Interactive comment on “Assessing the potential of amino acid 13C patterns as a carbon source tracer in marine sediments: effects of algal growth conditions and sedimentary diagenesis” by T.Larsen et al.

Replies to Anonymous Referee #1 (Received and published: 1 April 2015)

Major comment 1. p1621,L7-10: Isn’t the fractionation during derivitization likely to vary (in an uncontrolled way) between samples and standards dependent on the material matrix? More information is needed on the standards used, and justification for comparing δ13C-AA values between different sample matricies in the context of fractionation during derivitiazation.

REPLY (this reply was also posted in the Interactive Discussion 10 Apr 2015): The reviewer raises the important point of how a given sample matrix may affect derivatization and δ13C-AA values. We realize that we did not describe this part properly. In fact, we forgot to mention in ‘2.3 Stable isotope analyses’ that we purified the hydrolyzed sediment samples with Dowex 50WX8 cation exchange resin before derivatization. See paper by Larsen et al. Plos One, 8, e73441, 10.1371/journal.pone.0073441, 2013 for methodological details. The purification ensures that non-amino acid compounds are removed this avoiding co-elution between unknown compounds and protein amino acids during chromatographic separation. To address the question of how the sample purification affect δ13C-AA values, we will publish test results with yeast samples and amino acid standards in the Supplementary. The tests were done in 2011 but not published until now. We found that purification generally did not alter δ13C-AA values except for significantly enriching δ13C-Asx values by ~2‰. In some cases, Lys, Met and Thr were affected as well, but not consistently for the two sample matrices. Thus, we conclude that sample purification can affect δ13C-AA values, but that these effects are relatively minor to be of practical significance except for Asx.

Minor comments 2. Abstract: There seem to be a number of words missing in the abstract, and elsewhere in the manuscript (e.g. p1615,L6: “for tracing THE biosynthetic origin”; p1616,L28-29: “as well as THE direct bacterial role”? 3. L7: “natural occurring” –> “naturally occurring”? 4. p1617,L14: “largely independent OF variation”. 5. p1619,L22: “collected IN 2008”. 6. p1622,L10: “subdivided INto the following. . .”

REPLY: Corrected.

Replies to Anonymous Referee #2 (Received and published: 19 May 2015)

1) I guess that the authors are well aware of the pitfall of the carbon isotopic measurement of individual amino acids from natural samples; I mean, isotopic fractionation associated with the acetylation. You may not meet serious difficulties when comparing the data from the samples whose amino acid composition is similar (like diatom samples). However, when comparing the diatom data with those of the sediment samples, you might be in trouble, because in case of acetylation, isotopic fractionation seems to depend on the composition of amino acids, fatty acids, etc. How did you overcome this issue? IF you think that you correctly overcome this issue, I strongly ask the authors to clearly describe the pitfalls of the measurement of d13C of amino acids to make a caution to the followers in the future.

REPLY: If we understand the question correctly, the reviewer asks how molar composition may affect acetylation, i.e. derivatization, of the amino acids. Neither the molar composition nor the total concentration of amino acids will affect isotope fractionation during derivatization because the reagents are in excess. However, fractionation or incomplete derivatization can become an issue if the matrix contains an abundance of non-amino acid compounds such as humic substances, lipids, carbonates etc. This can certainly become an issue with complex matrices such as sediments. We provided an initial answer to how we cleaned our sample matrix in our reply to Reviewer #1, and we would like to follow up on this issue here.

All sediment samples were purified with Dowex 50WX8 cation exchange 100–200 mesh resin, but we neglected to mention it under Material and Methods. This information is now added in lines 178-180. Since the purification was very efficient, we were not concerned by a sample matrix dependent isotope fractionation. (see Fig. S1). To demonstrate this, we have inserted a GC trace of a sediment sample in the Supplement showing that co-elution of non-amino acid compounds is minimal. We were unsure whether loss of amino acids during the purification process would affect isotope values. For that reason, we tested potential isotope effects on freeze-dried yeast samples and a mixture of amino acid standards, respectively. We conclude (see 1.3 Stable isotope analyses) that for "both sample types, asparagine/aspartate (Asx) was significantly enriched by approximately 1.5‰ compared to the controls (see Fig. S2). For the remaining amino acids, we found no consistent isotope effects of purification indicating that d13C-AA values of purified and non-purified samples are comparable except for Asx". We have added these new contents in lines 182-192.

2) How archaeal degradation can be assessed? In the subsurface water, archaea rather than bacteria are dominant microbes that catalyze the degradation of organic matter in the water column and subsurface sediments.

REPLY: While we recognize that archaea are likely play an important role for degradation in subsurface waters and sediments, it is a topic beyond the scope of the current study. We have added a remark about archaea in lines 437-439. Here we would like to add that obtaining pure laboratory cultures of archaea are needed to obtain a d13C-AA fingerprinting training dataset for archaea. A second line of research could be controlled degradation experiments comparing d13C-AA fingerprints after bacterial and archaeal degradation. Ideally, both methods should be employed including a variety of taxa and conditions to make the d13C-AA fingerprinting a truly universal proxy for following degradation in the sediment (and the water column).

3) One of your conclusions is that ONLY 10-15% of sedimentary amino acids are contributed from microbes. For me, 10 to 15% is rather surprisingly small number. Does it mean 85-90% of organic matter produced in the surface water remain intact after many years?

REPLY: We estimated that the fraction of bacterial derived amino acids ranged between 10-30% in the sediment. Lomstein et al. (2006) found for sediments from Chilean coastal upwelling areas that a large fraction of their measured D-amino acids derived from diatom empty cell sacs and cell wall fragments, including peptidoglycan. The fact that these remains persisted in the sediment after cell death indicates that microbial degradation of diatoms was relatively modest after reaching the sea floor. We have added this information to the paragraph where we discuss the influence of water column processes (lines 508-512). We would also note that the only prior study compound-specific amino acids from a high/productivity marine anoxic sediment by Batista et al. (2014) also reached very similar conclusions based on δ^{15}N-AA patterns from shallow sediment cores. These δ^{15}N-AA patterns, as well as degradation parameters derived from them, indicated almost exclusively algal-derived amino acids.

4) Related to above comments. In the sediment, it has been known that the extractable form of amino acids substantially reduced (10%) (e.g., Keil, R.G., E. Tsamakis, and J.I. Hedges. 2000. Early diagenesis of particulate amino acids in marine systems. In: "Perspectives in Amino Acid and Protein Geochemistry" (G. A. Goodfriend, M. J. Collins, M. L. Fogel, S. A. Macko, and J. F. Wehmiller, eds.). Oxford University Press).

The degradation processes of amino acids in the water column seem to transform most of them to “amino acid complex” that cannot be extracted with the normal procedure. If this is the case, your conclusion was lead by the analysis of only a small portion of sedimentary amino acids. You need to point out this and should carefully discuss how the analytical result can be extended to the non-extractable amino acids.

REPLY:

The reviewer makes a good point here, and one that we agree should be addressed at least briefly in the manuscript. In general, while only one paper is specified, the reviewer here references a perennial observation in organic geochemistry: that the amount of major biochemicals *identifiable at the molecular level* decreases rapidly from surface production, to deep particles, and then into sediments (e.g., (Cowie and Hedges, 1994;Wakeham et al., 1997); and many others over the years). The implicit assumption the reviewer makes in the comment, however, is that all the *other* organic nitrogen preserved in sediments - but not measured by hydrolytic methods as being in the THAA pool- is in fact also composed of amino acids, but simply hydrolysis resistant ones.

However, while the observation of lower THAA yields is perennial, how to interpret low AA/ total organic N ratios as much less clear. Without going into a full literature review on this long standing issue, there are certainly hypotheses that support the idea that all other organic N is hydrolysis resistant amino acid (ie, various theories about protection by mineral surfaces, or “encapsulation” in hydrolysis resistant cell structures or organic associations.) However, there are other ideas about condensation and transformation reactions, which would suggest this material is simply not proteinaceous- at least not in its original form- and yet others that suggest other nitrogenous biochemicals (pyrol nitrogen, see for example the XANES study by Vairavamurthy and Wang (2002)) and amino sugars (which could equally account for amide functionality indicated by limited ^{15}N CPMAS- NMR data) simply become quantitatively more important in the refractory preserved nitrogen than (much more labile) proteinaceous material. Finally, the single study so far to directly compare stable isotope values of bulk sedimentary nitrogen vs. the hydrolyzable proteinaceous AA pool suggest that other, non-AA nitrogen species are almost certainly extremely important (Batista et al, 2014).

Taken together, we do agree that a caveate about this point is appropriate to include, and this would improve the context of the papers discussion. We have therefore now done this in our revision, with new text from line 490 to 496. However, as should be clear from the above, exactly what accounts for lower sedimentary AA recovery vs. total sedimentary organic nitrogen remains a complex and debated point. Solving this issue, or even addressing it in any significant detail, is clearly beyond the scope of the current study.

References

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ADDITIONAL CHANGES:

For the main text, no additional changes were made except for those mentioned above (note that additional citations were added as well).