

Molecular and bulk geochemical proxies in sediments from the Conwy Estuary, UK

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ABSTRACT

Owing to their restricted riverine flow and greater numbers, small estuaries (annual discharge < 2000 m³ s⁻¹) are important sites of organic carbon (OC) storage in sediments. The Conwy Estuary is an exemplar small rural-agricultural catchment which delivers a diverse pool of terrestrial OC to the Irish Sea. Thirty-nine surface sediments, covering a distance of 10.5 km, were taken transversal as well as longitudinal to the estuary to examine local and distant catchment inputs. Biomarkers (branched Glycerol Dialkyl Glycerol Tetraethers - brGDGTs, *n*-alkanes) and molecular and bulk proxies (BIT, P_{aq} , TAR, $\delta^{13}C$, C/N), showed sediments were dominated by terrestrial inputs, with only sediments of the three most seaward sites showing stronger physical and geochemical characteristics of a marine influence (e.g., sandier sediments and lower BIT). Biomarker concentrations in the Conwy were comparable to the more urbanised Thames and the Clyde estuaries. The three estuaries showed similar fractional abundance of the brGDGTs, recording an inverse trend between the hexamethylated and the tetramethylated brGDGTs, and showing the pentamethylated brGDGTs as the dominant group. A systematic decrease in terrestrial biomarkers from bank to the main channel confirmed either a strong localised erosion/runoff process or a decrease in water velocity leading to a higher deposition.

Keywords: Biomarkers, brGDGTs, *n*-Alkanes, Stable isotopes, $\delta^{13}C$, C/N, sediments

1. Introduction

Rivers and estuaries transport vast amounts of carbon from land to oceans and play an important role in the global carbon cycle through their high rates of carbon respiration and sequestration (Galy et al., 2015). The amount, origin and fate of carbon transported globally

through rivers and estuaries has been the subject of numerous studies (e.g., Schlesinger and Melack, 1981; Galy et al., 2015). The majority of these investigations have focused on large river-estuaries such as the Amazon (Kim et al., 2012). However, others have argued that small river-estuaries also affect the global carbon cycle and that their importance has gone unrecognised even though they are more numerous than their high-volume counterparts (Coynel et al., 2005; Hotchkiss et al., 2015). Consequently, if the sedimentary transfer of carbon from land to open ocean is to ever be fully understood, there is a need to track carbon flows and also quantify carbon stored in smaller river-estuaries.

This study focuses on the application of molecular and bulk geochemical proxies in sediments of a small estuary system, the Conwy estuary, located in the north-west of Wales, UK. It is the third largest river that discharges into the Irish Sea with a catchment area of 678 km², a mean annual discharge of 20 m³ s⁻¹ (range 0.5 to 500 m³ s⁻¹ due to seasonal variation) and a spring tidal range of 7 m (Mudge and Norris, 1997). In total, the main channel covers a distance of 55 km crossing blanket bogs, grazing lands, conifer plantations, extensive flood plains, several towns and meeting the tidal limit at approximately 20 km inland from the estuary mouth (Mudge and Norris, 1997). The estuary is of national importance supporting tourism and commercial fisheries. It is also of international importance (Aber Afon Conwy) being a site of Special Scientific Interest, GB due to its exemplar terrestrial and marine biology. The sediment and water quality of this estuary has been widely reported. For example toxic heavy metals and organic sewage pollution have been previously identified in its sediments (Elderfield et al., 1979; Mudge and Norris, 1997; Zhou et al., 2003; Bussi et al., 2017). However, the source and fate of natural organic matter within the Conwy catchment has not been reported.

Biomarkers, whose structures can be traced to specific biological sources, together with

the bulk parameters have been used to successfully track the origin and fate of organic carbon in estuarine sediments (Lopes dos Santos and Vane, 2016; 2019). Bulk organic parameters, such as C/N and stable carbon isotopes ($\delta^{13}\text{C}$) have been routinely applied to track carbon in estuarine environments (e.g., Bristow et al., 2013; Milker et al., 2015). However, the widespread range of potential end-member compositions and preferential remineralisation of N relative to C can confound the interpretation of these data (Khan et al., 2015). These complications can be overcome by the complementary use of source-specific biomarkers. One group of biomarkers commonly applied to track the source of organic carbon in estuaries is *n*-alkanes, derived from epicuticular waxes present in vascular plant leaves (Eglinton and Hamilton, 1963). Major *n*-alkanes from higher plants range from C_{27} to C_{33} while typical *n*-alkanes from algae and aquatic plants range from C_{15} to C_{25} (Bourbonniere and Meyers, 1996). The proxy ratio (P_{aq}) and the terrigenous/aquatic ratio (TAR), that expresses the relative proportion of mid/short-chain length to long-chain length of the *n*-alkanes in a particular soil or sediment can therefore be used to indicate vegetation source (Bourbonniere and Meyers, 1996; Ficken et al., 2000). However, *n*-alkane distributions are prone to bias from petroleum contamination that is often present in urban river-estuaries (Vane et al., 2019).

Another source-specific group of biomarkers used to trace the terrestrial input into aquatic environments are the branched glycerol dialkyl glycerol tetraethers (brGDGTs) (Hopmans et al., 2004). These compounds are ubiquitous, but are generally found at higher abundance in soil and peat compared to marine sediments (Tierney et al., 2010; De Jonge et al., 2014a; 2015; Sinninghe Damsté, 2016; Xiao et al., 2016; Freymond et al., 2017; Kirkels et al., 2020). It has been suggested that they are produced by soil anaerobic and heterotrophic bacteria with the phylum Acidobacteria as a likely producer (Weijers et al., 2006a; Sinninghe Damsté et

al., 2014; 2018). BrGDGTs and the “Branched and Isoprenoid Tetraether” index (BIT index: ratio between brGDGTs mainly derived from soil bacteria and the isoprenoid GDGT, crenarchaeol, mainly produced by aquatic Thaumarchaeota; Hopmans et al., 2004) has been previously applied in large river systems such as the Amazon to trace soil organic carbon input (Kim et al., 2012). More recently, this proxy has been applied in small estuaries (with annual discharge $< 2000 \text{ m}^3 \text{ s}^{-1}$) in the UK (Lopes dos Santos and Vane, 2016; 2019). In the Clyde estuary (Scotland, UK), brGDGT concentrations and BIT index successfully tracked the distribution of soil organic matter in the river over a distance of 100 km (Lopes dos Santos and Vane, 2019). Similarly, brGDGT concentrations in the Thames estuary (England, UK), tracked soil organic carbon distribution but the BIT index was disrupted by elevated anthropogenic inputs in and around London that sourced allochthonous crenarchaeol to the estuary (Lopes dos Santos and Vane, 2016).

This study aims: (1) to characterise the type of terrestrial OC entering the Conwy estuary by identifying and quantifying the amount of lipid biomarkers and to compare these results to other similar size river-estuaries, (2) to compare these molecular proxies to bulk proxies such as TOC, $\delta^{13}\text{C}$ and C/N to assess the changes in the contribution of marine versus terrestrial OC along the length of the estuary, (3) to determine which variables and components are most representative of the chemical distribution of Conwy sediments and (4) to access changes in the OC distribution between different transverses (bank to channel to bank) of the estuary.

2. Material and methods

2.1. Sample collection and grain size analyses

Conwy estuary surface sediments (~10 cm depth) were collected in August 2017 (Fig. 1).

The sites were accessed by the vessel “*Four Reasons*” using pre-determined GPS co-ordinates to accurately locate each position ± 3 m. Thirty-nine samples were collected, at each site 3×2 L Van-Veen grabs were combined in a bucket and the contents transferred to a polyethylene bag. The samples were immediately stored in a coolbox at ~ 4 °C and transported back to the laboratory where they were frozen at ~ -20 °C. All samples were then freeze-dried, and a representative sub-sample stored for grain size testing whilst the remainder was sieved through a mesh aperture of 2 mm and the < 2 mm fraction ground to a fine powder for geochemical analyses (Vane et al., 2007).

Grain size analysis was performed using a Malvern Mastersizer 2000. Each sediment was placed in a beaker with 20 ml H₂O and stirred for 2 min to achieve a vortex depth in the liquid of approximately 50% of the liquid level at the beaker wall. The suspension (0.5 ml) was transferred by glass pipette and analysed using either a HydroS with pump/stir speed of 2700 rpm or a HydroG with pump speed 1600 rpm and stir speed 700 rpm. In order to provide the absolute particle size, organic material was not removed. The proportions of particles at each size class (100 groups, from 0.1 μm to 1000 μm) were calculated using the Fraunhofer model and further grouped in Excel using established size term categories (e.g., clay-silt-sand; Folk and Ward, 1957).

2.2. Total organic carbon (TOC), stable carbon isotope ($\delta^{13}\text{C}$) and % nitrogen

Prior to analysis, samples for TOC and $\delta^{13}\text{C}$ were treated with 1 M HCl, left overnight, washed with deionized water and oven dried at 60 °C. Analyses for TOC, $\delta^{13}\text{C}$ and % N were performed at Iso-Analytical Limited Laboratories using a Europa Scientific Elemental Analyser. Reference and quality control samples were also analysed during the batch analysis. All

standards were calibrated against and traceable to IAEA-CH-6 (sucrose, $^{13}\text{C}_{\text{V-PDB}} = -10.43\text{‰}$), an inter-laboratory comparison standard distributed by the International Atomic Energy Agency (IAEA), Vienna. Replicate analysis of samples indicated an average standard deviation of 0.02% for TOC, 0.05% for $\delta^{13}\text{C}$ and 0.002% for N.

2.3. *n*-Alkane and brGDGT analyses

Freeze-dried sediments were extracted using an Accelerated Solvent Extractor (ASE) 200 (Dionex), operated at 100 °C and 7.6×10^6 Pa with a mixture of dichloromethane (DCM): methanol (MeOH) (9:1, v/v) to obtain a total lipid extract (TLE). Internal standards squalane and C_{46} GDGT were added to the TLE, which was subsequently separated into apolar and polar fraction using an alumina oxide column (Al_2O_3), using *n*-hexane/DCM (9:1, v/v), and methanol/DCM (1:1, v/v) as eluents. *n*-Alkanes contained in the apolar fraction were analysed using a Hewlett Packard 6890 series GC-FID system fitted with an Agilent DB-1ms UI column (60 m length, 0.25 mm i.d., 0.1 μm film thickness). The oven temperature program was: 60 °C (isothermal for 1 min) to 320 °C (isothermal for 15 min) at 10 °C/ min. 1 μL was injected at 280 °C in splitless mode for 0.7 min, split 1:30 thereafter. Helium carrier gas flow was 1 mL/min. The *n*-alkanes were identified based on correlation of retention times to a standard mixture. P_{aq} and TAR were calculated using the equation from Ficken et al. (2000) and Bourbonniere and Meyers (1996), respectively (see Supplementary Information for the formulae).

The polar fractions were filtered through a polytetrafluoroethylene filter (PTFE: 0.45 μm) and analysed using a Thermo TSQ Quantiva coupled to an Ultimate 3000 series U-HPLC following a slightly modified method of Schouten et al. (2007). Unfortunately, it was not possible to use the new U-HPLC method to separate the 5- and the 6- methyl brGDGTs

(Hopmans et al., 2016). Chromatographic separation was achieved on a Prevail Cyano column (2.1 × 150 mm, 3 Å; Alltech, Deerfield, IL, USA), maintained at 30 °C. Tetraethers were eluted isocratically with 99% A and 1% B for 5 min, followed by a linear gradient to 1.8% B in 45 min, where A is hexane and B is isopropanol. Flow rate was 0.2 mL/min. Detection was achieved using atmospheric pressure positive ion chemical ionization mass spectrometry (APCI-MS) of the eluent. Conditions for APCI-MS were as follows: sheath gas 20, auxiliary gas 2, ion transfer tube temperature 325 °C, vaporizer temperature 400 °C, positive ion discharge 2. Relative GDGT distributions were determined by integrating the summed peak areas in the respective $[M+H]^+$. The m/z values of the GDGTs integrated were: 1292, 1050, 1048, 1046, 1036, 1034, 1032, 1022, 1020, 1018 (Supplementary Fig. S1). The BIT index was calculated according to Hopmans et al. (2004). pH was calculated according to Peterse et al. (2012) (see Supplementary Information for formulae). The instrumental reproducibility of the BIT index and pH estimation were determined by duplicate measurement of all samples with an average standard deviation of ± 0.01 for BIT and 0.03 for pH. For the concentrations, the average standard deviation of each compound was less than 1 ng/g. The weighted average of cyclopentane moieties was calculated for the tetramethylated brGDGTs according to Sinninghe Damsté (2016).

2.4. Statistical analyses

Pearson correlation and principal component analysis (PCA) were performed using Sigma Plot software. Concentrations of brGDGTs and *n*-alkanes, and all the molecular and bulk proxies were used as variables in the Conwy Estuary.

3. Results

3.1. Longitudinal and transversal distribution of TOC and biomarkers: *n*-alkanes, brGDGTs, crenarchaeol

The highest value of TOC (2.4%) occurred upstream (site 1; Figs. 1 and 2). This value decreased to approximately half for the next three downstream sites (2–4) and then to values below 0.5% in the majority of the seaward sites. Generally, only sites located close to the banks (sites 9, 19, 30, 32) still had values above 0.5% (Fig. 3). Site 15 had a TOC value of 0.5% but is not close to the banks. The lowest value of TOC was ~ 0.03% recorded in many of the sites located downstream. brGDGT, *n*-alkane and crenarchaeol concentrations ranged from ~ 6 to 480 ng/g, 69 to 7108 ng/g and 1 to 195 ng/g, respectively. All of the three biomarkers (brGDGTs, *n*-alkanes and crenarchaeol) had a very similar distribution to the TOC with the higher concentrations (ng/g) in the four first sites and the sites from transects closer to the banks (Figs. 2 and 3). The lowest concentrations of the biomarkers were also recorded at the same sites that recorded the lowest TOC values, i.e. around mid-estuary and towards the mouth of the estuary.

Pearson correlation coefficients between the biomarkers and TOC were very high, $r^2 > 0.9$ (Table 1). BrGDGTs and *n*-alkane distributions showed the higher correlation with r^2 of 0.993. The lowest correlation was found between crenarchaeol vs TOC ($r^2 = 0.932$) and between crenarchaeol and *n*-alkanes ($r^2 = 0.938$). When the transverse sites were included in the correlation (numbers in parentheses, Table 1), the coefficients slightly decreased with the highest still between brGDGTs vs *n*-alkanes of $r^2 = 0.981$. The lowest correlation, however, changed to $r^2 = 0.787$ between crenarchaeol vs *n*-alkanes.

3.2. Bulk and molecular proxy distributions

The BIT index based on the brGDGTs and crenarchaeol, ranged from 0.4 to 0.8 with highest recorded values in the middle of the estuary. The lowest recorded values were in the estuary mouth and in transects with high concentrations of biomarkers and TOC (Fig. 4). The longitudinal distribution of the BIT did not follow the same distribution as the biomarkers/TOC and was quite variable going downstream. Similarly, inspection of the four transverse transects, revealed that the BIT values did not co-vary with the biomarker concentrations. P_{aq} values ranged from approximately 0.17 to 0.77 with higher values around the middle of the estuary and lower values upstream and in certain areas in the middle of the estuary (Fig. 4). TAR ranged from approximately 2.5 to 29 with highest values at site 27 and lower values around the middle and at the mouth of the estuary (Fig. 4). The $\delta^{13}C$ values ranged from -26‰ to -22‰ with the highest values at the estuary mouth and the lowest values upstream at site 6. No clear pattern was recorded in the $\delta^{13}C$ distribution and the average of all sites is -24‰ . Unfortunately, it was not possible to obtain $\delta^{13}C$ values for all the sites in the estuary due to low organic carbon content and instrumental detection limit. C/N values ranged from 3.0 to 8.7 and showed a step-wise decrease downstream (Fig. 4). The highest values ($C/N = \sim 8.7$) were recorded at sites 1 and 3 and, thereafter, decreased to an average of ~ 6 with exception of sample 19 ($C/N = \sim 8$) and 32 ($C/N = \sim 8.5$). The more marine sites (sites 37–39) recorded a C/N value of ~ 3 . Pearson correlation between the different proxies are stated in Table 2. BIT values have a high negative correlation with the $\delta^{13}C$ with an r^2 of -0.85 . The p value for BIT and the other proxies were > 0.05 suggesting no correlation between them. $\delta^{13}C$ values showed a strong correlation with all the proxies, showing a negative correlation with BIT, C/N and TAR and a positive correlation with P_{aq} . C/N and P_{aq} showed $p > 0.05$ suggesting no correlation between them. TAR showed a high negative correlation with $\delta^{13}C$ and P_{aq} and some positive correlation with C/N.

4. Discussion

4.1. Terrestrial biomarkers in the Conwy Estuary

The concentrations (ng/g) of the terrestrial biomarkers (*n*-alkanes, brGDGTs) and TOC consistently decreased downstream which suggests a reduction in terrestrial OC input (Fig. 2). The first four sites (1–4) had the greatest concentration of terrestrial biomarkers possibly because they are located at the landward (narrower end) of the estuary which has less marine influence. The lowest terrestrial biomarker concentrations were found further downstream at sites in Conwy Bay (sites 37–39) supporting the physical characteristics of these samples that are sandier and have more shells (Fig. 5) and, therefore, expected to show a decrease in terrestrial OC and an increase in marine OC. Apart from the four upstream sites that displayed high inputs of terrestrial OC, it is possible to identify a few locations along the river that are point sources of terrestrial OC. For example, sites 8, 15 and 24 showed increased values of the terrestrial biomarkers and TOC. Sample 15 was taken slightly downstream of the tributary Nan Garred-Ddu, therefore it may also be influenced by the input of OC coming from the connecting stream.

The individual distribution of the *n*-alkanes at sites 8, 15 and 24 were dominated by C₂₉ and C₃₁, a situation repeated throughout the estuary (with the exception of sites 37, 38 and 39), confirming a terrestrial influence up to and including Conwy and Deganwy Marinas (Fig. 1; Supplementary Fig. S2). However, when the concentrations are normalised to OC (Fig. 6), specific changes are revealed for the biomarkers. The highest *n*-alkane concentrations (µg/g OC) occurred around sites 16 and 17 and the highest brGDGT concentrations (µg/g OC) around site 26. These changes are not driven by variations in TOC content corresponding to distance to land but are probably related to higher in situ production of these biomarkers. Indeed, the *n*-alkanes at

site 16 presented the highest P_{aq} value of 0.77 in the estuary suggesting the presence of submerged/floating plants (Ficken et al., 2000).

In situ production of brGDGTs in rivers has been suggested previously (Zell et al., 2013; De Jonge et al., 2014a) and one approach to unveil potential in situ production of brGDGTs is by evaluating their degree of cyclisation (Sinninghe Damsté, 2016; Dearing Crampton-Flood et al., 2019). The degree of cyclisation of brGDGTs has been reported to be related to a change in soil pH (Weijers et al., 2007). Reconstruction of soil pH in the Conwy sediments using CBT (Peterse et al., 2012) revealed a pH of 6.5–7 for sites 1–36 and a value of 7.3 for sites 37–39. Given the CBT error of 0.8 (Peterse et al., 2012), these results suggest that the upstream and mid-estuary pH slightly differed from those in the marine zone (Conwy Bay). On the other hand, the consistent pH observed upstream and mid-estuary suggests that the sudden changes in concentration of the terrestrial biomarkers along the estuary does not represent a change in the source of the OC entering the estuary but just a change in the absolute amount entering.

To trace changes in the source of brGDGTs in the environment, some studies have calculated the weighted average of cyclopentane moieties for tetra and pentamethylated brGDGTs (Peterse et al., 2009; Sinninghe Damsté, 2016; Dearing Crampton-Flood et al., 2019). In the Conwy sediments, #ringtetra fell to 0.3–0.4 (Supplementary Table S2) with the two more-marine samples showing values of 0.5. This pattern of increasing #ringtetra with increasing distance from shore was also noted by Sinninghe Damsté (2016) for the Berau delta and by Dearing Crampton-Flood et al. (2019) for the Svalbard fjords. The authors explained the increased values of #ringtetra as an increase in the in situ production of brGDGTs. However, Sinninghe Damsté (2016) specified that when the #ringtetra > 0.7, sources other than soil must contribute to the brGDGT concentrations because no global soil has been shown to have

#ringstetra > 0.7. This confirms that soil contributes to most of the brGDGTs in the surface sediments of the Conwy estuary since #ringstetra values are all < 0.7. In addition, the more-marine samples in this study were collected around 10 m water depth which is much shallower than the 50 m water depth reported where in situ brGDGT production start to be more pronounced (Sinninghe Damsté, 2016; Dearing Crampton-Flood et al., 2019). Finally, when the fractional abundances of the summed tetra-, penta- and hexamethylated brGDGTs of the Conwy sediments are plotted in a ternary diagram (Fig. 7) to assess the provenance of sedimentary brGDGTs, the Conwy sediments present a very similar distribution as the global soils dataset. This again supports the idea that brGDGTs in the Conwy Estuary are primarily sourced from soils and that the hotspot of brGDGTs concentrations at site 26 is probably still related to the amount of terrestrial material entering in the Conwy, possibly through the small tributary adjacent to this site. Further work should focus in the separation of the 5-methyl and 6-methyl brGDGTs that have been shown to provide further insight into in situ production of brGDGTs (De Jonge et al., 2015; Dearing Crampton-Flood et al., 2019). Analyses of the Conwy Estuary catchment soils covering different land-use types to provide terrestrial end-members may also help constrain the origin of the brGDGTs.

A comparison with other small estuarine systems in the UK shows that the Conwy Estuary average concentrations of *n*-alkanes (847 ng/g) are similar to the inner estuary of the Clyde with concentrations varying from 1700 ng/g to 4000 ng/g. However, the outer estuary of the Clyde had a much higher *n*-alkane concentration (8000 ng/g) and was also characterised by an unresolved complex mixture (UCM) hump from anthropogenic hydrocarbon pollution (Vane et al., 2019). Therefore, comparison of *n*-alkanes from urban industrial estuaries with those from rural-agricultural should be considered with caution. The brGDGT average concentration of 27

$\mu\text{g/g}$ OC and maxima of $48 \mu\text{g/g}$ OC in the Conwy estuary are comparable to the Clyde estuary (average of 17, maxima of $53 \mu\text{g/g}$ OC: Lopes dos Santos and Vane, 2019) and slightly higher than the Thames estuary (average of $5 \mu\text{g/g}$ OC, maxima of $15 \mu\text{g/g}$ OC: Lopes dos Santos and Vane, 2016).

The fractional distribution of the brGDGTs in the River Conwy proceeds in the order pentamethylated > tetramethylated > hexamethylated brGDGTs (Figs. 7 and 8). A similar fractional abundance is also recorded in the Thames (Lopes dos Santos and Vane, 2016) and in the Clyde (Lopes dos Santos and Vane, 2019) with the dominant group being the pentamethylated followed normally by the tetramethylated group (Fig. 8). These three small estuaries all plot together with the European soils in the ternary plot (Fig. 7). A dominant pentamethylated group was also found, for instance, in the Yenisei River Gulf sediments although the difference to the hexamethylated was minimal (De Jonge et al., 2015). This may reflect the temperatures of the study areas as the UK has warmer temperatures than the Yenisei River area in Russia. A higher amount of hexamethylated groups is expected in colder areas and a possible explanation is that the addition of methyl groups to sustain membrane fluidity is a mechanism used by the bacteria to adapt to colder temperatures. Interestingly, the hexa- and tetramethylated groups show an inverse trend (mirroring each other) in all the samples of the three rivers in UK (Fig. 8). At the moment, it is not possible to explain this opposing trend due to lack of data for comparison but it can be a reflection of the little variability of the pentamethylated group

4.2. Molecular and bulk proxy variations in the Conwy Estuary

Those soils and sediments strongly dominated by terrestrial OC show BIT values of 0.9–1 whereas those from more open marine environments are characterised by BIT values < 0.3

(Hopmans et al., 2004). In this current study, the maximum BIT of 0.8 was recorded more upstream suggesting a terrestrial OC influence with minor marine OC input whereas the three more-seaward sites situated in Conwy Bay show a lower BIT of ~ 0.45 confirming a mixed terrestrial and marine OC source. The BIT profile presented in Fig. 4 shows that the maximal values did not correspond to the four upstream sites as described by the brGDGT concentrations. This mismatch may be explained by heterogeneity in the sources of crenarchaeol. Crenarchaeol can be produced in soils, rather than from a purely aquatic origin (Weijers et al., 2006b). In the Conwy estuary, crenarchaeol shows a strong correlation to the terrestrial biomarkers: *n*-alkanes and brGDGTs, suggesting that crenarchaeol has multiple sources. In fact, looking at the normalised concentrations of the biomarkers (Fig. 6), it is possible to see that crenarchaeol changes are an important driver of the BIT variations. Therefore, although the BIT shows a decrease of terrestrial input from land to sea, it does not fully represent the areas where most terrestrial input occurs. This may partly explain the lack of correlation between BIT and the C/N, TAR and P_{aq} . Nevertheless, when taken together, the BIT index, #ringstetra, and the terrestrial biomarker concentrations suggest that the estuary is mainly influenced by terrestrial OC.

Commonly used proxies to track the changes of terrestrial vs aquatic OC sources are the P_{aq} and TAR. Although P_{aq} values are all > 0.1 (Fig. 4) suggesting no terrestrial plant inputs in the estuary, sites 1–4 and other areas in the estuary show boundary values of ca. 0.2 that have been reported previously as a terrestrial input of *n*-alkanes (Ficken et al., 2000; Mead et al., 2005). Therefore, P_{aq} shows a trend of decreasing terrestrial input from upstream to downstream with a strong increase in submerged/floating vegetation around the middle of the estuary. The TAR distribution in the Conwy complement the P_{aq} and BIT showing higher terrestrial input upstream and higher aquatic input at the Conwy Bay. High values of TAR (> 20) are recorded in

the Conwy Estuary. This may be explained by TAR over-representing the absolute amounts from terrigenous sources because contributions of land-derived organic matter typically contain higher proportions of *n*-alkanes than do those from aquatic algae (Bourbonniere and Meyers, 1996). Nevertheless, this ratio is clearly showing the distribution of terrestrial input in the estuary.

The third proxy used, the $\delta^{13}\text{C}$ profile, was quite variable with values ranging from -22‰ to -26‰ . In estuaries and coasts, terrestrial OC ranges from -25‰ to -28‰ whilst that from marine environments ranges from -19‰ to -22‰ (Vane et al., 2013; Khan et al., 2015). Using these broad criteria, sites 1–36 are mainly comprised of terrestrial OC whereas the three more downstream sites (37–39) contain marine OC. Similarly, some sites more upstream or close to banks presented values between -25‰ to -26‰ suggesting an enhanced terrestrial influence. However, most of the estuary showed values from -24‰ to -25‰ which may represent a mixture of the two contributions. The $\delta^{13}\text{C}$ shows a strong negative correlation to BIT, TAR and C/N and a positive correlation to P_{aq} supporting the trend of decreasing terrestrial input from land to Conwy Bay (because of $\delta^{13}\text{C}$ negative values). It is the only proxy that correlates well with all of the other proxies possibly because of the lower number of samples analyzed for this proxy.

C/N suggests a marine organic input throughout the estuary with values < 9 in all the sites. Previous studies show a typical terrestrial environment to have values ≥ 15 or ≥ 20 (Saito et al., 1989; Sampei and Matsumoto, 2001) whereas coastal and river-estuarine sediments receiving mixtures of marine and terrestrial organic matter yield values of 8–16 (Vane et al., 2013; 2020). In the current study, the high C/N associated with abundant terrestrial input were not recorded in the Conwy sediments. However, the longitudinal down-stream C/N profile (Fig. 4) shows a clear decrease from inland to the river mouth supporting the other proxies. One

plausible explanation for the low values recorded in the Conwy sediments is that additional anthropogenic sources of N such as treated sewage or possibly application of fertilisers in the catchment reduced the C/N ratio (Sgouridis and Ullah, 2014; Bussi et al., 2017). Indeed, the %N values have a similar distribution to the TOC and the terrestrial biomarkers (*n*-alkanes and brGDGTs) for the whole estuary apart the most seaward samples of Conwy Bay confirming the notion that at least a proportion of the N has a terrestrial origin. In contrast, we attribute the slight %N increase at the more-seaward sites (37–39) to incorporation of N-rich marine organic matter supporting the slight increase in the marine crenarchaeol biomarker. C/N only showed significant correlation to $\delta^{13}\text{C}$ and TAR possibly due to the reasons explained above.

Although the proxy's longitudinal variations (BIT, P_{aq} , TAR, $\delta^{13}\text{C}$ and C/N; Fig. 4) do not fully reproduce the changes of the biomarker's concentrations, they still indicate the same downstream trend, namely a predominance of terrestrial carbon sources up to and including site 36. The close agreement of the proxies for a stronger terrestrial impact through the estuary is supported by the Pearson correlation between some of them (Table 2) where r^2 varied from 0.528 to 0.853 when only the longitudinal sites are considered.

To determine the factors controlling the molecular and bulk proxies, PCA was applied to the dataset. Most variation is explained by Factor 1 (PC1: 41.08%) followed by Factor 2 (PC2) explaining approx. 30.84% (Fig. 9). In the PCA, it is clear that the bulk parameters tend to have positive values while the molecular parameters tend to have negative values in Factor 2. On the other hand, $\delta^{13}\text{C}$ and the P_{aq} give negative values in Factor 1. A sample score plot of the PCA is used to examine the relationships between Factor 1 and 2 and the samples. The component scores of the 39 sites shows that those with greatest marine influence as well as all the samples closer to the river banks and situated further upstream, plot on the positive axis of Factor 2. This

distribution may suggest a difference in grain size between the samples because visually the more terrestrial and more marine samples were coarser. Inspection of the grain size data (Fig. 5) shows that the samples closer to the banks and the three more downstream samples (37–39) are dominated by sand while samples from the middle of the estuary have a finer grain size. These results support the notion that Factor 2 mainly indicates the influence of grain size on the geochemical proxies. This is supported by a previous study that found a dissimilar association of brGDGTs to different grain sizes (Peterse and Eglinton, 2017). Based on the position of the samples in the component scores plot, it can be assumed that Factor 1 corresponds to the location and/or indirectly to the type and content of OC because the marine group of samples (sites 37–39) presents the most negative values whereas the more upstream samples (e.g., site 1) had the highest positive value. This suggests that the opposing trajectories of the $\delta^{13}\text{C}$ and P_{aq} compared to the BIT and TAR in the component loadings plot are due to a stronger influence of terrestrial sources on the BIT and TAR. This opposite trend between the $\delta^{13}\text{C}$ and the BIT in the PCA has also been observed in Changjiang Estuary, China (Yao et al., 2015). Similarly, the authors described OC source and decay signals, and grain size as the two main factors explaining the variations of their sample set.

Fig. 9 shows that the $\delta^{13}\text{C}$ and the BIT for the Clyde estuary also have this opposing trend and that the bulk and molecular proxies are also controlled by different factors (Lopes do Santos and Vane, 2019). The sample score of the whole river Clyde system shows three distinct groups: the upper river samples, the estuary and the river sediments around Glasgow. Grain size and type of OC are possible factors in Factor 1 and 2 controlling these scores.

4.3. Transverse (bank to bank) variation of OC in the Conwy estuary

One of the aims of this study was to assess how the OC is distributed transversally within the Conwy estuary. Although it is established that higher concentrations of terrestrial OC are found in the banks of estuaries, patterns of water velocity and suspended sediments can vary considerably over decadal time-scales as well as within and between estuaries (Vane et al., 2020). Therefore, if the distribution and source of organic carbon hosted in river-estuarine sediments is to ever be fully realised, sampling schemes need to consider both longitudinal (upstream to downstream) and multiple transverse (bank to bank) sampling approach for a better characterisation of the sedimentary OC.

Fig. 3 clearly shows that higher TOC and, *n*-alkane and brGDGT concentrations (ng/g) indicative of terrestrial OC sources are located closer to the bank, most probably due to higher deposition of run-off material. This higher deposition of terrestrial material is expected during autumn/winter months due to greater frequency of winter storms and higher precipitation (45% of annual rainfall between October to January; DEFRA, 2012). The hypothesis that sedimentary carbon in the Conwy is mainly delivered in the autumn and winter seasons is supported by the air temperature estimations of the Conwy Estuary sediments based on the brGDGTs (see Supplementary Information). Crenarchaeol concentrations and %N also follow the same transverse spatial pattern as the terrestrial biomarkers supporting the assumption discussed above of crenarchaeol and N having a terrestrial source. Higher concentrations near the banks coincided with areas of large deposition of sediments due to tidal and hydrodynamic changes (sites 9 and 30) or areas of higher terrestrial input from a tributary discharging in the estuary (site 19) and the Conwy marina (site 32). However, none of the calculated proxies supported enhanced terrestrial input at the banks. For example, the BIT index showed an opposing trend to the brGDGT concentrations with lower values near the banks and higher values mid-estuary. This is most

likely due to an increase in crenarchaeol concentrations at the banks as it possibly has a terrestrial origin and, as reported above, crenarchaeol seems to be an important driver of the BIT.

The *n*-alkane based proxies, P_{aq} and TAR, only showed an increased terrestrial input near the banks for transect A, where values of P_{aq} and TAR changed from 0.2 to 0.4 and 14 to 2.5, respectively, going from the banks to the middle of the estuary. The other transects showed a slight increase of terrestrial material in middle of the estuary or no significant change throughout the transect suggesting no big difference of types of OC in these transects. Evaluation of the C_{max} in transects C and D shows that the dominant chain length is 29 or 31 in all the sites, whereas in transects A and B the dominant chain length was lower in the middle sample of transects (20 and 25 respectively). The predominance of the lower chain length *n*-alkanes at these sites may represent greater marine/bacteria input. This latter interpretation is also supported by lower $\delta^{13}C$ values ($< -25\%$) in the middle sites. Unfortunately, it was not possible to obtain a reliable $\delta^{13}C$ measurement from several transect sites limiting the interpretation of this proxy. C/N also showed considerable variation across transects possibly due to terrestrial rather than marine origin of the N as suggested for the crenarchaeol. Overall, evaluation of bank-channel and bank to bank transect chemistry in the Conwy estuary reveals clear systematic changes in absolute concentrations whereas the proxies show greater variability and non-systematic changes.

5. Conclusions

The Conwy estuary, UK, receives a diverse range of natural OC sourced from moorland and blanket bog, agricultural pasture and woodlands (e.g., natural deciduous, coniferous forest plantations). The results of the molecular and bulk geochemical analyses show that most of the sedimentary OC accumulating in the Conwy estuary originates from terrestrial sources. For

example, biomarker (brGDGTs, *n*-alkanes) concentrations reflect multiple OC sources from soil and plant origins with only the three more-seaward samples showing a stronger marine-derived signal. The application of the proxies (P_{aq} , TAR, BIT, C/N and $\delta^{13}C$) also supported this conclusion but care should be taken when interpreting the results due to heterogeneity of the sources of different components used in these ratios. The physical characteristics of the sediments and PCA of the entire data-set suggests that grain size and type of OC were possible factors controlling the molecular values and bulk proxies. The results also demonstrate that sediments in three UK small estuaries show similar brGDGT concentrations and fractional distributions. A clear tendency of the pentamethylated brGDGTs to be the dominant group in the Conwy, the Clyde and also the Thames estuaries suggests that similar fractional abundance and distribution may occur in other estuarine environments in UK. Similarly, an inverse distribution pattern is recorded for the tetra- and hexamethylated brGDGTs along the three estuaries that, to the best of our knowledge, has not been reported before. Future work should focus in analysing catchment soils and more small estuarine systems to understand factors controlling the brGDGT abundance. Finally, it can be concluded that erosion and runoff is the main source of sedimentary OC in the Conwy Estuary with sedimentation mainly occurring during winter months. High variability of the sedimentary OC deposition is observed transversally to the estuary with considerably higher accumulation of terrestrial OC near the banks.

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References

- Bourbonniere, R.A., Meyers, P.A., 1996. Sedimentary geolipid records of historical changes in the watersheds and productivities of Lakes Ontario and Erie. *Limnology and Oceanography* 41, 352–359.
- Bristow, L.A., Jickells, T.D., Weston, K., Marca-Bell, A., Parker, R., Andrews, J.E., 2013. Tracing estuarine organic matter sources into the southern North Sea using C and N isotopic signatures. *Biogeochemistry* 113, 9–22.
- Bussi, G., Whitehead, P.G., Thomas, A.R.C., Masante, D., Jones, L., Cosby, B.J., Emmett, B.A., Malham, S.K., Prudhomme, C., Prosser, H., 2017. Climate and land-use change impact on faecal indicator bacteria in a temperate maritime catchment (the River Conwy, Wales). *Journal of Hydrology* 553, 248–261.
- Coyne, A., Etcheber, H., Abril, G., Maneux, E., Dumas, J., Hurtrez, J.E., 2005. Contribution of small mountainous rivers to particulate organic carbon input in the Bay of Biscay. *Biogeochemistry* 74, 151–171.
- Dearing Crampton-Flood, E., Peterse, F., Sinninghe Damsté, J.S., 2019. Production of branched tetraethers in the marine realm: Svalbard fjord sediments revisited. *Organic Geochemistry* 138, 103907.

- DEFRA (Department for Food, Environment and Rural Affairs), 2012. A climate change risk assessment for Wales. (GA0204). British Government.
- De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Sinninghe Damsté, J.S., 2014a. In situ produced branched glycerol dialkyl glycerol tetraethers in suspended particulate matter from the Yenisei River, Eastern Siberia. *Geochimica et Cosmochimica Acta* 125, 476–491.
- De Jonge, C., Hopmans, E.C., Zell, C.I., Kim, J.H., Schouten, S., Sinninghe Damsté, J.S., 2014b. Occurrence and abundance of 6-methyl branched glycerol dialkyl glycerol tetraethers in soils: Implications for palaeoclimate reconstruction. *Geochimica et Cosmochimica Acta* 141, 97–112.
- De Jonge, C., Stadnitskaia, A., Hopmans, E.C., Cherkashov, G., Fedotov, A., Streletskaya, I.D., Vasiliev, A.A., Sinninghe Damsté, J.S., 2015. Drastic changes in the distribution of branched tetraether lipids in suspended matter and sediments from the Yenisei River and Kara Sea (Siberia): Implications for the use of brGDGT-based proxies in coastal marine sediments. *Geochimica et Cosmochimica Acta* 165, 200–225.
- Eglinton, G., Hamilton, R.J., 1963. The distribution of *n*-alkanes. In: T. Swain (Ed.), *Chemical Plant Taxonomy*. Academic Press, pp. 197–217.
- Elderfield, H., Hepworth, A., Edwards, P.N., Holliday, L.M., 1979. Zinc in the Conwy River and Estuary. *Estuarine and Coastal Marine Science* 9, 403–422.
- Ficken, K.J., Li, B., Swain, D.L., Eglinton, G., 2000. An *n*-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. *Organic Geochemistry* 31, 745–749.
- Folk, R.L., Ward, W.C., 1957. A study in the significance of grain-size parameters. *Journal of*

- Sedimentary Petrology 27, 3–26.
- Freymond, C.V., Peterse, F., Fischer, L.V., Filip, F., Giosan, L., Eglinton, T.I., 2017. Branched GDGT signals in fluvial sediments of the Danube River basin: Method comparison and longitudinal evolution. *Organic Geochemistry* 103, 88–96.
- Galy, V., Peucker-Ehrenbrink, B., Eglinton, T., 2015. Global carbon export from the terrestrial biosphere controlled by erosion. *Nature* 521, 204–207.
- Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2016. The effect of improved chromatography on GDGT-based palaeoproxies. *Organic Geochemistry* 93, 1–6.
- Hopmans, E.C., Weijers, J.W.H., Schefuss, E., Herfort, L., Sinninghe Damsté, J.S., Schouten, S., 2004. A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. *Earth and Planetary Science Letters* 224, 107–116.
- Hotchkiss, E.R., Hall, R.O., Sponseller, R.A., Butman, D., Klaminder, J., Laudon, H., Rosvall, M., Karlsson, J., 2015. Sources of and processes controlling CO₂ emissions change with the size of streams and rivers. *Nature Geoscience* 8, 696–700.
- Khan, S.N., Vane, C.H., Horton, B.P., Hillier, C., Riding, J.B., Kendrick, C., 2015. The use of bulk stable carbon isotope geochemistry in the reconstruction of Holocene relative sea levels and paleoenvironments, Thames UK. *Journal of Quaternary Science* 30, 417–433.
- Kim, J.H., Zell, C., Moreira-Turcq, P., Perez, M.A.P., Abril, G., Mortillaro, J.M., Weijers, J.W.H., Meziane, T., Sinninghe Damsté, J.S., 2012. Tracing soil organic carbon in the lower Amazon River and its tributaries using GDGT distributions and bulk organic matter properties. *Geochimica et Cosmochimica Acta* 90, 163–180.
- Lopes dos Santos, R.A., Vane, C.H., 2016. Signatures of tetraether lipids reveal anthropogenic overprinting of natural organic matter in sediments of the Thames Estuary, UK. *Organic*

- Geochemistry 93, 68–76.
- Lopes dos Santos, R.A., Vane, C.H., 2019. Tracking natural organic carbon in the River Clyde, UK using glycerol dialkyl glycerol tetraethers. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh* 108, 289–298.
- Mead, R., Xu, Y.P., Chong, J., Jaffé, R., 2005. Sediment and soil organic matter source assessment as revealed by the molecular distribution and carbon isotopic composition of *n*-alkanes. *Organic Geochemistry* 36, 363–370.
- Milker, Y., Horton, B.P., Vane, C.H., Engelhart, S.E., Nelson, A.R., Witter, R.C., Khan, N.S., Bridgeland, W.T. 2015. Annual and seasonal distribution of intertidal foraminifera and stable carbon isotope geochemistry, Bandon Marsh, Oregon, USA. *Journal of Foraminiferal Research* 45, 146–166
- Mudge, S.M., Norris, G.E., 1997. Lipid biomarkers in the Conwy Estuary (North Wales, UK): A comparison between fatty alcohols and sterols. *Marine Chemistry* 57, 61–84.
- Peterse, F., Kim, J. H., Schouten, S., Kristensen, D. K., Koç, N., Sinninghe Damsté, J.S., 2009. Constraints on the application of the MBT/CBT palaeothermometer at high latitude environments (Svalbard, Norway). *Organic Geochemistry* 40, 692–699.
- Peterse, F., van der Meer, J., Schouten, S., Weijers, J.W.H., Fierer, N., Jackson, R.B., Kim, J.H., Sinninghe Damsté, J.S., 2012. Revised calibration of the MBT-CBT paleotemperature proxy based on branched tetraether membrane lipids in surface soils. *Geochimica et Cosmochimica Acta* 96, 215–229.
- Peterse, F., Eglinton, T.I., 2017. Grain size associations of branched tetraether lipids in soils and riverbank sediments: Influence of hydrodynamic sorting processes. *Frontiers in Earth Science* 5. doi:10.3389/feart.2017.00049

- Saito, Y., Nishimura, A., Matsumoto, E., 1989. Transgressive sand sheet covering the shelf and upper slope off Sendai, northeast Japan. *Marine Geology* 89, 245–258.
- Sampei, Y., Matsumoto, E., 2001. C/N ratios in a sediment core from Nakaumi Lagoon, southwest Japan – usefulness as an organic source indicator. *Geochemical Journal* 35, 189–205.
- Schlesinger, W.H., Melack, J.M., 1981. Transport of organic carbon in the worlds rivers. *Tellus* 33, 172–187.
- Schouten, S., Huguet, C., Hopmans, E.C., Kienhuis, M.V.M., Sinninghe Damsté, J.S., 2007. Analytical methodology for TEX₈₆ paleothermometry by high-performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry. *Analytical Chemistry* 79, 2940–2944.
- Sgouridis, F., Ullah, S., 2014. Denitrification potential of organic, forest and grassland soils in the Ribble-Wyre and Conwy River catchments, UK. *Environmental Science – Processes & Impacts* 16, 1551–1562.
- Sinninghe Damsté, J.S., 2016. Spatial heterogeneity of sources of branched tetraethers in shelf systems: The geochemistry of tetraethers in Berau River delta (Kalimantan, Indonesia). *Geochimica et Cosmochimica Acta* 186, 13–31.
- Sinninghe Damsté, J.S., Rijpstra, W.I.C., Hopmans, E.C., Foesel, B.U., Wust, P.K., Overmann, J., Tank, M., Bryant, D.A., Dunfield, P.F., Houghton, K., Stott, M.B., 2014. Ether- and ester-bound iso-diabolic acid and other lipids in members of Acidobacteria Subdivision 4. *Applied and Environmental Microbiology* 80, 5207–5218.
- Sinninghe Damsté, J.S., Rijpstra, W. I. C., Foesel, B. U., Huber, K. J., Overmann, J., Nakagawa, S., Kim, J.J., Dunfield, P.F., Dedysh, S.N., Villanueva, L., 2018. An overview of the

- occurrence of ether- and ester-linked iso-diabolic acid membrane lipids in microbial cultures of the Acidobacteria: Implications for brGDGT paleoproxies for temperature and pH. *Organic Geochemistry* 124, 63–76.
- Tierney, J.E., Russell, J.M., Eggermont, H., Hopmans, E.C., Verschuren, D., Sinninghe Damsté, J.S., 2010. Environmental controls on branched tetraether lipid distributions in tropical East African lake sediments. *Geochimica et Cosmochimica Acta* 74, 4902–4918.
- Vane, C.H., Harrison, I., Kim, A.W. 2007. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in sediments from the Mersey Estuary, UK. *Science of the Total Environment* 374, 112–126.
- Vane, C.H., Kim, A.W., Moss-Hayes V, Snape C.E, Castro-Diaz, M., Khan, N.S., Engelhart S.E., Horton, B.P. 2013. Mangrove tissue decay by arboreal termites (*Nasutitermes acajutlae*) and their role in the mangrove C cycle (Puerto Rico): Chemical characterisation and organic matter provenance using bulk $\delta^{13}\text{C}$, C/N, alkaline CuO oxidation-GC/MS and solid-state ^{13}C NMR. *Geochemistry, Geophysics, Geosystems* 14, 3176–3191.
- Vane, C.H., Moss-Hayes, V., Kim, A.W., Edgely, K.E., Cave, M.R., Bearcock, J.M., 2019. Mercury, *n*-alkane and unresolved complex mixture hydrocarbon pollution in surface sediment across the rural-urban-estuarine continuum of the River Clyde, Scotland, UK. *Transactions of the Royal Society of Edinburgh* 108, 315–326.
- Vane, C.H., Turner, G.H., Chenery, S.R., Richardson, M., Cave, M.R., Terrington, R., Gowing, C.J.B., Moss-Hayes, V., 2020. Trends in heavy metals, polychlorinated biphenyls and toxicity from sediment cores of the inner River Thames estuary, London, UK. *Environmental Science – Processes & Impacts* 22, 364–380.
- Weijers, J.W.H., Schouten, S., Hopmans, E.C., Geenevasen, J.A.J., David, O.R.P., Coleman,

- J.M., Pancost, R.D., Sinninghe Damsté, J.S., 2006a. Membrane lipids of mesophilic anaerobic bacteria thriving in peats have typical archaeal traits. *Environmental Microbiology* 8, 648–657.
- Weijers, J.W.H., Schouten, S., Spaargaren, O.C., Sinninghe Damsté, J.S., 2006b. Occurrence and distribution of tetraether membrane lipids in soils: Implications for the use of the TEX₈₆ proxy and the BIT index. *Organic Geochemistry* 37, 1680–1693.
- Weijers, J.W.H., Schouten, S., van den Donker, J.C., Hopmans, E.C., Sinninghe Damsté, J.S., 2007. Environmental controls on bacterial tetraether membrane lipid distribution in soils. *Geochimica et Cosmochimica Acta* 71, 703–713.
- Xiao, W.J., Wang, Y.H., Zhou, S.Z., Hu, L.M., Yang, H., Xu, Y.P., 2016. Ubiquitous production of branched glycerol dialkyl glycerol tetraethers (brGDGTs) in global marine environments: a new source indicator for brGDGTs. *Biogeosciences* 13, 5883–5894.
- Yao, P., Yu, Z.G., Bianchi, T.S., Guo, Z.G., Zhao, M.X., Knappy, C.S., Keely, B.J, Zhao, B., Zhang, T., Pan, H., Wang, J., Li, D., 2015. A multiproxy analysis of sedimentary organic carbon in the Changjiang Estuary and adjacent shelf. *Journal of Geophysical Research—Biogeosciences* 120, 1407–1429.
- Zell, C., Kim, J.H., Moreira-Turcq, P., Abril, G., Hopmans, E.C., Bonnet, M.P., Lima Sobrinho, R., Sinninghe Damsté, J.S. (2013). Disentangling the origins of branched tetraether lipids and crenarchaeol in the lower Amazon River: Implications for GDGT-based proxies. *Limnology and Oceanography* 58, 343–353.
- Zhou, J.L., Liu, Y.P., Abrahams, P.W., 2003. Trace metal behaviour in the Conwy estuary, North Wales. *Chemosphere* 51, 429–440.

Figure captions

Fig. 1. Location of (a) Wales, (b) Conwy Estuary and (c) the sites analysed in this study. Letters A–D show location of transects.

Fig. 2. Longitudinal distribution (from land to sea) of TOC and biomarkers (ng/g) along the Conwy Estuary.

Fig. 3. Biomarkers distribution along transects A–D.

Fig. 4. Terrestrial vs aquatic carbon input proxies along the Conwy Estuary.

Fig. 5. Grain size distribution of samples from the Conwy Estuary.

Fig. 6. Longitudinal distribution (from land to sea) of TOC and normalized concentrations ($\mu\text{g/g}$ OC) of biomarkers along the Conwy Estuary.

Fig. 7. Ternary plot of fractional abundances of the brGDGTs from the Conwy Estuary together with the global soils (De Jonge et al., 2014b) and sediments from the Thames and the Clyde (Lopes dos Santos and Vane 2016, 2019).

Fig. 8. Longitudinal variations of the fractional abundance of the tetra-, penta- and hexamethylated brGDGTs in the Conwy Estuary, the Clyde river-estuary and the Thames

Estuary.

Fig. 9. Component loading and scores for the Conwy Estuary and Clyde river-estuary (the Clyde river dataset is from Lopes dos Santos and Vane, 2019).

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Highlights

- *n*-alkane and brGDGT analysed to assess terrestrial C input in the Conwy Estuary
- Biomarkers in sediments showed a higher terrestrial influence compared to marine OC
- Molecular and bulk proxies compromised due to mixed source of compounds
- Similar concentration and fractional distribution of brGDGT in three UK estuaries

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Table 1. Pearson correlation between the biomarkers (ng/g) and TOC (%). The numbers show the r^2 values for only the longitudinal sites while the numbers in parentheses also includes sites from transects.

Variables	sum brGDGT	<i>n</i> -alkanes	TOC	Crenarchaeol
				0.966 (0.867)
sum brGDGT	1	0.993 (0.981) $p < 0.05$	0.990 (0.965) $p < 0.05$	$p < 0.05$
				0.938 (0.787)
<i>n</i> -alkanes		1	0.989 (0.978) $p < 0.05$	$p < 0.05$
				0.932 (0.814)
TOC			1	$p < 0.05$
Crenarchaeol				1

Table 2. Pearson correlation between molecular and bulk proxies. The numbers show the r^2 for only the longitudinal sites while the numbers in parentheses also includes sites from transects.

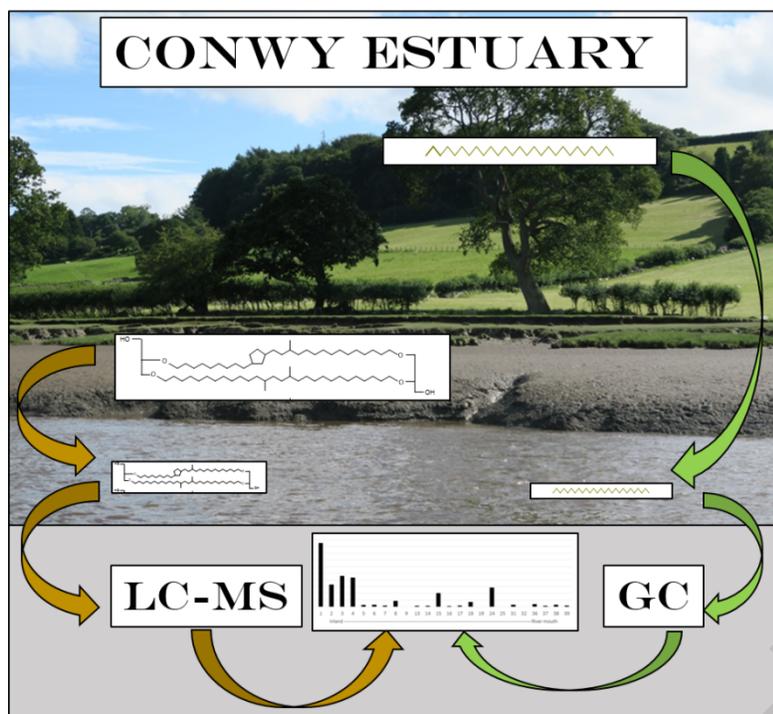
Variables	BIT	$\delta^{13}\text{C}$	C/N	P_{aq}	TAR
BIT	1	-0.853 (-0.751) p < 0.05	0.316 (0.055) p > 0.05	0.241 (0.3) p > 0.05	-0.164 (-0.249) p > 0.05
$\delta^{13}\text{C}$		1	-0.644 (-0.547) p < 0.05	0.78 (0.509) p < 0.05	-0.722 (-0.529) p < 0.05
C/N			1	-0.358 (-0.23) p > 0.05	0.528 (0.313) p < 0.05
P_{aq}				1	-0.694 (-0.631) p < 0.05
TAR					1

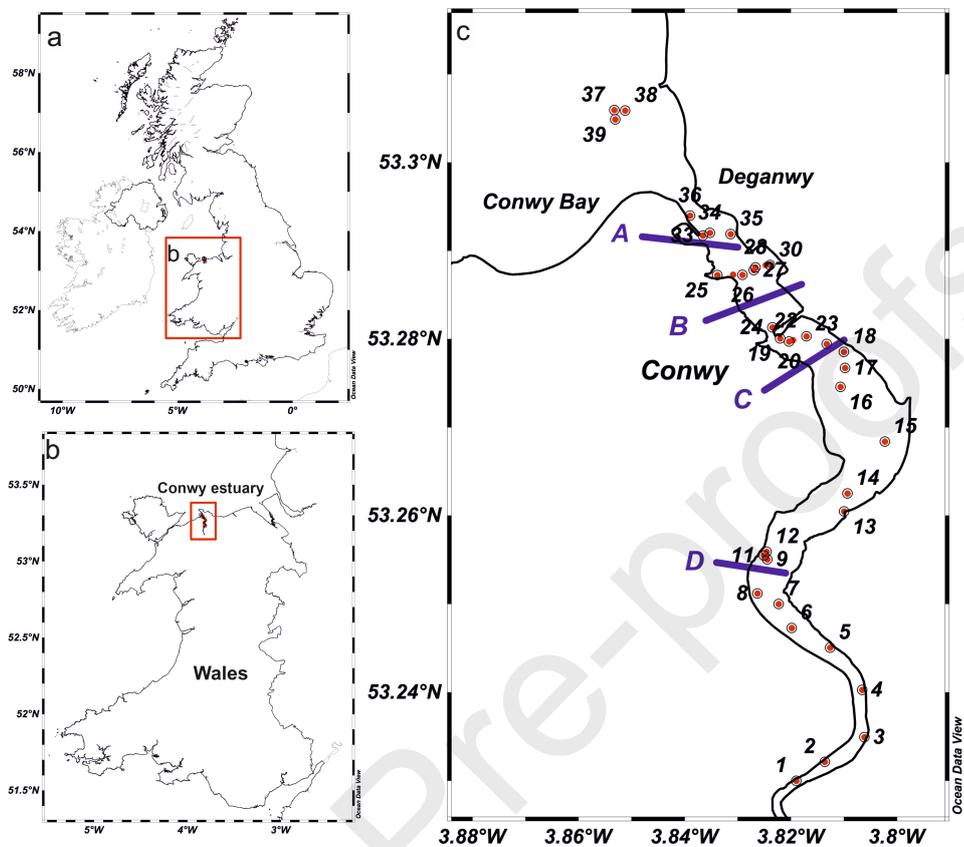
Declaration of interests

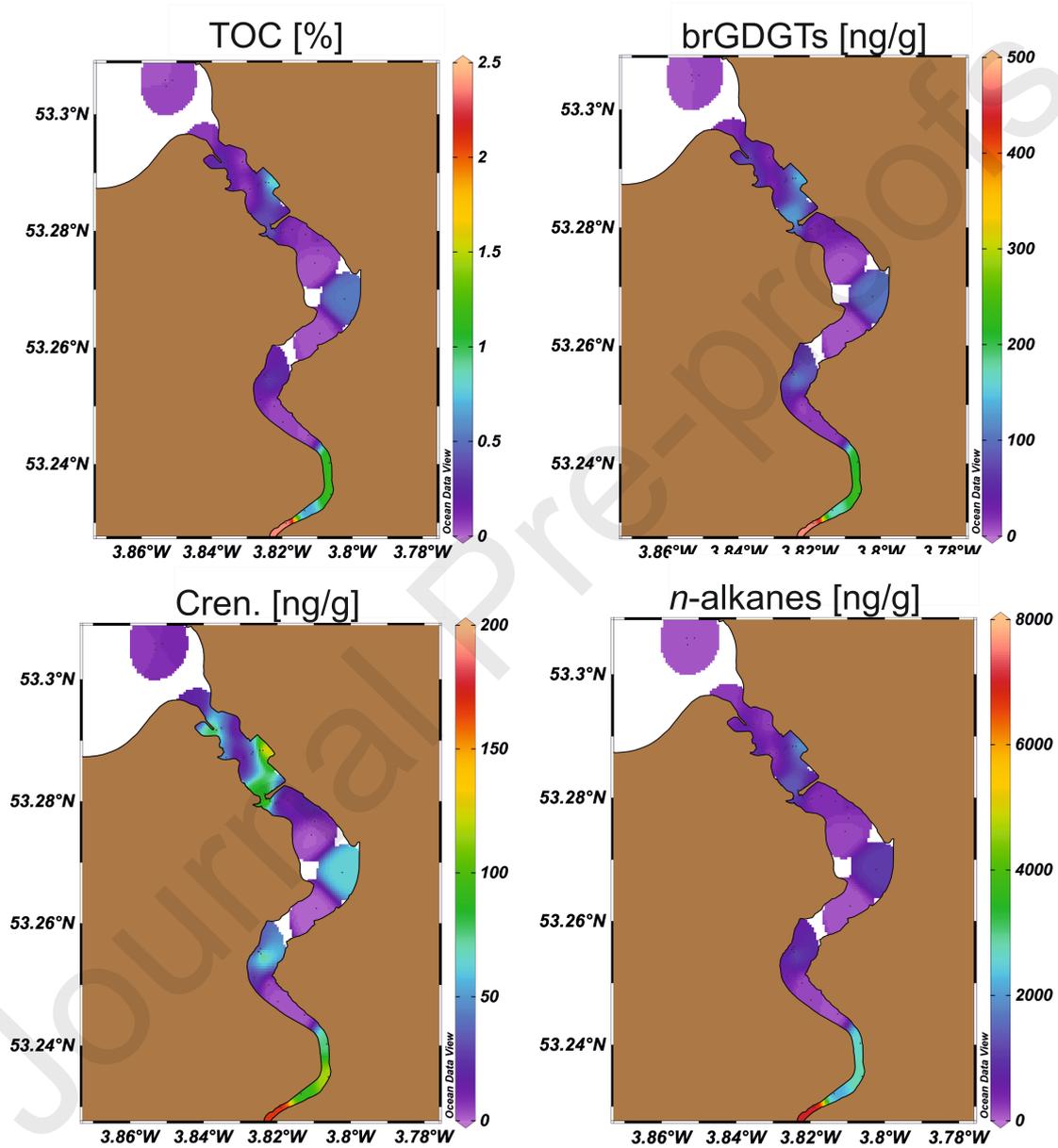
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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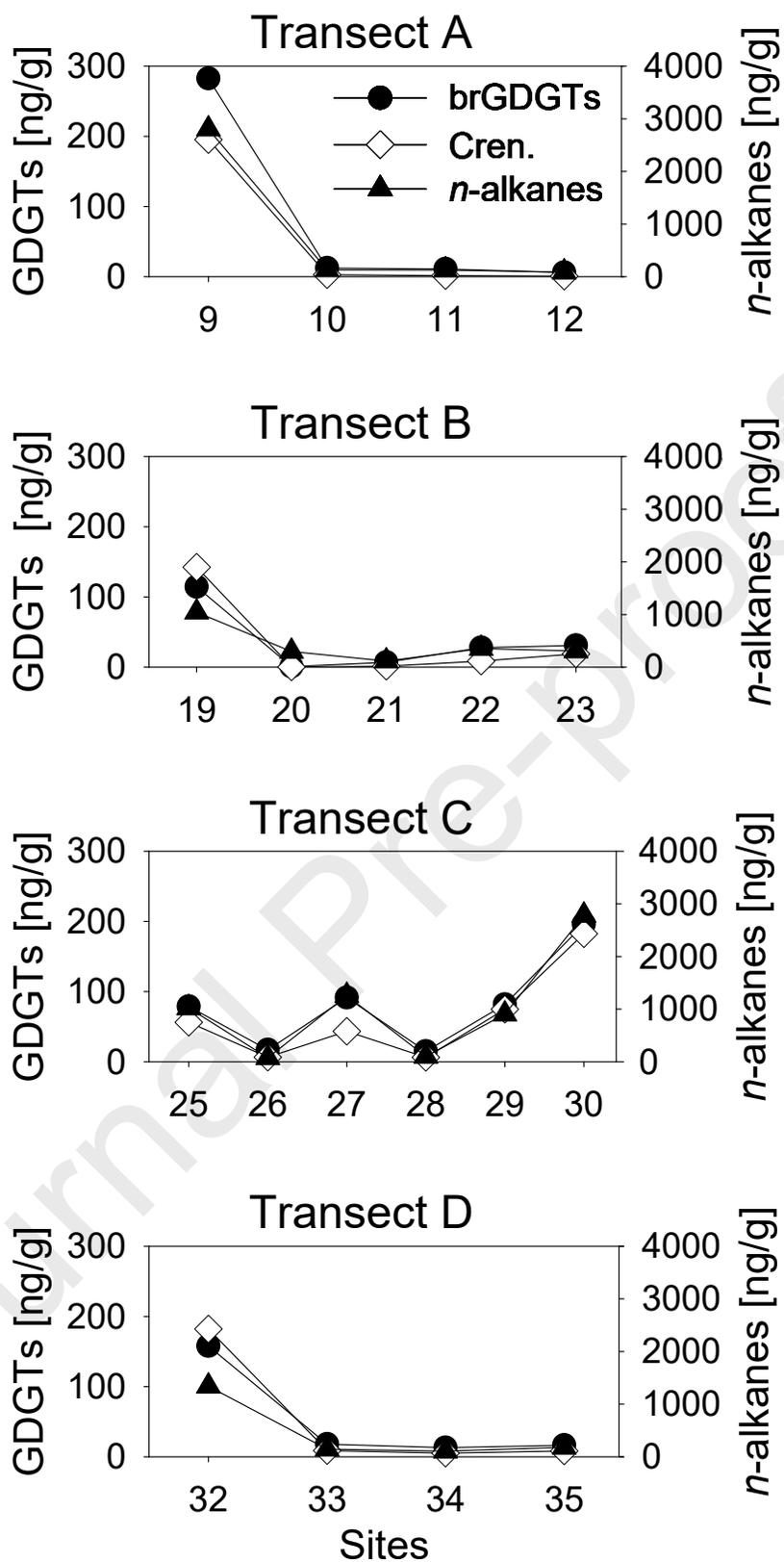
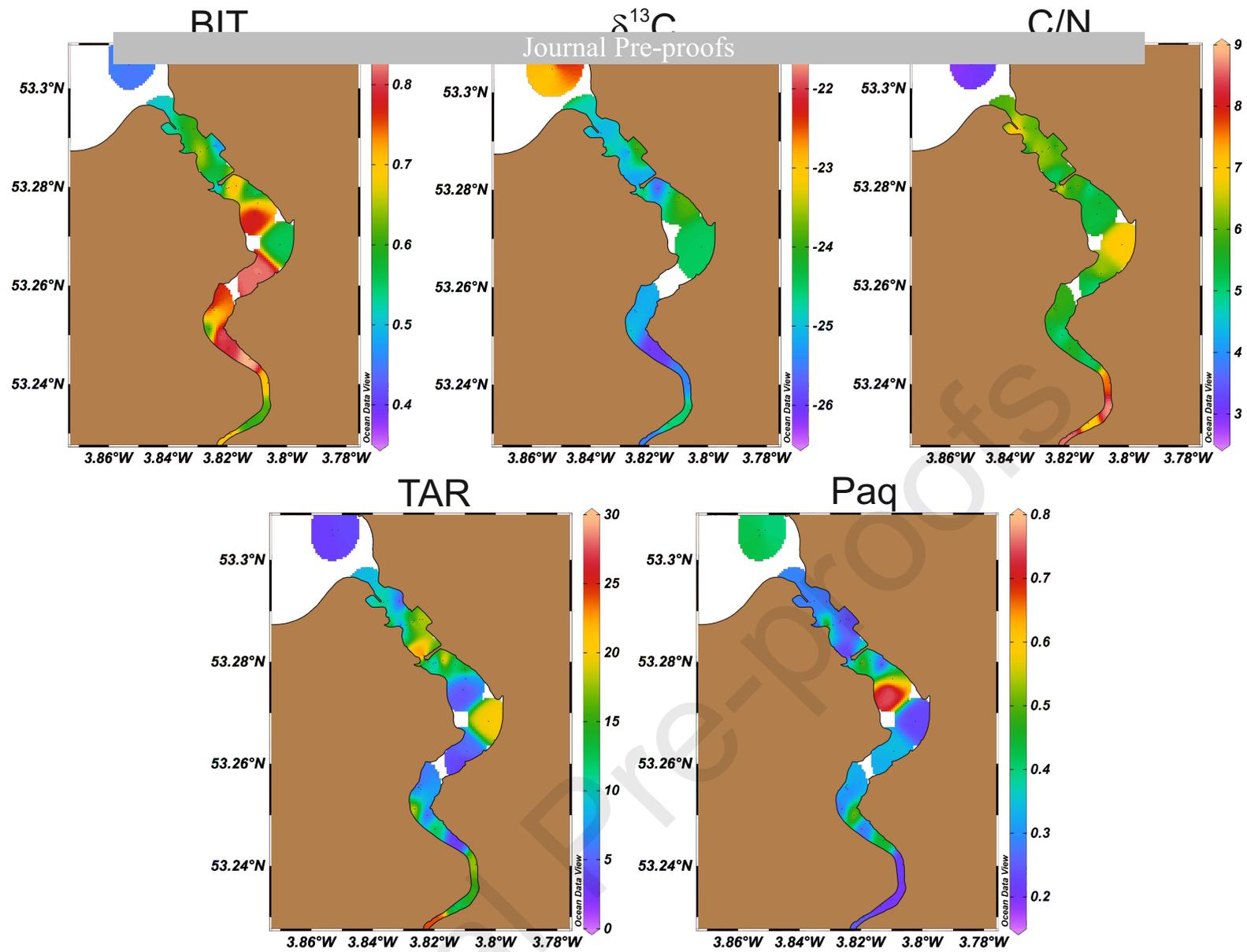
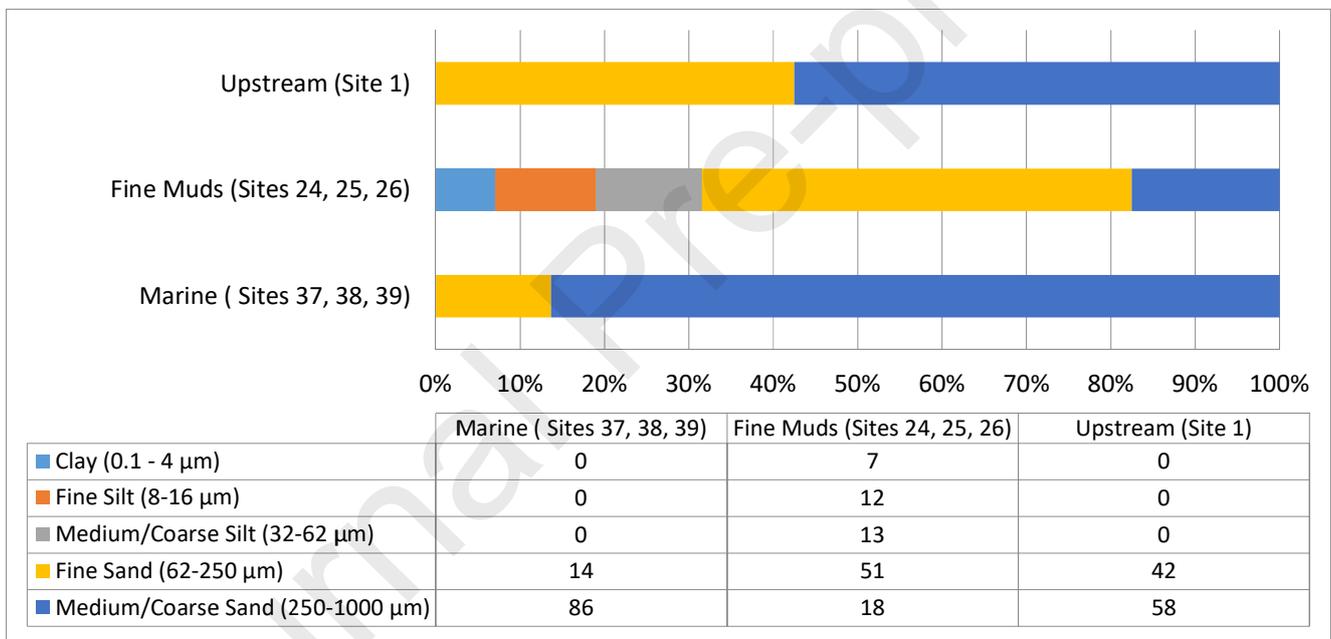
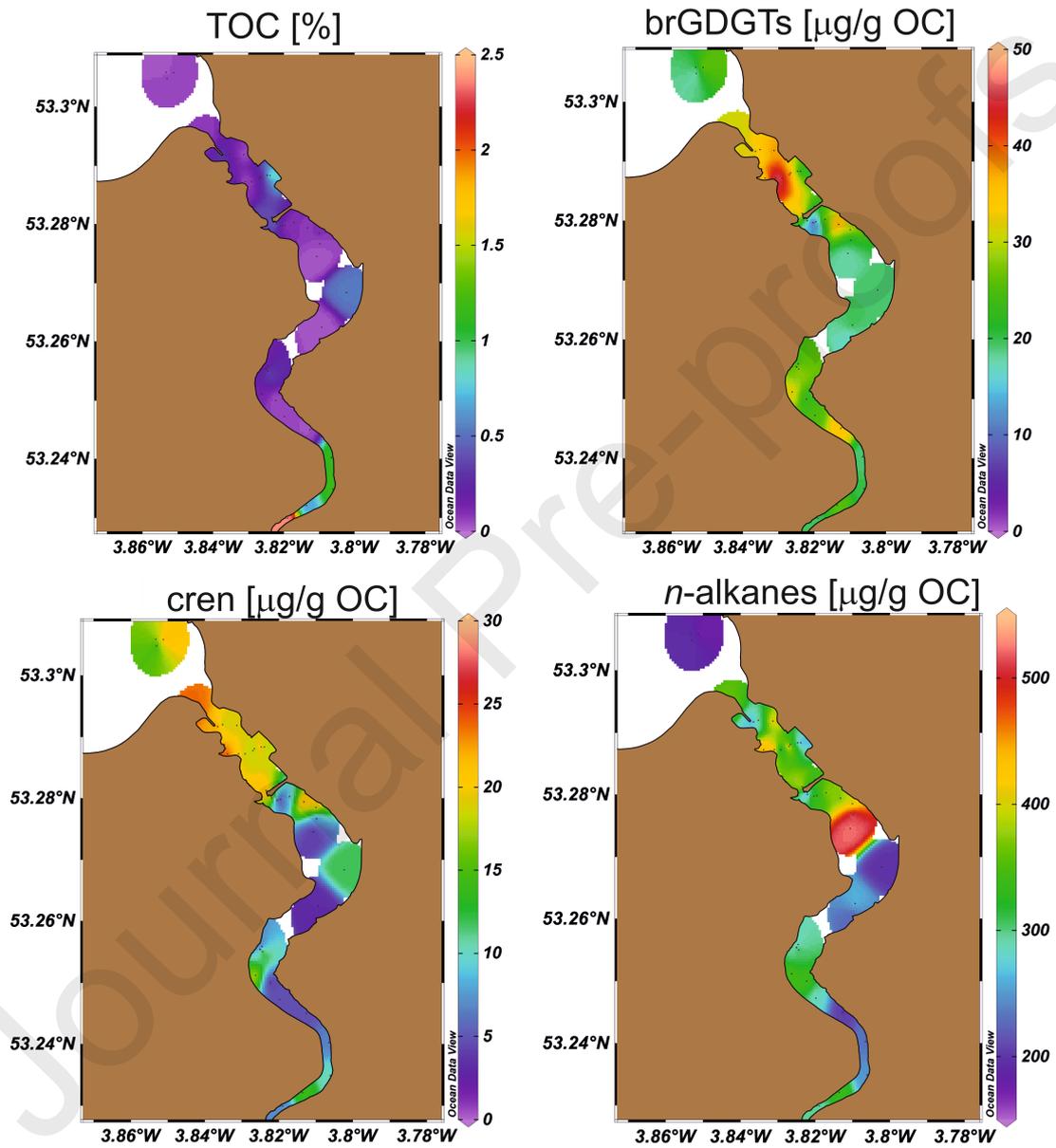


Figure 4







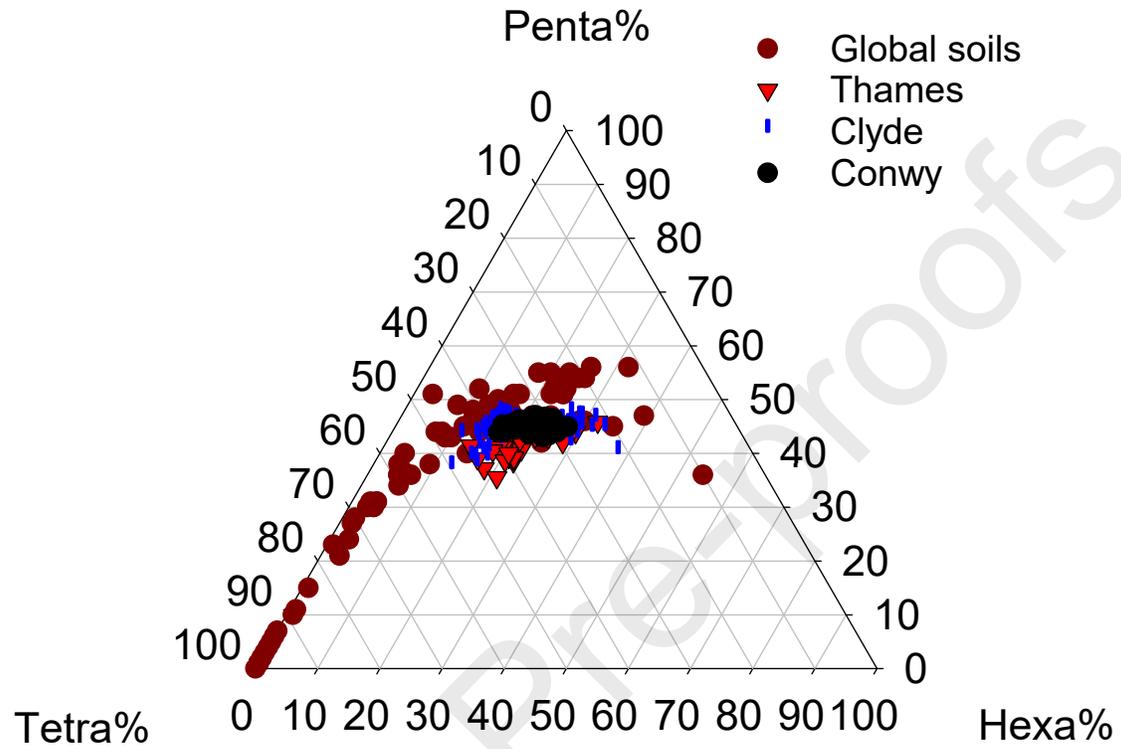
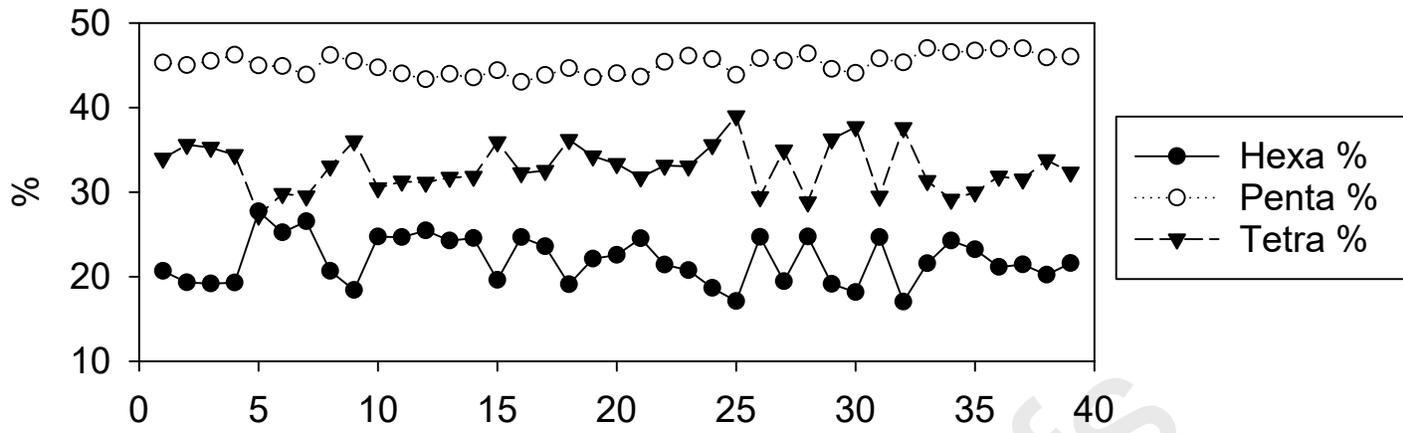
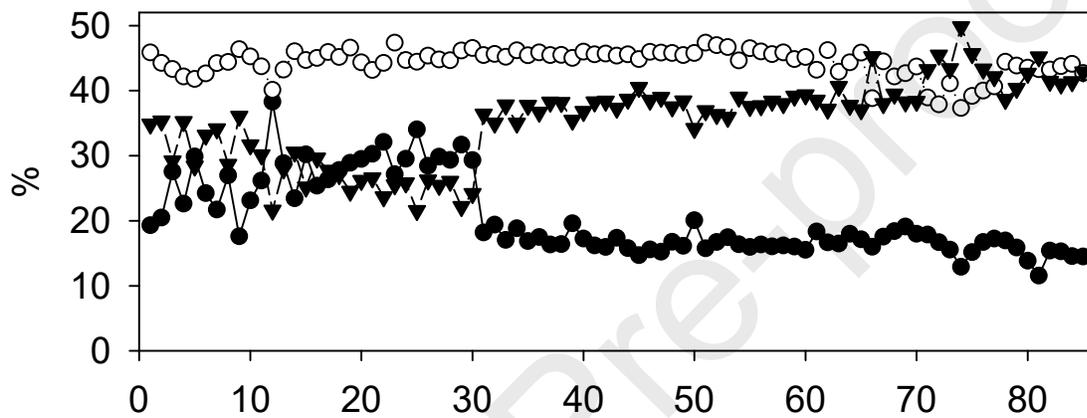


Figure 8



Clyde



Thames

