

1 **Evidence for bias in C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of bulk organic matter, and on**
2 **environmental interpretation, from a lake sedimentary sequence by pre-analysis acid**
3 **treatment methods.**

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12

13 **Abstract**

14 There is a known bias in C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of organic matter (OM) due to pre-
15 analysis acid treatment methods. We report here, for the first time, the results of a pre-
16 analysis acid treatment method comparison of measured C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in bulk
17 OM from a sedimentary sequence of samples to illustrate this bias. Here we show that acid
18 treatment significantly reduces the accuracy (between method biases) and precision (within
19 method bias) of C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of OM, suggesting a differential response of
20 sample OM between methods and sample horizons, and in some cases inefficient removal of
21 inorganic carbon. We show that different methods can significantly influence environmental
22 interpretation in some of our sample horizons (i.e. interpretation of aquatic vs. terrestrial OM
23 source; C₃ vs. C₄ vegetation). Specifically, there are unpredictable and non-linear differences
24 between methods for C/N values in the range of ~ 1 – 100; $\delta^{13}\text{C}$ values in the range of 0.2 –
25 6.8 ‰ and; $\delta^{15}\text{N}$ values in the range of 0.3 – 0.7 ‰. Importantly, these ranges are mostly
26 much greater than the instrument precision (defined as the standard deviation of replicate
27 analysis of standard reference materials; for this study, ± 0.5 for C/N values, ± 0.1 ‰ for
28 $\delta^{13}\text{C}$ values and; ± 0.1 ‰ for $\delta^{15}\text{N}$). The accuracy and precision of measured C/N, $\delta^{13}\text{C}$ and
29 $\delta^{15}\text{N}$ values of bulk OM is not just dependent upon environmental variability, but on acid pre-
30 treatment, residual inorganic carbon and organic matter state and composition. Collectively,
31 this makes the correlation between samples prepared in different ways, including those from
32 down core reconstructions, highly questionable.

33

34 **Keywords:** C/N ratios, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, organic matter; pre-analysis acid treatment methods,
35 environmental interpretation, palaeoclimate.

36

37 1.1 Introduction

38 Bulk organic matter (OM) in lacustrine sediments is a heterogeneous composition of organic
39 materials derived from aquatic (e.g. phytoplankton; macrophytes) and terrestrial origins (e.g.
40 trees; shrubs; grasses; animals; see reviews in Meyers and Ishiwatari, 1993; Meyers, 1997;
41 Sharpe, 2007). A number of factors contribute to the structure and isotopic composition of
42 OM in lake sediment: the contribution of C and N from different source end-members; the
43 state and availability of C and N in the environment; carbon fixation pathways; lake
44 productivity; pre- and post-burial diagenetic processes (aerobic and anaerobic); dissolved
45 CO₂ concentration, *p*CO₂, light, temperature, changes in palaeoenvironmental controls on
46 OM C and N budget, and species composition (e.g. Stuiver, 1975; Meybeck, 1982; Hedges et
47 al., 1986; Ehleringer and Monson, 1993; Hayes, 1993; Meyers and Ishiwatari, 1993; Meyers,
48 1994, 1997; Ehleringer et al., 1997; Krishnamurthy et al., 1999; Turney, 1999; Lehmann et
49 al., 2003; Lucke et al., 2003; Perdue and Kopribnjak, 2007). *Prima facie*, these factors make
50 the evaluation of the palaeoenvironmental and palaeoclimatic influence on sedimentary OM
51 difficult. However, the investigation of lake sediment sequences with highly resolved age-
52 depth models and high signal-to-noise ratios can still provide high amplitude
53 palaeoenvironmental information (e.g. Lucke et al., 2003; Wei et al., 2010).

54
55 Despite the complexity of these processes on sediment OM, weight ratios of elemental carbon
56 to nitrogen (C/N), and stable isotope ratios of C and N ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) from bulk OM, have
57 been widely used to interpret OM provenance (e.g. aquatic versus terrestrial source) and
58 vegetation type (e.g. C₃ versus C₄ plants; Meyers and Ishiwatari, 1993; Thornton and
59 McManus, 1994; Meyers, 1997; Sampei and Matsumoto, 2001; Lamb et al., 2004, 2007;
60 Street-Perrott et al., 2004; Wilson et al., 2005; Zong et al., 2006; Mackie et al., 2007). These
61 proxies have subsequently underpinned palaeoenvironmental research and been used as a tool
62 for understanding biogeochemical processes in a range of sedimentary sequences (Talbot and
63 Johannessen, 1992; Street-Perrot et al., 1997; Holmes et al., 1997; Turney, 1999; Huang et al.,
64 2001; Fuhrmann et al., 2003; Lucke et al., 2003; Baker et al., 2005; Lamb et al., 2007; Galy
65 et al., 2008; Mampuku et al., 2008; Domingo et al., 2009; Langdon et al., 2010; Scholz et al.,
66 2010; Yu et al., 2010; Wei et al., 2010).

67
68 In general, C/N ratios of OM tend to range from 3 – 9 (dominated by aquatic biomass; protein
69 rich, lignin poor), 10 – 20 (admixture of aquatics, including emergent aquatics, and terrestrial
70 sources) and > 20 (dominated by terrestrial biomass; protein poor; lignin rich) (e.g. Meybeck,

71 1982; Hedges et al., 1986; Tyson, 1995; Meyers, 1997; Sharpe, 2007). The $\delta^{13}\text{C}$ of OM is
72 broadly used as an indicator for carbon sources, productivity and photosynthetic pathways in
73 plants. Values for land plants range from -6 to -35 ‰ (see overviews in Tyson, 1995;
74 Meyers, 1997; Sharpe, 2007), and can differentiate between C_3 plants ($\delta^{13}\text{C} \approx -22$ to -35 ‰)
75 and C_4 plants ($\delta^{13}\text{C} \approx -6$ to -15 ‰) in certain environments (e.g. estuaries, sea floors, lakes,
76 soils; e.g. Smith and Epstein, 1971; O'Leary, 1988; Tyson, 1995; Meyers, 2003; Street-Perrot
77 et al., 2004; Sharpe, 2007; Mampuku et al., 2008; Scholz et al., 2010). The $\delta^{13}\text{C}$ of OM has
78 also been used for a range of other investigations including (1) assessment of carbon reservoir
79 turnover times and soil C dynamics (Harris et al., 2001), (2) determination of trophic levels in
80 environmental systems (Bunn et al., 1995; Pinnegar and Poulnin, 1999; Kolasinski et al.,
81 2008), (3) primary productivity reconstructions and estimation of carbon burial rates and, (4)
82 to understand mineralisation processes (Midwood and Boutton, 1998; Freudenthal et al., 2001;
83 Leng and Marshall, 2004). $\delta^{15}\text{N}$ has been used to understand trophic pathways in food webs
84 (Bunn et al., 1995; Pinnegar and Polunin, 1999; Ng et al., 2007; Kolasinski et al., 2008);
85 animal dietary tracers (e.g. Koch et al., 2007; Lee-Thorpe, 2008); OM provenance and
86 degradation (Thornton and McManus, 1994; Meyers, 1997; Hu et al., 2006; Barros et al.,
87 2010); denitrification in the water column (Altabet et al., 1995; Ganeshram et al., 2000);
88 nitrate utilisation (Calvert et al., 1992; Teranes and Bernasconi, 2000); N_2 -fixation (Haug et
89 al., 1998) and; eutrophication (Owens, 1987; Vob et al., 2005). In addition, C/N values are
90 used to support $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, for example through bi-plots providing a structure within
91 which OM provenance and type can be broadly identified (e.g. Talbot and Johannessen, 1992;
92 Thornton and McManus, 1994; Meyers, 1997; Meyers and Teranes, 2001; Krull et al., 2002;
93 Lucke et al., 2003; Wilson et al., 2005; Lamb et al., 2006; Zong et al., 2006; Mackie et al.,
94 2007; Sharpe, 2007; Yu et al., 2010). Investigators have subsequently deduced changes in
95 environmental and/or climatic processes through interpretation of changing states of the
96 system under investigation (e.g. terrestrial vs. aquatic biomass; C_3 vs. C_4 vegetation).

97

98 However, interpretations of C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are predicated on the production of reliable
99 proxy data, and the ability to disentangle the complex processes leading to OM preservation
100 in the sedimentary record. This necessitates a complete understanding of the precision on the
101 measured data, which, for C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from bulk sediment OM, are not widely
102 discussed in the literature beyond instrument precision (reported as one standard deviation
103 (1σ) of replicate runs of elemental and isotopic reference materials). Further, the instrument
104 precision on C/N values is rarely (if at all) discussed.

105

106 The accurate determination of C/N and $\delta^{13}\text{C}$ composition of OM requires the complete
107 removal of any IC from the total carbon, commonly achieved through acid treatment. There is
108 a variety of pre-analysis acid treatment methods that have been used in the published
109 literature, from which it is clear there is no consensus on standard practice (see Brodie et al.,
110 2011a for an overview). Research has shown significant non-linear bias on measured C/N,
111 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values directly associated with these pre-analysis acid treatment methods,
112 which can undermine an environmental interpretation of the data (Froelich, 1980; Yamamuro
113 and Kayanne, 1995; Bunn et al., 1995; King et al., 1998; Lohse et al., 2000; Schubert and
114 Nielsen, 2000; Ryba and Burgess et al., 2002; Kennedy et al., 2005; Schmidt and Gleixner,
115 2005; Galy et al., 2007; Fernandes and Krull, 2008; Brodie et al., 2011a). For example,
116 Brodie et al. (2011a) noted a C/N value range of $\sim 6 - 13$, a $\delta^{13}\text{C}$ range of -27.0‰ to -28.4‰
117 and a $\delta^{15}\text{N}$ range of 0.8‰ to 1.8‰ for a terrestrial land plant (Broccoli) across pre-analysis
118 acid treatment methods. These C/N values suggest OM derived largely from aquatic sources,
119 or from an admixture of aquatic and terrestrial sources. More importantly, all of these offsets
120 were shown to be non-linear and unpredictable within and between pre-analysis acid
121 treatment methods (Brodie et al., 2011a; 2011b).

122

123 Differences in %C measured from modern and ancient, terrestrial and aquatic organic
124 materials, as a function of acid treatment, have been reported at $5 - 78\%$ and for %N at $0 -$
125 50% , either as a loss of C and N (Froelich, 1980; Yamamuro and Kayanne, 1995; Bunn et al.,
126 1995; King et al., 1998; Lohse et al., 2000; Schubert and Nielsen, 2000; Ryba and Burgess,
127 2002; Schmidt and Gleixner, 2005) or as an artificial gain in C and N (Brodie et al., 2011a).
128 Shifts in $\delta^{13}\text{C}$ are variable, ranging from enrichment in $\delta^{13}\text{C}$ of $0.2 - 8.0\text{‰}$ (e.g. Schubert and
129 Nielsen, 2000; Kolasinski et al., 2008; Brodie et al., 2011a), a depletion in $\delta^{13}\text{C}$ of $0.1 - 1.9\text{‰}$
130 (Kennedy et al., 2008; Komada et al., 2008; Brodie et al., 2011a) and no change (e.g.
131 Midwood and Boutton, 1998; Kennedy et al., 2005). This is similar for $\delta^{15}\text{N}$, where results
132 range from an enrichment of $0.1 - 3\text{‰}$ (Bunn et al., 1995; Brodie et al., 2011b), to a
133 depletion of $0.2 - 1.8\text{‰}$ (Bunn et al., 1995; Harris et al., 2001; Kennedy et al., 2005; Ng et al.,
134 2007; Fernandes and Krull, 2008), and no significant change (Serrano et al., 2008). The bias
135 on OM from acid treatment, alongside the complex processes that can influence OM prior to,
136 and during, sedimentary preservation, suggests that reliance on the commonly reported
137 instrument precision alone is unrealistic for robustly interpreting measured C/N, $\delta^{13}\text{C}$ and
138 $\delta^{15}\text{N}$ values.

139

140 1.1.1 Unresolved Issues

141 Despite the considerable potential for acid treatment method to alter the bulk OM signal prior
142 to C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis, the potential bias in a sedimentary sequence of samples has
143 hitherto never been investigated. In addition, the influence of inorganic carbon (IC) and
144 inorganic nitrogen (IN; Hoefs, 1973; Sharpe, 2007); sample homogenisation (Baisden et al.,
145 2002; Hilton et al., 2010) and sample size (Brodie et al., 2011a) can contribute additional
146 inaccuracy and imprecision to measured data. There is an increase in the application of “dual-
147 mode” isotope analysis (where C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are measured simultaneously from the
148 same pre-treated sample), implying an acidification of sample material prior to analysis. We
149 note it is not common to acidify samples prior to $\delta^{15}\text{N}$ analysis, but acidification is required
150 for dual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. It is clear, therefore, that the assumption that instrument
151 precision alone accounts for the absolute imprecision on measured C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values
152 is questionable. Moreover, assumptions on the accuracy of the measured C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
153 are also questionable.

154

155 The aim of this study was to compare, for the first time, the effect of pre-analysis acid
156 treatment methods on C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of OM from a sedimentary sequence. We
157 investigate an ancient lake cored sequence using the capsule and rinse methods alongside that
158 of untreated materials (after Brodie et al., 2011a; 2011b). We test the null hypothesis that
159 there is a significant difference between methods on the same sample horizon, implying that
160 data precision exceeds the commonly discussed instrument precision. Specifically, the
161 following research questions are addressed:

- 162 1. Are there significant differences between the results of pre-analysis acid treatment methods
163 for C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bulk OM on a stratigraphical sequence of samples? (i.e. above
164 instrument precision)
- 165 2. Can different pre-analysis acid treatment methods influence environmental interpretation
166 of C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bulk OM?

167

168 1.2 Materials and Methods

169 1.2.1 Core material

170 A sedimentary sequence was extracted from Lake Tianyang (20°31'1.11" N, 110°18'43.02"
171 E), south China, in January 2008, for multi-proxy palaeoenvironmental reconstruction,
172 including C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of OM. For this comparison, we sub-sampled 20 horizons in

173 the core from 7.00 – 10.24 m (16 cm resolution). This section of the core material was
174 selected due to the significant change in the lithology (Figure 1) from a brown clayey silt bed,
175 with few very fine sands and silts, and a low organic content (~0.3 – 1% OC; 10.24 m to 8.06
176 m), to an organic rich (amorphous) clay bed (~28 – 32% OC; 8.06 m to 7.09 m). In addition,
177 the $\delta^{13}\text{C}$ of bulk OM derived from some pilot samples showed that this section produced an
178 overall $\delta^{13}\text{C}$ range of ~ 15 ‰ and, in particular, there is a ~ 12 ‰ shift across the lithologic
179 boundary. Unfortunately, low levels of N precluded a full $\delta^{15}\text{N}$ record across all of our
180 selected sample horizons so we only report $\delta^{15}\text{N}$ values from 7.00 m to 7.46 m.

181

182 **Figure 1:** Lake Tianyang core lithology and description from 7.00 m to 10.20 m. The ^{14}C age
183 is reported in ^{14}C yrs BP (uncalibrated).

184

185 1.2.2 Cleaning Protocol

186 Prior to sample treatment, all sub-sampling equipment and glassware were thoroughly
187 washed in 1% nitric acid, rinsed in deionised water, followed by a wash in 2% soap solution
188 (neutracon®), a final deionised water rinse and then fired at 550°C for 3 hours. Ag capsules
189 were fired at 550°C for 3 hours prior to use and Sn capsules were submerged in methanol for
190 24 hours and then air dried. Cleaned capsules were then sealed in pre-cleaned containers and
191 stored until use.

192

193 1.2.3 Acidification methods

194 1.2.3.1 Rinse method

195 We compared the capsule and rinse methods using 5% w/w and 20% w/w HCl as the
196 acidifying reagents based on Brodie et al (2011a) who showed HCl tended to produce the
197 most coherent and reliable data. For the rinse method, approximately 250 mg of sample was
198 acidified in 50 ml of the chosen acid reagent for 24 h. Depending on the IC content additional
199 acid was added to maintain an acidic solution (checked with litmus paper) and left for a
200 further 24 h if necessary. After digestion, the sample material was sequentially rinsed 3 times
201 with deionised water, allowing 24 h between rinses to allow the sample to settle, using an
202 overall minimum of 1200 mls of deionised water. After the final decanting, the excess water
203 (50 – 100 mls) was allowed to evaporate off in a drying oven at ~50°C. Once dry, the sample
204 was loosened from the base of the beaker with a clean plastic spatula and transferred to an
205 agate pestle and mortar, ground, and a known quantity weighed into a Sn capsule (to provide

206 ~500 µg C after acid treatment). Capsules were then crimped ready for elemental and $\delta^{13}\text{C}$
207 analysis.

208

209 **1.2.3.2 Capsule Method**

210 Sample material was weighed into open Ag capsules and recorded (to provide ~500 µg C
211 after acid treatment). The capsules were then transferred on a metal tray to a cold hotplate
212 and 10 µl of distilled water was added to moisten the samples, reducing the potential of an
213 initial vigorous reaction from IC bearing materials. After moistening, 10 µl of the chosen acid
214 reagent was added to the cold sample before the hotplate temperature was slowly increased to
215 ~50°C. Additional acid was then added in steps of 10 µl, 20 µl, 30 µl, 50 µl and 100 µl
216 without allowing the sample to dry out between additions. The samples were monitored for
217 IC reaction by visual inspection but as the effervescence reduced, the reaction was checked
218 using a binocular microscope at 50x magnification. The stepped addition of acid described
219 here reduced problems associated with the ambiguous effervescence end-point, however, we
220 also added a final 200 µl of acid to act as a “fail safe”. After the addition of the final aliquot
221 of acid the capsules were left on the hotplate for c.1 hour to dry thoroughly. Once dry, the
222 capsules were removed from the hotplate, left to cool before being crimped. All capsule
223 method samples (traditionally analysed in Ag capsules only) were further wrapped in Sn
224 capsules to ensure complete combustion (see Brodie et al., 2011b).

225

226 **1.2.3.3 Untreated**

227 Sample $\delta^{15}\text{N}$ is traditionally measured on untreated sample material (e.g. Muller, 1977;
228 Altabet et al., 1995; Schubert and Calvert, 2001; Sampaei and Matsumoto, 2008), assuming a
229 negligible influence from inorganic nitrogen (e.g. nitrates, ammonia; e.g. Sampei and
230 Matsumoto, 2008). Therefore, in addition to the rinse and capsule methods we also prepared
231 untreated sample materials for C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis, which involved directly weighing
232 an untreated sample aliquot (500 µg for C/N and $\delta^{13}\text{C}$ and 15 µg for $\delta^{15}\text{N}$) into a prepared Sn
233 capsule, crimping and analysing.

234

235 **1.2.4 Analytical Methods**

236 The %C, %N and $\delta^{13}\text{C}$ values of sample OM were analysed using an online system
237 comprising a Costech ECS4010 elemental analyser (EA), a VG TripleTrap, and a VG Optima
238 mass spectrometer at the NERC Isotope Geosciences Laboratory (NIGL), with data reduction

239 carried out using DataApex Clarity ver 2.6.1 software package. Each analytical run contained
240 three control materials: external standard SOILB (2 replicates), internal NIGL standard
241 BROC (10 replicates) and independent external standard SOILC (2 replicates). All standards
242 returned values that were statistically indistinguishable from known sample values (p -value $>$
243 0.05) indicating the instrument measurements were accurate (in comparison to the long term
244 values) and precise (within reported σ of known values), where $C/N \leq 0.2$ and $\delta^{13}C \leq$
245 0.2 ‰. From knowledge of the laboratory standard's $\delta^{13}C$ value versus V-PDB (derived from
246 regular comparison with international calibration and reference materials NBS-18 and NBS-
247 19 and cross checked with NBS-22). $^{13}C/^{12}C$ ratios of the unknown samples were converted
248 to δ values versus V-PDB as follows: $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$ (‰), where R = the
249 measured ratio of the sample and standard respectively (for carbon and nitrogen).

250

251 Nitrogen isotope analyses were performed using a FlashEA 1112 elemental analyser linked to
252 a Delta+XL isotope ratio mass spectrometer (EA-IRMS) via a Conflo III interface. Samples
253 were combusted at $900^{\circ}C$ with all samples acidified in the capsule method further wrapped in
254 Sn capsules. Limits on analytical precision are mainly determined by conditions of
255 combustion and chromatography in the elemental analyser. Within-run precision for $\delta^{15}N$ is \leq
256 0.13 ‰ (1σ for $n = 13$ samples).

257

258 Measurements of background C and N concentrations from capsules and acid reagents were
259 below instrument detection limits suggesting contamination did not contribute to variability
260 within our results (e.g. see Brodie et al., 2011a, 2011b for further details on acid methods and
261 analytical methods).

262

263 1.2.5 Data Analysis

264 We compare our data using a one-way ANOVA, at the 95% confidence limit, to determine
265 differences within (i.e. acid reagent) and between (i.e. untreated versus capsule method
266 versus rinse method) the pre-analysis acid treatment methods, and take a p -value < 0.05 to
267 indicate a significant difference. All data were tested for normality using an Anderson-
268 Darling normality test, and tested for homogeneity of variances using a Bartlett's test (which
269 assumes data are normally distributed) and a Levene's test (which assumes data are non-
270 normally distributed). ANOVA comparisons for C/N and $\delta^{13}C$ were carried out on data
271 derived from acid treated samples but are not compared with untreated samples (untreated

272 measurements for C/N and $\delta^{13}\text{C}$ is not a common approach due to the potential for inorganic
273 carbon contamination, hence the necessity for acid pre-treatment). For $\delta^{15}\text{N}$, comparisons
274 were made on data derived from acid treated samples *and* untreated samples as there is no
275 consensus on the most appropriate method for N analysis (see Brodie et al, 2011b for an
276 overview).

277

278 1.3 Results

279 The %C, %N, C/N and $\delta^{13}\text{C}$ data are presented in Figure 2 and ANOVA comparisons for C/N
280 and $\delta^{13}\text{C}$ data from each pre-treatment method and reagent investigated are presented in
281 Table 1.

282

283 1.3.1 %C and %N

284 From 7.00 to 7.52 m, the core material is characterised by high %C and %N values relative to
285 the sample horizons below 7.52 m, where %C and %N are very low. For %C and %N, the
286 rinse method samples above 7.52 m are consistently ~ 20% higher than capsule and untreated
287 method samples. With the exception of the 5% HCl rinse method samples at 8.76 m for %C
288 (probably a residual inorganic C signal), and 20% HCl capsule method sample from 7.80 –
289 8.60 m for %N, the data below 7.52 m are relatively coherent.

290

291 1.3.2 C/N and $\delta^{13}\text{C}$

292 C/N values between methods are highly variable within specific sample horizons, especially
293 within the capsule method samples (e.g. 7.48 m, 8.76 m, 9.24 m for 5 % HCl capsule method;
294 8.12 m, 8.60 m for 20 % HCl capsule), and between the capsule method samples and rinse
295 method samples. An overall range of ~1 – 100 was evident between methods on some sample
296 horizons. For example, at 8.76 m the capsule method samples returned C/N values of 81 to
297 122, the rinse method samples returned C/N values of ~ 34, and untreated values were ~174.
298 At 9.08 m, samples in the capsule method return C/N values of ~19, and rinse method
299 samples ~ 11. In general, data from the rinse method appear more coherent than data from the
300 capsule method.

301

302 $\delta^{13}\text{C}$ data from samples between 7.00 m and 7.64 m across all methods tested are
303 indistinguishable from one another (i.e. appear to be within instrument precision limits).

304 Between 9.00 m and 9.24 m, all measured values (i.e. within and between all acid treatment

305 methods) converge over a significant shift in the data, but are divergent above 9.00 m and
306 below 9.24 m. However, our data also show sample horizons with incoherency between the
307 methods (i.e. greater than instrument precision by a minimum of ~0.2 ‰), in particular from
308 7.64 m to 8.12 m and 8.60 m to 9.00 m. The greatest divergence in the data are from 7.64 m
309 to 8.12 m and between the 20% HCl capsule method and 20 % HCl rinse method (~ 2.5 ‰
310 (7.64 m)), and from 8.60m to 9.00m which is caused by the 5 % HCl capsule method (~6 – 7 ‰
311 (8.60 m)). Between the remaining three methods at 8.60 m, the difference range is between
312 0.4 – 3.5 ‰. We note that the divergence in $\delta^{13}\text{C}$ data, within and between methods, appear
313 to become more evident in samples with relatively lower OC, but not in all instances (e.g.
314 9.00 m to 9.24 m). Given the evident differences between methods on any one sample
315 horizon, a general trend in C/N and $\delta^{13}\text{C}$ values between methods remains apparent, though
316 the amplitude of the signal is variable.

317

318 **Figure 2:** Down-core plots of %C, %N, C/N and $\delta^{13}\text{C}$ for data derived from the capsule
319 method, rinse method and untreated samples. Instrument precision is not visible on these
320 scales, but is 0.3% for %C, 0.3% for %N, 0.5 for C/N and 0.2 ‰ for $\delta^{13}\text{C}$. The embedded
321 legend indicates the data for each method.

322

323 **Table 1:** ANOVA comparison results for C/N and $\delta^{13}\text{C}$ for acid treated sample horizons only.
324 P-values and r-squ values are based on comparisons of all measurements from a specific
325 sample horizon after acid treatment, with a p-value < 0.05 deemed to represent a statistically
326 significant difference. “nd” indicates no significant difference in measured values within and
327 between acid treatment methods.

328

329 1.3.3 $\delta^{15}\text{N}$

330 $\delta^{15}\text{N}$ values were only measureable between 7.09 m and 7.46 m due to extremely low %N.
331 The $\delta^{15}\text{N}$ data and ANOVA comparisons for each method and reagent are shown in Figure 3
332 and Table 2 respectively. Our results show that all acid treated samples produced lower $\delta^{15}\text{N}$
333 in comparison to untreated samples, with the largest range in values between methods of ~
334 0.8 ‰ (at 7.16 m). In general, capsule method samples produced lower values than rinse
335 method samples, with the exception of 5 % HCl capsule samples at 7.09 m and 7.16 m
336 (Figure 3). Overall, the rinse method samples produced more coherent results than the
337 capsule method (< 0.2 ‰ overall range for all rinse method samples). ANOVA results

338 indicate statistically significant differences within the capsule method and between the
339 capsule and rinse method data. In addition, all data derived from acid treated samples
340 produced lower $\delta^{15}\text{N}$ in comparison to untreated values for all sample horizons, but
341 highlighted no differences within the rinse method (i.e. no difference between samples
342 acidified in 5% HCl or 20% HCl within the rinse method). We note a fractionation in $\delta^{15}\text{N}$ in
343 all sample horizons, within and between methods, but no concomitant change in mass %N.

344

345 **Figure 3:** Down-core plots of $\delta^{15}\text{N}$ for data derived from the capsule method, rinse method
346 and untreated samples. The scale bar in the plot represents instrument precision ($1\sigma =$
347 0.13‰), and the embedded legend indicates the data for each method.

348

349 **Table 2:** ANOVA comparison results for $\delta^{15}\text{N}$ for all tested sample horizons. P-values and r-
350 squ values are based on comparisons of all measurements from a specific sample horizon
351 after acid treatment, and also in comparison to untreated samples.

352

353 1.4 Discussion

354 1.4.1 Method differences

355 The pre-analysis acid treatment approach is underpinned by the assumption that the OM
356 fraction is either unaltered during the process, or that any changes are at least systematic and
357 proportional (i.e. predictable), and that all IC present is completely removed. This clearly
358 suggests that, within instrument precision, results from any method followed should be
359 indistinguishable from one another. Our results from the Lake Tianyang sedimentary
360 sequence indicate an inconsistency in the application of any single pre-analysis acid treatment
361 method in a down-core context for C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. There is evidence for significant
362 differences in measured C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within and between pre-analysis acid
363 treatment methods (Table 1 and 2). Differences between each acid treatment method in C/N,
364 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within and between sample horizons are highly variable, and not always
365 in the same direction. For some sample horizons, differences between acid treatment methods
366 for C/N values can be as high as ~ 90 (e.g. at 8.76 m) and as low as 0.2 (e.g. at 7.32 m; 8.44
367 m). Likewise, differences in $\delta^{13}\text{C}$ between acid treatment methods can be as high as 6.8 ‰
368 (e.g. 8.76 m) but for other horizons be within instrument precision (e.g. $< 0.2\text{‰}$; 7.09 m –
369 7.48 m). This may, in part, be a function of the overall %C and %N of the sample material,
370 including organic and inorganic components. For example, our $\delta^{13}\text{C}$ data are generally in

371 good agreement with high %C. However, the imprecision on the data tends to increase within
372 and between methods as %C in the sample material becomes lower (e.g. 7.64 m to 9.00 m),
373 but this is not always the case (e.g. 9.00 m to 9.24 m). This suggests sample materials with
374 low %C may be more susceptible to acid method bias (and of greater magnitude), but this is
375 not a general rule (Brodie et al., 2011a, 2011b).

376

377 Where there is a high range of C/N values apparent between treatment methods, this can
378 fundamentally alter the support for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from cross-plots of these data. For example,
379 at 7.80 m, the 5% HCl capsule method points towards an environment dominated by
380 terrestrially sourced OM (C/N value ~ 32), whereas C/N values from all other methods
381 suggest an environment with a significant aquatic biomass contribution to total OM (C/N
382 value ~ 12). This contradictory position clearly indicates a serious discrepancy regarding the
383 interpretation of elemental and isotopic C and N proxies derived from bulk OM, both from
384 these cross-plots and in a down-core context. It also suggests that the assumptions
385 underpinning pre-analysis methods are invalid (Brodie et al., 2011a, 2011b). In addition,
386 there are known biases from IN contamination, which can lower C/N values below the true
387 organic C/N value (e.g. Muller, 1977; Schubert and Calvert, 2001; Sampei and Matsumoto,
388 2001; Meyers, 2003; Mampuku et al., 2008). For example, Muller (1977) reported C/N
389 values <4 from deep sea sediments as a consequence of inorganic ammonia. Furthermore, the
390 range of C/N values, as discussed in the context of marine versus terrestrial OM provenance,
391 is also more complex than the standard interpretation suggests; for example, C/N values
392 (weight ratio) of submerged aquatic macrophytes have documented ranges of 6 – 60 (e.g.
393 Atkinson and Smith, 1983) and macroalgae ranges from 16 – 68 (brown macroalgae; Fenchel
394 and Jørgensen, 1977). Brodie et al (2011a) also report a C/N range of $\sim 6 – 13$ for broccoli (a
395 terrestrial plant) which has a “typical” C_3 $\delta^{13}\text{C}$ value of -27.4 ‰.

396

397 In addition, we also find that %C and %N are artificially concentrated (but not proportionally)
398 in samples from 7.00 m to 7.52 m analysed from the rinse method relative to untreated values
399 and capsule method samples. Brodie et al (2011a) suggested that this was likely a function of
400 the loss of fine grained inorganic material (e.g. clays) in the supernatant relative to the
401 amount of sample material treated with respect to that in other methods, despite the potential
402 losses of C and N through solubilisation (e.g. Schubert and Nielsen, 2000; Galy et al., 2007)
403 and absorption onto fine grained particles. We note that there is no concomitant shift in $\delta^{13}\text{C}$

404 values, though C/N values are disproportionally increased. Within the 20% HCl rinse method,
405 and for %N only (from 8.12 m to 8.60 m), the %N values are substantially higher (Figure 3).
406 Given the very low amounts of N within sample material, and the biasing effect of the acid
407 treatment, the results are likely to be unreliable as %N is very close to instrument baseline
408 conditions. Collectively, these factors point to a serious problem in the general theory on OM
409 provenance as interpreted through C/N.

410

411 **1.4.2 Residual inorganic carbon**

412 In addition to the problems highlighted for C/N values, at 8.76 m, the 5 % HCl capsule
413 method returned a $\delta^{13}\text{C}$ value of -12.5‰ , $\sim 6.8\text{‰}$ more enriched than data from all other
414 acidification methods (the overall $\delta^{13}\text{C}$ range between treatment methods is -21.3 to $-$
415 12.5‰). Our measurements on untreated material from this sample horizon suggests the
416 presence of a major IC component ($\sim 1.8\%$ inorganic carbon by weight with $\delta^{13}\text{C} = -1.6\text{‰}$),
417 suggesting the 5% HCl capsule method is less efficient at IC removal in comparison to other
418 methods for this sample, although it is widely assumed 5% HCl should efficiently remove
419 calcite. Between all other methods at 8.76 m, the difference in $\delta^{13}\text{C}$ value was $\sim 2 - 3\text{‰}$.
420 However, in the context of the overall data trend (e.g. the $\sim 12\text{‰}$ shift from 7.00 m to 7.52 m;
421 see Figure 2), the value returned for the 5 % HCl capsule method would not look out of place
422 had this been the only method followed. This, subsequently, could have led to a
423 misinterpretation of the core data in the context of sample OM: the 5 % HCl capsule data at
424 8.76 m suggest an environment dominated by C_4 type vegetation (both high C/N and $\delta^{13}\text{C}$
425 values) and could be interpreted as being representative of a more arid environment, whereas
426 the 3 other acid treatment methods tested produced more consistent results (though still
427 potentially imprecise), suggesting a C_3 dominated environment, which could be interpreted
428 as being representative of a more humid environment. We therefore do not recommend the
429 use of 5 % HCl in the capsule method. These differences between the acid reagents and
430 methods investigated here have three possible explanations: (i) the different effect of acid
431 treatment on the IC component(s) within the sample material and (ii) non-linear and
432 unpredictable offset on the OC component(s) within the sample material, or (iii) a
433 combination of both. At 8.76 m, the offset in $\delta^{13}\text{C}$ value in the 5% HCl capsule method is
434 caused by inefficient removal of IC (see above), an offset not recorded in the other methods.
435 This suggests that different methods and reagents (even at 5% HCl) have differential rates of
436 removal of what is probably calcite (i.e. 5% HCl appeared to remove the IC in the rinse

437 method, likely due to the increased time of exposure of the sample to the acid in this method
438 relative to the capsule method). Therefore, this problem is likely to be exacerbated where less
439 soluble forms of IC exist in sample materials, such as dolomites and siderites, which can
440 produce as large an offset to the $\delta^{13}\text{C}$ value as calcite. Moreover, an admixture of different
441 IC components can further complicate the digestion process due to different rates of removal
442 (i.e. stoichiometry of each IC component and combined stoichiometry, relative to dissolution
443 reagent) and IC component grain size (Al-Aasm et al., 1990; Yui and Gong, 2003).

444

445 Where there is an IC contamination on $\delta^{13}\text{C}$ values, enrichment is usually expected in the
446 $\delta^{13}\text{C}$ value due to the assumed relatively high $\delta^{13}\text{C}$ values of IC material; however, some
447 freshwater, marine, authigenic, diagenetic and detrital carbonates can have very negative $\delta^{13}\text{C}$
448 values (Hoefs, 1973; Hangari et al., 1980; Mozley and Carothers, 1992; Mozley and Burns,
449 1993; Chow et al., 2000; Coniglio et al., 2000; El-ghali et al., 2006; Sharpe, 2007; Pierre et
450 al., 2009). The $\delta^{13}\text{C}$ values of different forms of IC have been reported in the range of +30 ‰
451 to -51 ‰, a range which completely overlaps with the commonly cited $\delta^{13}\text{C}$ ranges for OM.
452 For example, Pierre et al (2009) reported values as low as -51 ‰ for calcite/aragonite and -
453 38 ‰ for dolomite measured in marine authigenic carbonate, Chow et al (2000) reported a
454 range of -22 ‰ to +8 ‰ for early diagenetic Mn-Fe carbonates, Hangari et al (1980) reported
455 $\delta^{13}\text{C}$ values of between -12 ‰ to -30 ‰ for freshwater siderite, and Mosley and Burns
456 (1993) provide an overview of $\delta^{13}\text{C}$ values of marine calcite, dolomite and siderite minerals
457 illustrating the common nature of very depleted $\delta^{13}\text{C}$ values (≤ -15 ‰).

458

459 If we take a hypothetical sample material, containing 3% OC with a $\delta^{13}\text{C}$ value of -14 ‰,
460 and 1% IC with a $\delta^{13}\text{C}$ value of -30 ‰, then, by mass balance, the overall sample $\delta^{13}\text{C}$ value
461 would be -18 ‰ (i.e. a 4 ‰ depletion in $\delta^{13}\text{C}$ due to IC contamination, not an enrichment
462 that is commonly assumed), tending an interpretation towards C_3 vegetation (e.g. more
463 humid environment). Given the potential for more robust forms of IC to have very low $\delta^{13}\text{C}$
464 values, such as dolomite and siderite which are not readily digested by acid, then the potential
465 for the depletion of measured $\delta^{13}\text{C}$ values as a result of residual IC is real, but largely
466 unrecognised!

467

468 Our data illustrate a depletion of the $\delta^{13}\text{C}$ value, e.g. 7.64 m - 7.80 m, which suggests that the
469 data may not only be affected by the inefficient (and disproportional) removal of IC from the
470 sample (assuming an enrichment in $\delta^{13}\text{C}$ within this core from residual IC), but also by the

471 effect of acidification on the OC component (assuming the untreated values at these depths
472 are not representative of IC contamination). We therefore suggest that the sample IC
473 component should be identified and quantified to ensure no residual IC remains after
474 treatment, or, where the IC exists as a more robust form (e.g. dolomite, siderite), the size of
475 the offset can at least be partly accounted for and an investigation into the bias associated
476 with the OC component can be undertaken. Sample OC must be understood in the context of
477 IC within the same sample and alongside acid treatment biases: data presented without this
478 explicit quantitative understanding are potentially unreliable. In addition, differences in
479 interpretation of $\delta^{13}\text{C}$ from OM as an indicator for changes in C_3 and C_4 vegetation are also
480 questionable, where the $\delta^{13}\text{C}$ of C_3 plant tissue has been reported in the range of -13 to -29 ‰
481 (e.g. Hedges et al, 1986), and C_4 plants in the range of -7 to -23 ‰ (e.g. Schilowski, 1987).
482 This is counterintuitive relative to the widely used C_3 v C_4 interpretation, suggesting an
483 additional environmental consideration, *inter alia*, in interpretation.

484

485 The structure and composition of C and N in OM from a down-core sedimentary sequence
486 can vary substantially (e.g. relative proportions of lipids, lignins, proteins, amino acids, and
487 cellulose; Fernandes and Krull, 2008), and may subsequently respond disproportionately
488 under different acid treatment methods (i.e. differences in proportions of refractory and labile
489 organic components). This suggests that C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are likely to be a relative
490 proxy for the overall chemistry of the core material, but the degree with which it reflects the
491 true OM value of the core, and thus a specific process, after acid treatment is highly variable
492 and makes interpretation more difficult. In addition, where sample material is low in %C
493 and %N, the effect of acidification on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ could be significantly magnified (e.g.
494 Brodie et al., 2011a, 2011b) which may be due to C and N isotopes becoming highly
495 heterogeneous within the OM at these low levels. These factors add unpredictable, non-linear
496 biasing to the dataset within sample horizons and with varying magnitude and proportions
497 between sample horizons (i.e. suggesting the underlying trend of the data can be biased in a
498 non-systematic fashion).

499

500 For $\delta^{15}\text{N}$, we note a fractionation between untreated and acid treated samples (~ 0.8 ‰), and
501 between acid treated samples but with no concomitant loss in %N (no difference in %N
502 values between treated and untreated data). The mechanisms for this are unclear; however,
503 there seems to be a systematic shift across all acid treated samples towards lower $\delta^{15}\text{N}$ with

504 samples in the rinse method tending to produce the lowest $\delta^{15}\text{N}$. This shift towards more
505 depleted $\delta^{15}\text{N}$ values may reduce the certainty on interpretations of water column
506 denitrification, for example, and biases of the order of $\sim 0.8\text{‰}$, or $\sim 1.7\text{‰}$ (Brodie et al.,
507 2011b), can account for between ~ 15 and 40% of the variability in some records with an
508 overall range of $\sim 5\text{‰}$ (e.g. Altabet et al., 1995). Additionally, the isotopic signature of IN is
509 not significantly dissimilar to that of organic N, making the overall interpretation of the $\delta^{15}\text{N}$
510 of OM in the presence of IN difficult (e.g. Knies et al., 2007). This illustrates the importance
511 of fully understanding OM structure and composition, and the IC and IN components, within
512 the system under investigation where a bulk organic matter approach is adopted.

513

514 These findings have significant implications for the comparison of records that are (i) derived
515 in different laboratories following differing pre-treatment methods (or variations of the same
516 method), and (ii) derived from different environments where the amounts and relative
517 proportions of C and N in sample OM varies, and the amount, type and nature of OM, IC and
518 IN varies. The assumption that data are reliable (and the subsequent interpretation robust)
519 because of our ability to produce extremely high instrument accuracy and precision is a *non*
520 *sequitur*. Our data suggest the necessity to account for the acid treatment bias in full and
521 determine the size of the offset to ensure that the interpretation is more robust and
522 acknowledge the full range of “error” in the analysis (see section 1.6 for more detail). We
523 suggest that the biasing of the true OM signature during pre-analysis acid treatment is
524 inevitable, but unpredictable. The environmental interpretation of elemental and isotopic
525 values of OM is not necessarily dependent upon an environmental shift, but can be
526 significantly affected by both IC and IN, pre-analysis acid treatment method and the structure
527 and composition of OM across the land-sea gradient. It is imperative that the effect of pre-
528 analysis acid treatment methods on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values be pursued at the molecular level to
529 improve our understanding of the mechanisms controlling the bias evident in our data (and
530 most likely in other down-core records).

531

532 **1.5 Implications for interpretation of C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of bulk OM**

533

534 Our findings have significant implications for the interpretation of measured C/N, $\delta^{13}\text{C}$ and
535 $\delta^{15}\text{N}$ values of bulk OM in the context of the established theory in the literature (e.g. OM
536 provenance and vegetation type), the estimation of organic and inorganic carbon burial and/or
537 accumulation rates (e.g. Twichell et al., 2002) and interpretation of carbon bi-plots (e.g.

538 Thornton and McManus. 1994; Meyers, 1997; Meyers, 2003; Lamb et al. 2006; Zong et al.
539 2006; Mackie et al, 2007; Yu et al., 2010). We show that the interpretation of C/N and $\delta^{13}\text{C}$
540 data is not just dependant on an environmental shift, but can also be dependent on the bias
541 due to pre-analysis acid treatment method. This is likely to be underpinned by the
542 complexities in the structure and composition of OM within and between environments.
543 Specifically, it suggests that small changes in the down-core records (i.e. < 4 ‰) may provide
544 less reliable interpretations in comparison to much larger shifts (i.e. of the order of 10 ‰, or
545 greater). Interpretations of C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values have been underpinned by the
546 assumption that we can reliably determine C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of sample OM. We have
547 shown that this assumption is highly problematic, and that a detailed discussion and
548 investigation on the potential source of bias, above that of the standard instrument precision,
549 is essential for a robust interpretation of the data. It is clear that additional bias on C/N, $\delta^{13}\text{C}$
550 and $\delta^{15}\text{N}$ measurements in OM can derive from inorganic carbon (IC) and inorganic nitrogen
551 (IN) content, pre-analysis acid treatment method followed and OM composition of the
552 sample material.

553

554 However, our data also show sample horizons with no difference in results within and
555 between methods, highlighting the inconsistency in any one method down-core. This
556 suggests that the accuracy and precision with which C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from any one
557 acid treatment method reflects sample OM is highly variable and unpredictable. Therefore,
558 instrument precision should be interpreted as an absolute minimum precision on measured
559 data (e.g. Brodie et al., 2011a, 2011b). The fact that pre-analysis treatment method can
560 significantly influence the environmental interpretation of sedimentary OM is worrying, and
561 cautions against the over interpretation of the minutiae of the data acquired. For example, we
562 report differences within and between methods on our down-core record in the region of 2 –
563 3.5 ‰ for $\delta^{13}\text{C}$ (excluding the excursion at 8.76 m which has a substantial IC contamination
564 signal). A precision range of this magnitude can account for the overall range of some down-
565 core studies (e.g. Turney et al., 1999; Zong et al., 2006; Mackie et al., 2007; Bertrand et al.,
566 2010; Scholz et al., 2010; Yu et al., 2010). It is therefore critical the extent of bias due to acid
567 treatment on elemental and isotopic measurements in OM is understood to ensure that any
568 interpretation is grounded on a robust dataset reflecting sample OM, especially where
569 inferences on climate variability and mechanisms are being proposed. In addition, these

570 findings suggest that the correlation of C/N and $\delta^{13}\text{C}$ values of bulk OM derived from
571 different sedimentary archives is highly problematic.

572

573 Given the current drive in the community to derive annual – centennial resolution from
574 down-core records of past environmental change, and in the context of increasing use of data
575 transformation techniques, such as spectral and wavelet transforms used to understand
576 periodicities (e.g. Baker et al., 2005), it is imperative that the inaccuracy and imprecision of
577 the data is fully understood and the subsequent limitations to interpretation acknowledged.
578 For example, differences within and between methods of the order of $\sim 2 - 3.5 \text{ ‰}$ would
579 significantly alter the amplitude and potentially change the frequency of a down-core record,
580 which may be misinterpreted as being environmentally significant (i.e. the amplitude of
581 environmental variability compared with the amplitude of variability in the data caused by
582 inaccuracy and imprecision of the data). It may artificially cause high-frequency signals to
583 manifest as significant periodicities in the core data during analysis, which may lead to
584 incorrect interpretation. In addition, the bias due to acid treatment can also affect the
585 underlying trend in the record, which can further undermine data analysis.

586

587 This suggests C/N and $\delta^{13}\text{C}$ values from bulk OM are a less reliable tool for reconstructing
588 environmental events with low amplitude variability. This is likely to have implications for
589 the high resolution, high frequency reconstructions favoured in the recent literature. We did
590 not carry out time-series analysis on our data – the analysis itself, in addition to the acid
591 treatment bias, would have been undermined by the low resolution sampling and poor dating
592 constraint across the data in the first instance, and made *a priori* assumptions about the
593 system and climatic processes responsible for the geochemical OM signature (e.g. Wunsch,
594 2010).

595

596 **1.6 Implications for accuracy and precision**

597

598 Based on our findings, we preliminarily assess the sources of inaccuracy and imprecision on
599 C/N (Σ_E), $\delta^{13}\text{C}$ (Σ_C) and $\delta^{15}\text{N}$ (Σ_N) values from sample bulk OM as follows:

600

601 Σ_E (on individual C/N values) = $e_d + e_{pic} + e_{pin} + e_{sh} + e_{ss} + e_{an}$

602

603 Σ_C (on individual $\delta^{13}\text{C}$ values) = $c_d + c_{ic} + c_{sh} + c_{ss} + c_{an}$

604

605 Σ_N (on individual $\delta^{15}\text{N}$ values) = $n_d + n_{in} + n_{sh} + n_{ss} + n_{an}$

606

607 The inaccuracy and imprecision associated with each component of the above equations are
608 summarised in Table 3. The diagenesis component (e_d ; c_d ; n_d) can contribute a significant
609 bias relative to the organic signal of the original source OM from pre- and post deposition
610 processes, such as oxidation and microbial reworking, the extent of which will vary
611 depending on the length of time OM takes to reach the sediment, the productivity in the water
612 column and dissolved oxygen concentrations (Meyers, 1994; Krull et al., 2002; Lehmann et
613 al., 2003). For example, Meyers and Ishiwatari (1993) noted that a diagenetic decrease in C/N
614 values could occur in lake sediments of the order of ~ 26 . A loss of non-lignin compounds
615 from a C_4 marsh plant depleted $\delta^{13}\text{C}$ by ~ 4 ‰ (Benner et al., 1987), though in sediments
616 evidence for diagenetic bias is contradictory. Spiker and Hatcher (1984) noted a 4 ‰
617 depletion in lake sediments which they attributed to the loss of ^{13}C -rich carbohydrates,
618 whereas Rea et al (1980) and Jasper and Gagosian (1989) noted no bias due to diagenesis.
619 For $\delta^{15}\text{N}$, the effect of diagenetic processes on the primary sedimentary OM signal is also
620 contradictory. Altabet and Francois (1994) reported a 5 ‰ enrichment in $\delta^{15}\text{N}$ and Sigman et
621 al (1999) noted an increase of ~ 4 ‰ in $\delta^{15}\text{N}$ from Southern Ocean sediments. However, de
622 Lange et al (1994) reported a decrease of ~ 1 ‰ in $\delta^{15}\text{N}$, and Freudenthal et al (2001) noted a
623 ~ 1 ‰ bias in eastern Atlantic Ocean sediments with no clear trend towards an increase or
624 decrease in $\delta^{15}\text{N}$. The degradation of organic compounds, which have distinctive isotopic
625 signatures, appears to be non-discriminatory, implying that diagenetic processes must be
626 accounted for on a system by system basis. For example, the loss of readily degradable amino
627 acids and hydrocarbons, relative to terrestrially sourced compounds such as lignins and lipids,
628 would deplete $\delta^{15}\text{N}$ (loss of ^{15}N and ^{13}C). These contradictory results clearly imply that there
629 is no emergent generalised affect on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signature of bulk OM, and this can
630 differ markedly between oxic and anoxic conditions (Tyson, 1995). However, a detailed
631 molecular level investigation may allow the estimation of this bias on bulk OM.

632

633 The influence of IC and/or IN (e_{pic} ; e_{pin} ; c_{ic} ; n_{in}) is dependent upon the efficiency of removal
634 during acid treatment, and the structure and composition of the inorganic component (e.g. Al-
635 Aasm et al., 1990; Yui and Gong, 2003; Knies et al., 2007). The bias on bulk $\delta^{13}\text{C}$ in this
636 study was shown to be an enrichment of ~ 6.8 ‰ at 8.76 m, though it was noted that residual
637 IC could result in an overall depletion of the bulk $\delta^{13}\text{C}$ value (e.g. Hoefs, 1973; Hangari et al.,

1980; Mozley and Burns, 1993; Pierre et al., 2009). Additionally, and similar to $\delta^{13}\text{C}$, the extent of the bias on $\delta^{15}\text{N}$ is less obvious owing to the similar values in both organic and inorganic N (Knies et al., 2007). The homogeneity of the sample material (e_{sh} ; c_{sh} ; n_{sh}) can also contribute additional imprecision, likely increasing substantially on samples poorly homogenised or with significantly low amounts of C and N (e.g. Basiden et al., 2002). Sample size can also contribute significant bias, particularly those low in C and N before acid pre-treatment is undertaken (e_{ss} ; c_{ss} ; n_{ss}). Where sample size becomes very small (especially in conjunction with an acid pretreatment), %C and %N can increase by over 50% (see Brodie et al., 2011a) and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values tend to become more positive, suggesting at least a ~ 1 ‰ deviation in the OM value (our analysis were carried out on sample sizes significantly above machine baseline conditions and therefore do not carry this additional inaccuracy). Finally, the analytical term (e_{an} ; c_{an} ; n_{an}), comprising the bias from acid treatment and the instrument precision. Pre-analysis acid treatment has been shown here, and elsewhere, to substantially bias the elemental C and N values of sample OM through degradation of the OC fraction and/or inefficient removal of the IC fraction (and differential rates of removal linked to IC stoichiometry). For this core, we estimate this error to be in the region of $\sim \pm 2 - 3.5$ ‰ associated with the OC fraction and ~ 6.8 ‰ (enrichment) associated with the IC fraction. The instrument precision, which is inherent to all measurements in this study, is of the order of ± 0.5 for C/N values, ± 0.1 ‰ for $\delta^{13}\text{C}$ values and ± 0.1 ‰ for $\delta^{15}\text{N}$ values.

657

We caution, however, that whilst these equations are more representative of the absolute inaccuracy and imprecision on measured C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than instrument precision alone, the terms are by and large inherently non-linear and unpredictable, implying the absolute inaccuracy and imprecision is unobtainable. An assumption of linearity of these terms would be seriously flawed. We conclude that the unpredictable, non-linear biasing to the data within sample horizons and with varying magnitude and proportions within and between sample horizons can undermine a robust interpretation of the data, with the size of bias varying considerably between different cores.

666

Table 3: Summary of inaccuracy and imprecision on C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values measured from bulk OM. The size of bias is estimated from past literature (see section 1.6 for a discussion and evaluation) and biases due to pre-analysis acid treatment reported in this study.

670

671 1.7 Summary and Recommendations

672

- 673 1. This study has clearly demonstrated significant *non-linear* bias on bulk C/N, $\delta^{13}\text{C}$ and
674 $\delta^{15}\text{N}$ values of OM associated with pre-analysis acid treatment method in a stratigraphical
675 sequence of samples. We show that there is an inconsistency in the use of any one method
676 within and between sample horizons and that where this bias is evident it is significantly
677 above instrumental precision. The differences appear to be the result of (i) differential
678 rates of removal of IC and (ii) disproportionate biasing to OC fraction of the sample
679 material.
- 680 2. In light of our findings, we recommend that researchers do not interpret the minutiae of
681 the bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of OM data, but restrict interpretations and discussions to those
682 shifts significantly greater than a robust estimate of the inaccuracy and imprecision on the
683 data (i.e. $\sim 4\%$ on this down-core data). The estimation presented here is considerably
684 greater than is normally assumed (i.e. standard instrument precision) and underlines the
685 importance of determining the size of the bias on C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data in a down-core
686 record. Consequently, $\delta^{13}\text{C}$ data should be used as a first-order indication of potential
687 changes in sample OM, which could be further investigated for environmental and
688 climatic change at the molecular level.
- 689 3. The biases discussed here make the environmental interpretation of C/N values (e.g.
690 terrestrial versus aquatic) and $\delta^{13}\text{C}$ values (e.g. C_3 versus C_4 vegetation) problematic. For
691 example, at 7.80 m C/N values range from 12 – 32 between methods and at 8.60 m $\delta^{13}\text{C}$
692 values range from -21.3 to -12.5% . In addition, it also makes the interpretation of C/N
693 versus $\delta^{13}\text{C}$, C/N versus $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ bi-plots questionable.
- 694 4. The rinse method can artificially elevate %C and %N values and significantly undermine
695 the integrity of C/N values. We recommend including a centrifugation step in this method,
696 but warn that this will not guarantee resolution of the problems associated with decanting.
- 697 5. The 5% HCl capsule method appears to be less efficient in the removal of IC leading to
698 more enriched $\delta^{13}\text{C}$ values (e.g. 8.76 m), and so we therefore do not recommend the use
699 of this reagent within the capsule method. However, we warn that the assumption that
700 residual IC causes an overall enrichment of the measured bulk $\delta^{13}\text{C}$ value is invalid,
701 where $\delta^{13}\text{C}$ values of IC can be very negative. This suggests a comprehensive
702 understanding of sample IC alongside sample OC is required without which C/N and $\delta^{13}\text{C}$
703 values of OM may be unreliable.

704

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709

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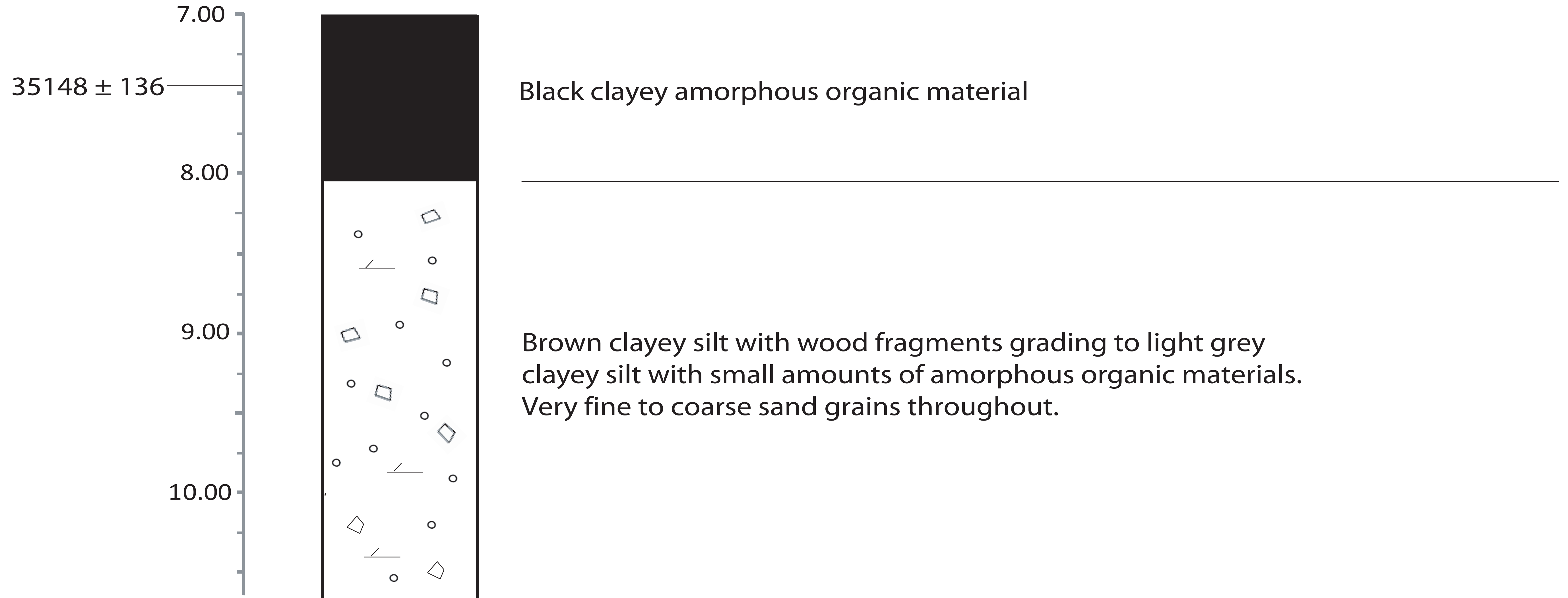
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Lithology Key

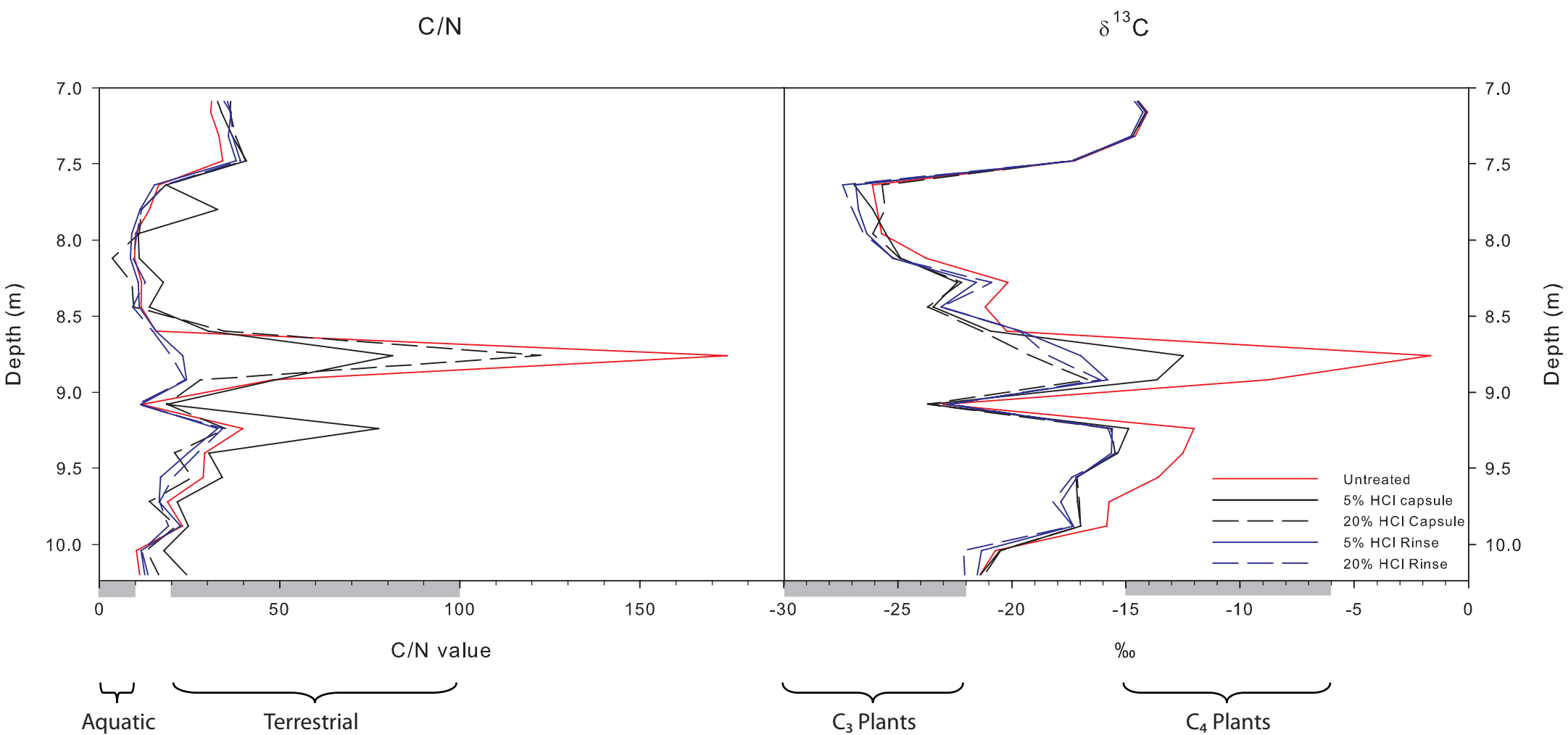
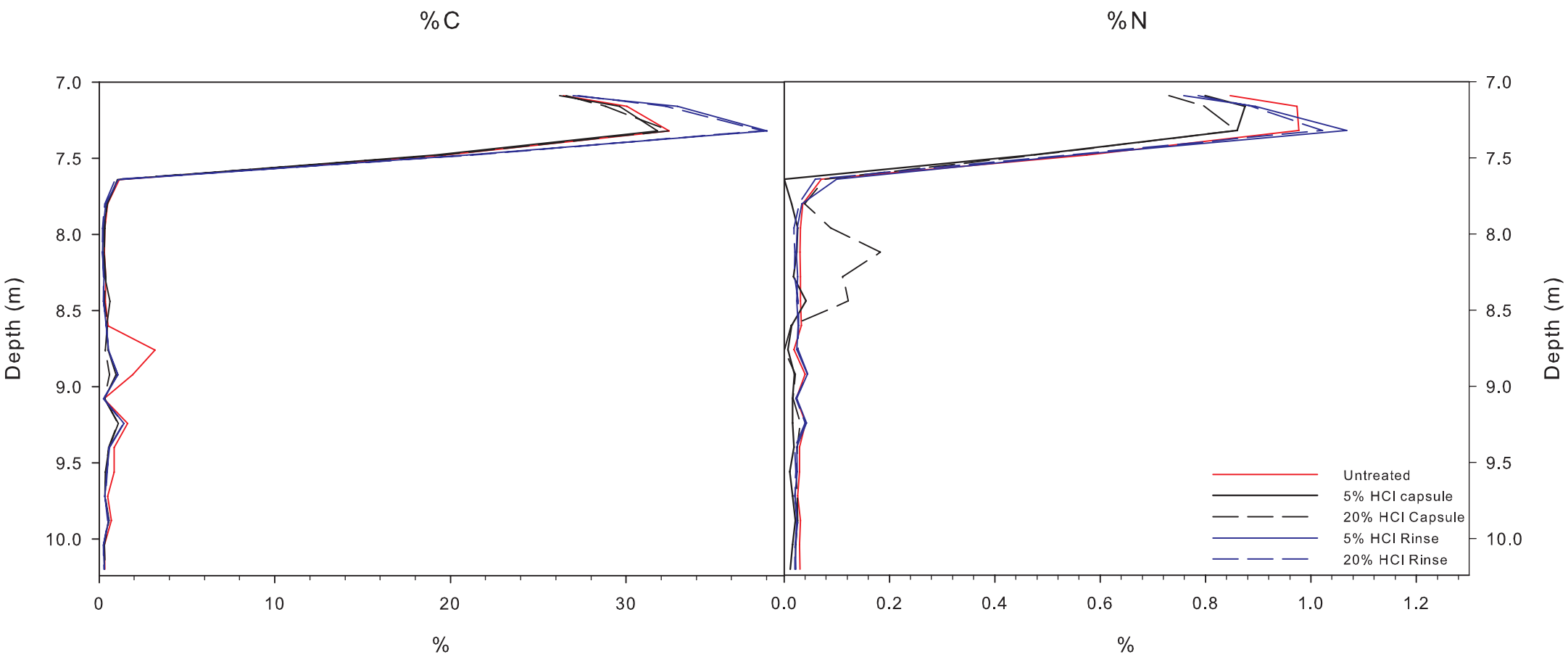
Wood Fragment

Sand

Amorphous Organic Material

Clayey Silt

Black amorphous organics



Depth (m)	$\delta^{13}\text{C}$			C/N		
	P-value (R-Squ)	Difference (‰)	Cause	P-value (R-Squ)	Difference	Cause
7.09	0.197 (20.98)	nd	-	0.00 (74.05)	3 – 4	5% and 20% HCl capsule higher than 5% rinse
7.16	0.183 (22.52)	nd	-	0.18 (23.18)	nd	-
7.32	0.04 (49.01)	0.2	5% HCl more enriched than 20% HCl in rinse method	0.29 (11.69)	nd	-
7.48	0.55 (0.00)	nd	-	0.143 (27.67)	nd	-
7.64	0.00 (94.69)	1.0	20% HCl capsule sample more enriched than 20% HCl rinse sample	0.97 (0.00)	nd	-
7.80	0.00 (98.25)	0.6 – 1.5	All samples differ over 1.5 ‰ range, with more enriched values in the capsule method.	0.00 (95.63)	22	5% HCl capsule higher than all other samples
7.96	0.01 (64.38)	0.3 – 0.7	5% HCl capsule more enriched than all other methods	0.94 (0.00)	nd	-
8.12	0.01 (60.68)	0.4	Capsule method samples more enriched than rinse method samples	0.02 (58.67)	4 – 6	5% and 20% capsule method different and 20% HCl capsule different from rinse samples
8.28	0.00 (87.85)	0.2	Capsule method slighted more depleted than rinse method	0.08 (38.60)	nd	-
8.44	0.01 (65.66)	0.4 – 0.6	20% HCl capsule more enriched all other samples	0.52 (0.00)	nd	-

8.60	0.00 (98.83)	0.4	5% HCl capsule more enriched than 20% HCl capsule, and both rinse samples	0.00 (87.59)	16	Capsule method samples higher than rinse method samples
8.76	0.00 (95.72)	1.2 – 6.8	5% HCl capsule more enriched than all other samples. 20% HCl capsule most depleted.	0.00 (66.52)	98 – 103	5% HCl and 20% HCl capsule method higher than rinse method samples.
8.92	0.01 (81.77)	2.6 – 3	5% HCl more enriched than all other samples.	0.00 (94.19)	20 – 25	5% HCl capsule higher than other samples
9.08	0.00 (97.53)	0.6 – 1.0	Capsule samples more depleted than rinse samples. 5% HCl rinse more enriched than 20% HCl rinse.	0.00 (87.87)	7	Capsule method samples higher than rinse method samples
9.24	0.00 (97.71)	1.0	5% HCl capsule more enriched than all other samples	0.04 (47.69)	20	5% HCl capsule higher than other samples
9.40	0.07 (40.58)	nd	-	0.03 (54.35)	10	5% and 20% HCl capsule method samples different
9.56	0.74 (0.00)	nd	-	0.31 (9.77)	nd	-
9.72	0.00 (88.05)	1.0 – 1.2	Rinse method samples more depleted than capsule method samples. 20% HCl capsule more enriched than all other samples	0.06 (41.74)	nd	-
9.88	0.09 (35.93)	nd	-	0.04 (49.52)	7	5% HCl capsule different from 20% HCl rinse
10.02	0.00 (94.86)	1.0 – 1.8	Rinse samples more depleted than capsule samples. 5% HCl rinse	0.04 (49.95)	8	5% HCl different from both rinse samples

			most depleted			
10.20	0.00 (94.72)	0.3 – 0.6	Rinse method sample more depleted than capsule samples	0.00 (74.84)	6 – 8	5% HCl capsule higher than other samples

	$\delta^{15}\text{N}$		
Depth (m)	P-value (R-Squ)	Difference (‰)	Cause
7.09	0.00 (84.07)	0.46	Acid treated samples lower than untreated samples. 20% HCl rinse lowest.
7.16	0.00 (89.14)	0.77	Acid treated samples lower than untreated samples
7.32	0.02 (51.33)	0.46	Acid treated samples lower than untreated samples. Capsule method samples lowest.
7.48	0.00 (79.94)	0.42	Capsule method and 5% HCl rinse lower than untreated samples and 20% HCl rinse.

Process		C/N Bias	$\delta^{13}\text{C}$ Bias	$\delta^{15}\text{N}$ Bias
Diagenesis	Bias variable	e_d	c_d	n_d
	Explanation	Bias associated with breakdown, oxidation and reworking of initial OM.	Bias associated with breakdown, oxidation and reworking of initial OM.	Bias associated with breakdown, oxidation and reworking of initial OM.
	Size of Bias	$\sim 5 - 26$	$\sim 0.2 - 4 \text{ ‰}$	$\sim 0.1 - 5 \text{ ‰}$
Inorganic Carbon	Bias variable	e_{pic}	c_{in}	N/A
	Explanation	Bias associated with the structure, composition and quantity of sample IC.	Bias associated with the structure, composition and quantity of sample IC.	
	Size of Bias	$\pm 1 - 60$	$\pm 3.4 \text{ ‰}$	
Inorganic Nitrogen	Bias variable	e_{pin}	N/A	n_{in}
	Explanation	Bias associated with the structure, composition and quantity of sample IN.		Bias associated with the structure, composition and quantity of sample IN.
	Size of Bias	$\pm 1 - 5$		indefinable
Analytical Bias	Bias variable	e_{an}	c_{an}	n_{an}
	Explanation	Bias associated with acid treatment of sample (and instrument precision).	Bias associated with acid treatment of sample (and instrument precision).	Bias associated with acid treatment of sample (and instrument precision).
	Size of Bias	$\pm 0.5 - 15 (\pm 0.2)$	$\pm 4 \text{ ‰} (\pm 0.1 \text{ ‰})$	$\pm 0.7 \text{ ‰} (\pm 0.1 \text{ ‰})$
Sample Size	Bias variable	e_{ss}	c_{ss}	n_{ss}
	Explanation	Bias associated with the amount of C and N supplied for analysis.	Bias associated with the amount of C and N supplied for analysis.	Bias associated with the amount of C and N supplied for analysis.
	Size of Bias	$\pm 0.5 - 100$	$0.5 \text{ ‰ (or greater)}$	$0.5 \text{ ‰ (or greater)}$
Sample Homogenisation	Bias variable	e_{sh}	c_{sh}	n_{sh}
	Explanation	Bias associated with homogenisation of sample material prior to treatment.	Bias associated with homogenisation of sample material prior to treatment.	Bias associated with homogenisation of sample material prior to treatment.
	Size of Bias	$\pm 0 - 0.2$	$\pm 0.0 - 0.1 \text{ ‰}$	$\pm 0.0 - 0.1 \text{ ‰}$