

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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Thank you very much for your constructive comments and suggestions for further improvement of our manuscript. Below, we list our detailed replies.

General comment: COMMENT: This study attempts to analyze the contribution of various food sources in the nutrition of estuarine sediment-dwelling meiofauna (nematodes and harpacticoid copepods) using stable isotope (13C, 15N) signals from potential food items and selected consumer organisms. The major merit of the manuscript is the fact that most of the consumers have been identified to genus- and all to family-level. This makes a comparison between assumptions on the diet and the results from the present

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isotope study possible and leads to a critical appraisal of so far published assignments to trophic levels and resource use. Several of the results contradict (or at least seem to contradict) these assignments and the authors plead for caution in using e.g. the buccal morphology classification used in nematode ecology since Wieser's extensively cited paper. The scarcity of data on meiofauna nutrition makes this study valuable and publication should be envisioned. Unfortunately the authors try to do too much at the same time with the effect that many of the conclusions remain only weakly supported. Instead of focusing on a single habitat and season (where enough material could have been collected for all the planned analyses) they attempt to include the role of different food sources comparing vegetated and non vegetated habitats and two seasons. Sentences such as ". . .suggesting they either utilize a mix of more 13C -depleted (e.g. SPOM) and more 13C -enriched (e.g. seagrass detritus) food sources or, more likely feed predominantly on MPB and/or epiphytes" leave me puzzled. As a consequence, a number of simplifications had to be made especially in using the mixing models and interpreting the results. I share all the reservations expressed in the detailed review of Michel Loic and do not want to add to these.

RESPONSE: Of course we agree that our manuscript is unique in the amount of genuslevel information on resource utilization of meiobenthos presented. We partly agree with the suggestion that we tried to do too much: hence, as also suggested by the first reviewer, we have now omitted any reference to seasonal differences, because our isotopic data do not demonstrate differences between seasons, and pooling the data from both seasons greatly adds to the power of our statistical analysis. On the other hand, our main aim was to contrast the relative importance of different resources for meiobenthos between vegetated and non-vegetated sediments. We felt that doing this at two different field locations greatly added to the conclusiveness of our study. The specific sentence which appeared unclear merely points out that intermediate δ 13C signatures can point at two contrasting conclusions: 1) consumers with such intermediate isotopic signatures indeed predominantly utilize resources with intermediate isotopic signatures, which in the present study is microphytobenthos and/or epiphytes; 2) consumers may have intermediate carbon isotopic signatures because they utilize a mixture of more depleted (here SPOM or chemoautotrophic carbon) and more enriched (here seagrass derived carbon) resources. We have rephrased the pertinent sentence to clarify this issue, but feel it needs mentioning to avoid that readers would consider it self-evident that the resource with the most similar isotopic signature to that of the consumer automatically has to be its main food source.

Specific comments:

COMMENT : I would have appreciated a more clear description of the sampling site (position with regard to tide level, tidal amplitude during the sampling dates).

RESPONSE: A more clear description of the sampling site, including information on tidal amplitude, has been included in the revised ms: Lines 115-117: "...a semidiurnal tidal regime (amplitude 1-3 m during neap and spring tides, respectively). It has a single channel, 5–10 m deep and up to 400 m wide. Tidal influence extends 40 km upstream." and Line 128: "during low tide (amplitude of 3 m)".

COMMENT : Sample collection is said to be "random" – how has this been achieved? Or was it just "haphazard"?

RESPONSE: You are correct that we did not sample random points from a sampling grid, but collected samples haphazardly. Hence, the word 'randomly' has been replaced by the word 'haphazardly' in Lines 135 and 148 of the revised ms.

COMMENT : Pooling the meiofauna samples before sorting and identification lead to a loss of information on within-station variability, which could have been considerable. One could have pooled the meiofauna later to obtain enough biomass for the analyses.

RESPONSE: Indeed, the pooling of meiofauna samples obscures within-station heterogeneity. Such heterogeneity can indeed exist. However, since our main goal was to compare vegetated vs non-vegetated stations (and this at two locations), we decided that our sampling should (a) cover a representative community from vegetated and

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from unvegetated sediments, and (b) should preferably contain sufficient biomass from a good number of meiofauna taxa. Had we collected separate cores and sorted meiofauna separately from each core, we would have ended up with very few taxa for which we had replicates within stations, and a majority of taxa for which we had one or two samples obtained by pooling specimens from the various samples of a given station. Particularly for nitrogen isotopes, the biomass requirements are such that a sampling strategy which would adequately and consistently (over all taxa reported here) present within-station variability based on true replicate samples, is practically not or hardly feasible. This is evidenced by the lack of studies in the literature presenting replicated data at genus level. One should also not forget that each meiobenthos sample analysed for stable isotopes contained from a few to a few hundred individuals. Since these individuals were drawn from a pooled sample composed of 7 samples taken haphazardly in a station, we consider such sampling strategy adequate for our main aim, i.e. to compare resource use of taxa between vegetated and non-vegetated stations.

COMMENT: The fact that the nematode Terschellingia and the Cletodidae have stable carbon isotope signatures that suggest feeding preferentially on chemosynthetic bacteria is very interesting. In my pet group of nematodes, the Stilbonematinae, this seems to be the case (see Ott et al. 1991. PSZN Marine Ecology 12) and is plausible considering their symbiosis with chemosynthetic bacteria. In the case of Terschellingia, where no such symbiosis has so far been described, it would imply a strong selectivity in its bacteriovorous habit. How can one explain a 13C of -41.7 and a contribution of 0.81 in the worm assuming a 13C of -35 for chemosynthetic bacteria - all other possible food items being less depleted?

RESPONSE: This is a very logical and pertinent question, with which we have initially struggled ourselves. The problem is inherent to Bayesian isotope mixing models. When a consumer has isotopic values which are outside the range of measured resources, the Bayesian mixing model nevertheless try to find the solution which better fits to real data, it tries to find a source which would fit better to this 'outsider' consumer. In this case most solutions return a very high contribution of chemosynthetic carbon, but such solutions do not yield a very strong fit with the real data, and hence have broad credibility intervals. Other mixing models (for instance the least-squares mixing models) compute no solution to our system, because the consumer is outside the isotope polygon defined by the resources (i.e. more negative than the lowest food source). However in Bayesian models (like the MixSIR used in this study) multiple possible solutions are computed, in a wide range of credibility. For our study this means that a solution with a high chemosynthetic contribution is more probable than a solution with a low chemosynthetic contribution. However, this more probable solution does not fit the data very well (lower reliability, broader credibility intervals), but it is the best one that could be found for this case.

COMMENT : Furthermore, SOM, SPOM and SLD represent a mixture of the original photosynthetic organic matter (in part mainly the nutrient poor and indigestible structural matter) and of microorganisms that have utilized the more valuable compounds and converted them into their biomass. Therefore the isotopic signature of bulk SOM, SPOM and SLD will be determined by discrete fractions, which may differ significantly in 13C and 15N. Due to their small size meiofauna could selectively graze the microbial component that is more depleted in the heavier isotopes. Even when ingested, the fraction representing the original photosynthetic tissue might not be easily digestible. This would make the results of the mixing model ambiguous and could be the cause of some of the surprising discrepancies between buccal morphology and isotopic composition. This should at least be discussed.

RESPONSE: The comment has been accepted and seagrass utilization via microbes and their indirect input at the food web was further discussed in the revised ms.

Technical corrections:

Line 74: Kharlmamenko vs. Kharlamenko in references RESPONSE: The typing error in the reference to Kharlamenko has been corrected. Line 237: this is the first time

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that the acronym SOM is used. Therefore it should be explained what it stands for. RESPONSE: SOM is now introduced in Line 134 and mentioned in full there as well.

COMMENT: In conclusion, I think the Discussion section should be streamlined, emphasizing those results that are well substantiated by the data before the manuscript is published. RESPONSE: Several smaller changes have been made to the discussion in order to improve its flow and its focus on the core results. Among other things, any discussion on temporal variability in resource utilization has been removed.

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