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Interactive comment on "Optimizing sample pretreatment for compound-specific stable carbon isotopic analysis of amino sugars in marine sediment" *by* R. Zhu et al.

Anonymous Referee #2

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General Comments

The manuscript contains many ideas how to optimize and validate the various steps (hydrolysis, purification and derivatization) and each of them was tested carefully. Thus, the methodological tests look targeted and carefully performed and I'm glad about such a systematic method evaluation, which would be needed much more frequently in biogeochemistry. Due to this, in my view, this is a substantial contribution to scientific progress in the field of biogeoscience: working with well-developed and evaluated methods is the fundament of scientific work in that field and I strongly support the scientific relevance of method publications. However, finally it remains the editor's decision, whether to consider method comparison and evaluation studies for publica-

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tion in biogeoscience. Method evaluations are precisely described and well performed and I suggested only minor comments, how this could be additionally improved for further method studies. Some aspects have to be mentioned more clearly and I wrote a respective comment wherever I realized a gap in information (see specific comments). Especially, in the discussion, it has to set a bit more focus on the comparison of HPLC versus GC based method for isotope determination: HPLC has the clear advantage of a higher isotopic precision and accuracy (see Bodé et al and Dippold et al.). However, GC-method have a much lower detection limit and thus are the only option to measure amino sugars in trace amounts – as they occur in sediments. This has to be clearly stated, because if not it's not really clear, why you go back from new-developed LC methods towards the "old" GC-C-IRMS methods, for your amino sugar d13C determination. In addition, the opportunities which arise from the possibility to measure amino sugar d13C in sediment samples is not really worked out – without a final conclusion in this direction, the manuscript is missing it's highlight: the reader is not only interested in the fact, that he can now measure d13C of amino sugars in sediments, but also, which new perspectives arise from that possibility. I really enjoyed having a brief look into first results with a brief interpretation (and this is in my view fully sufficient for a method publication) but I missed a bit the perspective of your method (some ideas see in specific comments) – and this information will strongly improve the scientific relevance of your study.

Specific Comments: p. 595 l. 26: The comparison of GC versus LC methods here is very subjective: Surely, most LC-methods suffer from matrix effects and have to be adapted separately, to new matrix types. However, also GC-methods suffer from the "matrix" which frequently disturbes the dervatization step – and the reproducibility of this step is essential for d13C analysis in the range of natural abundance. In addition, an important disadvantage of GC-C-IRMS methods is not mentioned here: the introduction of derivatization C – which clearly restricts precision and accuracy of GC-C-IRMS methods for d13C determination. In addition, there are new instrument couplings like IC-O-IRMS (recently published in RCM), which overcome many of the

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classical problems of LC-O-IRMS (single run measurement, less matrix problems,. . .). However, I fully agree that LC-O-IRMS has further problems, e.g. the higher concentrations needed, which are even worse to be extracted from marine sediments compared to soils,... To summarize: the introduction section has to be more detailed at this point: the sentence: "GC-based method is advantageous. . ." is not true – here a more detailed introduction into that topic is needed.

p. 596 l. 19: Evaluation is not only needed with regard to recovery, but also regarding the following aspects: - amount of introduced derivatization C - reproducibility of the derivatization (matrix dependency) - fractionation caused by the derivatization p. 598 l.17: I strongly approve the use of an internal standard at this step: however, I don't think, myo-inositol is the best internal standard for amino sugars, as it contains no amino group and may behave strongly different to amino sugars in some of the purification steps. I don't know your samples, but in none of the samples I ever measured, methyl-Glucamine was present. This substance is an internal standard structurally and chemically more similar to amino sugars and thus might be the better choice for future samples.

p. 600 l. 9: Are the abbreviations already introduced? Nevertheless, I suggest to write here once the full name of the derivatives, because it's likely, they are no longer in the mind of the reader p. 601 l. 9 I'm not sure, this approach is helpful: although, there are no visible peaks in the sample, it is difficult to define a sample "MurA"-free – it is just under the detection limit. However, even small contributions to a mixture, if deviating strongly from the ïĄď13C of the spiked standard substance, can significantly contribute to the peak's ïĄď13C-value. Therefore, a standard-addition line, where by a linear regression the "true ïĄď13C-value of unspiked soil" and the ïĄď13C-value of pure standard can be fitted is more reliable.

p. 603 l. 20 This is not true: the reason for low monosaccharide recoveries with HCl is a dehydration reaction: HCl is water-attracting and thus causes a dehydration of the monosaccharides. This reaction doesn't occur with amino sugars – the NH2-group

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cannot be split of by this reaction.

p. 604 l.17 There's a newly published SPE column comparison paper from Indorf et. al out, where an SPE purification column is tested: you should briefly mention there result here and compare their SPE column with your resins

p. 604 l.25 This is one of the most important results: long-term stability is one of the most crucial requirements for an isotope determination in the natural abundance range (an aspect, that many people using e.g. silylation do not consider). You should emphasize this result also in conclusion and/or abstract!

p. 605 l. 9 Add here one sentence like: However, it has to be tested for new samples with deviating matrix peaks, which of the columns will give the best performance / peak separation / . . .

p. 606 l. 17 Add here the reason: the clear disadvantage, of GC-C-IRMS methods, is a higher total error which is mainly a result of the introduced derivatization C (and presumably matrix-dependent fractionation processes during derivatization). Although, you optimize a GC-IRMS method here, you clearly have to discuss, that this is the obvious disadvantage of GC-C-IRMS compared to LC-O-IRMS

p. 607 l. 1-11 It's not clear what the "ng" refer to: if it is ng in the final sample before injection or the amount that is injected by one injection (i.e. underlying the peak)?

p. 609 l. 8 I fully agree – and this is also an highly important aspect of your manuscript which you should strongly highlight: it's not only the fact, that you are focusing on other members of the microbial community with amino sugars compared to lipid biomarkers, but in addition: 1) you have biomarkers which are preserved by a completely different mechanism (polymerization versus hydorphobicity) – and this may be a great advantage for specific research questions 2) you have biomarkers, which are derived from a completely different biosynthetic pathway (gluconeogenesis versus lipid synthesis) and consequently other metabolic precursors – consequently a big potential lies in the com-

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bined use of “hydrolysable sugar-derived biomarkers” with “lipid biomarker” → and this study is the prework for such approaches in sediment samples → this should clearly be highlighted here! Mention these aspects either here or in the conclusion section!

Fig. 1 Figure Caption is misleading: I expected the ANA-derivative of muramic acid as structure V – but it was pure muramic acid: I suggest: first show the four structures of underivatized amino sugars – then show the structure of one derivatized hexosamine and then add the structure of the ANA-derivative of muramic acid

Unfortunately, I couldn’t open page 622, 623 and 620 – so I cannot comment these figures

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Interactive comment on Biogeosciences Discuss., 11, 593, 2014.