1 **Supplementary material 2**

2 In supplement to:

3 Loss of nitrogen via anaerobic ammonium oxidation (anammox) in the California current

4 system during the Quaternary

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6 **S2.1 Age model**

7 Sediment material for this study was obtained from ODP Site 1012 Hole C (32°16.970'N, 118°23.039'W) at

8 a water depth of 1784 mbsf (Lyle et al., 1997). Sampling resolution (ca. 2 kyrs between 0 and 180 kyr) was

9 based on the composite depths and age model reported in the initial report of Lyle et al. (1997). Prior studies

10 from Site 1012 are predominantly from Hole B with high resolution. The initial sampling request was done

11 based on the information available from Site 1012 Hole B, however, the material used in this study is

12 predominantly from Hole C and Hole A with the intention of having a sampling resolution of ca. 2 kyr for

13 the first 160 kyr. Once the initial bulk sediment $\delta^{15}N$ data was obtained and compared to the much higher

14 resolution dataset from Liu et al. (2005) we found the necessity to revise the composite depths and the age

15 model (Fig. S2.1). A revised age model for Site 1012 Hole C was finally generated for the last 160 kyr (see

16 table S1.1) using bulk sedimentary $\delta^{15}N$ data from which the age *vs* depth tie points were selected by fine-

17 tuning using Analyseries (last version in 2022) with higher resolution $\delta^{15}N$ data of Liu et al (2005). For

18 sediments >160 ka cal ka BP, with no $\delta^{15}N$ data, the composite depths are reported with estimated age (see

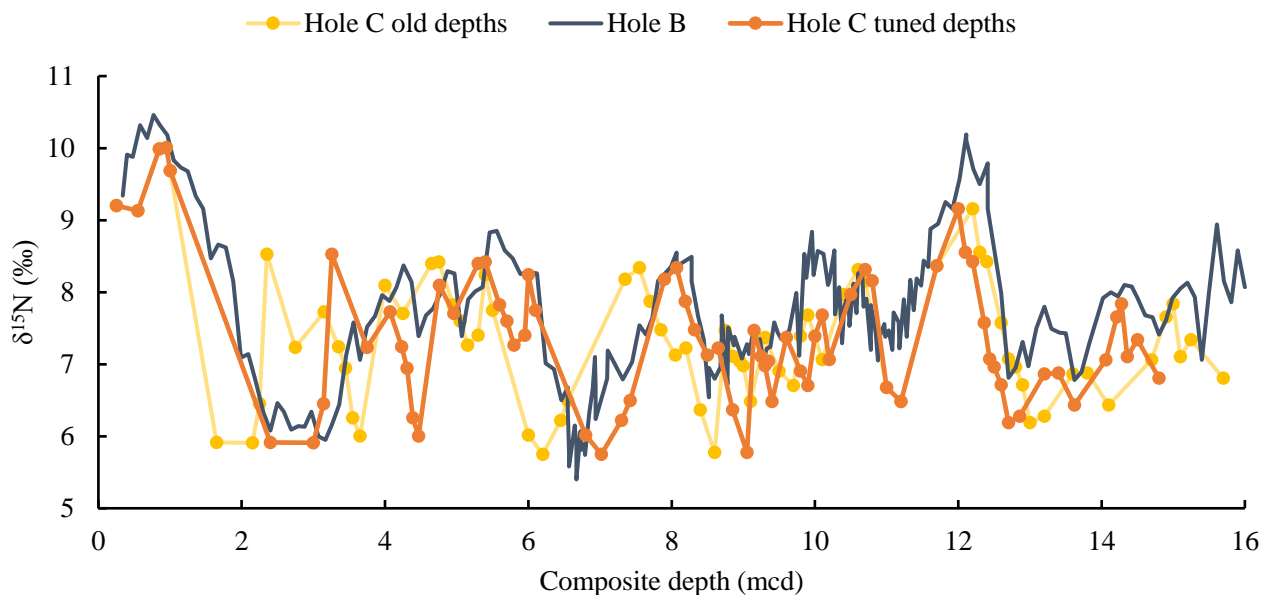19 table S1.1) according to the age model of Liu et al., (2005).

Fig. S2.1: Sedimentary $\delta^{15}N$ *vs* composite depth of Site 1012 Hole B and Hole C. We show here both the composite depth (in yellow) from the initial report (Lyle et al.,1997) and the revised composite depths (in orange) in comparison with the high-resolution data from Hole B (Liu et al., 2005).

S2.2 Comparison of ladderane analysis methods

Previously, ladderane FAME quantification has been conducted using calibration curves of in-house isolated $C_{20}[3]$- and [5]-ladderane standard (Hopmans et al., 2006). However, this quantification method does not correct for any variability in ion intensity, due to e.g., matrix effects and/or changes in the instruments functioning. Therefore, we further optimised this quantification method to include a response correction using an internal standard. A commercially available deuterated $C_{20}[5]$-PUFA (Reagecon Diagnostics Ltd.) was methylated using the same procedure as for the ladderane FAs (see section 3.4 of the manuscript), and added as an internal standard to the FAME fractions. Ladderane concentrations were calculated with and without the use of the internal standard (see section 3.5 of the manuscript for the equations).

Supplement 1, Table 8a provides the $C_{20}[3]$-ladderane, $C_{20}[5]$-ladderane and $C_{20}[5]$-PUFA calibration curves. The resulting relative response factor (RRF) between the C_{20}-ladderanes and $C_{20}[5]$-PUFA calibration curves was 1.3 for [3]-ladderanes and 1.2 for [5]-ladderanes, which were used to calculate their respective concentrations for the method using the internal standard. The two quantification methods showed a strong positive linear relationship ($R^2 = 0.88$ and 0.89 for [3]-(SC-)ladderanes and [5]-(SC-)

38 ladderanes, respectively) when plotted against each other. (Fig. S2.2) Absolute concentrations (supplement

39 1, Table 8b) with the use of the internal standard were higher than without the use of an internal standard

40 (factor 1.2 and 1.3 for [3]-(SC-)ladderanes [5]-(SC-)ladderanes, respectively). This shows that both

41 methods are compatible when comparing relative concentrations within a sample set. Since the method

42 using the internal standard corrects for any variability in ion intensity, such as reduced ionisation due to

43 sample matrix effects, these slightly higher concentrations are likely more accurate. In addition, by using the

44 RRF provided in this paper, use of the commercially available deuterated $C_{20}[5]$-PUFA standard removes

45 the necessity for a ladderane standard, of which the isolation is labour intensive. We therefore decided to

46 report the ladderane concentrations using our approach to quantification, which includes the use of the

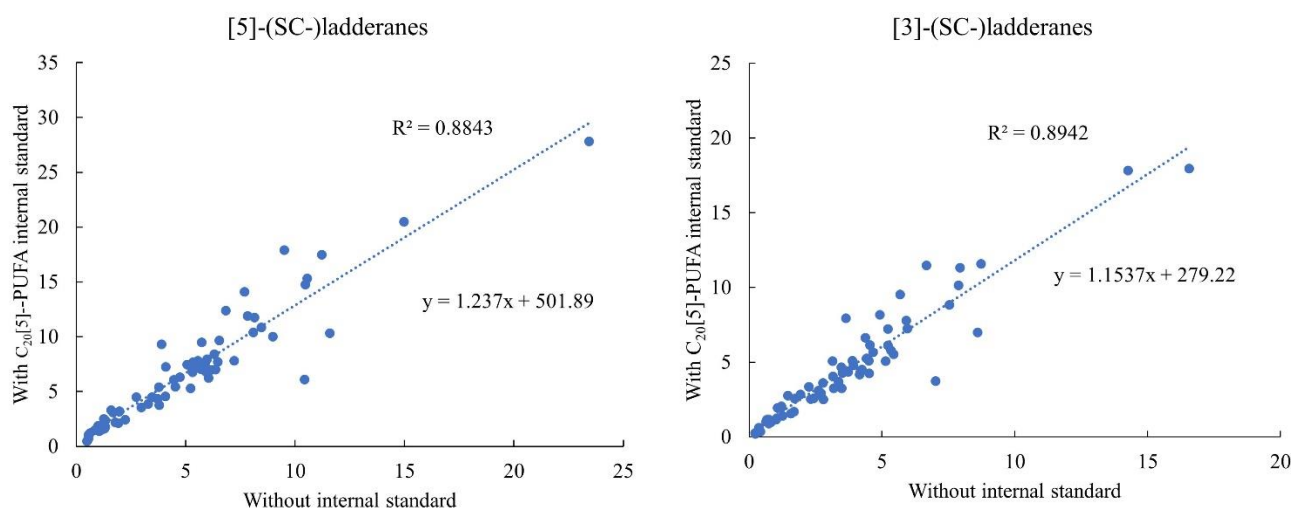47 internal deuterated $C_{20}[5]$-PUFA standard.

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49 **Fig. S2.2**: Relationship between calculated concentrations of ladderanes with a [5]-configuration (left) or a

50 [3]-configuration (right), using the two different methods. Concentrations with the use of the internal

51 deuterated $C_{20}:5$-PUFA standard are plotted on the y-axis (pg g^{-1} dry weight) and concentrations without the

52 use of the internal standard are plotted on the x-axis (pg g^{-1} dry weight).

53 **References**

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