# Evidence for bias in C/N, $\delta^{13}C$ and $\delta^{15}N$ values of aquatic and terrestrial organic materials due to acid pre-treatment methods

Chris R. Brodie[1,2]

[1]Department of Geography, Durham University, UK
[2]Department of Earth Sciences, Hong Kong University, Hong Kong: brodie@hku.hk

**Acid treatment of organic materials, necessary to remove inorganic carbon prior to isotopic analysis, adds an unpredictable and non-linear bias to measured C/N, $\delta^{13}C$ and $\delta^{15}N$ values questioning their reliability and interpretation.**

## C/N, $\delta^{13}C$ and $\delta^{15}N$ as paleoenvironmental proxies

The analysis of organic matter (OM) from modern and paleoenvironmental settings has contributed to the understanding of the carbon biogeochemical cycle at a variety of spatial and temporal scales. Specifically, the concentrations of carbon (C) and nitrogen (N), from which the C/N ratio is derived, and stable C and N isotopes ($^{12}C/^{13}C$, quoted as $\delta^{13}C$ relative to Vienna Pee Dee Belemnite (V-PDB) and; $^{14}N/^{15}N$, quoted as $\delta^{15}N$ relative to $N_2$-AIR) of OM have been used to understand processes from biological productivity through to paleoenvironmental interpretations. For example, C/N ratios are widely used as an indicator of OM origin (C/N < 10 interpreted as aquatic; C/N > 20 as terrestrial source) and $\delta^{13}C$ can be used to, among other things, understand broad-scale changes in vegetation type (e.g., photosynthetic pathways; $C_3$ and $C_4$ plant types; Smith and Epstein, 1971; Meyers, 1997; 2003; Sharpe, 2007). $\delta^{15}N$ has also been used to investigate OM origin (Thornton and McManus, 1994; Meyers, 1997; Hu et al., 2006), but is more commonly used to understand nitrate utilization, denitrification and N deposition in aquatic systems (e.g., Altabet et al., 1995). These interpretations are based on the assumption that we can reliably determine C/N, $\delta^{13}C$ and $\delta^{15}N$ values in OM.

## Acid pre-treatment methods: The "free for all"

In the natural environment, carbon is commonly considered in two major forms—organic and inorganic (OC and IC). Both forms can act as a contaminant in the measurement of the other due to their distinctive isotopic signatures (e.g., IC is assumed to be enriched in $^{13}C$ relative to OC: Hoefs, 1977; Sharpe, 2007). Therefore, the accurate determination of C/N and $\delta^{13}C$ of OM necessarily involves the removal of IC from the sample material. This is commonly achieved by acid pre-treatment. A number of fundamentally different acid pre-treatment methods exist, within which a range of acid reagents and strengths, types of capsule and reaction temperatures are used. There is no consensus on "best practice". An inherent, and widely unrecognized, assumption of these acid pre-treatment methods is that their effect on sample OM is either negligible or at least systematic (and small), implying that, within instrument precision, all measured values should be indistinguishable from one another regardless of the method followed. The type and strength of the acid reagent, and type of capsule the sample is combusted in, are assumed to have no effect on measured values. However, these assumptions have hitherto never been systematically investigated, implying that the scientific approach remains to be validated.

We examined three common acid pre-treatment methods for the removal of IC in OM: (1) Rinse Method: Acidification followed by sequential water rinse, the treated samples from which are combusted in tin (Sn) capsules (e.g., Midwood and Boutton, 1998; Ostle et al., 1999; Schubert and Nielsen, 2000; Galy et al., 2007); (2)

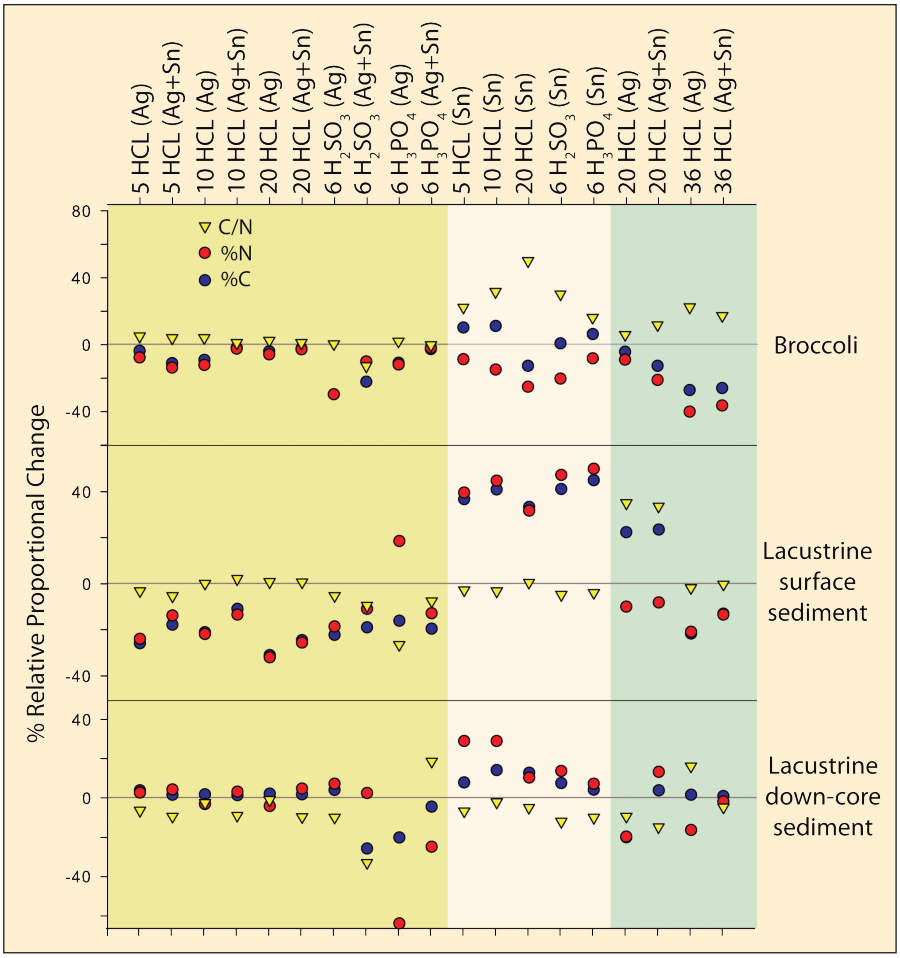


*Figure 1: Relative offset in %C (**blue circles**), %N (**red circles**) and C/N (**yellow triangles**) for a selection of materials sampled (Details of additional samples in Brodie et al., 2011a), and all combinations of acid pre-treatment methods (varying concentrations of HCl, $H_2SO_3$ and $H_3PO_4$). Broccoli was calculated relative to known values. Of note, broccoli C/N results suggest either aquatic (<10) or aquatic/terrestrial (>10) origin. The lacustrine surface sediment (Newstead Abbey Lake, Nottingham, UK) and lacustrine down core sediment (Lake Tianyang, South China) were calculated relative to their overall means from all measured acidified samples. Background shading represents pre-treatment method: Yellow = capsule, white = rinse, green = fumigation (figure modified from Brodie et al., 2011a).*

Capsule Method: In-situ acidification in silver (Ag) capsules (e.g., Verardo et al., 1990; Nieuwenhuize et al., 1994a, b; Lohse et al., 2000; Ingalls et al., 2004); and (3) Fumigation Method: Acidification by exposure of the sample to an acid vapor in silver (Ag) capsules (e.g., Harris et al., 2001; Komada et al., 2008). $\delta^{15}N$ is often measured from untreated sample aliquots weighed directly into Sn capsules, assuming negligible influence of inorganic nitrogen (e.g., Müller, 1977; Altabet et al., 1995; Schubert and Calvert, 2001; Sampei and Matsumoto, 2008). However, the application of "dual-mode" isotope analyses (the simultaneous measurement of C/N, $\delta^{13}C$ and $\delta^{15}N$ from the same pre-treated sample; e.g., Kennedy et al., 2005; Jinglu et al., 2007; Kolasinski et al., 2008; Bunting et al., 2010) is increasing. It was therefore also necessary to test whether acid pre-treatment had an effect on $\delta^{15}N$ results. Hydrochloric (HCl), sulfurous ($H_2SO_3$) and phosphoric ($H_3PO_4$) acid, at varying strengths have been compared (e.g., Kennedy et al., 2005; Brodie et al., 2011a).

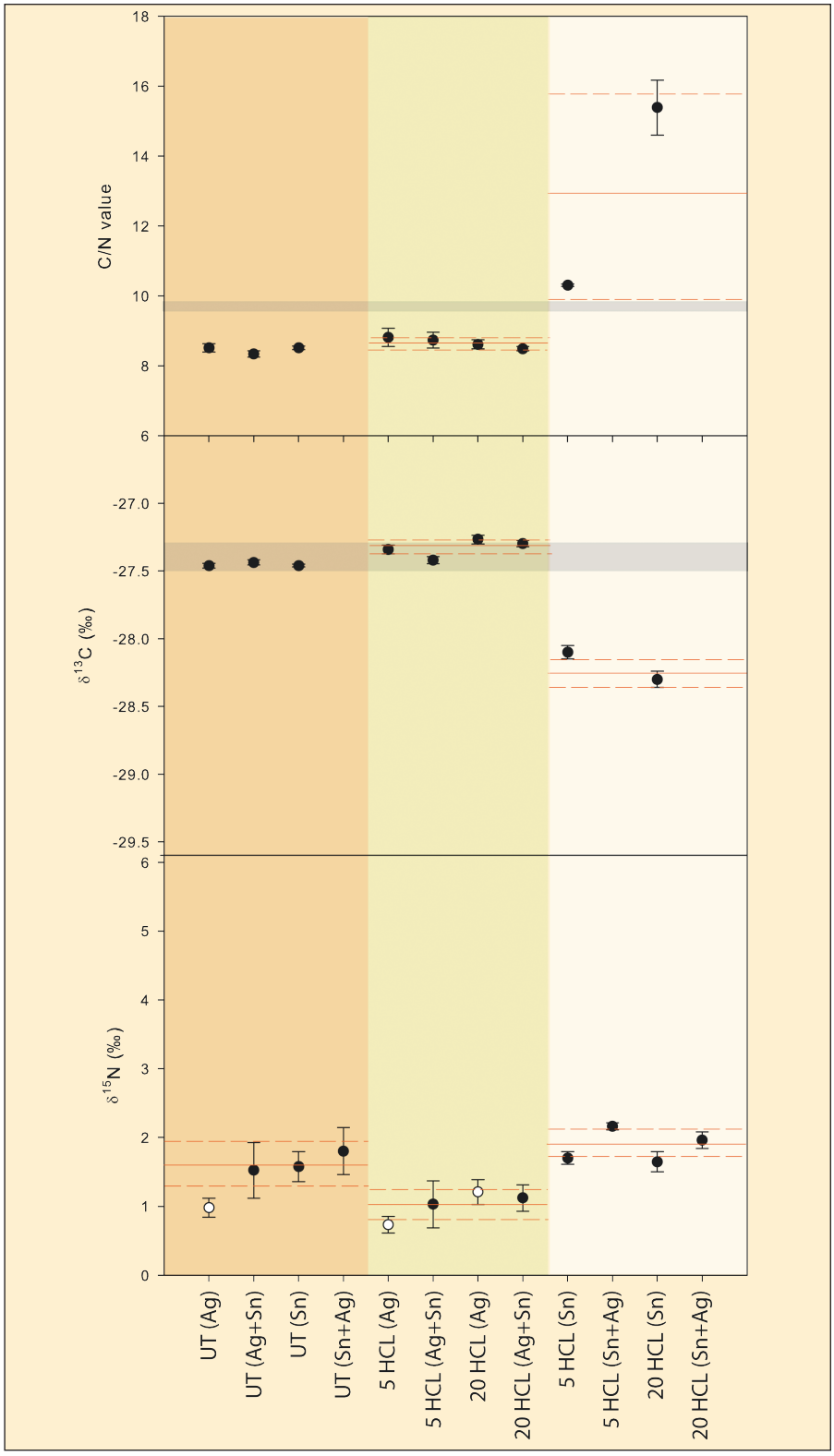


*Figure 2: Broccoli C/N, $\delta^{13}C$ and $\delta^{15}N$ values for each pre-treatment method showing that measured C/N, $\delta^{13}C$ and $\delta^{15}N$ values vary in a non-linear, unpredictable manner within and between acid pre-treatment methods. Horizontal red lines indicate mean values for each method, and perforated red lines 1σ. Background shading represents pre-treatment method: Yellow = capsule, white = rinse, orange = untreated. Horizontal gray shaded bars represent known values. Error bars are calculated as standard deviation (1σ) of triplicate measurements. Unfilled circles represent samples analyzed in Ag capsules only (figure modified from Brodie et al., 2011b).*

## Non-linear, unpredictable bias to organic matter

Measured C/N, $\delta^{13}C$ and $\delta^{15}N$ values vary in a non-linear, unpredictable manner within (capsule type and acid reagent) and between ("capsule", "rinse" and "fumigation") acid pre-treatment methods (Fig. 1 and 2). In addition, the coherency of any one method or acid reagent is highly variable between the materials tested (i.e., high variability in accuracy and precision). This suggests that the measured C/N, $\delta^{13}C$ and $\delta^{15}N$ values of OM are not only dependent on environmental process, but also on analytical procedure, reducing the reliability of the data to the point of questioning the strength of the subsequent interpretation. Across all of the materials and pre-treatment methods tested, biases in C/N were in the range of 7 – 113; $\delta^{13}C$ in the range of 0.2 – 7.1 ‰; and $\delta^{15}N$ in the range of 0.2 – 1.5 ‰, resulting directly from bias to sample OM by acid treatment and in some instances residual IC (see Brodie et al., 2011a, b for a detailed discussion). The range and magnitude of these treatment-induced biases indicate that the assumption that there is negligible or systematic effect from acid pre-treatment is seriously flawed.

The range and magnitude of these biases are influenced by a number of factors. For example, %C and %N can be artificially concentrated by weight in the "rinse" method due to a loss a fine colloidal materials in the discarded supernatant; and C/N, $\delta^{13}C$ and $\delta^{15}N$ values can be biased due to loss of fine colloidal organic in the supernatant and solubilization of OC (Brodie et al., 2011a). These values can similarly be influenced in the "capsule" and "fumigation" methods due to volatilization of OC and residual IC. Furthermore, the type of acid reagent (e.g., HCl, $H_2SO_3$ or $H_3PO_4$) and strength of acid reagent (e.g., 5% HCl, 10% HCl or 20% HCl) within and between pre-treatment methods can affect the accuracy and precision of measured values. In addition, the capsule within which the sample is combusted can influence results due to the fundamental difference in combustion temperatures (Sn is 232°C and Ag is 962°C). Sample size, C and N homogeneity and the type, amount and nature of OM, can further influence the analysis. The underlying mechanisms causing these biases, however, remain unclear.

### Implications for interpretation of C/N, $\delta^{13}C$ and $\delta^{15}N$ values

Bias by acid pre-treatment on OM can significantly undermine C/N values as indicators of OM provenance. For example, Figures 1 and 2 show that although broccoli (*Brassica oleracea*) is a terrestrial $C_3$ plant, an aquatic or aquatic/terrestrial combination could be concluded from the data, depending upon the method and/or acid reagent (see Brodie et al., 2011a). In addition, C/N values can also vary considerably depending on whether they are calculated with %N from treated or untreated sample aliquots (see Brodie et al., 2011a, b). For $\delta^{13}C$, biasing in the range of 0.2 – 7.1‰ can undermine $C_3$ vs. $C_4$ plant type interpretations, and together with C/N undermine bi-plot interpretations of C/N, $\delta^{13}C$ and $\delta^{15}N$ values. This clearly demonstrates that the data are inherently unreliable as a function of the analytical approach. Although the underlying mechanisms require further research, it is clear the biases represented here across a range of terrestrial and aquatic, modern and ancient organic materials has direct implications for paleo reconstructions: understanding and reducing the uncertainty on the data is an essential prerequisite for reliable interpretations and reconstructions.

### Concluding Remarks

The systematic comparisons of Brodie et al. (2011a, b) clearly demonstrate non-linear and unpredictable biasing of OM due to acid pre-treatment, and concomitantly indicate that complete IC removal (the purpose of acid pre-treatment) is not guaranteed. It is concluded that these biases are inherently not correctable but inevitable, and have a direct consequence for the accuracy and precision of measured values (i.e., significantly greater than instrument precision). Moreover, environmental interpretations of the data in both modern and paleo systems could be highly questionable.

### Acknowledgements

The author is grateful to J. Casford, J. Lloyd, Z. Yongqiang, T. Heaton, M. Bird and M. Leng for their support and discussions. Financial support came from the NERC through PhD studentship (NE/F007264/1), a NERC isotope Facility grant (IP/1165/0510), and the Dudley Stamp Memorial Fund.

### References

Brodie, C.R., Leng, M.J., Casford, J.S.L., Kendrick, C.P., Lloyd, J.M., Zong, Y.Q. and Bird, M.I., 2011a: Evidence for bias in C and N concentrations and $d^{13}C$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods, *Chemical Geology*, **282**: 67-83.

Brodie, C.R., Heaton, T.H.E., Leng, M.J., Kendrick, C.P., Casford, J.S.L. and Lloyd, J.M., 2011b: Evidence for bias in measured $d^{15}N$ values of terrestrial and aquatic organic materials due to pre-analysis acid treatment methods, *Rapid Communications in Mass Spectrometry*, **25**: 1089-1099.

Kennedy, P., Kennedy, H. and Papadimitriou, S., 2005: The Effect of acidification on the determination of organic carbon, total nitrogen and their stable isotopic composition in algae and marine sediment, *Rapid Communication in Mass Spectrometry*, **19**: 1063-1068.

Meyers, P.A., 2003: Applications of organic geochemistry to palaeolimnological reconstructions: a summary of examples from the Laurentian Great lakes, *Organic Geochemistry*, **34**: 261-289.

Sampei, Y. and Matsumoto, E., 2008: C/N ratios in a sediment core from Nakaumi Lagoon, southwest Japan, Usefulness as an organic source indicator, *Geochemistry Journal*, **35**(3): 189-201.

For full references please consult:

http://www.pages-igbp.org/products/newsletters/ref2011_2.pdf

# Extending glacier monitoring into the Little Ice Age and beyond

Michael Zemp[1], H.J. Zumbühl[2], S.U. Nussbaumer[1,2], M.H. Masiokas[3], L.E. Espizua[3] and P. Pitte[3]

[1]World Glacier Monitoring Service, Department of Geography, University of Zurich, Switzerland; michael.zemp@geo.uzh.ch
[2]Institute of Geography and Oeschger Centre for Climate Change Research, University of Bern, Switzerland; [3]Argentine Institute of Snow Research, Glaciology and Environmental Sciences, (IANIGLA), CCT-CONICET Mendoza, Argentina

**Reconstructions of glacier front variations based on well-dated historical evidence from the Alps, Scandinavia, and the southern Andes, extend the observational record as far back as the 16th century. The standardized compilation of paleo-glacier length changes is now an integral part of the internationally coordinated glacier monitoring system.**

Glaciers are sensitive indicators of climatic changes and, as such, key targets within the international Global Climate Observing System (GCOS, 2010). Glacier dynamics contribute significantly to global sea level variations, alter the regional hydrology, and determine the vulnerability to local natural hazards. The worldwide monitoring of glacier distribution and fluctuations has been well established for more than a century (World Glacier Monitoring Service, 2008). Direct measurements of seasonal and annual glacier mass balance, which are available for the past six decades, allow us to quantify the response of a glacier to climatic changes. The variations of a glacier front position represents an indirect, delayed, filtered and enhanced response to changes in climate over glacier-specific response times of up to several decades (Jóhannesson et al., 1989; Haeberli and Hoelzle, 1995; Oerlemans, 2007). Regular observations of glacier front variations have been carried out in Europe and elsewhere since the late 19th century. Information on earlier glacier fluctuations can be reconstructed from moraines, early photographs, drawings, paintings, prints, maps, and written documents. Extensive research (mainly in Europe and the Americas) has been carried out to reconstruct glaciers fluctuations through the Little Ice Age (LIA) and Holocene (e.g., Zumbühl, 1980; Zumbühl et al., 1983; Karlén, 1988; Zumbühl and Holzhauser, 1988; Luckman, 1993; Tribolet, 1998; Nicolussi and Patzelt, 2000; Holzhauser et al., 2005; Nussbaumer et al., 2007; Zumbühl et al., 2008; Masiokas et al., 2009; Nesje, 2009; Holzhauser, 2010; Nussbaumer and Zumbühl, 2011). However, the majority of the data remains inaccessible to the scientific community, which limits the verification and direct comparison of the results. In this article, we document our first attempt towards standardizing reconstructed glacier front variations and integrating them with in situ measurement data of the World Glacier Monitoring Service (WGMS).

### Standardization and database

The standardization of glacier front variations is designed to allow seamless comparison between reconstructions and in situ observations while still providing the most relevant information on methods and uncertainties of the individual data series. The standardized compilation of in situ observations is straightforward: the change in glacier front position is determined between two points in time and supplemented by information on survey dates, methods and data accuracies. The reconstruction of paleo-glacier front positions and their dating is usually more complex and based on multiple sources