

## ***Interactive comment on “Resource utilization and trophic position of nematodes and harpacticoid copepods in and adjacent to *Zostera noltii* beds” by A.-M. Vafeiadou et al.***

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Reply to Referee 1

Thank you very much for the constructive comments and suggestions. We have adopted most of them and have included them in the revised version of our manuscript. Admittedly, they have helped us to further improve the quality of this manuscript. Below there is a detailed reply to your specific comments:

COMMENT 1: MixSIR was run without taking into account the most "extreme" food items in terms of carbon isotopic ratios. Seagrass leaves and roots (the most positive items, roughly -11 to -13 ‰) were left out based on the assumption that none of the

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studied organisms were able to consume living seagrass tissues. Chemoautotrophic bacteria (the most negative item, -35‰) were included for only 3 of the studied taxa. Although it is not stated explicitly, I suppose it was assumed that it could only be a major food source for the most  $^{13}\text{C}$ -depleted animals. A priori exclusion of sources likely has an important influence on model outputs, and is in my opinion a valid option only if authors are sure that these food sources do not contribute to the diet of any consumer, either directly or indirectly. Here, I highly doubt that it is the case. For example, examination of figure 3 reveals that some *Metachromadora* and one *Daptonema* samples had  $\delta^{13}\text{C}$  values compatible with important reliance on living seagrass tissues. Moreover, results suggest that several of the studied organisms are secondary consumers. In the absence of supporting data, I don't think it is reasonable to assume that none of their prey could rely on either seagrass tissues or chemoautotrophic bacteria. Actually, the authors themselves state that some nematods could indirectly depend on the latter source (p.1290, l. 25-28 & p.1291, l. 1-2). In this context, I don't think it is wise to exclude those "extreme" food sources. I therefore suggest that authors run the model again, including them. Of course, adding extra sources could impair model performance, but this can be taken into account by using diagnostic tools (e.g. correlation between sources in model-estimated proportions distributions).

RESPONSE: In the previous version of our manuscript, we indeed excluded living seagrass materials (roots and fresh leaves; these also happened to be the resources with the highest  $\delta^{13}\text{C}$ -values) from the potential food sources because there are no indications in the literature that nematodes or harpacticoid copepods would be capable of directly grazing on living macrophyte tissues. We also did not systematically include chemoautotrophic bacteria, except for three meiofauna taxa which had carbon isotopic ratios more depleted than all resources included in our model. Based on your comments, we have reconsidered the resources to be included in our model. Since we have no a priori reason to exclude chemoautotrophic bacteria as a resource for any of the nematode or copepod taxa analysed here, we have now systematically included this resource in our model runs. Similarly, although we do not expect any direct graz-

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ing on seagrass roots by meiofauna, root exudates may directly or indirectly (through grazing on bacteria which utilize these exudates) contribute to the diets of meiofauna. Hence, we have now included seagrass roots in the model runs for all meiofauna from inside seagrass beds, but not for meiofauna from bare sediments. Seagrass detritus has been included in all model runs, as in the previous manuscript version (but see below). Living seagrass leaves have not been included as a resource, since we only analysed meiofauna from inside the sediments, which are both physically separated from, and incapable of grazing on living seagrass leaves. The power of isotope mixing models to discriminate between contributions of resources with highly similar isotope ratios is limited, particularly so when several other resources are also included. In order to limit the number of 'informative' resources in our model, we decided to pool seagrass detritus and seagrass roots as one resource for the model runs on meiofauna from inside seagrass vegetation. Both sources partly overlap isotopically, and their most likely route of carbon transfer to meiofauna is through meiofaunal grazing on associated bacteria, or meiofaunal uptake of dissolved material leaching from, or exudated by, the seagrass material. Secondly, given the strong overlap between isotopic ratios of microphytobenthos and epiphytes, we combined these two resources into one (see also the authors' reply to Comment 2 below). Combining two resources implied that we calculated mean values and standard deviations of the delta-values of both. In doing so, we were able to limit the number of resources in our model to four: seagrass-derived (seagrass detritus in bare sediments; seagrass roots + seagrass detritus in vegetated sediments), microalgae (microphytobenthos + epiphytes), suspended particulate matter (SPOM) and chemoautotrophic bacteria.

COMMENT 2: Authors ran MixSIR twice: once including seagrass epiphytes as a food item, and once without epiphytes (p. 1284, l. 21-23). It is not clear to me what they were trying to achieve by doing so, but it seems like a rather arbitrary way to decrease the number of sources. In addition, it is conceptually wrong, because animals had access to seagrass epiphytes regardless of their inclusion as food items in the model. I think that seagrass epiphytes should be included in MixSIR inputs. If authors want to lower

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the number of "overlapping" (i.e. isotopically similar) sources, like microphytobenthos and seagrass epiphytes in this case, I think that aggregating them (i.e. computing a single mean and SD for all values of these two sources) constitutes a more objective, and therefore more suitable approach. I don't expect it to cause a significant loss of model predictive power, because the model will not be able to efficiently discriminate between isotopically similar sources anyway.

RESPONSE: As mentioned under Reply to comment 1, we combined microphytobenthos and epiphytes into one resource because of their strongly overlapping isotopic ratios. In the discussion of our manuscript, we argue that microphytobenthos is considerably more likely to importantly contribute to the diets of benthic meiofauna than epiphytes.

COMMENT 3: Models estimates are presented by giving the median and 95th percentile, with the full distribution of solutions as electronic supplementary material. I think that presenting model outputs as credibility intervals (e.g. 95% intervals, with lower and upper limits) would be more intuitive because it would allow the reader to estimate dispersion on both sides of the solution distribution.

RESPONSE: We have accepted the comment about the presentation of model data. Hence, proportional contributions are now given as upper and lower limits of 95% credibility intervals, instead of as median and 95th percentile, in Table 3 of the revised ms.

COMMENT 4: In some cases (e.g. tables 1 and 3), authors give sample numbers, but in some cases they do not (e.g. table 2 or model input data). Moreover, the pooling strategy is not described in detail, and only partial information is given on p. 1282 (l. 21-23). I think it would add value to the manuscript if authors provided a synthetic table giving sample numbers and (if applicable) pooling strategy for each food item and studied taxon, and for each measured parameter ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). For example, it would help the reader to quickly and conveniently understand what is meant by "We used  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of all replicate samples per taxon separately [...] as input data"

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(p. 1284, l. 17-19).

RESPONSE: We have not presented an additional table because it ended up being rather complex, but we have provided additional information, as requested, such as the number of samples analysed per case in Table 1 of the revised ms (which is the transformed table 2 of the previous version). We also have better explained the model input data in the materials and methods section of the revised ms. (In Line 222-225 of the revised ms: We used as input data for consumers:  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of each replicate sample per taxon separately, including data only of those samples from whom we obtained both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and for potential sources: mean (and SD) of  $\delta^{13}\text{C}$  and of  $\delta^{15}\text{N}$  of all replicate samples per source.)

COMMENT 5: Table 1 (seasonal variation of food items isotopic composition) and the associated results section (3.1, p. 1285) do not seem very informative to me. In some cases, inter-season comparisons are impossible (e.g. seagrass detritus that were only sampled in one season), and in other effectiveness are very low, questioning the validity of comparisons (e.g. epiphytes, where  $n=2$  for one of the seasons). Regardless of the outcome of these comparisons, source values measured in both seasons were pooled for elaboration of figure 3 and for modeling purposes. Distinction between sampling events is almost never made for consumers. In this context, I don't think that the sampling strategy was adequate to assess seasonal variation among the studied food web, and the discussion statements focusing on this issue (p. 1292, l. 27-29 and p. 1293, l. 1-6) seem rather arbitrary, as there is not enough data to either support or infirm them. I'd advocate taking these results out of the paper to focus on findings that are more relevant to this study's aims.

RESPONSE: Our rationale for sampling in two different seasons was to come to more robust conclusions about the relative importance of different carbon sources to meiofauna than what could have been obtained based on a single sampling event. Admittedly, analyzing the data from both sampling events separately reduces the sample size, and since no statistically significant differences were obtained in isotopic signa-

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tures between sampling events, we decided to analyse these data together, omitting 'season' as a factor in our statistical analysis. We have now consistently done so and have therefore also excluded comparison between seasons from the results and discussion of the revised ms. Additionally, Table 1 of the previous version, which listed the food source information of both sampling seasons separately, has been now excluded from the revised ms. Instead, we included mean source and SD delta-values of both sampling moments in previous Table 2, which is now Table 1 in the revised ms.

COMMENT 6: On p. 1281 (l. 1-3), authors mention that the studied system is still recovering from a major collapse that happened in 2008. I have concerns about potential impacts of meadow structure alteration on trophic relationships among the associated communities. Besides lower shoot density, are major perturbations of the ecosystem still visible? If so, this should be explicitly mentioned and discussed.

RESPONSE: It is still unclear what caused the *Zostera* collapse. Except for an obviously lower shoot density, we have not observed other major perturbations of the studied ecosystem.

Technical corrections:

- p. 1284 l.28 and p. 1285 l. 1-2: This sentence seems redundant with p. 1284 l.2-4. Response: The sentence was deleted. - p. 1294 l.3: Parentheses are lacking in the unpublished citation. Response: Indeed, it was a wrong typeset and was corrected. - p. 1294 l.12: Use of a common name without stating the organism's scientific name first should be avoided, especially when, like "cordgrass", it can relate to several taxa. Response: The word 'cordgrass' was corrected to 'Spartina spp.'

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