Sea. The two squares represent the sampling sites at the macroscale in the western basin and the eastern basin. A, B, C, areas used for the mesoscale analysis within each basin; A1, A2, A3, sampling stations at the local scale in the western Mediterranean; R1, R2, R3, replicated samples at the small scale in the western and eastern Mediterranean; vertical bar, bathymetric ranges in metres.

Our sampling design was defined to address the following questions: (1) What is the spatial scale at which most of the variability in nematode species richness, turnover and functional diversity occurs? and (2) What are the factors that drive the different attributes of nematode biodiversity at the different spatial scales?

MATERIALS AND METHODS

The Mediterranean Sea

The Mediterranean Basin is *c.* 3680 km long, with an average width of only 700 km. It is conventionally divided into the western and eastern basins, which are geographically separated by the strait of Sicily. These two basins are also characterized by different benthic topographies, with the western Mediterranean consisting of two distinct deep basins (namely the Algero-Provençal and the Tyrrhenian Sea basins) and the eastern Mediterranean including several distinct deep basins that are located below the Ionian, South Adriatic and Aegean Seas, and the Levantine Basin (Sardà *et al.*, 2004). The deep Mediterranean Sea is characterized by a trophic gradient that decreases from the western to the eastern basin (Danovaro *et al.*, 2008b). This pattern reflects the different values of surface primary productivity: *c.* 163 ± 7 gC m^{-2} $year^{-1}$ in the western Mediterranean and *c.* 121 ± 5 gC m^{-2} $year^{-1}$ in the eastern Mediterranean (Bosc *et al.*, 2004). Two bathyal plains (*c.* 700 km in diameter) have been identified at *c.* 1500 km distant from each other (one in the western Mediterranean and one in the eastern Mediterranean). These two plains are located within a very narrow bathymetric range (2760–2854 m depth), thus excluding the potential effects of hydrostatic pressure (which is nonetheless controlled as a covariable in statistical analyses). For each plain, a triangle of areas located *c.* 30 km apart was identified. In the western Mediterranean basin, for each of these three areas, three stations were also identified.

Hierarchical sampling strategy

The variability of deep-sea nematode biodiversity and the potential drivers was estimated at the following spatial scales: (1) the macroscale, between the western and eastern basins, at a distance of > 1500 km apart; (2) the mesoscale, among areas within the basins *c.* 30 km apart; (3) the local scale (only in the western Mediterranean Basin), among stations about 7 km apart; and (4) the small scale, with tens of metres between samples collected from independent deployments within each station (Fig. 1; Appendices S1 & S2 in Supporting Information). Considering the size of the ship and the potential drift during sampling, we estimate that the different deployments were of the order of tens of metres apart from each other, and were distributed randomly.

To account for temporal variability at the seasonal scale, replicated sediment samples for the analysis of nematode diversity and the available food sources were collected in two periods: April and November 2001, which can be considered as representative of spring and autumn, respectively, given the absence of short-term temporal fluctuations in environmental conditions at these depths (Stavrakakis *et al.*, 2000; Heussner *et al.*, 2006).

Nematode biodiversity

The laboratory procedures are summarized in Appendix S2. For each sampling site we determined the number of species present (species richness, as the sum of the number of species of three replicated samples) and the evenness of the nematode assemblages through Pielou's index (J'). To have a gross estimate of variability, we also estimated the maximum to minimum ratio of species richness at all spatial scales, from macroscale to small scale, in both basins. Since diversity indices are strongly influenced by the number of individuals identified, to standardize the values of nematode diversity and to facilitate comparisons with other studies, species-abundance data were converted into rarefaction diversity indices (Sanders, 1968; as modified by Hurlbert, 1971). The expected number of species for a theoretical sample of 51 nematode specimens, ES(51), was thus calculated (Soetaert & Heip, 1990; Danovaro *et al.*, 2008a,b).

We measured turnover (beta) diversity to examine the changes in species composition at the different spatial scales, using the PERMDISP permutational multivariate dispersion analysis (Anderson *et al.*, 2008). Prior to the analysis, the Bray–Curtis similarity matrix, which is based on species-abundance data (as presence/absence), was transformed into Euclidean distance by principal coordinates analysis. Beta diversity was measured as the multivariate dispersion from the group of the centroid (mean dispersion ± standard error). As PERMDISP cannot be applied for fewer than two replicates, SIMPER analyses were used to estimate the dissimilarities in the species compositions at the small scale (among replicates) and to identify the presence of exclusive species at each sampling site. All of the diversity indices, including the turnover diversity, were calculated using the DIVERSE, PERMDISP, PCO and SIMPER routines included in PRIMER version 6+ software (Clarke & Gorley, 2006).

The functional diversity of the nematodes was estimated using the index of trophic diversity (ITD) as 1 – ITD, with $ITD = g_1^2 + g_2^2 + g_3^2 \ldots + g_n^2$, where according to (Wieser, 1953), g is the relative contribution of each trophic group to the total number of individuals and n is the number of trophic groups (Gambi *et al.*, 2003). The nematodes were divided into four groups, as follows: 1A, selective (bacterial) deposit feeders; 1B, non-selective deposit feeders; 2A, epistrate feeders; and 2B, predators/omnivores. The value of 1 – ITD ranged from 0.00 (lowest diversity, where one trophic guild accounts for 100% of the nematode abundance) to 0.75 (highest trophic diversity, where four trophic guilds account for 25% each).

Potential food sources

The protocols for the analyses of the biochemical composition of the organic matter (chlorophyll *a* and phaeopigments, protein, carbohydrate, lipid) are reported in Appendix S2. As we hypothesized that the benthic biodiversity is dependent not only on the amount of trophic resources but also on the relative importance of the different resources, we calculated an index of resource evenness using the equation: index of resource diversity (IRD) = Σ(C protein/biopolymeric C)^2 + (C carbohydrate/biopolymeric C)^2 + (C lipid/biopolymeric C)^2, based on the C equivalents of the protein, carbohydrate and lipid contents of the sediments and the biopolymeric carbon ratios, respectively. This index ranges from 0.33 (all of the biochemical components are equally represented in the biopolymeric C) to 1.0 (the biopolymeric C is theoretically made up of only one of the three components). In addition to the IRD as an indicator of the diversity of the food items, we also used the chlorophyll *a* to total phytopigment (Chl*a*/Phyt) ratio and the protein to total phytopigment (Prt/Phyt) ratio, as indicators of the nutritional quality of the trophic resources. The Chl*a*/Phyt ratio is indicative of the relative freshness of the organic detritus of algal origin that is deposited on the deep-sea floor, while the Prt/Phyt ratio is indicative of the availability of organic N for heterotrophic consumption.

Statistical analyses

We used regression models that are common to the spatial analysis of ecological data to evaluate the scales of variation of the abiotic variables and the diversity measures, and to quantify the degree of association between nematode diversity and the environment. The environmental variables (i.e. total phytopigments, biopolymeric C, IRD, Chl*a*/Phyt and Prt/Phyt ratios) were standardized prior to the analysis. For each of these variables, we fitted a linear mixed effect (LME) model with macroscale, time and macroscale × time interactions as fixed terms and with mesoscale and local scale nested in mesoscale as random effects. We started with a full model fitted with restricted maximum likelihood (REML) to determine the optimal error structure. The full model included time as a covariate in the random part, to allow for possible time × mesoscale interactions. A series of reduced models was obtained by sequentially removing time, the local scale and finally the mesoscale from the random part of the model. The full and reduced models were compared sequentially through likelihood ratio tests. These tests also served to assess the significance of the variance components. Normalized residuals were extracted from the selected model and inspected for normality and variance homogeneity using quantile–quantile plots, and through plots of the residuals versus the fitted values. We then estimated the fixed coefficients and their standard errors through maximum likelihood. The data present maximum likelihood estimates for fixed effects and REML estimates for variance components.

To determine the importance of broad-scale variations in the environment, we also fitted a generalized additive model (GAM) to each abiotic variable, based on thin-plate splines of geographic coordinates, and we report the coefficient of determination to indicate the importance of broad-scale variation. This approach removes the broad-scale spatial structure, so the remaining variation in the residuals reflects the fine-scale variation and autocorrelation. We evaluated the importance of fine-scale variation through Moran's I on the GAM residuals, with lag distance determined from a semi-variogram of these residuals. The significance of Moran's I was determined from 999

permutations of the original observation for the given spatial weighting scheme.

LMEs were also used to examine the scales of variation in the nematode diversity [i.e. species richness, ES(51), evenness, index of trophic diversity], as described for the environmental variables. The potential importance of the environmental variables in determining changes in nematode diversity was evaluated through a comparison of the outcomes of the models that accounted for the spatial dependences at different scales, following the approach described by Lichstein *et al.* (2002). We started by fitting an OLS multiple regression model that included all of the environmental variables and time as covariates, with both large-scale spatial trends and fine-scale variations ignored. We then examined how these models changed when allowing for broad-scale spatial trends. This was achieved through GAMs that included the same covariates as the OSL regressions, but that modelled spatial trends through thin-plate splines of geographic coordinates. Finally, we evaluated how the OLS models change when accounting for fine-scale autocorrelation, which was assessed through conditional autoregressive (CAR) models. These models incorporate spatial autocorrelation using neighbourhood matrices that specify the relationships between the response variable in each sample and those in neighbouring samples. We chose an appropriate neighbourhood size for each response variable from semi-variograms of OLS residuals, and we compared the model fits for three distance–decay functions of spatial weights using the corrected Akaike information criterion (AICc): $w_{ij} = 1$, $1/\text{distance}_{ij}$ and $(1/\text{distance}_{ij})^2$; where w_{ij} is the weight that determines the relative influence of sample j on sample i. We selected $w_{ij} = 1$ for species richness and ES(51), and $w_{ij} = 1/\text{distance}_{ij}$ for evenness and functional diversity.

As our goal was to assess how much of the variation in the nematode diversity was accounted for by the different environmental variables and at what scale(s) these associations occurred, we omitted the categorical spatial variables (i.e. macroscale, mesoscale and local scale) from these models. Assumptions were evaluated for all of the models, as described in the analysis of the environmental data, and collinearity among covariates was assessed through the variance inflation factor (Fox & Monette, 1992).

To make the statistical tests more comparable among the OLS, GAM and CAR models, we assessed the significance of individual covariates through likelihood ratio tests for nested models, where a reduced model without a specific environmental covariate was compared with a full model that included all of the environmental predictors, as described in Lichstein *et al.* (2002). Thus, an environmental variable explains large-scale spatial variation in diversity if it is significant in the OLS regression but loses significance in GAM, which already accounts for broad-scale spatial structures. Similarly, an environmental variable explains small-scale spatial variation in diversity if it is significant in the OLS regression, but loses significance in CAR, which accounts for fine-scale spatial autocorrelation. Of course, some environmental variables can explain variations in nematode diversity at both small and large spatial scales. In this case, a covariate that is significant in the OLS regression should become non-significant both in GAM and CAR. Finally, covariates that are significant in the OLS regression can still be significant in GAM and/or CAR analyses. This suggests that the scales of variation accounted for by these analyses do not match that (those) of the environmental covariate(s). In this case, assessing the scale(s) of influence of the environmental variable independently from the outcomes of OLS, GAM and CAR, as we have done here, will aid in the interpretation of the data. Univariate analyses were performed in R2.11 (R Development Core Team, 2011).

Distance-based permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001; McArdle & Anderson, 2001) was used to test for the presence of significant differences among nematode assemblages at different spatial scales, from macro- to meso- and local scales in spring and autumn (the local scale was only tested in the western basin). The analysis was based on Bray–Curtis dissimilarities that were calculated on presence/absence transformed data, and each term of the analysis was tested using 999 random permutations of the appropriate units (Anderson *et al.*, 2008). The analysis consisted of four factors: macroscale, at two fixed levels (western and eastern basin); time, at two fixed levels (spring and autumn); mesoscale, at three random levels (area: A, B and C) nested in the macroscale; local scale (only for the western basin), at three random levels (station: 1, 2, 3), with $n = 3$ as the combination of factors. Pair-wise tests were carried out for the fixed factors of macroscale and time. All of the analyses were performed using PRIMER version 6 (Clarke & Gorley, 2006), including the add-on PERMANOVA+ package (Anderson *et al.*, 2008).

Finally, we assessed the linkages between the beta diversity and the environmental variables through semi-parametric multiple regression analysis based on Euclidean distances. This analysis was carried out using the routine DISTLM_forward.exe (Anderson, 2003), which allows the forward selection of the predictor variables using tests with permutation. *P*-values were obtained using 4999 permutations of raw data for the marginal tests (tests of individual variables), while for all of the conditional tests, the routine used 4999 permutations of residuals under a reduced model. To run this test, the following were considered as explanatory variables: depth, latitude, longitude, total phytopigments, biopolymeric C and IRD, Chl*a*/Phyt and Prt/Phyt ratios as indicators of the diversity of food items and the quality of the trophic resources.

RESULTS

Trophic characteristics of the sediments

There were significant macroscale × time interactions for the total phytopigments and biopolymeric C and Prt/Phyt ratio (Table 1). The total phytopigments and biopolymeric C concentrations were larger in the western Mediterranean than the eastern Mediterranean in spring, whereas the Prt/Phyt ratio followed the opposite pattern (Appendix S1). Hence, the differences in these abiotic variables at the macroscale varied considerably with season. GAMs confirmed the presence of significant broad-scale spatial variations in the total phytopig-

Table 1 Scale of influence of environmental variables. Shown are *F* values for fixed effects and variance components from mixed effect models, according to the sampling design of Fig. 1. R^2 values are from generalized additive models (GAM) on geographic coordinates to quantify variation at large spatial scales and Moran's *I* on GAM residuals to quantify small-scale variation. Significances of variance components are based on likelihood ratio tests comparing a full model with a reduced model that excluded a particular random source of variation. Fixed and random effects were estimated through maximum likelihood and restricted maximum likelihood methods, respectively. Significant terms reflect the potential scale of influence of environmental variables.

	Total phytopigments	Biopolymeric C	Index of resource diversity	Chlorophyll *a*/total phytopigments	Protein/total phytopigments
Macroscale	6.9	16.8*	0.3	0.6	1.4
Time	43.2***	20.6***	0.1	3.9	1.9
Macroscale × time	35.3***	28.7***	1.1	3.5	22.6***
Variance components					
$S^2_{mesoscale}$	0.000	0.000	0.000	0.000	0.000
S^2_{local}	0.235**	0.050	0.178	0.000	0.253**
$S^2_{residuals}$	0.323	0.440	0.877	0.933	0.602
R^2 (from GAM)	0.15**	0.15**	0.17*	0.00	0.03
Moran's *I*	0.15	0.16	-0.30	-0.24	0.17

Models that included time as a covariate for mesoscale effects were never selected by the corrected Akaike information criterion, so the corresponding covariance term is not reported.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

ments and biopolymeric C, and also for the IRD, for which GAM on the geographic coordinates explained a significant fraction of variation (as indicated by the significant R^2 in Table 1). Variance components highlighted significant variation at the local scale for the total phytopigments and Prt/Phyt ratio (Table 1). The residual variance was large for all of the abiotic variables, which indicates considerable variation at the small scale. None of the Moran's *I* tests were significant, which indicates that there is no fine-scale spatial autocorrelation in the abiotic variables. The variance at the mesoscale was negligible in all cases.

Nematode species richness and evenness, and their potential drivers

Overall, 3562 nematodes were analysed and 182 species were identified, which belonged to 35 families and 127 genera. The logistic difficulties in determining the exact positioning of the multiple corer resulted in unintended systematic differences in depth between the basins. The samples in the western Mediterranean were slightly, but significantly, deeper than those in the eastern Mediterranean (average depths of 2852 m and 2786 m in the western and eastern basins, respectively; $F_{1,4} = 3.5 \times 10^4$, $P < 0.001$). For this reason, we included depth as a covariate in all of the analyses of nematode diversity.

Nematode species richness was significantly greater in the western basin than the eastern basin, and this difference was greater in spring than autumn, which resulted in a significant macroscale × time interaction (Table 2, Fig. 2, Appendix S3). In contrast, there were no significant variations in species richness at the mesoscale and local scale, although there was a trend that suggested that the variance at the mesoscale was larger than zero (likelihood-ratio test = 3.53, 1 d.f., $0.05 < P < 0.07$). Depth was significant in the OLS regression, but not in GAM, which confirmed macroscale differences in this variable. However, depth effects did not obscure the macroscale × time interaction in the analysis (Table 2, LME). The nematode species richness was positively associated with biopolymeric C and negatively related to IRD and the Prt/Phyt ratio. It should be noted that biopolymeric C was not significant in GAM, which is an indication of the potential effects of this variable on the broad-scale spatial variation in nematode richness. In contrast, IRD and the Prt/Phyt ratio retained significance in all of the analyses; thus, no specific scale of influence can be ascribed to these variables, although analysis of the data in Table 1 suggests that they should operate mostly at large and small scales, respectively.

The results for ES(51) were very similar to those of species richness, although in this case there was significant variation also at the mesoscale (Appendices S3 & S4).

The western Mediterranean had larger evenness than the eastern Mediterranean, and this difference was greater in spring than autumn, which results in a significant macroscale × time interaction (Table 3, Appendix S3). Evenness also showed large and significant variation at the mesoscale, but not at the local scale. The macroscale × time interaction persisted after allowing for depth effects (LME analysis), the latter being significant in OLS and CAR. Evenness was positively associated with biopolymeric C and negatively related to the Prt/Phyt ratio. These environmental variables accounted for the large-scale spatial variation in evenness, although neither GAM nor CAR analyses captured their spatial structure adequately.

Nematode species composition and turnover (beta) diversity

Only 27 families, 46 genera and 56 species were observed in both of the basins, whereas 73 genera and 119 species, and 5 genera

Table 2 Regression coefficients (and standard error) of linear mixed effect (LME), ordinary least squares (OLS), generalized additive (GAM) and conditional auto regressive (CAR) models of nematode species richness.

	LME	OLS†	GAM	CAR
Source of variation				
Intercept	10.52 (4.03)*	26.56 (1.01)***	27.99 (0.87)***	26.55 (1.02)***
Macroscale: western vs. eastern	24.00 (5.22)**			
Time	6.44 (2.49)*	1.52 (1.67)	0.24 (1.43)	1.42 (1.55)
Macroscale × time	−11.49 (2.96)***			
Depth	0.71 (2.28)	6.42 (0.82)***	−1.43 (1.78)	6.30 (0.85)***
Total phytopigments		−0.35 (1.11)	−0.98 (0.94)	−1.45 (1.04)
Biopolymeric carbon		4.72 (1.01)***	3.79 (0.87)	5.65 (0.94)***
Index of resource diversity		−1.56 (0.83)*	−0.70 (0.71)*	−1.58 (0.76)*
Chlorophyll *a*/total phytopigments		1.12 (0.76)	1.00 (0.65)	0.56 (0.66)
Proteins/total phytopigments		−2.02 (0.84)*	−2.73 (0.72)***	−2.14 (0.77)**
$S^2_{mesoscale}$	4.25			
S^2_{local}	2.23			
$S^2_{residuals}$	27.2			
R^2		0.74	0.82	
AICc	423.9	421.7	401.1	418.3

Models that included time as a covariate for mesoscale effects were never selected by the corrected Akaike information criterion (AICc), so the corresponding covariance term is not reported. Variance components are restricted maximum likelihood estimates from LME.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
†Akaike information criterion obtained by refitting the model through maximum likelihood estimation.

and 6 species, were exclusively reported for the western and the eastern basin, respectively. In both basins the most abundant families (representing > 10% of the total nematode abundance) were Oxystominidae (14%), Chromadoridae (12%) and Xyalidae (12%). The families Draconematidae, Ethomolaimidae, Haliplectidae, Pandolaimidae, Paramicrolaimidae, Siphonolaimidae and Trefusidae were exclusively observed in the western Mediterranean, whereas only the family Lauratonematidae was exclusive to the eastern Mediterranean. The dominant nematode species differed between the two basins: *Halalaimus* sp4 (7%), *Amphymonhystrella* sp1 (5%) and *Syringolaimus* sp1 (4%) in the western Mediterranean, and *Viscosia* sp1 (21%), *Halalaimus* sp4 (10%) and *Syringolaimus* sp1 (6%) in the eastern Mediterranean. The PERMDISP results revealed that the beta diversity was significantly higher at the macroscale (average dispersion 44.3 ± 3.1, *t*-test $P = 0.01$) than the mesoscale (average dispersion 39.1 ± 1.6 for the western basin; average dispersion 36.5 ± 3.2 for the eastern basin) which, in turn, was significantly higher than the local scale (average dispersion 33.5 ± 1.1, *t*-test $P = 0.001$) (Fig. 2). The high species dispersion was already evident from the preliminary analysis of the taxonomic composition of nematode assemblages, with the presence of exclusive nematode species (and even exclusive families) inhabiting each basin, sampling area and station. Since PERMDISP is not applicable for the small scale, SIMPER analyses was used, which revealed the presence of high variability in species composition also among the replicates (percentage of dissimilarity, from 29 to 72% for the western basin, and from 40 to 100% for the eastern basin). The results of PERMANOVA showed significant interactions for macroscale × time and mesoscale × time, while there were no differences among nematode assemblages at the local scale (Table 4). The pairwise tests revealed significant differences in nematode species composition at the macroscale in both sampling periods ($P = 0.001$ and $P = 0.003$ in spring and autumn, respectively), and significant differences between spring and autumn ($P = 0.029$) only in the western basin.

Functional diversity and its potential drivers

Functional diversity, as 1 – ITD, was positively related to the species richness in both basins (Appendix S5). The trophic diversity index was larger in the western Mediterranean than the eastern Mediterranean in spring (Appendix S3), whereas the opposite pattern was observed in autumn, which resulted in a significant macroscale × time interaction (Table 5). This index did not vary at the mesoscale or local scales, and showed no significant relationships with any of the environmental covariates, apart from depth, which nevertheless did not obscure the macroscale effects (LME analysis).

To further characterize the functional diversity in the nematode assemblages, we also examined the patterns of variation in the abundance of the four trophic guilds (Appendices S6–S9). Selective deposit and epistrate feeders were more abundant in the western basin than the eastern basin, and this difference was greater in spring than autumn; this resulted in a significant macroscale × time interaction, while non-selective deposit feeders were also more abundant in the western basin, consistently in both sampling periods. The abundance of predators was similar between the basins, and declined slightly between spring and autumn. The differences between the basins at the macroscale persisted after accounting for depth effects (LME analysis), which were significant for all of the trophic guilds except the

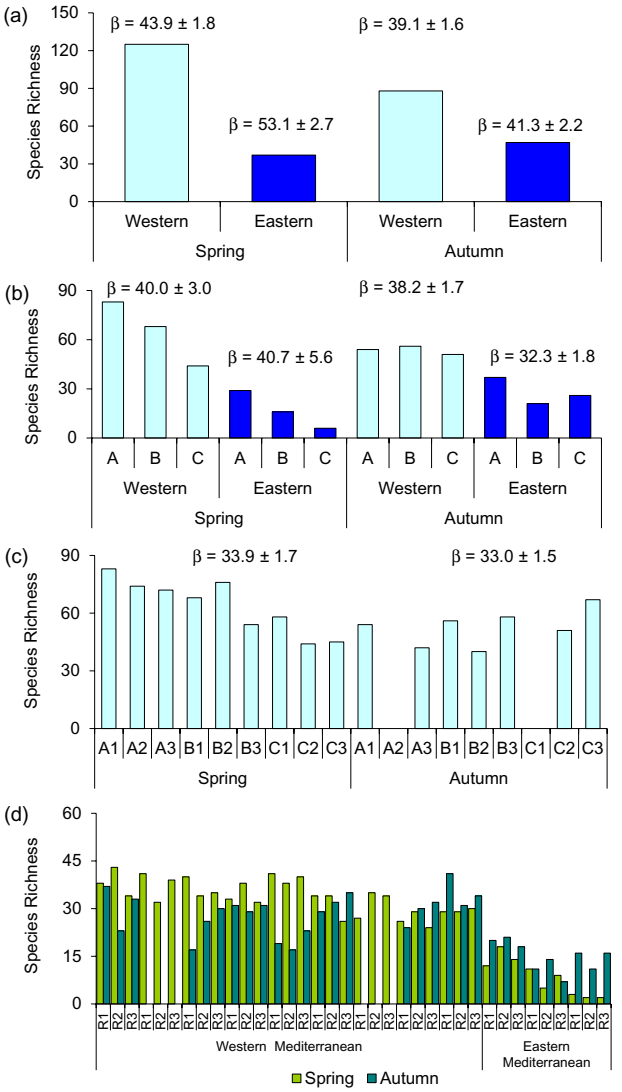

Figure 2 Nematode species richness and beta diversity (as mean dispersion ± SE) at the macroscale (a), mesoscale (b), local scale (c) (only in the western Mediterranean basin), and smaller scale (d) in the western basin and the eastern basin in spring and autumn. The values of the diversity for the macroscale and mesoscale are based on the same number of sampling sites.

predators. Similarly, all of the trophic guilds except the predators were positively associated with biopolymeric C, whilst epistrate feeders were also negatively associated with IRD. These environmental variables accounted for large-scale spatial variations in the nematode trophic guilds, although neither GAM nor CAR analyses captured their spatial structure adequately.

DISCUSSION

Spatial variability of nematode diversity

Early quantitative investigations on biodiversity conducted at large spatial scales have shown that deep-sea ecosystems are not, as previously hypothesized, biological deserts; rather, they are among the most diverse systems on the globe, with values of expected species numbers comparable to, and second only to, coastal tropical systems (Sanders & Hessler, 1969; Grassle & Maciolek, 1992). This is probably also true for the deep Mediterranean Sea (Ramirez-Llodra *et al.*, 2010; Coll *et al.*, 2012). Indeed, although the biodiversity of the macrofauna and megafauna in the deep Mediterranean Sea is apparently lower than that in other deep-sea regions (Danovaro *et al.*, 2010), we show here that the nematode diversity is comparable to values reported world-wide (Fig. 3). Despite the low abundance of individuals, nematode diversity, as ES(51), is also high in the sediments of the deep Mediterranean Sea. Comparing the biodiversity at depths ranging from *c.* 200 to 5000 m, the values reported at the shelf break are no different from those reported along the slope or at abyssal depths. Our findings show that the data from the deep western Mediterranean and eastern Mediterranean at 3000 m in depth cover the widest ranges of biodiversity that have been reported in the literature for deep-sea ecosystems world-wide. This suggests that the differences observed for nematode fauna among oceanic regions are more important than the bathymetric gradients. It is now evident that high values of biodiversity (ES(n), with n varying from 51 to 100 depending on the taxon and the study; Danovaro *et al.*, 2010) characterize most of the deep-sea regions world-wide. However, the available information on the combined spatial and temporal variability of deep-sea biodiversity is very scant. Our data indicate that the variability in biodiversity (as species richness, ES(51), and evenness) is largest at the macroscale, while the largest maximum to minimum ratio of species richness is observed in the eastern Mediterranean at both the small scale and mesoscale (Fig. 4a). The maximum to minimum ratio of species richness reported here for the small scale is similar to, or even higher than, several other deep-sea regions, at both deeper and shallower depths (Fig. 4b). These findings are based on a dataset that was obtained using the same, or similarly performing, sampling devices and conducted in the same laboratory by intercalibrated operators; these suggest that deep-sea environments share similar ranges of biodiversity variability (the maximum to minimum ratios ranged from 10 to 50% at all scales in the Mediterranean Sea), with the average value of the maximum to minimum ratio of species richness at the small scale of 1.3 ± 0.2. For the first time, our data enable the assessment of the confidence level of the maximum to minimum ratio in extrapolating data based on analyses from small to large spatial scales (e.g. mesoscale), and they reveal that this value is consistent at all of the investigated spatial scales in the deep Mediterranean Sea, and is similar to that reported at the small scale world-wide. We report also that, on average, the differences observed in terms of the species richness at the macroscale (western to eastern species richness ratio = 3) are lower than those reported for both total meiofaunal and nematode abundance (western to eastern abundance ratio = 5, faunal abundance from Gambi & Danovaro, 2006), which suggests different responses of the abundance and diversity to environmental/trophic changes that occur at the macroscale.

Table 3 Regression coefficients (and standard error) of linear mixed effect (LME), ordinary least squares (OLS), generalized additive (GAM) and conditional auto regressive (CAR) models of nematode evenness.

	LME	OLS†	GAM	CAR
Source of variation				
Intercept	0.44 (0.06)***	0.73 (0.01)***	0.74 (0.01)***	0.74 (0.01)***
Macroscale: western vs. eastern	0.43 (0.08)***			
Time	0.08 (0.03)**	−0.02 (0.02)	−0.04 (0.02)**	−0.03 (0.02)
Macroscale × time	−0.22 (0.03)***			
Depth	0.03 (0.03)	0.13 (0.01)***	0.001 (0.04)	0.13 (0.01)***
Total phytopigments		0.004 (0.02)	−0.003 (0.01)	−0.02 (0.01)
Biopolymeric carbon		0.06 (0.01)***	0.04 (0.01)***	0.08 (0.01)*
Index of resource diversity		−0.01 (0.01)	0.004 (0.01)	−0.01 (0.01)
Chlorophyll *a*/total phytopigments		0.002 (0.01)	0.001 (0.01)	−0.01 (0.01)
Proteins/total phytopigments		−0.04 (0.01)***	−0.05 (0.008)***	−0.04 (0.01)***
$S^2_{mesoscale}$	2.3×10^{-3}***			
S^2_{local}	8.4×10^{-4}			
$S^2_{residuals}$	3.7×10^{-3}			
R^2		0.83	0.94	
AICc	−157.2	−137.1	−193.8	−142.0

Models that included time as a covariate for mesoscale effects were never selected by the corrected Akaike information criterion (AICc), so the corresponding covariance term is not reported. Variance components are restricted maximum likelihood estimates from LME.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
†Akaike information criterion obtained by refitting the model through maximum likelihood estimation.

Table 4 Results of the permutational multivariate analysis of variance carried out to ascertain multivariate differences in nematode species composition at each investigated spatial scale in spring and autumn. The percentage of variance explained by each source of variability is also reported.

	Nematode species composition			
Source	d.f.	MS	*P*	% explained variance
Macroscale	1	19876	**	
Time	1	10503	**	
Mesoscale (macroscale)	4	3059	*	5
Macroscale × time	1	7505.1	*	
Local [mesoscale (macroscale)]	6	1881.5	n.s.	4
Time × mesoscale (macroscale)	4	3541.7	**	16
Time × local [mesoscale (macroscale)]	4	1811.8	n.s.	5
Residual	44	1507.1		70
Total	65			

d.f., degree of freedom, MS, mean square; *P*, probability level (*$P < 0.05$; **$P < 0.01$; n.s. = not significant).

We also found that species richness increased moving westwards, although the ratio between the western basin and the eastern basin is about two- to three-fold lower than that shown by the abundance. These data allow us to hypothesize that the observed differences in the nematode abundance at the macroscale are in a large part due to the different recruitment success of the species that inhabit the two basins, rather than to their different ingression from adjacent regions (i.e. from the Atlantic Ocean, through the Gibraltar Strait for the western basin, and from the Black Sea and Red Sea for the eastern basin). This implies that differences in availability of food sources at the macroscale are more relevant in the control of the abundance than the species richness.

At the same time, both species richness, as ES(51), and evenness showed significant spatial variability also at the mesoscale, which suggests that in regions characterized by similar trophic status the heterogeneity of the topographic and environmental characteristics has importance in determining the high variability in the biodiversity and evenness. We can thus conclude that the spatial variability of biodiversity at the macroscale is controlled by different processes from those acting at smaller spatial scales (see below for further discussion).

As reported for species richness, the values of turnover (beta) diversity in the deep Mediterranean Sea are also typically high at all of the spatial scales, and the highest source of variability is encountered at the macroscale and mesoscale. These findings are supported by the data from the random subsampling in the western basin, which avoids any bias for the spatial distribution of the species richness and beta diversity due to the different sampling efforts carried out in the two basins (Appendix S10).

The beta diversity decreases with the decrease in spatial scale, especially in the western basin, although the variability in species composition still remains relatively high at the smallest spatial scale (tens of metres). Similar results have been reported recently from the deep Arctic Sea, where, however, pseudoreplicates were considered (Gallucci *et al*., 2009). Altogether these

Table 5 Regression coefficients (and standard error) of linear mixed effect (LME), ordinary least squares (OLS), generalized additive (GAM) and conditional auto regressive (CAR) models of nematode index of trophic diversity.

	LME	OLS†	GAM	CAR
Source of variation				
Intercept	0.40 (0.04)***	0.38 (0.01)***	0.38 (0.01)***	0.38 (0.01)***
Macroscale: western vs. eastern	−0.01 (0.06)			
Time	−0.12 (0.03)***	−0.042 (0.02)*	−0.04 (0.02)	−0.04 (0.02)*
Macroscale × time	0.11 (0.04)**			
Depth	−0.05 (0.02)	−0.02 (0.01)*	−0.03 (0.03)	−0.02 (0.010)*
Total phytopigments		−0.004 (0.014)	−0.01 (0.01)	−0.00 (0.01)
Biopolymeric carbon		−0.01 (0.01)	−0.01 (0.01)	−0.01 (0.01)
Index of resource diversity		0.00 (0.01)	−0.00 (0.01)	−0.00 (0.01)
Chlorophyll *a*/total phytopigments		−0.01 (0.01)	−0.01 (0.01)	−0.01 (0.01)
Proteins/total phytopigments		0.01 (0.01)	0.01 (0.01)	0.01 (0.01)
$S^2_{mesoscale}$	0.00			
S^2_{local}	0.00			
$S^2_{residuals}$	4.2×10^{-3}			
R^2		0.15	0.17	
AICc	−162.6	−155.6	−154.8	−154.3

Models that included time as a covariate for mesoscale effects were never selected by the corrected Akaike information criterion (AICc), so the corresponding covariance term is not reported. Variance components are restricted maximum likelihood estimates from LME.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
†Akaike information criterion obtained by refitting the model through maximum likelihood estimation.

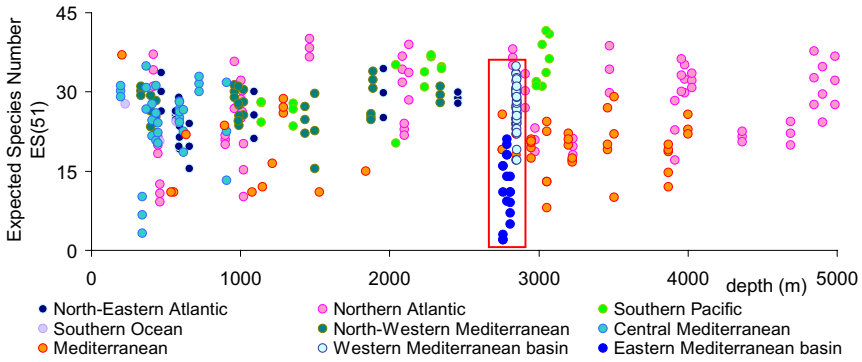

Figure 3 Nematode expected species number, ES(51), along a bathymetric gradient. The data from the north-eastern Atlantic, northern Atlantic, southern Pacific, Southern Ocean and Mediterranean Sea are from Danovaro *et al.* (2008a), and the data from the north-western and central Mediterranean Sea are from Danovaro *et al.* (2009). The rectangle includes the data from the present study.

data led us to hypothesize that the processes of nematode species aggregation at small spatial scales (less than a few metres) has a key role in influencing/driving the variations in species composition within deep-sea nematode assemblages at all spatial scales. Consistent with what has been observed for terrestrial ecosystems (Huston, 1999), these findings support the hypothesis that in the deep sea also the species interactions are important in setting the changes in species composition (beta diversity) at the smaller spatial scales, and they contribute to the effects determined by other scale-dependent factors (such as, among others, habitat heterogeneity at the smallest scale).

The data presented here suggest that species richness and beta diversity have opposite patterns in the deep Mediterranean Sea: species richness is significantly greater in the western Mediterranean than the eastern Mediterranean, whereas beta diversity is higher within the eastern Mediterranean than the western Mediterranean. These patterns might be the result of the effects of different environmental characteristics (topography and trophic conditions), the history and environmental events (episodic events and climate change, with different spatial extension and intensity) that characterize these two basins (Danovaro *et al.*, 2004, 2008b; Canals *et al.*, 2006; Gambi & Danovaro, 2006; Pusceddu *et al.*, 2010, 2013). All of these environmental factors probably have different effects on the biodiversity attributes in the western and eastern Mediterranean basins. The functional diversity in the deep Mediterranean Sea shows significant spatial variability at the macroscale, especially in spring, when the values of the trophic diversity index are significantly lower in the eastern than the western Mediterranean. This is the result of significant variability at the macroscale of the abundance of all of the nematode trophic groups except for the predators. The larger skewness of the functional diversity of the nematode assemblages that inhabit the eastern Mediterranean basin is associated with a higher percentage of predators, and in particu-

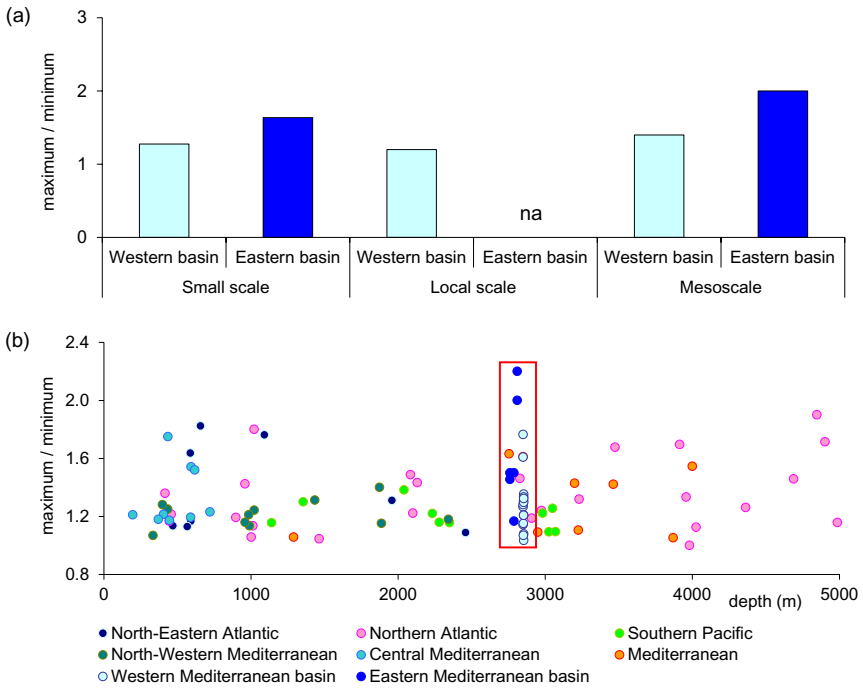

Figure 4 Variability of nematode species richness (as the maximum to minimum ratio) from the small to mesoscale in the western Mediterranean and eastern Mediterranean (a), and at the small scale in the different deep-sea sites (b). The data from the north-eastern Atlantic, northern Atlantic, southern Pacific, Southern Ocean and Mediterranean Sea are from Danovaro *et al.* (2008a), and the data from the north-western and central Mediterranean are from Danovaro *et al.* (2009). The rectangle includes the data from the present study. na, not available.

lar the genus *Viscosia*, which dominates the nematode assemblages in spring (Appendix S11). This is also confirmed by the SIMPER analyses, and these findings suggest that the quantitative importance of predators is a consistent and typical feature of the deep-sea nematode assemblages of the eastern Mediterranean Sea (Danovaro *et al.*, 2008b). These percentages of predators are the highest reported so far in deep-sea investigations (Soetaert & Heip, 1995; Gambi *et al.*, 2003), and this led us to hypothesize a key role for this trophic group that can influence the relationship between biodiversity and ecosystem functioning in these systems, and which deserves further investigation.

The spatial patterns in nematode functional diversity agree well with those reported for species richness, but not with those of beta diversity. This is surprising, as changes in species composition of nematode assemblages might be related to changes in functional diversity, as different species have different functional traits (i.e. they belong to different trophic groups). The variability of functional diversity might conceivably imply differences in ecological interactions among the trophic levels, with consequences for the structure and functioning of the communities (Giere, 2009) and, consequently, for the relationships between biodiversity and ecosystem functioning in deep-sea ecosystems (Danovaro *et al.*, 2008a). On the other hand, any changes in community composition – and thus in beta diversity – might be associated with changes in the structure and functioning of the benthic food webs, and therefore of the functional diversity.

Previous studies have hypothesized a rapid response (i.e. days to weeks) of deep-sea fauna to temporal variability in the supply of food inputs, not only in terms of abundance and biomass but also in terms of biodiversity (Danovaro *et al.*, 2004; Smith *et al.*, 2008; Gooday *et al.*, 2010; McClain & Barry, 2010). The data from the present study reveal that the variations at both the macroscale and mesoscale in species richness, evenness, composition and functional diversity are larger in spring than in autumn, which suggests that the food inputs reaching the sea floor as a result of the enhanced productivity processes that occur in the euphotic zone in spring have a significant impact upon, and indeed increase, all of the attributes of deep-sea biodiversity.

Factors controlling the spatial patterns of biodiversity

Previous investigations have revealed the important role of available energy in controlling the spatial distribution of biodiversity at the large spatial scale in deep-sea ecosystems (Levin *et al.*, 2001; Lambshead *et al.*, 2002; Snelgrove & Smith, 2003; Rex *et al.*, 2005; Smith *et al.*, 2008; Pusceddu *et al.*, 2009; Rex & Etter, 2010; McClain *et al.*, 2012; Brault *et al.*, 2013). Our analyses emphasize the importance of the quantity of food sources (i.e. biopolymeric C) as a driver of spatial variability in nematode species richness and evenness at the macroscale and mesoscale. We also report that total phytopigments, the food quality (Prt/Phyt and Chl*a*/Phyt ratios) and the diversification of the food sources have key roles, and these explain statistically up to 100% of the biodiversity variations at larger spatial scales (especially for beta diversity; Table 6). The total phytopigments include chlorophyll *a* and phaeopigments (the degradation products of chlorophylls). Therefore, the Chl*a*/Phyt ratio represents an estimate of the freshness of the algal material that is deposited on the deep-sea floor, whereas the Prt/Phyt ratio is an estimate of the relative importance of the organic material of a detrital/heterotrophic nature versus organic material associated

Table 6 Summary of the conditional tests of the DISTML (distance-based multivariate analysis for a linear model using forward selection) analyses performed at macroscale, mesoscale, local and small scale for beta diversity in spring and autumn. Explanatory variables for the analyses are depth, latitude (Lat), longitude (Long), biopolymeric carbon (BPC), total phytopigments (Phyt), index of resource diversity (IRD), chlorophyll *a*/total phytopigments (Chl*a*/Phyt) and protein/total phytopigments (Prt/Phyt) ratios.

Spatial scale	Season	Explanatory variable	*F*	*P*	Var(%)	Cum(%)
Macroscale	Spring	Prt/Phyt	1226.4	*	72	72
		Phyt	382.0	*	23	95
	Autumn	Phyt	202.9	*	84	84
		Depth	38.0	*	16	100
Mesoscale: western basin	Spring	IRD	966.7	***	92	92
		Chl*a*/Phyt	44.7	**	4	96
	Autumn	Chl*a*/Phyt	276.8	*	56	56
		Phyt	203.4	***	41	97
		IRD	6.5	***	1	98
Mesoscale: eastern basin	Spring	Prt/Phyt	2742.6	**	68	68
		Chl*a*/Phyt	926.2	**	23	91
		Depth	266.5	**	7	98
		Phyt	46.8	***	1	99
	Autumn	Depth	145.4	*	50	50
		Chl*a*/Phyt	79.1	*	27	77
		Prt/Phyt	68.4	***	23	100
Local scale: western Mediterranean	Spring	Depth	509.4	*	54	54
		IRD	260.3	*	27	81
		Lat	105.0	*	11	92
		Long	63.4	*	6	98
	Autumn	Phyt	38.7	*	15	15
		Chl*a*/Phyt	12.5	*	5	20
Small scale: western Mediterranean	Spring	Depth	1522.9	***	42	42
		IRD	804.3	***	23	65
		Lat	223.0	*	6	71
		Prt/Phyt	215.8	*	6	77
	Autumn	BPC	529.3	**	33	33
Small scale: eastern Mediterranean	Spring	Prt/Phyt	2500.7	*	62	62
	Autumn	Depth	145.3	*	50	50

F, *F* statistic; *P*, probability level; Var(%) is the percentage of the variance explained by that explanatory variable; Cum(%) is the cumulative percentage of variance explained by the explanatory variables.

Reported are those variables that display a *P* level $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

with algal detritus (Pusceddu *et al.*, 2009). We show that the nematode species richness and evenness are negatively related to the Prt/Phyt ratio, while the total phytopigments and Chl*a*/Phyt ratio explain large fractions of the beta diversity variation at the macroscale and mesoscale. These findings suggest that the quality (and freshness) of the organic inputs supplied from the water column drive the species composition. Conversely, large inputs of aged detritus of heterotrophic origin, with lower nutritional quality, were associated with lower species richness and evenness. Recent studies based on linear inverse modelling (LIM) exercises have postulated a limited role of labile detritus (i.e. phytopigments) in explaining the functioning of benthic trophic webs along several continental margins and deep-sea plains (Van Oevelen *et al.*, 2012). The same simulations have postulated that the functioning of deep-sea benthic trophic webs is mainly dependent upon the availability of semi-labile detritus (i.e. the biopolymeric C; *sensu* Pusceddu *et al.*, 2009). Our data allow the identification of the pivotal role of biopolymeric C in controlling also the deep-sea biodiversity at a depth of 3000 m, while the availability and freshness of the sedimentary organic matter (i.e. phytopigments) have key roles in driving nematode beta diversity.

When the analysis is performed at local and small scales, the proportion of variation in beta diversity explained by the quality and composition of the food sources decreases to a large extent (Table 6). These data indicate that at smaller scales drivers such as species interactions and habitat heterogeneity are also important, as well as the availability of food. Our data also reveal that the sampling depth explains some of the variations in the biodiversity at all of the spatial scales, although the effects of this covariate are not robust enough to obscure the macroscale patterns observed. Overall, these data provide new insights into the scales and the processes driving the spatial and temporal variability of biodiversity in the deep sea.

Also in light of the episodic events that can dramatically change the transport and distribution of resources from the upper water column to the deep sea (Smith *et al.*, 2009), global changes are expected to influence the quantity, quality and distribution of the food supply to the deep-sea benthos (Danovaro *et al.*, 2004; Coma *et al.*, 2009; Pusceddu *et al.*, 2010, 2013). Since

these variables are crucial in the determination of the variability of the biodiversity distribution, based on the results of our investigation, we can anticipate that global changes will have a significant impact on the distribution of species richness, as well as on the beta and functional diversity of the deep-sea fauna.

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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 Sampling sites and potential food sources in the deep western and eastern Mediterranean Sea.

Appendix S2 Details on sampling activities, laboratory procedures for the analysis of nematode diversity and potential food sources.

Appendix S3 Deep-sea benthic biodiversity in the Mediterranean Sea at different spatial scales.

Appendix S4 Regression coefficients (and standard error) of linear mixed effect (LME), ordinary least squares (OLS), generalized additive (GAM) and conditional autoregressive (CAR) models of standardized nematode species richness as ES(51).

Appendix S5 The relationship between expected species number, ES(51), and functional diversity in the western and eastern Mediterranean Sea.

Appendix S6 Regression coefficients (and standard error) of linear mixed effect (LME), ordinary least squares (OLS), generalized additive (GAM) and conditional autoregressive (CAR) models of nematode (bacterial) selective deposit feeders.

Appendix S7 Regression coefficients (and standard error) of linear mixed effect (LME), ordinary least squares (OLS), generalized additive (GAM) and conditional autoregressive (CAR) models of nematode non-selective deposit feeders.

Appendix S8 Regression coefficients (and standard error) of linear mixed effect (LME), ordinary least squares (OLS), generalized additive (GAM) and conditional autoregressive (CAR) models of nematode epistrate feeders.

Appendix S9 Regression coefficients (and standard error) of linear mixed effect (LME), ordinary least squares (OLS), generalized additive (GAM) and conditional autoregressive (CAR) models of nematode predators/omnivores.

Appendix S10 The importance of the sampling effort.

Appendix S11 Nematode trophic structure in the Mediterranean Sea at different spatial scales.

BIOSKETCHES

The Marine Biology research team of the Department of Life and Environmental Sciences of the Polytechnic University of Marche includes a highly interdisciplinary and multidisciplinary group of scientists who are devoted to the investigation of marine biology and ecology, with a special focus on the understanding of the links between biodiversity and ecosystem functioning, and of the management and protection of deep-sea habitats.

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Author contributions: R.D. and C.G. conceived the idea; C.G. and R.D. collected the data; C.G., A.P. and L.B.-C. analysed the data; C.G. and R.D. led the writing of the manuscript, and all of the other authors contributed with suggestions and corrections. All of the authors contributed to finalizing of the manuscript.

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