

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

Chris R. Brodie^{1,2}

¹Department of Geography, Durham University, UK

²Department 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, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values questioning their reliability and interpretation.

C/N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 ($^{13}\text{C}/^{12}\text{C}$, quoted as $\delta^{13}\text{C}$ relative to Vienna Pee Dee Belemnite (VPDB) and $^{15}\text{N}/^{14}\text{N}$, quoted as $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ can be used to, among other things, understand broad-scale changes in vegetation type (e.g., photosynthetic pathway); C_3 and C_4 plant types; Smith and Epstein, 1971; Meyers, 1997; 2003; Sharpe, 2007). $\delta^{15}\text{N}$ 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, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 (IOC 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, 1997; Sharpe, 2007). Therefore, the accurate determination of C/N and $\delta^{13}\text{C}$ 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 Meth-od; 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)

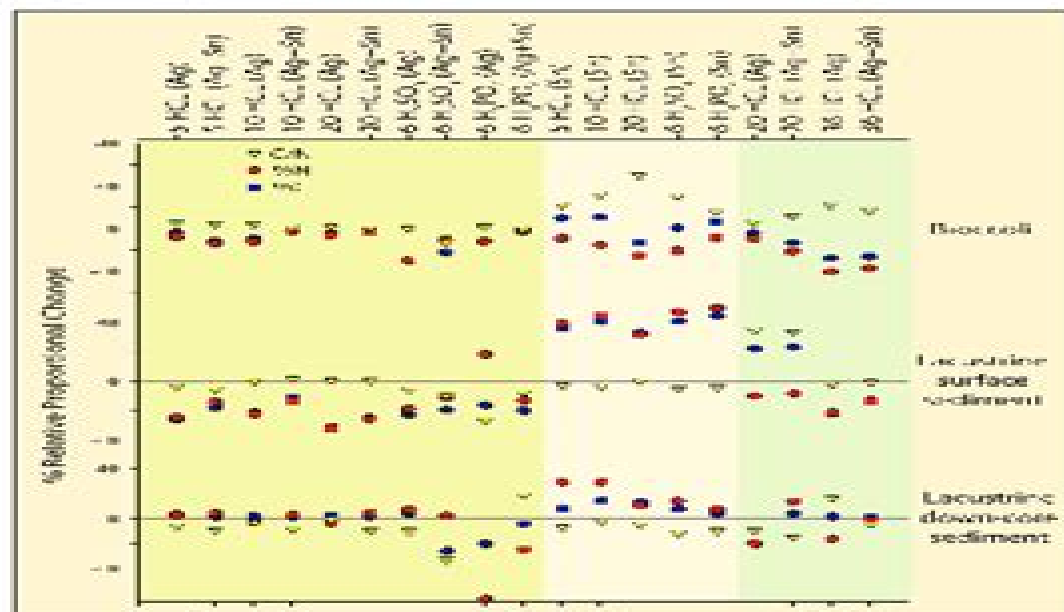


Figure 1: Relative offset in C/N (blue circles), $\delta^{13}\text{C}$ (red circles) and $\delta^{15}\text{N}$ (yellow triangles) for a selection of materials sampled. Details of additional samples in Brodie et al., 2011a, and all combinations of acid pre-treatment methods, varying concentrations of HCl , H_2SO_4 , and HNO_3 . Brodie et al. results are calculated relative to known values. C/N note: Brodie et al. results suggest either aquatic (< 10) or aquatic-terrestrial (> 10) origin. The lacustrine surface sediment (investigated by Lake, Nottingham, UK) and lacustrine down-core sediment (Lake Tanganyika, South China) were calculated relative to their overall means from all measured acidified samples. Background shading represents pre-treatment method: light blue – capsule with rinse, green – combustion (figure modified from Brodie et al., 2011a).

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