CHAPTER 2

REVEALING SPECIES ASSEMBLY RULES IN NEMATODE COMMUNITIES

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ABSTRACT

Species assemblages are not randomly assembled from a local species pool; they often show segregated or aggregated distribution patterns. These patterns may be attributed to both biotic and abiotic factors. On a large scale abiotic factors may be important, while on a smaller scale other factors such as species interactions may become essential. Here we will focus on small-scale patterns in nematode communities. Species patterns are generally revealed by null models based on presence/absence data. Since there is an increasing chance of falsely rejecting the null hypothesis of a random assembled community with increasing matrix size, we used an algorithm generating independent null matrices and applied a large number of swap attempts to build a null matrix. Moreover, we applied an additional test to reveal the susceptibility of the analyses of checker and the C-, T- and V-score to a Type I error for randomised data. To minimise the influence of the abiotic environment, we restricted the swapping algorithm of the null model to the replicate samples of one sampling event. Since stronger species interactions are expected for species of the same functional type, the nematode data was split according to the four feeding types defined by Wieser (1953). Our data indicate that species tend to aggregate and co-occur more often in some replicate samples than would be expected from a random species distribution of the local species pool. This is in accordance with the patchy distribution patterns known for nematode species. These aggregated patterns are also found for the different feeding types. The factors causing these aggregated patterns cannot be established since they are not included in the data, but the data do indicate that competitive exclusion is unlikely at the scale of a sample core.

Keywords

Null models, Nematoda, species assembly rules, aggregated pattern, patchiness

INTRODUCTION

Community ecology searches for repeated community patterns to investigate how communities are assembled from species pools (Wilson, 2001). The mechanisms behind these patterns can be mainly attributed to two factors: the abiotic environment and assembly rules. The latter focuses on community patterns due to interactions between species, such as competition, facilitation, mutualism and other biotic interactions (Wilson, 2001). Species assembly rules were first formulated by Diamond (1975). These rules imply that interspecific competition between species with similar niches results in non-random patterns of species distributions where certain species are competed out of the community. As a result, some species pairs may never be found together which leads to less species co-occurrences than expected by chance. These rules have been strongly disputed and there has been a proliferation of studies promoting, refuting and testing these ideas (Connor and Simberloff, 1979; Diamond and Gilpin, 1982; Weiher and Keddy, 1995; Bell, 2000; Hubbell, 2001; Weiher and Keddy, 2001; Bell *et al.*, 2006; Purves and Turnbull, 2010). In addition, it has been shown that neutral processes considering only birth, death, random dispersal and species richness may result in non-random patterns as well (Bell, 2000; Hubbell, 2001; Whitfield, 2002). However, it has been realised that neutral processes alone cannot explain the observed community patterns (Bell *et al.*, 2006; Purves and Turnbull, 2010). Moreover, the theory of Diamond (1975), stating that closely related species with similar attributes are more likely to outcompete each other, is contradicted by an alternative theory, which postulates that closely related species might have similar tolerances to environmental stressors, and would thus rather co-occur within the same communities (Webb, 2000). This theory has found support in marine communities, where species assemblages tend to be more closely related than would be expected by chance (Mouillot *et al.*, 2007; Somerfield *et al.*, 2009). This may indicate that environmental and evolutionary factors are determining marine community composition, rather than species interactions.

The observations of non-randomness in species assemblages have been tested extensively for terrestrial and freshwater ecosystems (Gotelli and McGabe, 2002). In marine habitats, null model analyses have been applied only recently (Mouillot *et al.*, 2007; Carranza *et al.*, 2010; Semmens *et al.*, 2010). Here we focus on the free-living marine nematodes. Many studies have investigated the biologic interactions between nematodes and other taxonomical groups (Schrijvers *et al.*, 1995; Debenham *et al.*, 2004; Kristensen, 2008; Braeckman *et al.*, 2011). However, here we will focus on interactions generating non-random distribution patterns of nematode species. Nematode communities are characterised by a high local diversity (Heip *et al.*, 1985) and within samples several species may belong to the same trophic group and may even be congeneric. Segregated patterns can be expected, since interspecific interactions between nematode species have been reported. In natural conditions, competition within nematode assemblages has been suggested (Heip *et al.*, 1985; Yodnarasri *et al.*, 2008) and significant interactions between species of the same

feeding type have been observed (Alongi and Tietjen, 1980; Joint *et al.*, 1982; Steyaert *et al.*, 2003). In experimental conditions, nematode species reveal complex interactions such as competition (Alongi and Tietjen, 1980), inhibition (De Mesel *et al.*, 2006a), facilitation (dos Santos *et al.*, 2009) and predation (Moens and Dos Santos, 2010). Since competitive interaction is mostly expected within groups of species feeding on the same food type, non-random patterns indicating segregation of species are often sought for within feeding types or guilds (Fox and Brown, 1993; Fox and Fox, 2000; Heino, 2009). Here, the data was subdivided into four feeding types (Wieser, 1953): selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A), and predators and omnivores (2B).

The aim of this study is to investigate species co-occurrence patterns within the nematode community, while (largely) excluding the effect of the environmental variables on the distribution patters. When these patterns are actually observed, they may then be attributed to species assembly rules (i.e. as a result of species interactions) rather than by the environmental conditions. To minimise the effect of the environment on the outcome of the analysis, the swapping algorithm was restricted to repeated samples taken at the same location at the same moment in time (further referred to as 'replicate samples'). Our null hypothesis states that for all the sampling stations, the species in the replicate samples are randomly assembled from the local species pool, with the local species pool being all the species found in the replicate samples from one sampling event. The presence of non-random distribution patterns are revealed by null models. In null model analysis, co-occurrence indices derived from the real species-samples matrix are compared with the indices derived from randomly assembled matrices. These random matrices can be assembled in many different ways (Gotelli, 2000). Here, we applied two null models for presence/absence data, the fixed-fixed and fixed-equiprobable model (Gotelli, 2000). Since, there is an increasing chance of falsely rejecting the null hypothesis of a random assembled community with increasing matrix size (Fayle and Manica, 2010), we 1) developed an algorithm generating independent null matrices in contrast to the generally used 'sequential' swap algorithm (Gotelli and Entsminger, 2003) and 2) applied a large number of swap attempts to build a null matrix. Moreover, we applied an additional test to reveal the susceptibility of the different analyses to a Type I error.

MATERIALS AND METHODS

Species data

To investigate assembly rules within nematode assemblages, data was drawn from the MANUELA database. Within the EU Network of Excellence MarBEF, MANUELA is a Responsive Mode Project focusing on the meiobenthos (metazoans passing a sieve of 1 mm and retained on a 38 µm sieve). A central MANUELA database was compiled comprising the available data on meiobenthos on a broad European scale (Vandepitte *et al.*, 2009).

The challenge in this study is to find non-random community patterns on a small spatial scale, but in such a way that these patterns are little or not influenced by environmental conditions. Replicate samples are obtained within a small spatial scale and at a certain moment in time. These replicate samples exhibit local variations in species composition, for similar environmental conditions. Thus, they can provide information on nematode assemblages on a limited time and spatial scale. Consequently, only those samples with at least two replicate samples are extracted from the MANUELA database. Time series were considered as different samples, since seasonal fluctuations may alter the species composition significantly (Vincx, 1989b; Vanaverbeke *et al.*, 2004a; Franco *et al.*, 2008).

In this way 911 replicate samples belonging to 338 sampling events (with each 2 to 4 replicate samples) were selected from the database (Fig. 2.1 and Fig. 2.4). Only those species found in more than one replicate sample are considered, resulting in a final dataset consisting of data on 450 different nematode species. The surface area of the samples varied between 3.8 cm² and 23.76 cm²: 44% of the samples had a surface area of 10 cm² and 24% had a surface area of 23.76 cm². A small proportion of the samples (about 4 %) had a surface area smaller than 6 cm² and for 28 % of the samples the surface area was not exactly known.

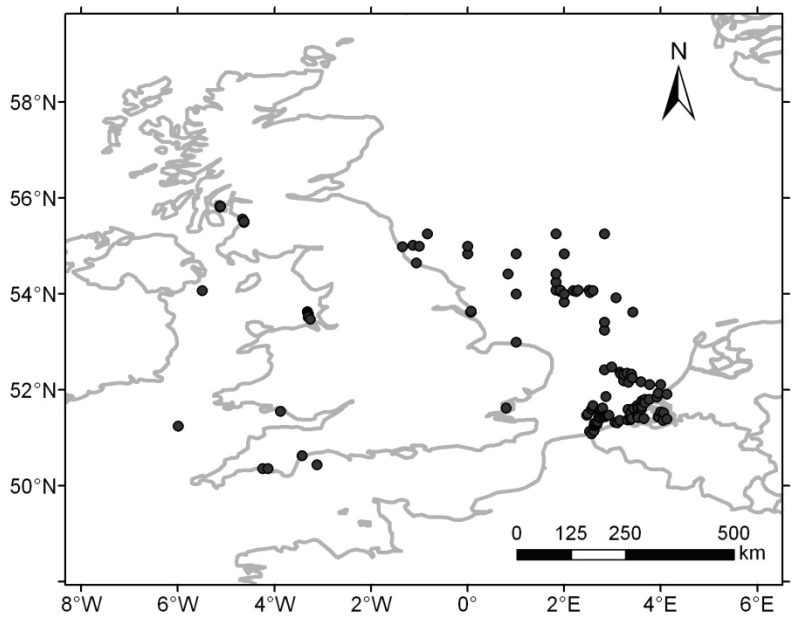

Fig. 2.1. Location of the sampling stations (•).

Tests for species assembly rules

There are two aspects in a null model test: the test statistic and the null model. The test statistic can be any parameter summarising a community aspect which might relate to species interactions, such as species aggregation or segregation. The main aspect in assembly-rule work is framing a valid null model (Wilson, 2001). The null model tests if the null hypothesis is valid. In this study, we test community patterns against a null hypothesis of random community assembly. Our null hypothesis states that for all the samples the species

in a core sample (i.e. a replicate sample) are randomly assembled from the local species pool with the local species pool being the species found in the replicate samples from one sampling event.

The null model tests whether the test statistic is significantly different from random, and should be chosen carefully to include every feature of the observed and thus realistic data, except the tested feature (Tokeshi, 1986). A systematic comparison of the dataset with different null models may help to reveal random or non-random distribution patterns in the species data. Therefore, two null models were developed using the Matlab software package.

Indices for revealing assembly rules

Several indices have been developed to summarise patterns in species distributions. Here, 4 indices based on presence/absence data are considered: Checker (Diamond, 1975; Gotelli, 2000), the C-score (Stone and Roberts, 1990), the T-score (Stone and Roberts, 1992) and the V-statistic (Pielou and Robson, 1972 *in* Schluter, 1984).

The C-score and Checker are commonly used indices (Gotelli and Rohde, 2002; Ulrich, 2004; Sanders *et al.*, 2007; Tomašových, 2008; Carranza *et al.*, 2010; Semmens *et al.*, 2010; Kamilar and Ledogar, 2011). Checker is the number of species pairs which never co-occur and form perfect checkerboard patterns (Diamond, 1975; Gotelli, 2000). The C-score indicates how species tend to avoid each other ('checkerboardness') (Stone and Roberts, 1992) and is a measure of species segregation (Stone and Roberts, 1990):

$$C - score = \frac{2\sum_{i=1}^{S}\sum_{j=i+1}^{S}(r_i - r_{ij})(r_j - r_{ij})}{S(S-1)} \qquad \text{(Eq. 2.1)}$$

where S is the number of species, r_i is the number of sites where species i occurs and r_{ij} is the number of sites where both species i and j occur. In a competitively structured community, the C-score should be significantly larger than expected by chance. The T-score on the other hand measures how species tend to aggregate ('togetherness') (Stone and Roberts, 1992):

$$T - score = \frac{2\sum_{i=1}^{S}\sum_{j=i+1}^{S} r_{ij}(N + r_{ij} - r_i - r_j)}{S(S-1)} \qquad \text{(Eq. 2.2)}$$

where N is the total number of samples.

The V-score is as an index for species association in samples. V is calculated as follows (Schluter, 1984):

$$V = \frac{\sum_{j=1}^{N}(T_j - \bar{T})^2}{N.\sum_{i=1}^{S}\left(1 - \frac{r_i}{N}\right).\frac{r_i}{N}} \qquad \text{(Eq. 2.3)}$$

with T_j the number of species in replicate sample j and $\bar{T}$ the observed mean number of species per replicate sample. A value larger than 1 indicates that the species co-vary positively, while if V is smaller than 1 the species co-vary negatively (Schluter, 1984).

Checker is most prone to measurements errors since a single occurrence can destroy a perfect checkerboard pair, while the C-score and the V-ratio are more robust and patterns can still be detected in noisy datasets (Gotelli, 2000).

The Null Models

Null models provide tools for testing non-standard hypotheses about patterns in ecological data (Gotelli, 2000). The general idea is that the original index of the real data matrix is compared with indices calculated from randomised data. There is always a trade-off between generalism and realism of the null model (Gotelli, 2000). A general null model without any constraints easily rejects the random null hypothesis. Thus, the null model fails to include obvious community features and reveals a non-random pattern. This is a Type I statistical error and should be avoided at all times. By introducing more of the original structure into the model, the model becomes ecologically more realistic. However, simulations will closely reflect the observed data and the null hypothesis will never be rejected if too much structure is incorporated in the model. In other words, the test is too conservative and produces a Type II error. Thus, the community is in fact different from random, but the null model is not capable of revealing this pattern (Wilson, 2001).

On a large scale, a pattern will always be discerned in the data due to environmental drivers (Chapter 1, Fig.1.3). Consequently, the influence of the environment should be excluded as much as possible when these patterns are investigated. This is achieved here by using replicate samples; replicate samples are obtained from the same station and are assumed to reflect similar environments. Thus, differences in species composition between replicate samples will be less influenced by the environmental conditions, but more likely by other factors.

Since more competitive interaction is expected within feeding groups, the original dataset was split according to the four feeding groups defined by Wieser (1953).

Due to data limitations the null model used in this study is based on the assumption that the volume of the individual replicate sample is small enough to allow species interactions, while the distance between the replicate samples is large enough to exclude species interactions and is still small enough to reduce the differences in environmental conditions. Because of the small size of the nematodes, this is an important consideration. Assumptions for interactions within a replicate sample cannot be deduced for this data since the nematode community is identified for the whole replicate sample and not for patches within the replicate sample. For some replicate samples data is available on the vertical species distribution at different depths in the sediment (slices). It is well known that nematode communities change with sediment depth: this may be attributed to both environmental conditions (Soetaert *et al.*, 1994; Steyaert *et al.*, 1999) and competitive or predatory interactions (Joint *et al.*, 1982; Steyaert *et al.*, 2003). Thus, these vertical environmental gradients will confound the distinction between the influence of abiotic and biotic factors on

the species distribution, while it is the purpose of this study to find patterns which are little or not influenced by environmental gradients.

The swapping algorithm

Null model analyses are based on binary presence/absence matrices where each row represents a species and each column a site, and each value indicates presence (1) or absence (0). Gotelli (2000) described nine different swapping algorithms, we applied two of them which are commonly used (Sanders *et al.*, 2007; Tomašových, 2008): the Fixed-Fixed (Swap1) and Fixed-Equiprobable (Swap2) approach.

For the first approach the matrix elements are reshuffled, but row and column totals of the original matrix are preserved (Connor and Simberloff, 1979; Gotelli and Entsminger, 2003; Kamilar and Ledogar, 2011) (Swap1). This has the following ecological background: setting a fixed row total will ensure that the number of occurrences of each species in the null communities is the same as in the original dataset (Gotelli, 2000). Thus, rare species will remain rare and common species remain common. Setting a fixed column total ensures that species poor sites, will remain species poor. Since the values of the number of species in a replicate sample (T_j) and the number of sites where species i occurs (r_i) remain the same with this algorithm, the V-score (Eq. 2.3) of the null models will have the same value as the V-score of the original data (Gotelli, 2000) and no pattern can be revealed. Therefore, the V-score was not considered for Swap1.

For the Fixed-Equiprobable approach, only the row totals are kept constant, and the replicate samples are considered to be equiprobable which can eliminate observed differences in species richness of replicate samples (Swap2). This null model approach is thus less conservative than the first one and it seems to produce good results for sample data collected in the field (compared to island data) (Gotelli, 2000).

For Swap1 the presence/absence data were transposed with the swap algorithm suggested by Gotelli (2000). In the data matrix submatrices of the form:

$$\begin{bmatrix} 0 & 1 \\ 1 & 0 \end{bmatrix} \text{ or } \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

are randomly chosen and zeros and ones are swapped. In this way row and column totals are kept constant, but the overall matrix changes. Here, the submatrices are chosen within the replicate samples of a sample. Thus, species can only shuffle between replicate samples and species from one sampling site cannot move to another sampling site. For each of the 338 samples (with each 2 to 4 replicate samples) 1000 swapping attempts were applied. Thus to create one null model 338 000 swapping attempts have been made. This is well above the commonly used value of 5000 (Gotelli and Entsminger, 2005) and the recommended value of 50 000 for large matrices (Fayle and Manica, 2010). Increasing the number of swaps decreases the Type I error (Fayle and Manica, 2010). Moreover, in our case every null model is developed independently, while the null models generated by the 'sequential' swap algorithm (Gotelli and Entsminger, 2003) are non-independent since each new null matrix is

generated based on the one before and differs from it by only 4 matrix elements (Gotelli and Ulrich, 2010). This implies that more null matrices are needed in case of a 'sequential' swap algorithm to obtain unbiased estimates of significance values (Fayle and Manica, 2011).

The above described indices were then calculated for each null matrix. This was repeated for 999 null matrices (Gotelli, 2000; Carranza *et al.*, 2010).

For Swap2, the swapping algorithm is somewhat easier. Only the row totals have to remain the same, thus a species can move from one replicate sample to another and the species richness of the replicate samples can change, but the number of observations for each species remains constant. One restriction is added to this swapping algorithm: if the swapping would result in replicate samples with no species, then the swap is not allowed, since degenerate matrices may increase the frequency with which the null hypothesis is rejected (Gotelli, 2000), and may thus enhance Type I error.

Gotelli (2000) showed that both swapping algorithms have good power for detecting non-random patterns in noisy datasets for Checker, the C-score and the V-ratio and have a low chance of falsely rejecting the null hypothesis. These swapping algorithms were applied to the complete dataset and to the datasets for the four feeding types.

An additional advantage of restricting the swapping algorithm to the replicate samples is that replicate samples were processed according to the same methodology. Thus, differences in sampling techniques between researchers and institutes will only have a minor influence on the final result.

Null Model Check

Recent research by Fayle and Manica (2010) showed that the probability of incorrectly detecting a signal in truly random data (Type I error) for the different indices increases with matrix size. They reviewed 47 publications, and it seems that our database is larger than any of the databases analyzed in these publications. Therefore, we developed an additional test, to check for Type I errors related to the structure of our database or related to the swapping algorithm. A reliable null model should only reveal significant differences caused by non-random distributions of species. Thus, if an artificial data matrix is supplied to the swapping algorithm, the null model should not reveal significant differences for the test statistics based on the randomised data matrix. Otherwise these significant differences should be attributed to other factors aside non-random structure in the species assemblage, such as errors inherent to the swapping algorithm or due to the specific structure of the data matrix. To keep the test within realistic constraints the artificial data matrix was composed by keeping the same number of species in a replicate sampling, but by randomly assigning species to a replicate sample. Thus, species aggregations or segregations should not be revealed for this matrix. For this artificial dataset, the test statistics are calculated and compared with the results of null models created with the swapping algorithm described above. To check the reproducibility of this test, it was repeated for three artificial data

matrices. Since the test is repeated three times and the computation of null models is time consuming, the number of null models was restricted to 500. No significant differences should be detected by this test.

RESULTS

Null model check

The null model check for the artificial datasets for the first swapping algorithm with presence/absence data (Swap1, Fixed-Fixed), reveals no significant difference for any of the test statistics (Table 2.1). The second swapping algorithm (Swap2, Fixed-Equiprobable) however, reveals significant differences for the C-, T- and V-score for the artificial data matrix (Table 2.1). The C-score is larger than expected for a random community, while the T- and V-score are smaller than expected by chance; indicating segregated species patterns. The Checker-index shows no unequivocal response. In one case it is significantly larger than expected by chance, thus indicating that the number of species pairs forming perfect checkerboard patterns is larger than expected for a random distribution, while in the two other cases it cannot be distinguished from random.

Swapping algorithm	Test statistic	Results for 3 artificial data matrices		
Fixed-Fixed	C-score	2260.0	2259.4	2259.1
(p/a)	T-score	2828.2	2827.6	2827.3
	Checker	2724.0	2730.0	2785.0
Fixed-Equiprobable	C-score	**2259.8** (>)	**2259.3** (>)	**2259.4** (>)
(p/a)	T-score	**2828.0** (<)	**2827.5** (<)	**2827.6** (<)
	V-score	**6.97** (<)	**6.97** (<)	**6.97** (<)
	Checker	**2803.0** (>)	2794.0	2800.0

Table 2.1. Values of the test statistic for the artificial data matrices; values in bold indicate that the test statistic of the artificial data matrix is significantly different from the null models ($p<0.05$ for a two-sided confidence interval), (>) and (<) mean that the test statistic of the artificial matrix is respectively significant larger or smaller than expected by chance.

Results for the real data

Presence/absence data

The two swapping algorithms applied for the presence/absence data are thoroughly investigated by Gotelli (2000). He found that these two swapping algorithms have the best properties concerning Type I and Type II errors. However, Fayle and Manica (2010) showed that the algorithm may be prone to Type I errors for large datasets. The most conservative

null models (Fixed-Fixed) reveal no significant difference for the C-score and the T-score (Fig. 2.2) indicating that no species segregation and aggregation is apparent in the data. On the other hand, the number of perfect checkerboard pairs is significantly higher compared to the

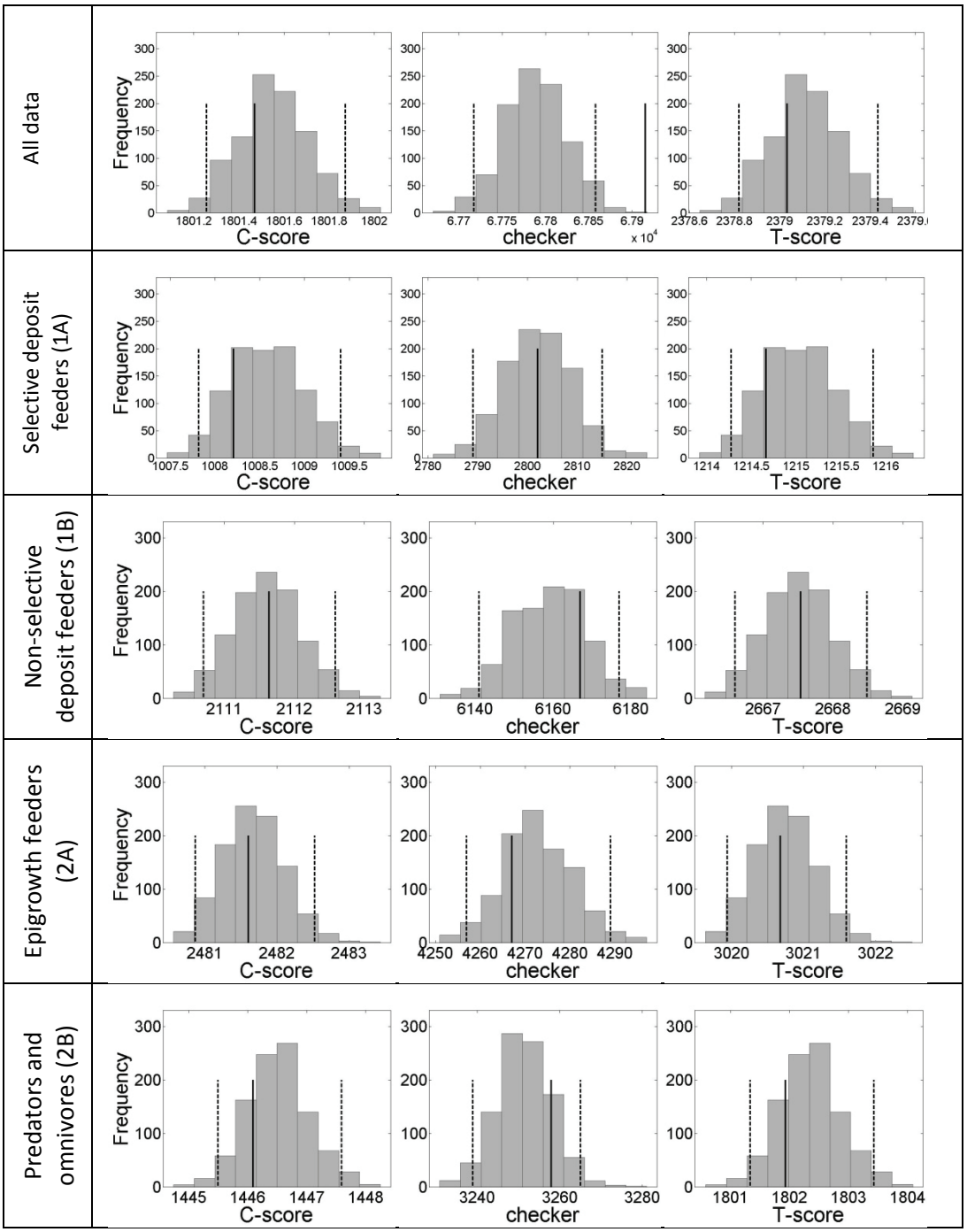

Fig. 2.2. Comparison of the two sided 95% confidence interval of the 999 null models (dotted lines) based on Swap1 (Fixed-Fixed) with the original value (full line) for the three community parameters (C-score, Checker and T-score) for all the data and the four feeding types.

null models. Thus, there are more checkerboard pairs in the real data matrix than would be expected by chance. Checker is a parameter which may be prone to Type II error (Gotelli, 2000), but it has good Type I characteristics and significant differences should be reliable (Gotelli, 2000; Carranza *et al.*, 2010).

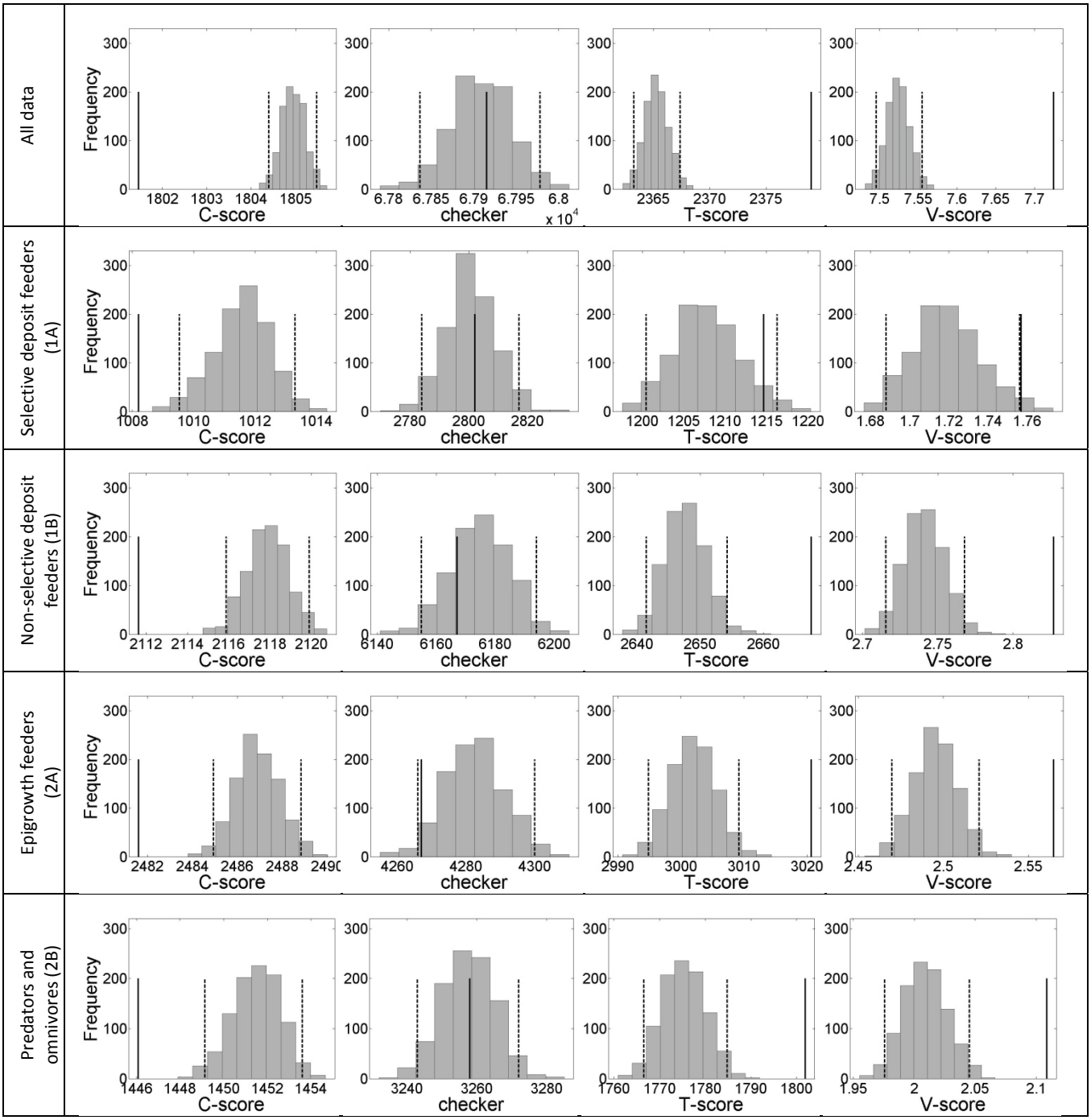

Fig. 2.3. Comparison of the two sided 95% confidence interval of the 999 null models (dotted lines) based on Swap2 (Fixed-Equiprobable) with the original value (full line) for the four community parameters (C-score, Checker, T-score and V-score) for all the data and the four feeding types.

The less conservative test (Fixed-Equiprobable) shows significant differences for the C-score, the T-score and the V-score, but not for Checker (Fig. 2.3): The C-score of the real data matrix is significantly smaller than the random values, while the T-score and V-score are higher than expected by chance. As demonstrated in the previous paragraph, this swapping algorithm results in Type I error for the C-, T- and V-score, thus caution is necessary when interpreting these results. However, for the real data matrix the opposite pattern is found compared to the artificial data matrices. Only the T-score for the 1A feeding type reveals no significant pattern.

The C-score and Checker indices quantify co-occurrence and can produce the same results (Gotelli, 2000), which is clearly not the case here. The C-score with Swap2 indicates that the species tend to aggregate more than expected by chance. In contrast, the Checker index with Swap1 indicates that there are more checkerboard pairs than would be expected by chance. However, Stone and Roberts (1992) found that a high 'checkerboardness' may be the result of aggregated species. To resolve this apparent contradiction they developed the T-score which in our case confirms the presence of aggregated communities. The overall V-score for the entire area is larger than one indicating that species co-vary positively.

DISCUSSION

Algorithm

The Fixed-Fixed algorithm for presence/absence behaves well for the artificial datasets: the indices calculated for the artificial data matrix are not significantly different from the indices calculated for the null models. However, the Fixed-Equiprobable algorithm for presence/absence data reveals that species tend to co-occur less than expected by chance, while such patterns are not supposed to be present in the artificial dataset. The presence of this Type I error may be due to the large size of our datasets (Fayle and Manica, 2010). The large amount of data triggers thus some unexpected problems. Fayle and Manica (2010) attributed these problems to 1) the 'sequential' swapping algorithm (resulting in non-independent null matrices) generally used in null model analysis and 2) the use of too few swappings to construct one null matrix. In our research we did not apply the 'sequential' swap: each null matrix was built independently from the previous null matrix and the number of swaps to construct one null model was increased from 5000 to 338 000 swapping attempts. This resulted in a time-consuming null model analysis, which we expected to be less prone to a Type I error for the different indices. Nevertheless, a significantly smaller T-score and a significantly larger C- and V-score are found for the randomised data. Remarkably, the opposite pattern, a higher co-occurrence than expected by chance, was found for the real data matrix. Thus, notwithstanding the bias of the swapping algorithm and the matrix structure towards segregated communities, the real data overrules this bias and indicates that species tend to co-occur in some replicate samples, forming aggregated patterns (Fig. 2.4).

This pattern is not found for the first swapping algorithm: when the total number of species in the replicate sample is kept constant, no co-occurrence patterns are revealed. This can be related to the fact that the algorithm is too conservative to reveal any non-random distribution patterns. However, it is possible that the co-occurrence patterns revealed by Swap2 are caused by the differences in species richness between the replicate samples and less by the presence of specific species pairs.

The V-score of the presence/absence data for the complete dataset is larger than 1 indicating that the species co-vary positively, which is not surprising since the species form distinct communities over the studied area, which can be ascribed to environmental gradients (Vincx *et al.*, 1990; Vanreusel, 1990; Vanaverbeke *et al.*, 2011). This is also reflected in the null models, where V is larger than one as well. It is evident that the null models also have V-scores larger than one, since swapping is only allowed within replicate samples. Thus, species will not appear in regions where they are not observed.

The analyses based on data concerning the feeding types generally display the same patterns as for the entire dataset. Hence, there is no evidence that species within feeding types interact stronger with each other. For the Fixed-Fixed algorithm for presence/absence data a significant difference for Checker was found for the overall data but not for the four feeding types apart. This may indicate that the checkerboard pairs are formed by species belonging to different feeding types. Yodnarasri *et al.* (2008) observed competitive interactions between epigrowth feeders (2A) and non-selective deposit feeders (1B). However, checking for patterns within the combined group 1B and 2A with the Fixed-Fixed algorithm resulted in a random pattern for all the test statistics (results not shown).

The tendency of species to aggregate does not necessarily imply that specific species pairs co-occur more often than expected by chance. Appointing individual species pairs which often co-occur could be an interesting starting point to set up future experiments. However, this is a statistical challenge (Sfenthourakis *et al.*, 2006) because even a small number of species results in a high number of species pairs. Many pairs will be significantly different from random just by chance at the 5% or 1% error threshold (Ulrich *et al.*, 2009). In our case, the entire dataset contains data on 450 species, which is an unusual high number, resulting in 101 025 unique species pairs. Thus, appointing non-random species pairs is statistically very precarious.

Sample size and patchiness

The effect of the sample size on the result of the null model is minimised by restricting the swapping algorithm to the replicate samples of one sampling event, which all have the same size. The most probable effect of the different sample sizes on the outcome of the analysis is an increase of a Type II error: although a non-random distribution pattern is present, it cannot be derived from the data. For instance, in case species tend to form aggregated patterns (Fig. 2.4), this pattern might be obscured by large samples because species might

be sampled at the edges of the large replicate sample. The different sample sizes might thus blur the distribution patterns.

It is a well-known phenomenon that nematode communities tend to form patchy distributions (Li *et al.*, 1997; Somerfield *et al.*, 2007; Gingold *et al.*, 2010a). On a small scale the horizontal distribution of nematodes shows a strong patchiness; within a range of a few centimetres nematode densities can drop with a factor 3 (Arlt, 1973). Horizontal patch size of meiofauna can vary between 0.3 to 700 cm² (Heip and Engels, 1977; Findlay, 1981; Blanchard, 1990). The distribution of most nematode species show strong aggregations (Blanchard, 1990) and species may show repeating patterns in densities of 8, 10 or 12 cm depending on the species (Blome *et al.*, 1999). The meiofauna sampling cores in our study have mostly a diameter of 3.6 cm (44 %) or 5.5 cm (24 %) and it is thus evident that the cores may sample at the middle of a dense nematode community or between these communities (Fig. 2.4). The swapping algorithm is based on presence/absence data, and patchiness is often associated with higher nematode densities. To validate the hypothesis that patchiness and thus higher densities are linked with higher species richness an additional test was done: for each sample (with more than 2 replicate samples) the Spearman rank correlation coefficient between the total density of the nematode community and the species richness in the replicate samples is calculated. This could only be done for the samples where the total density of the replicate sample is known (216 samples): 50% of the 216 samples have a Spearman rank correlation coefficient larger than 0.5 and only 12% have a Spearman rank smaller than -0.5. For small samples, it is easy to produce a strong correlation by chance and caution should be paid when interpreting these results, but it is clear that there is a strong tendency to find more species in replicate samples with higher densities (as represented in Fig. 2.4).

Ecology

The previous analyses indicate that the communities in the replicate samples are not randomly structured: species tend to aggregate in some replicate samples within a station and not in others. However, the mechanisms explaining the non-random pattern are difficult to assess. Even if the null hypothesis is rejected, it is impossible to leap to the conclusion that species interactions have led to these patterns (Simberloff and Connor, 1981). The actual mechanism behind the non-random patterns should be revealed by experiments (Gotelli, 2001). However, if competition and facilitation are at work, these mechanisms are expected to leave different signatures in the pattern of species co-occurrence.

Competition may result in a given species pair co-occurring less often than expected by chance, whereas facilitation may result in a given species pair co-occurring more often than expected by chance. Previous research of assemblages in marine environments show that any pattern can be found: random patterns for gastropods (Carranza *et al.*, 2010), strongly aggregated patterns for reef fish assemblages (Semmens *et al.*, 2010) or strongly segregated patterns for brachiopods (Tomašových, 2008).

On a large scale of meters to kilometres environmental gradients structure nematode communities (Soetaert *et al.*, 1994; Li *et al.*, 1997). When reducing the scale of observation, other factors may become more important, such as species interactions and patch dynamics (Levin *et al.*, 2001). According to our results nematode species tend to aggregate in some replicate samples more than would be expected by chance: this may be attributed to both a

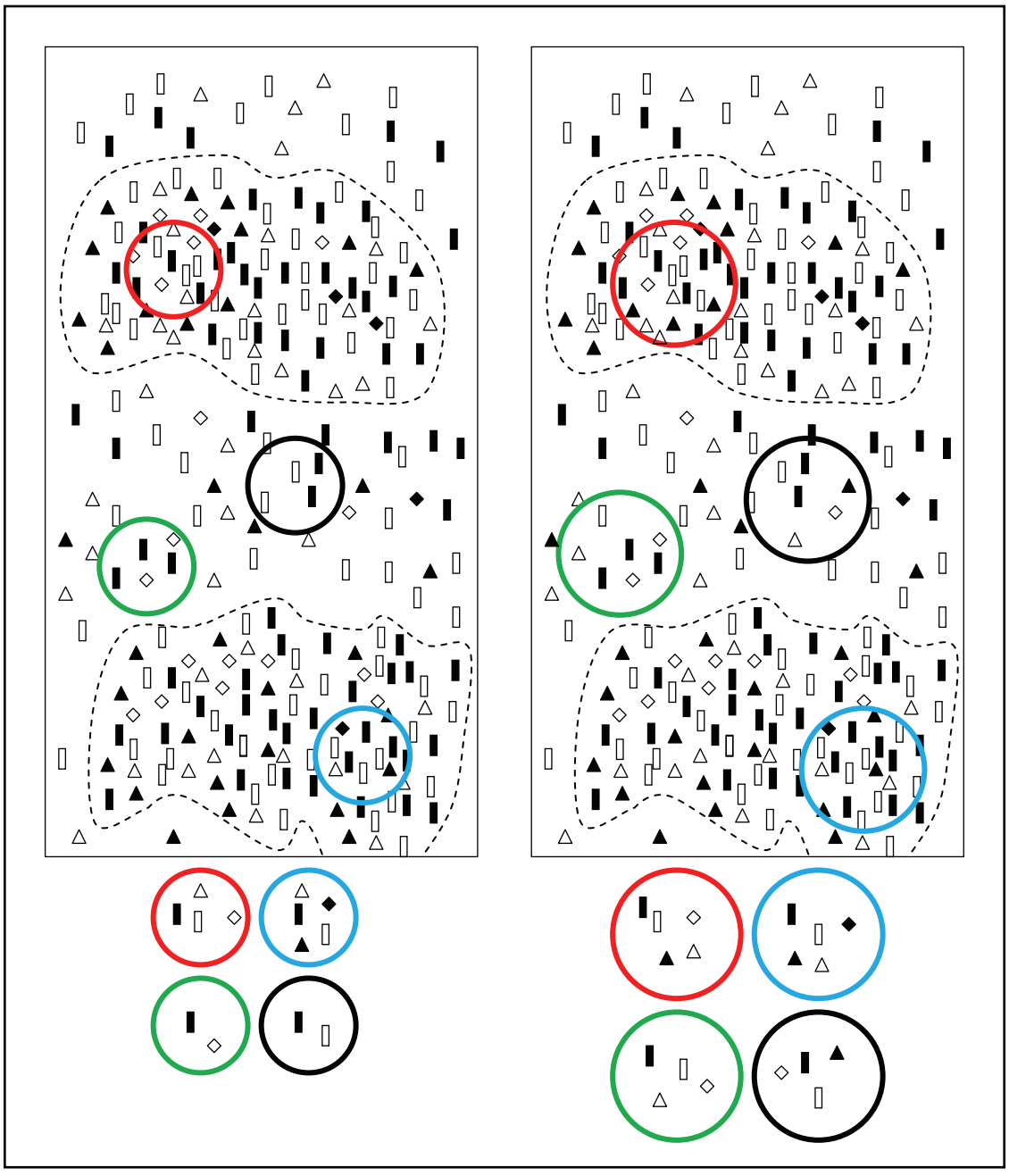

Fig. 2.4. Schematic representation of a sampling event where 4 replicate cores are taken with a small sampling core and a larger sampling core in the same community. Patches with higher densities of the nematode species are delineated by a dashed line. The species found in the replicate samples are represented in the circles at the bottom. The sampling design at the left is more likely to reveal the aggregated pattern of the species (blue and red core), while the sampling design at the right samples better the total species richness.

similar response to the environment (Sanders *et al.*, 2007) or to species interactions. Environmental differences between replicate samples may lead to different communities in these samples. The replicate samples are obtained at a certain moment in time at a certain sampling station. Therefore, the environmental circumstances in the replicate samples should be comparable. However, information about the actual distances and physical differences between the replicate samples is unavailable and it is possible that our original assumption of similarity of abiotic factors between replicate samples is idle.

Nematode communities often show a patchy distribution, a pattern which is confirmed here. Many factors may contribute to the origin of a patchy distribution: microtopography (Hogue and Miller, 1981; Sun *et al.*, 1993; Blome *et al.*, 1999), the presence of biogenic structures and macrofauna (Reise, 1981; Reidenauer, 1989; Braeckman *et al.*, 2011) or patches in food sources (Lee et al., 1977; Blanchard, 1990), and even (social) species interactions have been suggested for meiofaunal communities (Heip, 1975; Findlay, 1981; Chandler and Fleeger, 1987). Our study confirms the presence of small scale aggregations, with some replicate samples holding more species than others. However, the mechanisms behind this non-randomness cannot be unravelled with these null models.

The same aggregation patterns were found for the different feeding types. The current classification of nematodes by feeding groups is rather coarse and species belonging to the same feeding type may express different adaptations in the buccal cavity (Wieser, 1953; Deutsch, 1978). There has been some debate on this subdivision and more refined subdivisions have been suggested (Moens and Vincx, 1997; Moens *et al.*, 2004) but these differentiations are currently unknown for most nematode species. Thus, this refined subdivision could not be applied to our data. The aggregated patterns of the species belonging to the same feeding type may also be explained by the theory of Webb (2000) which postulates that closely related species are more likely to co-occur due to a similar response to the surrounding environment. This theory is supported by observations of co-existing closely related meiofaunal species (Heip *et al.*, 1985; De Mesel *et al.*, 2006b), and has been attributed at the time to the presence of microhabitats. Somerfield *et al.* (2009) suggested that in an open dynamic system such as the marine environment competition is most probably only operating on short time scales and small spatial scales. Indeed, species segregations have been found on a small vertical spatial scale between sediment slices of 1 mm or 5 mm (Joint *et al.*, 1982; Steyaert *et al.* 2003) where species interactions between two epigrowth feeders (Joint *et al.*, 1982), between predator and prey nematodes (Steyaert *et al.*, 2003) have been observed. But other factors such as food availability, oxygen distribution, physical disturbance and compaction of the sediment (Arlt, 1973; Joint *et al.*, 1982; Steyaert *et al.* 2003) may also contribute to these segregated patterns.

CONCLUSIONS

The results of our analysis are not unequivocal. Large databases may reveal non-random community patterns while they are not present (Fayle and Manica, 2010). This is also

supported by our analyses: randomizing the data revealed a Type I error for the different indices. When applying the swapping algorithms to large databases we therefore recommend an additional test which investigates the Type I error properties for the indices and the algorithms under study. It is clear that further research is needed to find the factors causing these errors and further adjustment of the algorithm is needed in such a way that co-occurrence patterns can be unequivocally revealed from large databases.

Nevertheless, our analyses indicate the presence of non-random community patterns at the level of replicate samples within a sampling station, suggesting locally aggregated nematode communities. Our analyses also indicate that patches with higher nematode densities generally have higher species richness. This is in accordance with previous research describing the patchy distribution of nematode assemblages which has been attributed to a variety of biotic and abiotic factors.

However, many questions remain unresolved: which factors contribute to this non-random distribution pattern? Is this pattern a general pattern for the entire area or do some regions or samples contribute strongly to the observed pattern?

Drawing conclusions regarding species interactions is impossible based on the algorithm; this is only achievable by carefully monitored experimental set-ups. However, our analyses do not suggest the presence of competitive interactions. Other factors may contribute to the observed aggregated pattern, such as the coarse subdivision of the feeding types, the large scale of the replicate samples compared to the interaction scale of nematodes, the unknown environmental differences between the replicate samples and the patchy distribution of the nematodes. Besides, in an open system such as the marine environment competitive interactions may only be present on a small temporal and spatial scale (Somerfield *et al.*, 2009) and due to environmental stressors closely related species may even co-occur more than expected by chance (Webb, 2000).

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