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Evaluating the influence of selected acid pre-treatment methods on C/N and $\delta^{13}C$ of temperate inter-tidal sediments for relative sea level reconstruction.

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Abstract

Inter-tidal environments play a central role in relative sea level (RSL) reconstruction studies and the use of composite records developed from elemental (organic carbon, total nitrogen) and isotopic ($\delta^{13}C$) data have been recently applied in these environments. Sample pre-treatment to remove inorganic carbon is a critical first step in this approach, but can distort the data. This paper presents the results of a comparative pilot study examining the performance of two acid preparation techniques (acid rinsing and acid fumigation) when applied to saltmarsh sediments from the Shannon estuary.

In high marsh to supratidal settings, acid rinsing caused depletion of both C_{org} and N_{tot}, though no significant change to C_{org}/N_{tot} (C/N) was recorded. At lower altitudes, increased N_{tot} losses led to distortion of C/N ratios. Both pre-treatment methods produced comparable $\delta^{13}C$ signatures in higher elevation contexts, but acid rinsing produced a depletion of 0.6‰ in low marsh and tidal flat samples. Whilst these differences between pre-treatment methods are observed, offsets are relatively small when compared to the range of elemental and isotopic values encountered across a saltmarsh and should not affect environmental interpretations for use in RSL reconstructions.

1 Introduction

The identification of saltmarsh environments preserved in sediment cores is a key component of research seeking to reconstruct past changes in relative sea level (RSL). Saltmarshes exist at the interface between the marine and terrestrial realms and their utility as sea level indicators arises from the strong environmental gradients that exist across this interface. Palaeoenvironmental information is routinely extracted from biological data, but in settings where taphonomic processes degrade identifiable floral and faunal remains, alternative methods of recognising the onset or removal of marine conditions are required.

Elemental (C_{org}/N_{tot}) and isotopic ($\delta^{13}C$) signatures preserved within bulk sediments provide one means of delimiting the transition from terrestrial to marine environment and a growing body of work is concerned with the potential application of such geochemical data in sea level research (e.g. Wilson *et al.* 2005a, 2005b; Lamb *et al.* 2006; Lamb *et al.* 2007; Da Cruz Miranda *et al.* 2009; Kemp *et al.* 2010; Kemp *et al.* 2012). In settings where the vertical zonation of saltmarsh flora engenders switches between plants utilising C_3 and C_4 photosynthetic pathways, large variations in $\delta^{13}C$ values occur and these have been used to distinguish intertidal to supra-tidal sub-environments (e.g. Törnqvist *et al.* 2004; Johnson *et al.* 2007). In regions where C_4 plants are absent from the historic record, there is a reduced variability in $\delta^{13}C$ source input and shifts between marginal marine and terrestrial conditions must be discerned by combining $\delta^{13}C$ signatures with C/N data (Mackie *et al.* 2005; Wilson *et al.* 2005a, 2005b; Lamb *et al.* 2007; Mackie *et al.* 2007). While organic matter (OM) C/N values converge during diagenesis, altitude-related variations useful for RSL reconstruction can still be discerned for the studies cited. The resulting carbon biplots (Figure 1) reflect shifts in the balance between saltmarsh (C_3) plants and inwashed particulate organic carbon (POC). Whilst the absolute values vary between sites, it is argued that the relative combined trends in C/N and $\delta^{13}C$ are robust and when used in association with lithostratigraphic data can delimit the onset and removal of marine conditions (see Edwards, 2013).

In common with other studies seeking to use C_{org} as an indicator of OM source, samples must be pre-treated with acid prior to analysis to remove inorganic carbon (IC) which will have a markedly different isotopic signature to the OM of interest. IC

from coastal locations is frequently assumed to be calcite or aragonite ($CaCO_3$), but could also contain varying proportions of dolomite ($Ca,Mg[CO_3]_2$), ankerite ($Ca[Fe,Mg,Mn][CO_3]_2$) and siderite ($FeCO_3$) that can be slower to react with acid during removal from the sample. Ideally, pre-treatment should completely remove IC without altering the elemental or isotopic ratios of the remaining material in a sample. In practice a range of pre-treatment methods are in use, all of which have some impact on the target variables ($\delta^{13}C$ and C/N), with the extent and significance of this impact varying with acidification method, laboratory protocol, and sample composition (Brodie *et al.* 2011a). The lack of a clear methodological consensus means that it is common for individual studies to replicate the pre-treatment approach employed in work on similar environments or sample material in an attempt to maximise the comparability of results.

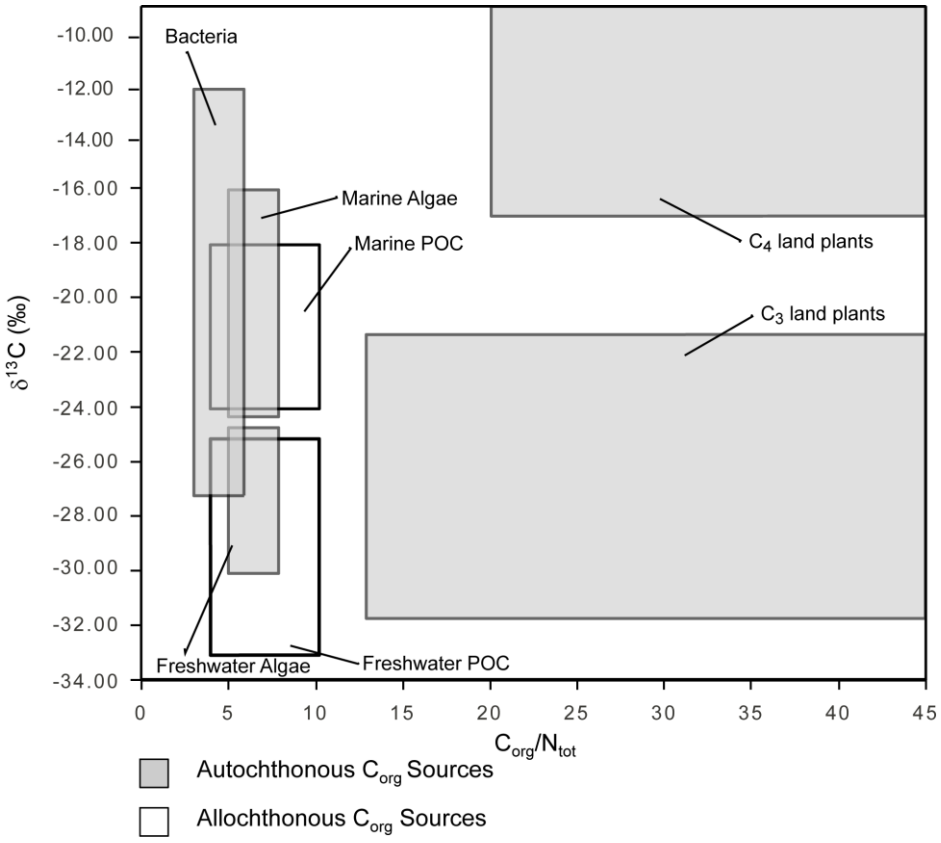

Figure 1: $\delta^{13}C$ and C/N of main OM sources to temperate saltmarsh sediments (Adapted from Lamb *et al.* 2006).

The three broad categories of pre-treatment methods to remove IC from sediment are acid rinsing, acid fumigating, and *in situ* acidification. Many bulk sediment studies

have used the acid rinse method where samples are treated with dilute acid (of various types and strengths) and rinsed with water prior to analysis (e.g. Froelich 1980; Midwood and Boutton 1998). This is the technique that has been employed in previous RSL-related studies using the kinds of sediments considered in this paper (Wilson *et al.* 2005a, 2005b; Lamb *et al.* 2007; Kemp *et al.* 2012). However, acid rinsing can cause the significant loss of acid soluble C_{org} (Froelich 1980; Yamamuro and Kayanne 1995; King *et al.* 1998; Harris *et al.* 2001) potentially altering $\delta^{13}C$ values (Harris *et al.* 2001), and causing the loss of N_{tot} (Midwood and Boutton 1998; Lohse *et al.* 2000; Ryba and Burgess 2002), possibly through a combination of hydrolysis, decarboxylation and oxidation. Many of these studies also show increasing loss of target components with increased reagent strengths (e.g. Midwood and Boutton 1998; Brodie *et al.* 2011a). Within C_3-only catchment areas, with reduced difference in isotopic signatures between samples from the marine and terrestrial end-members, these losses may distort C/N and $\delta^{13}C$ trends, resulting in erroneous RSL reconstructions by either amplifying or dampening the relative difference between terrestrial and marine sources.

In contrast, the acid fumigation technique has been described as having a reduced impact on elemental and isotopic variables as samples are exposed to concentrated HCl vapour rather than immersed in solution (Hedges and Stern 1984). This acid fumigation technique is suggested to minimise the loss of N_{tot} and C_{org}, whilst offering the added advantage of reduced labour in preparation (Yamamuro and Kayanne 1995; Harris *et al.* 2001; Lorrain *et al.* 2003). The third method of *in situ* acidification, where acid is added to sediments and subsequently dried within the same capsule used for isotopic analysis, is also considered to be conservative for N_{tot} and C_{org} as loss of labile OM is minimised because the acidic supernatant is not discarded (e.g. Nieuwenhuize *et al.* 1994; Kennedy *et al.* 2005; Brodie *et al.* 2011a). However, it is more labour intensive than the acid fumigation method and studies on similar coastal sediment have shown that results can be highly dependant on preparation procedures (Komada *et al.* 2008). This same study found fumigation to be preferential to *in situ* acidification for coastal sediments and provided over-exposure to acid did not occur, acid fumigation had low blank levels, removed dolomite, yielded accurate $\%C_{org}$ and $\delta^{13}C$ values; all of which are essential for RSL studies.

In comparative studies of acid pre-treatment methods applied to a range of sample materials, Brodie *et al.* (2011a; 2011b; 2011c) note considerable within- and between method variability in results for all pre-treatment methods. Consequently, they caution that the identification of changes in OM source based on C/N ratios and accompanying $\delta^{13}C$ data may be in error, especially where these variations are associated with a change in sediment matrix (lithology). Of the nineteen variants of acid pre-treatment method compared by Brodie *et al.* (2011a), no single approach consistently out-performed all others across the range of sample materials investigated, indicating that further context-specific assessment of pre-treatment method is advisable. This need is especially acute in the context of RSL/intertidal studies since: samples encompass material spanning the environmental gradient from terrestrial (freshwater) to marine conditions; these samples are associated with distinct changes in lithology/sedimentary character; and no intertidal samples were included in the test set of Brodie *et al.* (2011a).

Whilst $\delta^{15}N$ is not routinely considered in RSL studies, it has been used in other estuarine contexts to examine OM source and so is included in the analysis for completeness. Thornton and McManus (1994) statistically differentiated lacustrine and estuarine environments based on combined application of $\delta^{13}C$, C/N and $\delta^{15}N$. Muller and Voss (1999) used mean $\delta^{15}N$ values for terrestrial plants of 3‰ (range -5‰ to +18‰) and aquatic organisms of 7-10‰ to differentiate between these sources in low saline lagoons. However, nitrogen isotopes are more susceptible to diagenetic alteration than carbon isotopes, and although Thornton and McManus (1994) caution against their use in palaeoenvironmental reconstruction and indicate a general enrichment in ^{15}N with diagenesis, $\delta^{15}N$ has been used as organic tracers in palaeoenvironmental studies (e.g. Muller and Voss 1999, Da Cruz Miranda *et al*. 2009, Castro *et al*. 2010).

In light of the above, the objective of this paper is to investigate the potential for acid pre-treatment to generate spurious interpretations of RSL change based upon combined C/N and $\delta^{13}C$ data from bulk analyses of temperate intertidal- supratidal sediments. To this end, we analyse the isotopic and elemental compositions of 43 samples collected from three contrasting study sites that are typical of the kinds of material and locations targeted in RSL research. All samples are pre-treated by acid rinsing (the frequently-applied approach in RSL-focused applications following

Wilson *et al.* 2005a, 2005b; Lamb *et al.* 2006; Lamb *et al.* 2007; Kemp *et al.*, 2010, 2012) in which the sample is acidified with weak HCl before being rinsed repeatedly with distilled water.

To assess the extent to which acid rinsing may alter isotopic and elemental signatures, we perform replicate analysis on samples that are pre-treated by 'acid fumigation' in which the sample is acidified by exposure to concentrated acid vapour. As previous work indicates that this approach minimises the loss of N_{tot} and C_{org} in similar sediments (Komada *et al.* 2008), it provides a useful, conservative benchmark against which the effects of acid-rinsing are examined. This replicate set of analyses is further supplemented by additional duplicate analyses on non-acidified and IC-free sediment samples, coupled with targeted measurement of the dissolved organic carbon (DOC) and N_{tot} in the supernatant produced by acid rinsing. The other elementally conservative method of *in situ* acidification is not considered here as the primary aim of this paper is to investigate if acid rinsing using low concentration acid, as employed in previous studies, provides spurious isotopic results that could hinder RSL reconstruction.

2 Materials and Methods

2.1 Sample collection

Bulk sediment samples of approximately 20 cm^3 were recovered along transects extending from the supratidal to low intertidal (unvegetated) environments at three contrasting saltmarshes in Ireland (Figure 2). Altitudes of sampling levels at Portmarnock (PM), Ringmoylan (RMN) and Querrin (QN) were measured relative to known temporary bench marks with a Leica ® Runner 20 Automatic Optical Level and reported relative to Ordnance Datum Malin (OD Malin) which can be approximated to mean sea level. The bench mark altitudes had been previously measured using a Trimble® R6 GPS receiver with vertical precision <15mm.

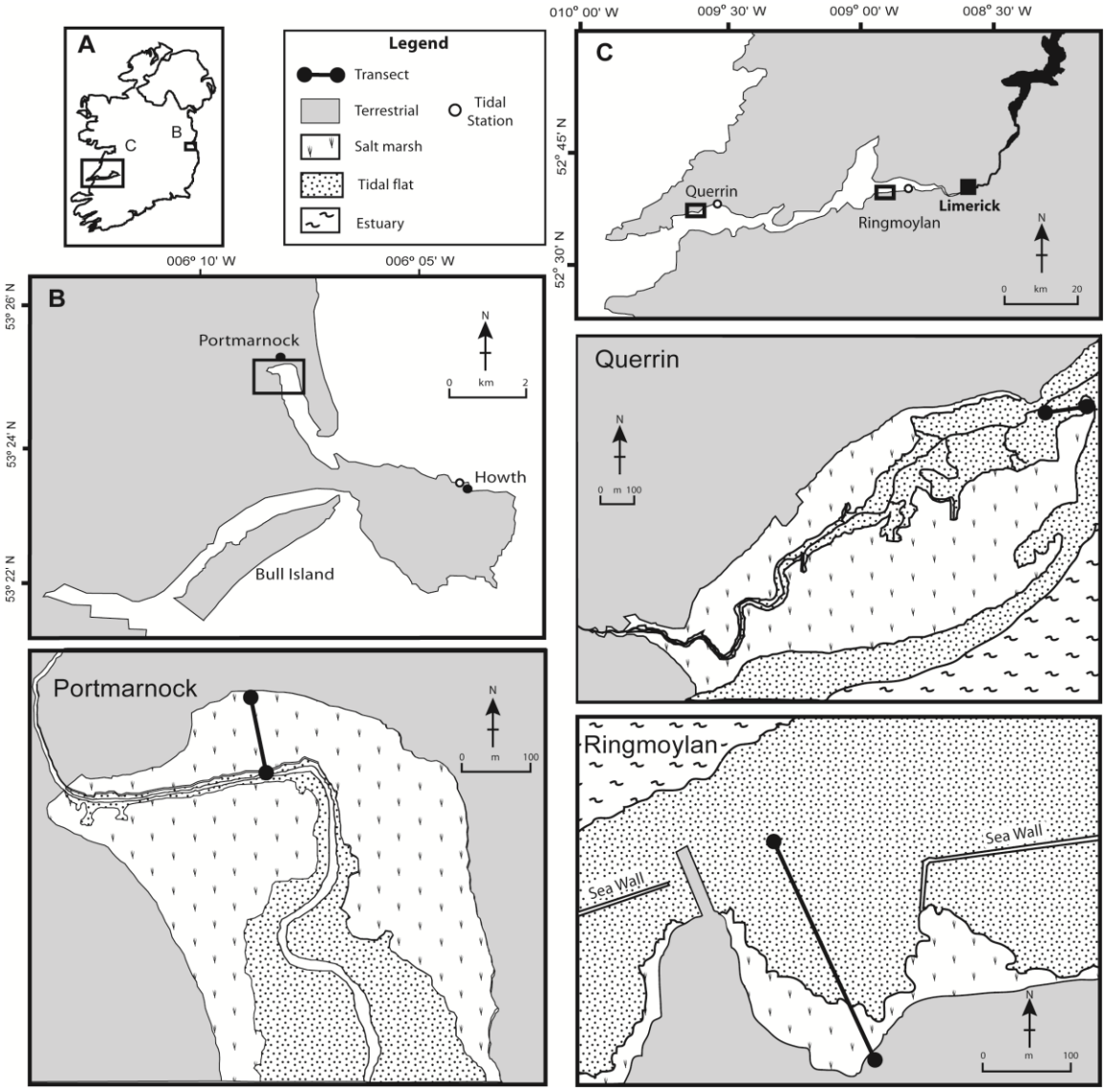

Figure 2: Location map of (A) Portmarnock and Shannon estuaries; (B) Portmarnock marsh; and (C) Shannon marshes with nearest tidal stations. Positions of transects shown on marsh plans.

The study site at PM is situated within a small tidal inlet (c. 3km long by 0.5km wide) on the east coast of Ireland through which the Mayne and Sluice Rivers drain into Irish Sea just to the north of Dublin Bay. The area is mesotidal with tidal range varying from around 2m on neap tides up to 3.6m during spring tides (Table 1). Above the unvegetated tidal flats, the low marsh is dominated by the C_4 plant *Spartina anglica*, before being replaced by common saltmarsh C_3 plants such as *Festuca rubra*, *Glaux maritima* and *Scirpus maritimus* in the high marsh. The highest elevation samples are associated with terrestrial grasses. While *Spartina* is a non-

native genus, introduced to Ireland in 1925 (Glavin, 1947) and therefore absent from the sedimentary record, its ubiquitous distribution means sampling contemporary Irish saltmarshes without its influence is not possible.

The two study sites of RMN and QN are located within the Shannon estuary on the west coast of Ireland (Figure 2c). The Shannon is macrotidal with spring tidal amplitude in the outer estuary of around 4m rising to almost 6m in the inner estuary (Table 1), whilst the tidal head extends up to Limerick city.

Given the variation in tidal ranges across the marshes, altitude values are normalised. We use the 'Standardised Water Level Index' (SWLI) to perform this normalisation (e.g. Horton *et al.* 1999; Edwards, 2001; Wright *et al.* 2011) in which the elevation in SWLI units is calculated as:

$$SWLI = [(Alt - MTL)/(MHWST - MTL) \times 100] + 100$$

where Alt is the altitude of the sample (m OD); MTL is the mean tide level (m OD); and MHWST is the mean high water spring tide (m OD).

Table 1: Tidal data from tidal stations closes to sample sites (in brackets, with distance from tidal station). SWLI is Sea Water Level Index. Source: Admiralty Tide Tables (2010).

Tidal Station	Units	Tidal Data				
		MHWST	MHWNT	MTL	MLWNT	MLWST
Howth (Portmarnock – 5.7km)	OD Malin (m)	1.6	0.8	-0.2	-1.2	-2.0
	SWLI	200	156	100	44	0
Mellon Point (Ringmoylan- 4.2km)	OD Malin (m)	2.8	1.4	0.1	-1.2	-2.7
	SWLI	200	149	100	53	-2
Kilrush (Querrin – 5.6km)	OD Malin (m)	2.0	0.7	-0.3	-1.3	-2.5
	SWLI	200	143	100	55	2

In contrast to PM, RMN and QN are minerogenic saltmarshes throughout, with varying abundances of *S. anglica*. Whilst both sites have similar high marsh vegetation to PM, the marsh at RMN has significantly less *S. anglica* than either PM or QN. These differences permit greater comparisons to be made regarding the applicability of the acid pre-treatment methods.

2.2 Sediment characteristics

2.2.1 Loss on ignition

Organic content was estimated based on loss on ignition (LOI) calculations 2cm^3 of sediment from each sampling level was weighed into a titanium crucible of known mass. Crucibles were placed in an oven at 50ºC for 48hrs and reweighed to estimate water content before transfer to a furnace at 550ºC for four hours. Samples were cooled in a desiccator and reweighed to estimate organic content as a percentage.

% Organic Matter = (A / B) x 100

LOI = (C-D) / (B-A)

Where A is the weight (g) of the post 550ºC ash and B is the weight (g) of the post 50ºC dry sample.

2.2.2 X-ray diffraction

X-ray diffraction (XRD) was conducted in the Geochemistry Laboratory at Trinity College Dublin to investigate IC phase. Approximately 0.6g of non-acidified surface sediment from each of the three sites were analysed between 2 and 55 degrees 2-theta in Al bulk cavity mounts. Results were calibrated against quartz at 26.66º and interpreted using the software package Traces v5.2.0. Identification of constituent parts and the presence/absence of IC (calcite, dolomite, ankerite and siderite) were determined. Acid rinsed samples were also analysed to investigate the removal success. Due to the reduced quantity of available sediment for acid rinsed samples, silicon-wafer mounts were used requiring around 0.05g of sediment. Acid fumigated samples were not analysed as sample sizes were too small to successfully identify mineral phases

2.3 Acid pre-treatment

On return to the laboratory, samples were dried at 40ºC for 48 hours before being ground to a fine powder using a pestle and mortar. Sediment samples were sub-divided into four replicate groups for subsequent analyses.

2.3.1 Acid Rinsing

The acid rinse pre-treatment method was conducted following the procedure of Harris *et al.* (2001), modified to include the centrifuging of sediment (as recommended by Brodie *et al.* 2011c) and a lower drying temperature. 13mL 0.5M HCl was added to 0.5g of dry, ground sediment weighed previously into a clean test tube. Samples were stirred three times over a 24-hour period and then centrifuged at 1200rpm for four minutes before the acidic supernatant was decanted and tested with litmus paper to ensure remaining acidity. The samples were rinsed by adding deionised water and stirring three times over a 24-hour period and centrifuged as before. This rinsing process was repeated a second time and on completion the remaining solid samples were dried at 40ºC overnight. Machine replicates of sediment from one acid treated sample were then weighed precisely into tin capsules (Elemental Microanalysis, 8mm x 5mm) using a high precision microbalance (Sartorius ME5, ±1μg). Individual sample masses varied between 1mg and 30mg depending on organic content (Table 2), with samples containing the lowest organic concentrations having the largest masses. Samples from PM were measured in triplicate, while RMN and QN were measured in duplicate.

2.3.2 Acid fumigation

The acid fumigation pre-treatment was conducted following the procedure of Harris *et al.* (2001). Although the original technique was recommended for samples containing less than ~50wt% $CaCO_3$ (Hedges and Stern 1984) and poor results have been reported at high carbonate concentrations (Schubert and Nielsen 2000), modifications of the technique by dampening sediment have been applied successfully to samples comprising >80wt% $CaCO_3$ with no loss of C_{org} or N_{tot} (Yamamuro and Kayanne 1995). Whilst completion of acid titration is difficult to ascertain and fumigation time can affect results (Komada *et al.* 2008) these temperate intertidal sediments tend to have relatively low carbonate concentrations and so should not be subject to this complication.

For each sample, replicates of between 1mg and 30mg were weighed precisely into silver capsules (Elemental Microanalysis, 8mm x 5mm), depending on organic content. Samples were moistened with deionised water (approx 15μL H_2O/10mg sample) to increase permeation by acid vapour and placed in a 10.5L Pyrex vacuum

desiccator with a beaker of 100mL 12M HCl. Grease (with a measured $\delta^{13}C$ value of -54‰) was applied carefully to the outer margins of the flange of a previously cleaned desiccator which was then placed under a vacuum of approximately $5x10^{-2}$ bar and left for 9 hours. Although Schubert and Nielson (2000) report contamination of samples through the use of silicon grease, they did not perform their acidification under vacuum and trials conducted during this study indicated that the presence of grease was necessary for the full retention of the vacuum during fumigation. Upon removal, samples were dried at 50ºC overnight and analysed via IRMS without reweighing.

2.3.3 Non-acidified (control) samples

At all levels of RMN and QN, and five sampling levels interspersed across the PM (02, 05, 09, 13 and 17) transect to provide indication of range of values found, sediment was analysed that did not undergo acid preparation. These samples provide a control to investigate loss of N_{tot} and change to $\delta^{15}N$ via the acid preparation techniques at changing elevations. They also provide total carbon content of samples, permitting estimation of carbonate content through comparison with acid-treated samples. Comparisons were made with acid fumigated samples to reduce the effects of C_{org} removal through acid rinsing using the equation: $wt\%CaCO_3 = 8.33 \times (\%C_{non\text{-}acidified} - \%C_{acid\text{-}fumigated})$. Carbonate content was not estimated via LOI due to the overlapping temperatures at which C_{org} and IC are released (Bisutti *et al.* 2004).

The previously determined absence of IC in the (terrestrial) sample PM02 permitted a direct comparison of pre-treatment influences on $\%C_{org}$ and $\delta^{13}C$. In addition, while the acidic supernatant for most sampling levels in PM was discarded after acid rinsing, it was retained from this level and analysed to quantify loss of C_{org} and N_{tot} (Froelich 1980). The dissolved C_{org} and N_{tot} concentrations of this acid were measured using an Elementar Q TOC (Total Organic Carbon) analyser.

2.3.4 Gravimetric correction of acidified samples

During acid rinsing, samples lost between 12% and 49% of their initial mass due to removal of IC, C_{org} and soluble material such as salts (Table 2). Correction for this mass change during acid rinsing is necessary to prevent an artificial inflation of sample $\%C_{org}$ and $\%N_{tot}$, although C/N ratios will not change. Carbon and nitrogen

concentrations of these acid rinsed samples are gravimetrically corrected to reflect the previous bulk values by a method similar to Hedges and Stern (1984) as follows:

$\%C_{org} = \%C_r * (W_f / W_o)$

$\%N_{tot} = \%N_r * (W_f / W_o)$

where: W_o = original weight of dried sample before acid addition, W_f = final weight of dried sample after acid addition and rinsing, $\%C_r$ = wt %C recorded by the mass spectrometer, and $\%N_r$ = wt %N recorded by the mass spectrometer

Acid fumigated samples were weighed directly into capsules prior to acid treatment and analysed without reweighing, so no gravimetric correction was necessary. Recording sample masses prior to acidification prevents complications arising from alterations caused by the formation of hygroscopic calcium salts ($CaCl_2.H_2O$) and silver chloride (AgCl) during the acidification process.

2.4 Isotopic and elemental analysis

The $\delta^{13}C$, $\delta^{15}N$, $\%C_{org}$ and $\%N_{tot}$ of all samples were measured simultaneously by combustion in a Flash 1112 series Elemental Analyser (EA) coupled to a Thermo Deltaplus continuous flow mass spectrometer in the Geochemistry Laboratory at Trinity College Dublin. The software package Eager-300 (Thermo Scientific) was used for performing on-line data acquisition and integration.

Combustion was facilitated with a 3 second O_2 pulse at a combustion furnace temperature of 1020ºC using Chromium sesquioxide (Cr_2O_2) as a catalyst and silvered cobaltosic oxide (Co_3O_4 + Ag) to remove halides. Carrier gas flow was set at a rate of 85 mL/min and the reduction tube was set at a temperature of 650ºC in the presence of elemental Cu.

Isotopic analysis runs were conducted with both international (IAEA-N-2, IAEA-NO-3, IAEA–CO-9 and USGS 24) and internal laboratory standards (calibrated using international standards) analysed in parallel with the samples. Isotopic ratios of standards ranged from -47‰ to -16‰ for $\delta^{13}C$ and -8‰ to +20‰ for $\delta^{15}N$. All results are reported in the standard delta (δ) notation as per mil (‰) deviations from accepted values for the international reference standards of VPDB (Vienna Pee Dee Belemnite) for $\delta^{13}C$, and air for $\delta^{15}N$ (both 0‰ by convention) as follows: $\delta = [(R_{sample} / R_{standard}) - 1] \times 10^3$, where R is the measured isotopic ratios of sample and standard respectively

(for carbon and nitrogen). The precision of analysis based on the standard deviation of these standards was <0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$. An internal standard with C_{tot} and N_{tot} concentrations of 40.44% and 15.73% respectively was used to ascertain elemental concentrations of samples to a precision of less than 0.9% for N_{tot} and 0.6% for C_{tot} (n=127).

Silver capsules were used during acid fumigation due to the rapid corrosion of tin, and the brittleness of aluminium caused by strong acid vapour. Silver capsules, unlike tin, do not react exothermically with oxygen during sample analysis. As a result, EA furnace temperature must be above the melting point of silver (962ºC) though flash combustion does not occur and combustion of encased samples is slowed with "tailing" of gas chromatographic peaks occurring. To compensate for this tailing, the time at which the reference CO_2 gas was administered was extended by 100 seconds to allow return of sample CO_2 levels to background before the reference gas was detected. As a safeguard, to ensure these silver capsules were not obstructing gas flow through the EA and causing carryover, blank analyses were inserted between sediment samples. A blank analysis was one where no sample or capsule was placed within the well of the autosampler, but the analysis was performed as normal. Sample blanks of empty capsules (silver and tin), including those subjected to the fumigation process, were also run and showed no indication of contamination, with carbon and nitrogen levels remaining below background.

One-way analysis of variance (ANOVA) followed by Tukeys test was used to assess the differences of sample preparation at level PM02 once normality and equal variance were confirmed. Paired T-tests were applied to $\delta^{15}N$ values once normality was confirmed. Results are reported as significant at the 95% confidence level with text and graphs reporting means ±1 standard deviation for samples, incorporating the accuracy of the mass spectrometer via root mean square calculations.

3 Results

3.1 Sediment characteristics

Sediment organic content increases with elevation at all three marshes. At PM, this rise is from 4% (by dry weight) in the tidal flats, to nearly 90% in uppermost samples

above the marine limit (Table 2). The upper marsh is therefore classified as organogenic (sensu Allen 1990). The carbonate content of the sediments is low throughout, never contributing more than 10% of the sediment (by dry weight).

Table 2: Relevant characteristics of sampling levels. Organic content is based on loss on ignition. Loss of mass calculated on dry masses before and after acidification. $CaCO_3$ concentrations are based on difference in %C of non-acidified and acid fumigated samples; n=2, wt%$CaCO_3$ = 8.33x ($TC-C_{org}$).

Saltmarsh	Environment	Sampling Level	Altitude (m OD Malin))	SWLI	Organic Content (wt %)	Loss of mass via acid rinsing (wt %)	$CaCO_3$ Content (wt %)
Portmarnock	Terrestrial	1	2.06	226	-	23	-
		2	1.80	217	87	13	0
	High Marsh	3	1.36	192	77	15	-
		4	0.82	162	19	21	-
		5	0.67	154	15	18	8
	Low Marsh	6	0.68	154	12	19	-
		7	0.59	149	12	19	-
		8	0.50	144	6	19	-
		9	0.40	139	10	18	10
		10	0.30	133	9	16	-
		11	0.20	128	7	15	-
		12	0.09	122	7	15	-
	Tidal Flats	13	-0.06	113	5	13	9
		14	-0.16	108	8	16	-
		15	-0.25	103	5	12	-
		16	-0.35	97	5	13	-
		17	-0.43	93	4	15	10
Ringmoylan	Terrestrial	1	3.25	217	20	16	7
		2	2.67	195	16	31	18
		3	2.45	187	16	29	21
		4	2.39	185	19	41	20
	High Marsh	5	2.55	191	17	27	20
		6	2.59	192	22	28	16
		7	2.31	182	12	31	34
		8	1.95	169	13	28	22
	Low Marsh	9	1.54	154	11	32	24
		10	1.49	152	9	34	29
	Tidal Flats	11	1.28	144	9	35	28
		12	1.11	138	10	34	25
Querrin		1	2.79	235	42	14	5
	Terrestrial	2	2.51	222	42	23	1
		3	2.29	213	58	15	13
		4	2.07	203	19	17	3
		5	1.78	190	19	17	19
	High Marsh	6	1.58	182	17	18	5
		7	1.26	167	25	25	4

Low Marsh	8	1.04	158	16	26	9
	9	1.00	156	15	21	5
	10	0.98	155	15	23	11
	11	0.63	140	6	39	30
Tidal Flats	12	0.61	139	5	39	34
	13	0.48	133	15	42	34
	14	-0.46	92	3	49	32

Both Shannon sites exhibit variations from PM, with significantly lower organic contents of marshes and higher carbonate content of tidal flats. Below the marsh, the tidal flat sediments of both RMN and QN are organic poor and therefore minerogenic (9% and 8% respectively) and have high carbonate content (27% and 33%). On average, the supratidal and vegetated marsh sediments at the inner estuary site of RMN have lower organic contents (16%), and higher carbonate contents (20%) than the corresponding samples at the outer estuary site of QN (25% and 10% respectively).

3.2 XRD composition

The minerals of quartz, feldspar, mica, chlorite and halite were found across all levels at all three sites. Gypsum (main peak at 11.59º 2θ) was found only at PM on the lower levels below PM7 (<0.59m OD). While peak analysis did indicate occurrence of calcite within the PM, RMN and QN samples (main peak at 29.43º 2θ), dolomite, ankerite and siderite were not present, or were below detection level (<1% of sample mass). No IC was found in acid-rinsed samples, indicating successful removal during acidification.

3.3 Sample carryover

Blank analyses run after sediment samples indicated the presence of carryover following analysis of certain acid fumigated samples from PM, but not from either RMN or QN. This carryover was largely restricted to sediment samples from level PM7 and below (from the tidal flats) with 17 of these 21 triplicates exhibiting carryover. Chromatographic peak CO_2 areas of carryover were compared to original sample peak areas to investigate the scale of the carryover. The peak area of carryover was 2.40±1.05% of the previous sample and the results reported below are corrected for this loss within the means and errors reported. Tidal flat samples contained less than 2% C_{org} and given the relatively homogenous input of OM at these low levels,

the loss of such limited amounts of low organic material during analysis is not considered to have affected isotopic ratios significantly for these samples. XRD failed to identify specific compounds that could result in carryover and so the exact cause of this localised phenomenon was never fully resolved. As gypsum was identified in (and restricted to) these samples, it is possible that H_2SO_3 formation through its combustion could affect sample OM combustion. However, other possibilities include: unidentified contaminants within the sediment; the increased adherence of organic compounds to clay minerals; or the absence of flash combustion causing refractive carbon moieties to remain in the combustion furnace.

3.4 Elemental analyses ($\%C_{org}$, $\%N_{tot}$)

The results of the bulk $\%C_{org}$ and $\%N_{tot}$ analyses are summarised by site and pre-treatment method in Figures 3a and 3b, with the calculated C/N ratio plotted in Figure 3c. A clear, height-related trend is apparent in the data at each site, with $\%C_{org}$, $\%N_{tot}$ and C/N increasing as sampling moves from the unvegetated tidal flats, through the vegetated marsh surface and into the terrestrial soils above the upper limit of marine influence. Whilst there are offsets between pre-treatment methods, their magnitude is predominantly small in comparison to the range of values experienced across the intertidal zone, and any deviations do not obscure the overall trends in the data. A single sample from the tidal flat at QN (QN1) plots as an extreme outlier in Figure 3c. The anomalous behaviour of this sample evident in Figure 4a, and the plot of $\delta^{13}C$ (Figure 6a) is best explained as arising from the incomplete removal of IC associated with the acid fumigation pre-treatment. In this instance, both acid fumigated replicates indicate incomplete removal of IC from this sample.

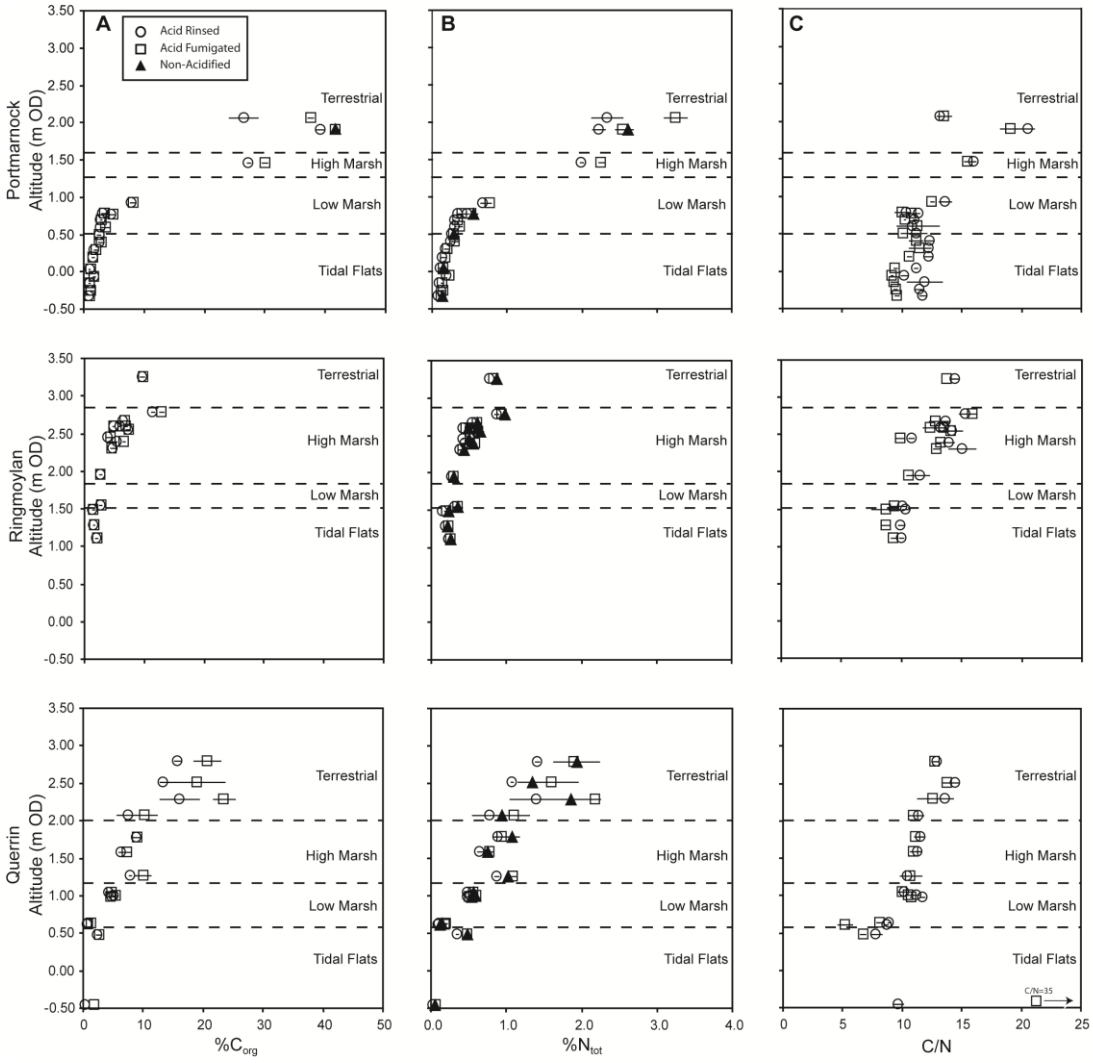

Figure 3: Mean elemental results of sediment samples (acid rinsed, open circles; acid fumigated, open squares; non-acidified, solid triangles). (A) C_{org} concentrations; (B) N_{tot} concentrations; (C) C/N ratios. Scales identical across each row and column. Error bars ±1s.d.; n=3 for PM replicates (n=2 for non-acidified), n=2 for RMN and QN. Altitudes relative to OD Malin and marsh zonations indicated.

To further explore the patterns in the elemental data, we combine the results from all sites after normalising the altitude values to account for differences in tidal range using the SWLI methodology. The $\%C_{org}$ data are replotted in Figure 4a as the percentage difference between the acid rinsed and acid fumigated samples. It is evident that at each site, acid rinsed samples show a greater reduction in C_{org} than their acid fumigated counterparts, with mean differences of 9%, 10% and 23% for the

tidal flats, saltmarsh and supratidal zones respectively, after removal of the outlier from the tidal flat at QN.

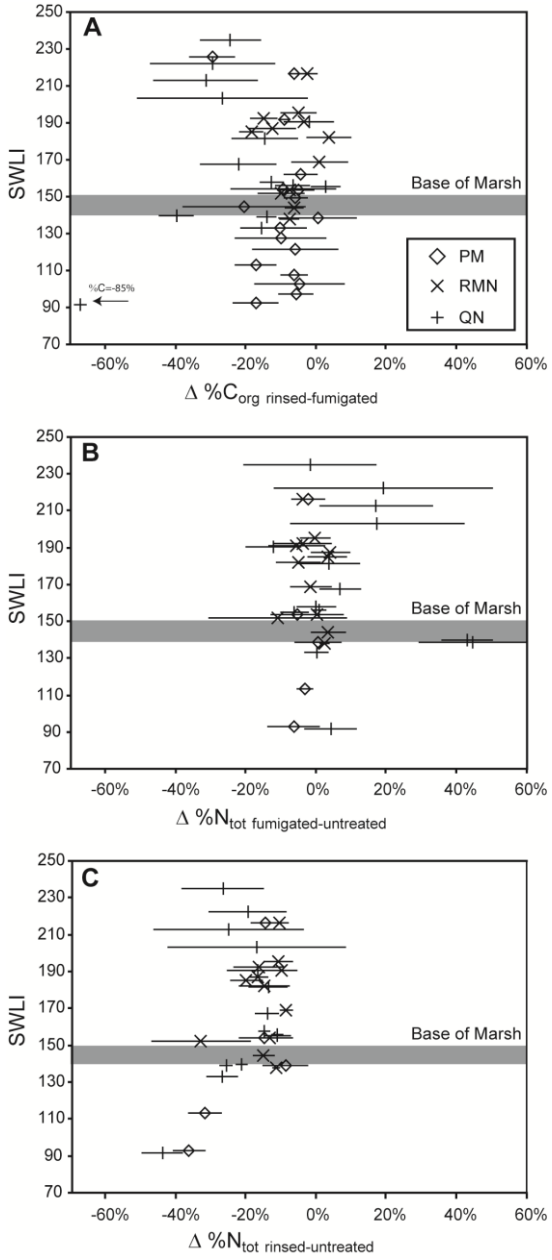

Figure 4: Mean (±1s.d.) difference of elemental compositions normalised to tidal range (PM = open diamonds, RMN = diagonal crosses; QN = crosses) (A) C_{org} of acid rinsed samples compared to fumigation; (B) N_{tot} of acid fumigated compared to non-acidified. (C) N_{tot} of acid rinsed and compared to non-acidified. Grey box designates range of SWLI values for transition between low marsh and unvegetated tidal flats for the three sampling sites.

The N_{tot} data are replotted in Figures 4b and 4c as the percentage difference between the acid pre-treated samples and their non-acidified counterparts. Once again, losses associated with acid rinsing are observed, averaging 26%, 15% and 19% for samples from the tidal flats, saltmarsh and supratidal zones respectively (Figure 4c). Whilst the percentage loss of N_{tot} associated with acid rinsing appears greatest in the tidal flat sediments, no such trend is apparent in the acid fumigated samples (Figure 4b). In fact, acid fumigated samples exhibited an apparent gain in N_{tot} of 4%, 1% and 8% in the tidal flats, saltmarsh and supratidal zones respectively although this is extremely small when considered in light of the low concentrations of N_{tot} and the overall variability in the data.

It should be noted that the size of the error bars in Figures 4b and 4c reflects to some extent the relatively high degree of variability apparent in some of the non-acidified samples against which the deviations associated with each pre-treatment method are expressed. This is particularly the case in the four terrestrial samples from QN, plus the two samples at the interface between the low marsh and tidal flats at the same site. This is possibly linked to sample heterogeneity which could indicate variable presence of plant fragments from this site.

The net result of these analyses is that for the vegetated marsh and the supratidal areas, the C/N ratios produced by both methods are essentially indistinguishable (Figure 3c) since acid rinsing promotes similar losses in C_{org} and N_{tot} whilst acid fumigation appears conservative for both. This situation changes for the lower elevation, intertidal flat samples however since the excessive loss of N_{tot} associated with acid rinsing results in elevated C/N ratios when compared to the replicates pre-treated by acid fumigation.

3.5 Isotopic analyses ($\delta^{13}C$ and $\delta^{15}N$)

The results of the $\delta^{13}C$ and $\delta^{15}N$ analyses are summarised by site and pre-treatment method in Figure 5. In the two sites from the Shannon estuary (RMN and QN), the $\delta^{13}C$ data exhibit a trend toward ^{13}C depletion with increasing height in the intertidal zone, although the data scatter at QN makes this pattern more equivocal (Figure 5a). In contrast, at the PM site, samples become increasingly enriched in ^{13}C moving from the tidal flat to low saltmarsh, before showing an abrupt shift to depleted values in the high marsh to supratidal environments. This anomalous pattern is due to the

dominance of the non-native C_4 plant *S. anglica* in the lower marsh at PM. Once again, when compared to the overall shifts in $\delta^{13}C$ values across the sites (10.6‰, 4.4‰ and 9.4‰ for PM, RMN and QN respectively), the differences between samples pre-treated by acid rinsing and acid fumigation are comparatively minor and do not materially alter the dominant patterns of change.

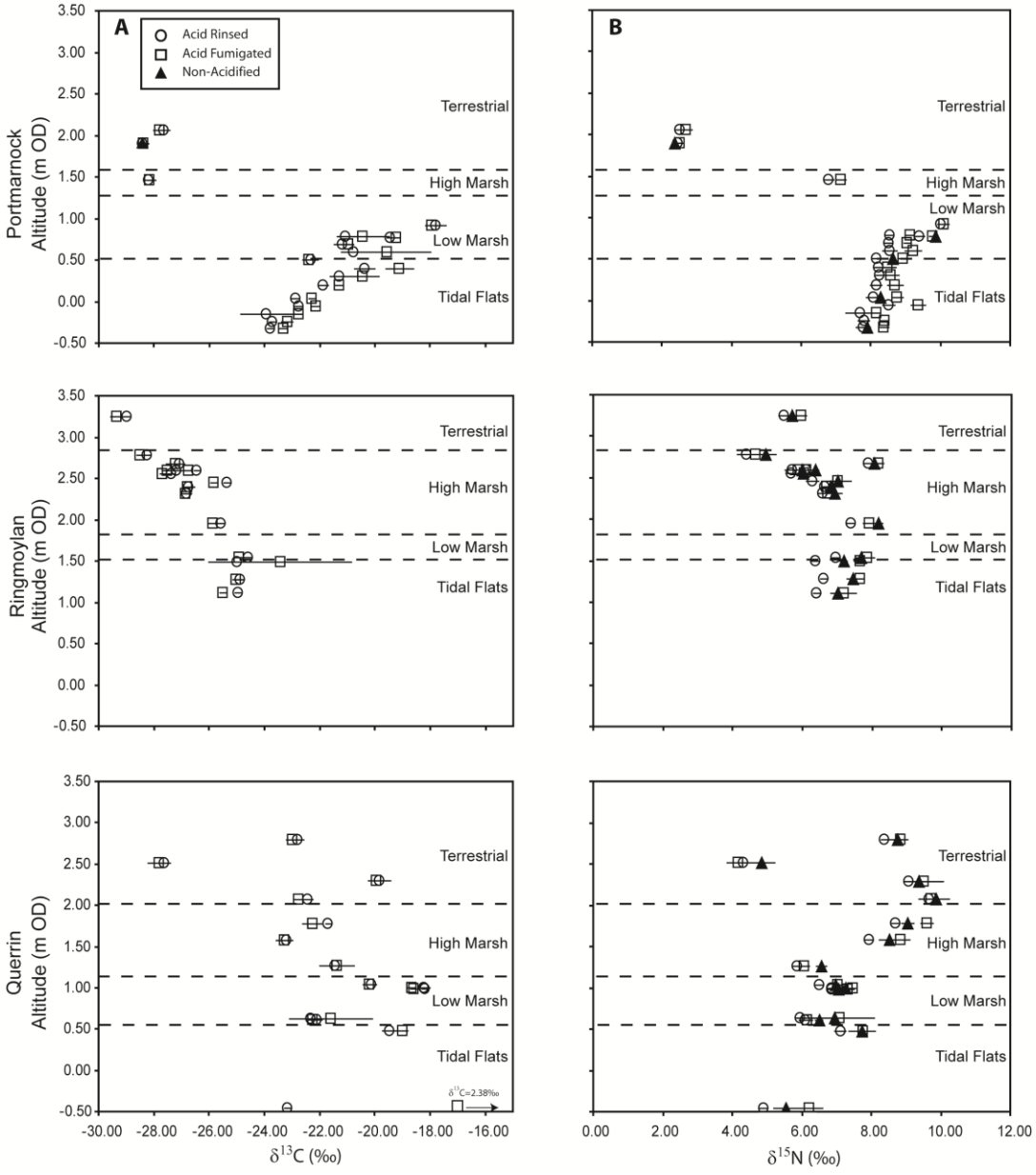

Figure 5: Mean isotopic results of acid treated sediment samples: (A) $\delta^{13}C$ values and (B) $\delta^{15}N$ values. Symbols defined in Figure 3.

In contrast to the other variables, the $\delta^{15}N$ values show much less systematic behaviour, both within and between sites. Whilst there is some indication for depletion of ^{15}N in the higher marsh to supratidal contexts of PM and RMN, any vertical relationship is weak and is not mirrored at QN (Figure 5b). The generally smaller range of $\delta^{15}N$ values means that the offsets between pre-treatment methods are larger in a relative sense.

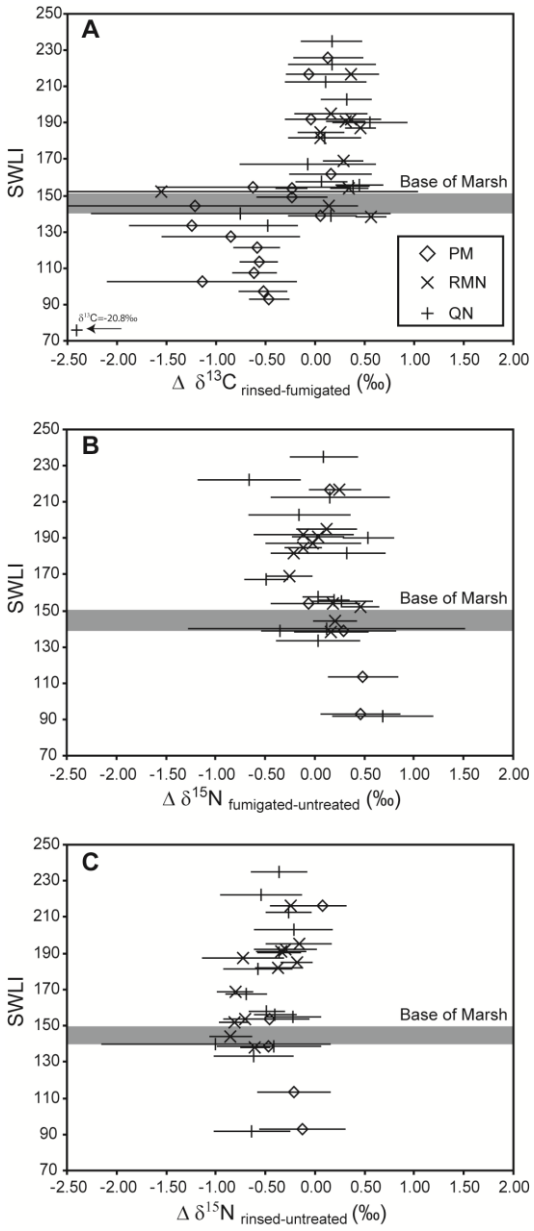

Figure 6: Mean differences (±1s.d.) of isotopic compositions normalised to tidal range: (A) $\delta^{13}C$ of acid rinsed samples compared to fumigation; (B) $\delta^{15}N$ of acid fumigated compared to non-acidified; (C) $\delta^{15}N$ of acid rinsed compared to non-acidified. Symbols defined in Figure 4.

Figure 6 presents the isotopic data replotted following SWLI normalisation of sample elevation. Once again, $\delta^{13}C$ values are expressed as the difference between the acid rinsed and acid fumigated samples (Figure 6a) whilst $\delta^{15}N$ is plotted as the deviation from the corresponding non-acidified control sample produced by acid fumigation (Figure 6b) or acid rinsing (Figure 6c).

In the vegetated marsh to supratidal environments at all sites, there is no clear difference in $\delta^{13}C$ values of acid rinsed versus acid fumigated samples (Figure 6a). However, as sampling moves onto the lower elevation, un-vegetated tidal flats, samples pre-treated by acid rinsing become depleted in ^{13}C by about 1‰ relative to their acid fumigated counterparts. A slight caveat to this general result is that the bulk of the lowest elevation data come from the tidal flats at PM.

In contrast to the height-related trend identified in $\delta^{13}C$ values, no systematic departures are noted in the $\delta^{15}N$ values produced by either of the pre-treatment methods. Samples pre-treated by acid fumigation show no significant change from the un-treated control samples ($T=-1.69$, $p=0.102$, $n=31$) (Figure 6b) while acid rinsing results in a minor depletion of 0.45±0.27‰ ($T=10.12$, $p<0.001$, $n=31$) (Figure 6c).

3.6 Non-calcareous soil

The results of the replicate analyses performed on the non-calcareous terrestrial soil sample from PM (Table 3) show that whilst acid rinsing causes a loss of 6.4±1.7% C_{org} compared to the control, the C_{org} content of the acid fumigated samples are unaltered ($F=51.38$, $p<0.001$, $n=9$). Analysis of the acid supernatant from the acid rinsing process revealed it contained 1593mg/L DOC. This corresponds to approximately 6% C_{org} of the sediment sample and is therefore in good agreement with the loss inferred from elemental analysis.

Table 3: Results of acid preparation techniques on the elemental and isotopic N_{tot} and C_{org} in a non-calcareous terrestrial soil from PM 02. Mean ± s.d.; n=3.

Acid Treatment	wt % C_{org}	wt % N_{tot}	$\delta^{13}C$	$\delta^{15}N$
Non-Acid	41.86 ± 0.34	2.62 ± 0.07	-28.44 ± 0.11	2.38 ± 0.16
Acid Fume	41.81 ± 0.41	2.55 ± 0.10	-28.37 ± 0.17	2.53 ± 0.06
Acid Rinse	39.17 ± 0.57	2.23 ± 0.09	-28.43 ± 0.12	2.45 ± 0.12

Similarly, acid rinsing causes a significant reduction in N_{tot} of 14.5±3.7% when compared to the untreated control ($F=19.83$, $p=0.02$, $n=9$) with no significant loss of nitrogen through acid fumigation. Analysis of the acid supernatant indicated a 4% loss of N_{tot} through acid rinsing. Low concentrations of nitrogen present in the samples could lead to the discrepancy between these two results. Although elemental concentrations vary, isotopic ratios exhibit no significant difference between acid preparation methods ($F=0.39$, $p=0.692$ for carbon; $F=1.49$, $p=0.298$ for nitrogen; $n=9$).

4 Discussion

4.1 Assessing the influence of acid rinsing on elemental and isotopic signatures

The pre-treatment method of acid rinsing has previously been identified as causing loss of both C_{org} and N_{tot} with varying degree of loss depending on the OM source (Yamamuro and Kayanne 1995; Midwood and Boutton 1998; Harris *et al.* 2001). Our results support these findings with acid rinsed samples being depleted in both C_{org} and N_{tot} when compared to their acid fumigated and non-acidified counterparts. The extent of C_{org} depletion associated with acid rinsing is not correlated with elevation (and hence to changing organic input). In contrast, the loss of N_{tot} is most pronounced in the lower marsh and tidal flats, particularly at PM and QN. This could indicate that the nitrogen fraction deposited at low elevations is more labile than that of higher elevations.

Whilst acid rinsing depletes C_{org} and N_{tot}, its impact on isotopic signatures is much less clear. Acid soluble fractions of C_{org} have been shown to have less negative $\delta^{13}C$ signatures than insoluble fractions (Hwang and Druffel 2003) which upon removal would result in lower isotopic values for the remaining bulk sediment. For example, Harris *et al.* (2001) report a reduction of 0.09 – 0.20‰ associated with acid rinsing in their analysis of calcareous soils. In contrast, in their study of terrestrial soils, Midwood and Boutton (1998) detect no change to $\delta^{13}C$ values associated with acid rinsing while Brodie *et al.* (2011a) found varying fractionation effects depending on the type of sample under investigation and the laboratory protocol employed.

Our analyses of intertidal (estuarine) and supratidal (terrestrial soil) samples indicates that the impact of acid rinsing on $\delta^{13}C$ values varies with environment / sediment type. Acid rinsed low elevation samples from the unvegetated tidal flats are depleted in ^{13}C with respect to their acid fumigated counterparts, possibly indicating increased removal of acid soluble C_{org}. In contrast, in the vegetated marsh to upland zones, there is no clear indication of such depletion. In fact it is possible that slight enrichment may occur although any shift is small and of comparable magnitude to the uncertainties inherent in the data. Thus, it appears to be the POC fraction, deposited in greatest quantities on tidal flats (Lamb *et al.* 2006) that is more labile with respect to acid treatment, while plant OM is more conservative.

Whilst $\delta^{15}N$ values are also altered by acid rinsing, no sample or environment-specific trend is discernible from our data. Instead, there is an overall depletion in ^{15}N of around 0.5‰ in samples from all sites and altitudes, although $\delta^{15}N$ values are not correlated with N_{tot} loss.

4.2 Does acid rinsing lead to erroneous environmental interpretations related to sea level change?

Saltmarsh sediments receive inputs of OM from both terrestrial and marine end-members in addition to the autochthonous material supplied from vertically-zoned saltmarsh plants. Consequently, shifts in the C/N and $\delta^{13}C$ values of bulk sediment samples reflect elevation-related variations in the relative contributions of these OM sources. These elevation-related signatures can be exploited by scientists interested in reconstructing past RSL in regions without historic occurence of C_4 plants since they permit increases or decreases in marine influence to be determined from suites of sediment samples (see Wilson *et al.* 2005a, 2005b for details). In this semi-quantitative approach, the identification of within-core trends is more significant than the precise determination of a particular isotopic or elemental value at a discrete sampling point. Of special interest are the trends across lithostratigraphic contacts between intertidal, minerogenic sediments and supratidal, organogenic deposits, since these are commonly used to establish sea level index points which pinpoint the former position of RSL in time and space (Shennan and Horton 2002; Edwards 2007). These lithological boundaries record the onset or removal of marine conditions but require supporting palaeoenvironmental data to confirm the nature of the transition.

In light of the above, the key question when assessing the significance of an acid pre-treatment approach is whether or not it exerts a non-systematic influence on isotopic or elemental signatures that is of sufficient magnitude to distort or conceal the environmentally-related trends in a dataset. Of particular concern is whether the pronounced changes in lithology and / or source of OM typically encountered in intertidal contexts may induce such non-systematic behaviour.

The results of our analyses indicate that the choice of acid pre-treatment method does influence the elemental and isotopic signatures extracted from coastal deposits and, in the case of N_{tot} and $\delta^{13}C$, this influence varies with sediment type / vertical position in the tidal frame. However, when compared with the range of values experienced across the transition from intertidal to supratidal environment, these distortions are comparatively small and do not conceal the dominant (diagnostic) trends within the data. This result holds across all the sites investigated, despite the fact that they were selected to provide contrasting sets of environmental conditions, encompassing differences in tidal range, estuary / catchment size, location within the estuarine system, and floral / sedimentological character. The lack of significant bias in $\delta^{13}C$ between pre-treatments also indicates that in regions where C_3 and C_4 plants have historically co-existed, acid pre-treatment choice similar to those described here should not alter conclusions based on isotopic signatures due to the large variations between these two end-members.

Combined $\delta^{13}C$ values and C/N ratios have been used in many studies to indicate OM provenance (e.g. Wilson *et al.* 2005b; Lamb *et al.* 2006; Lamb *et al.* 2007; Mackie *et al.* 2007). Brodie *et al.* (2011a) caution that this approach is susceptible to errors introduced during acid pre-treatment, particularly in relation to the C/N ratio. Figure 7 presents summary carbon biplots for each of the study sites and reiterates the overall similarity in the dominant trends between locations and the limited impact that pre-treatment method has on these patterns. Although low elevation acid rinsed samples exhibit higher C/N ratios than their acid fumigated counterparts, these distortions are not sufficient to obscure the provenance of OM when used in combination with the $\delta^{13}C$ values. Across all the sites, the range of C/N and $\delta^{13}C$ values encountered is broadly similar, although there is a notable shift toward more depleted $\delta^{13}C$ in the inner estuary site of RMN, suggesting significant inputs of freshwater POC from the large Shannon catchment.

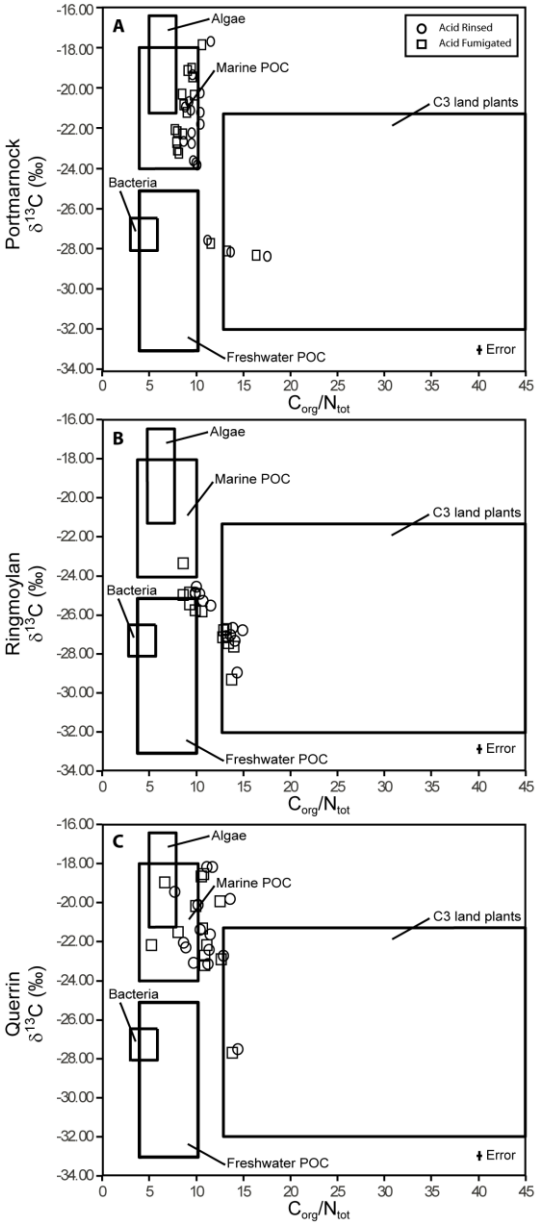

Figure 7: Carbon biplots of $\delta^{13}C$ and C/N values including main OM sources to C_3 saltmarshes. Mean values of acid treated data presented in Figures 3 and 5 for PM (A), RMN (B), QN (C). Error is ± 1s.d. Symbols defined in Figure 3.

In summary, our analyses provide no indication that the employed acid pre-treatment method of acid rinsing (using 0.5M HCl) significantly biases either C/N or $\delta^{13}C$ values to the extent that erroneous interpretations regarding the onset or removal or marine conditions associated with lithological contacts may arise.

However, acid-rinsing does influence both C/N and $\delta^{13}C$ values, particularly in the low elevation, intertidal flats where POC may make up a large constituent of OM. Consequently, any approach that relies on distilling precise, subtle variations in geochemical signatures will need to take steps to minimise or correct for this influence. In the temperate intertidal sediments analysed here, the main distorting influence on C/N was the N_{tot} loss during acid rinsing (Figure 4c) reflecting in part the very small initial N_{tot} concentration of the material (<1.0% by dry weight). Whilst the simultaneous measurement of C, N, $\delta^{13}C$ and $\delta^{15}N$ is an efficient and common approach, separate analysis of N_{tot} using non-acidified samples may be advisable for the kinds of low N_{tot} material analysed here, although the possible influence of inorganic N should also be assessed (see Brodie *et al.* 2011b). Alternatively, a different method of pre-treatment, such as acid fumigation (discussed below), or *in-situ* acidification may be appropriate in these circumstance, although each approach has its own set of limitations (e.g. Brodie *et al.* 2011a).

4.3 A comment on acid fumigation as a pre-treatment method for coastal sediments

Previous studies on coastal sediments and calcareous soils indicate that acid fumigation minimises loss or C_{org} and N_{tot} and does not substantially modify $\delta^{13}C$ values (Harris *et al.* 2001; Komada *et al.* 2008). A comparison of the results from acid fumigated versus non-acidified samples presented here provides general support for the essentially conservative nature of acid fumigation, at least within the limits of our available data and their associated precision. On average, both the N_{tot} and $\delta^{15}N$ values of acid fumigated samples are comparable to their non-acidified counterparts, with the analysis of the non-calcareous soil showing C_{org} to be unchanged by acid fumigation. Whilst it is impossible to rule out some loss of C_{org} from the other IC-bearing samples, the fact that acid rinsing consistently produces lower $\%C_{org}$ values than acid fumigation indicates that the latter pre-treatment is more conservative than the former.

We also find no evidence of unsystematic or irreproducible results in samples pre-treated by acid fumigation. Of the 103 individual machine replicates analysed, only four produced spurious results, leading to the large standard deviations associated with sampling levels PM10, RMN3, QN4 and QN1. In their comparative study, Brodie *et al.* (2011a) report the results of acid fumigation to be the least reproducible

of the pre-treatment methods they evaluated. This discrepancy could be linked to the kinds of sample material being analysed. Whilst Brodie *et al.* (2011a) found acid fumigation pre-treatments were associated with the most variable results of all the techniques they examined they did not use coastal sediments and alternative studies have shown acid fumigation to be more precise than other methods when dealing with these sediments (Komada *et al.* 2008). However, the discrepancy may also reflect the range of acid strengths and the use of both silver and tin capsules examined by Brodie *et al.* (2011a). In fact, if consideration is limited to the acid concentration / capsule combination we employ here (12M HCl and silver capsules) the repeatability of results and the resulting precision reported in Brodie *et al.* (2011a) is at least equal to that of other pre-treatment methods (their Figure 2), particularly for the sediments that most closely resemble the coastal material we consider here. Similarly, in their carbon biplots, acid fumigation produces results that consistently plot close to, or overlap with, the known value of the material being analysed (their Figure 6).

Whilst the acid fumigation approach may help to minimise loss of C_{org} and N_{tot} and distortion of isotopic values, the technique is associated with several practical limitations beyond the difficulty of verifying that IC has been fully removed before analysis. For example, acid fumigation is only applicable for EAs operating at temperatures >1000ºC due to the lack of flash combustion and the requirement for furnace temperature to be above the melting point of silver (962ºC). As observed here, even at temperatures above this, slow combustion of OM occurs that can result in sample gas carryover. Additional test analysis revealed that wrapping silver capsules in tin before combustion at 950ºC still resulted in carryover (Craven 2013). In addition, hygroscopic salts in the form of $CaCl_2$ are formed during the acidification process and introduced to the EA during analysis, which can be detrimental to the machine. When coupled with the fact that EA combustion columns and chemicals must be replaced at shorter intervals as a result of silver capsules accumulating and reducing gas flows, this increases the cost of each analysis. Therefore, the most precise method might not necessarily be the most desired method; but the chosen pre-treatment methodology should be always be considered in the context of each study.

5 Summary and Conclusions

Analysis of coastal sediments from temperate organogenic and minerogenic saltmarshes in Ireland indicates that variations in C/N and $\delta^{13}C$ can arise as a consequence of sample pre-treatment method. However, the magnitude of these differences is small when compared with the range of values typically encountered across the inter-tidal to supra-tidal environments of our study sites (C/N ranges from 4-9 and $\delta^{13}C$ ranges 3-6‰, compared to mean deviations due to acid pre-treatment method of up to 0.8 for C/N and 0.3‰ for $\delta^{13}C$). While isotopic results may vary slightly from true values in the rinsing process, the RSL-focused applications require the determination of relative trends, and not absolute values. Consequently, any bias associated with the choice of acid pre-treatment is not sufficient to obscure source provenance where C_3 plants dominant, and especially where C_3 and C_4 plants exist together. In addition, the use of tin capsules (for acid rinsing, the other methods require silver capsules on account of the concentrated HCl) permit improved sample combustion for analysis and the rinsing process removes halides that can be detrimental to the mass spectrometer, and allow more samples to be run before the chemicals used in combustion and reduction have to be replaced, thus reducing the cost of sample analysis. Therefore, the acid rinse methodology is demonstrated as being suitable for use in RSL contexts with these sediments.

It is possible that bias could become significant at sites where C/N or $\delta^{13}C$ ranges are much smaller than those associated with our study sites, or if subtle variations in signatures are the target of investigation. Under such circumstances, it would be prudent to conduct site-specific assessments of the possible influence of pre-treatment method, although we support the recommendation of Brodie *et al.* (2011c) which advocates a focus on significant shifts or trends in bulk elemental/isotopic values rather than interpretations based on the 'minutiae' of such data.

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