

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

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Reviewer#1: >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  $\delta^{13}\text{C}$ -AA values between different sample matrices in the context of fractionation during derivitiazation.

Reply: The reviewer raises the important point of how a given sample matrix may

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affect derivatization and  $\delta^{13}\text{C}$ -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 to avoid the problem of co-elution between unknown compounds and protein amino acids during chromatographic separation. To address the question of how the sample purification affect  $\delta^{13}\text{C}$ -AA values, we will publish test results with yeast samples and amino acid standards in the Supplementary (for now, find the results attached below). The tests were done in 2011 but not published until now. We found that purification generally did not alter  $\delta^{13}\text{C}$ -AA values except for significantly enriching  $\delta^{13}\text{C}$ -Asx values by  $\sim 2\%$ . 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  $\delta^{13}\text{C}$ -AA values, but that these effects are relatively minor to be of practical relevance except for Asx.

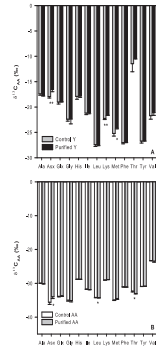
We would like to thank the reviewer for the comments.

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**Figure S1.** Effect of sample purification with Bio-Rad's 50W-X8 100–200 mesh resin on amino acid  $\delta^{13}\text{C}$  values. Below, results of sample purification are shown for yeast (Fig. S1A) and amino acid standards (Fig. S1B). For the yeast samples, we used 6 aliquots of 0.5 ml each from a 3 ml 6N HCl solution containing 15.0 mg freeze-dried yeast hydrolyzed at 110°C for 20 h. For the amino acid standard, we used 6 aliquots each containing 150  $\mu\text{l}$  0.1 N HCl solution. Before purification, the samples were dried and redissolved in 4 ml 0.05 M hydrochloric acid. The grey bars represent the control treatments and the black bars the purified samples (mean  $\pm$  SD of 3 samples; each sample was analyzed three times). Single asterisk (\*) represents significant of the 0.05 level and the double asterisk (\*\*) represent significant of the 0.01 level.



**Fig. 1.**

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