Research Article

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Stable isotope values (δ^{13}C, δ^{15}N) of macroalgal communities at Loch Creran and its relevance for elucidating sources of macroalgal organic carbon in fjordic sedimentary systems

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Abstract: Here, macroalgal isotopic values from Loch Creran, Western Scotland, were documented to determine the suitability of paired stable isotope analysis for identifying macroalgal-derived organic carbon sources in a fjordic sea loch. Variability in isotopic values (δ^{13}C and δ^{15}N) was evident within individual thalli of fucoid and kelp species, at the replicate level and between sampling localities. There were few consistent phylogenetic correlates in the isotopic values of macroalgae. The δ^{13}C ranges did, however, provide insight into differentiating between carbon sources more broadly, such as terrestrial from marine and between macrophyte lineages. As such, δ^{13}C could be indicative of the presence of macroalgal carbon sources within pools of organic matter but will likely be ineffective at separating these sources to lower taxonomic levels. Consequently, if these data are used alone to discriminate between macroalgal carbon sources and their relative contribution to a sedimentary pool of organic matter, the development of accurate conclusions will be challenging. The findings presented here demonstrate the need for complementary techniques or multi-tracer approaches to aid in the differentiation between macroalgal carbon sources to lower taxonomic levels rather than relying on stable isotopes as a biomarker alone.

Keywords: blue carbon; carbon; macroalgae; sediments; stable isotopes

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1 Introduction

Analogous to ‘green carbon’ and its association with terrestrial systems, ‘blue carbon’ refers to the sequestration of organic carbon (C_{org}) within marine environments (Tang et al. 2018). The recognition that vegetated coastal ecosystems sequester and store large amounts of C_{org} has driven the establishment of blue carbon (BC) strategies (Duarte et al. 2013). The acknowledgement of these ecosystems and their roles in climate change mitigation and adaptation, together with their potential for recognition within nationally determined contributions (NDCs) has led to widespread conservation and restoration initiatives (Herr and Landis 2016; Santos et al. 2021). However, efforts are largely limited to angiosperm-based ecosystems, such as seagrass meadows, salt marshes, and mangroves. This confined focus can be attributed to the process of fixating atmospheric CO_2 into a stable living biomass store and the extended storage of organic matter (OM) within sediments of the source ecosystem. Whilst this complete process of CO_2 fixation to long-term storage is perhaps limited to a few staple macrophytes (Mcleod et al. 2011), other habitats and ecosystems are components of global carbon sequestration, yet they are still excluded from climate change mitigation and BC strategies. A key example is macroalgal dominated habitats (Krause-Jensen et al. 2018).

Macroalgae are considered the most productive marine macrophytes globally, with an approximate sublittoral coverage of 3.5 million km^2 and an estimated net primary production (NPP) of 1.5 Gt C/yr^{-1} (Krause-Jensen and Duarte 2016). Many species, for example, those of the order Laminariales serve functional roles as habitat-forming species

where they are often keystones to the integrity of coastal ecosystems. Their biogenic structures alter surrounding environmental conditions through the dissipation of wave action and provide habitat for an array of floral and faunal assemblages, some of which are of socio-economic importance (Teagle et al. 2017). A large proportion of macroalgal biomass is released as detritus and may be exported further afield to recipient ecosystems, thus, forming trophic links between spatially disconnected habitats (Renaud et al. 2015). This is relevant when considering the pulsed release of post-meristematic growth by certain kelp species; a material that becomes increasingly synthesizable to consumers as it degrades (Frontier et al. 2021). Despite this, the low rates of consumption coupled with the refractory chemical nature of macroalgal tissue have led to the conjecture that roughly 89 % of this material remains in the coastal ocean (Krause Jensen and Duarte 2016).

Evidently, macroalgae possess a capacity to effectively store carbon within their living biomass however, most species grow on hard substrates where the long-term storage of C_{org} is precluded (Hill et al. 2015). BC assessments have predominantly been centred around carbon fixation and sequestration within the source habitat and because of this focus, macroalgae have largely been excluded from BC strategies (Krause-Jensen et al. 2018). Nonetheless, by evading grazing and remineralization, a significant fraction of C_{org} fixed by macroalgae is exported beyond its source of origin to depositional areas (Raven 2018). As such, macroalgae represent an important carbon donor to sedimentary systems further afield and exhibit the potential to contribute significantly to global carbon sequestration (Ortega et al. 2019).

Subject to both terrestrial and marine inputs, fjordic environments have recently been recognised for their important roles in global carbon sequestration (Smeaton et al. 2017). Characteristics of fjords including their deep, dark, and often low O_2 basins in line with minimal exposure to disturbance, and high sedimentation rates render them OM preservation hotspots. Whilst their prevalence and global coverage are limited, fjords are speculated to account for 11 % of global annual carbon sequestration, equating to 18 MT of C_{org} buried every year (Smith et al. 2015).

Through the provision of suitable substrate, fjords consistently support extensive beds of macroalgae, but the transport of macroalgal biomass between source and recipient locations has received little attention so that the fate of the C_{org} is unknown (Filbee-Dexter et al. 2018; Krause-Jensen and Duarte 2014). Assuming slow and incomplete decomposition of macroalgal detritus, fjords juxtaposed with dense macroalgal communities may allow for this material to reside in adjacent depositional basins, leading to the long-term burial and sequestration of macroalgal biomass (Pedersen et al. 2021). This speculation is supported by investigations in Arctic fjords, revealing a large proportion of sedimentary OM to be comprised of macroalgal detritus, and may thus be an extremely important yet unrecognized source of BC (Zaborska et al. 2018). Therefore, the productivity of macroalgae and the OM burial capabilities of fjord systems emphasizes the need for quantitative information that fully explores their potential in global carbon sequestration. As strategies to mitigate and adapt to climate change are paramount, recognising, conserving, and rebuilding the ocean's natural systems as a means of atmospheric CO_2 sequestration is indispensable.

Paired stable isotope analysis (SIA) of ^{13}C and ^{15}N has successfully been employed as a means of differentiating between carbon sources and their relative contributions to consumer diets or pools of OM within sedimentary systems (Kennedy et al. 2004; Zaborska et al. 2018). This application is founded on the assumption that primary producers exhibit marked differences in their isotopic 'signatures' that are relatively coherent in space and time, which allows for traceability. Concerning food web studies, carbon isotope ratios of $^{13}C/^{12}C$ (expressed as $\delta^{13}C$) are assumed to undergo negligible increases as they are transferred to higher trophic levels such that the $\delta^{13}C$ of consumers closely reflects those within their diet (De Niro and Epstein 1978). Nitrogen isotope ratios of $^{15}N/^{14}N$ ($\delta^{15}N$) undergo a greater but predictable consumer enrichment with each shift in trophic level and can therefore be used to construct trophic relationships where feeding interactions are elusive (Fredriksen 2003). For example, stable isotope studies in Arctic regions have verified the substantial contribution of macroalgal detritus to benthic food webs in locations geographically distant from the source of production (Renaud et al. 2015; Sokolowski et al. 2014). Analysis of nitrogen stable isotope ratios has also been used as a technique for identifying the origin of and tracing nitrogen in coastal ecosystems (Ochoa-Izaguirre and Soto-Jiménez 2014).

The underlying principles in using stable isotopes as biomarkers have rendered it to be a powerful tool not only for food web studies but for identifying sources of carbon that accumulate within sediments (Cloern et al. 2002; Kennedy et al. 2004). Though, regardless of its application, the use of stable isotopes as biomarkers often requires a precursory isotopic characterization of the sources of interest. In terrestrial environments, predictions of $\delta^{13}C$ variation in autotrophs are firmly established at both temporal and spatial scales (Mackey et al. 2015). Owing to the complexity of biogeochemical and physical mechanisms, stable isotopic compositions of marine autotrophs are highly variable over space and time. This was demonstrated in a

prominent survey of macrophytes, documenting a wide range in $\delta^{13}C$ (Raven et al. 2002). Specific to macroalgae, $\delta^{13}C$ values ranged from −3 to −35 ‰ which encompasses the majority of identified values for primary producers (Carvalho et al. 2009). Inherent factors such as the chemical composition, growth rate and physiological status of different tissues strongly influence macrophyte $\delta^{13}C$ values such that, variation can become significant within the blade of a single individual (Buchholz et al. 2019). Variation in isotope values can also become well pronounced at larger spatial scales, with differences emerging among sites and regions (Cornelisen et al. 2007; Ramshaw et al. 2017). Drivers of this variation may include environmental conditions like temperature, ambient nutrient levels, exposure to light and hydrodynamic processes (Drobnitch et al. 2018; Vanderklift and Bearham 2014). For example, the thickness of the diffusion boundary layer combined with the factors mentioned can govern macrophyte productivity and the kinetics of carbon discrimination (^{13}C vs. ^{12}C), which will ultimately modulate isotopic variability (Finlay et al. 1999; Raven et al. 2002). This variation in stable isotopes can distort differences between groups that are otherwise assumed to display isotopically distinct values (Vanderklift and Bearham 2014). If stable isotopes of ^{13}C and ^{15}N are to be used to differentiate between sources of OM in a marine context, it is fundamental to develop a sound understanding of the variation in the stable isotope composition of macroalgae.

The present study was therefore initiated to determine the suitability of paired SIA (^{13}C and ^{15}N) to elucidate sources of macroalgal C_{org} in fjordic sedimentary systems and associated consumer diets in subsequent studies. A baseline of stable isotope values was developed corresponding to macroalgal communities at Loch Creran, a sea fjord in Western Scotland recognized for its burial and storage of OM (Smeaton et al. 2017). Because research into the stable isotope compositions of macroalgal communities at Loch Creran is absent, this study aimed to gain a comprehensive understanding of the variability occurring in macroalgal isotopic values and whether consistent spatial patterns would emerge between species. Attention was directed towards the isotope variation between taxonomic groups and sampling sites. Intra-individual differences were also investigated by developing isotopic values corresponding to different sections of species thalli. As such, the following questions were addressed in this study: Is spatial variability of sampling locations reflected in the isotope values of macroalgae at Loch Creran? Do differences in isotope values exist at a species and/or higher taxonomical level to the extent that macroalgal C_{org} sources are distinguishable? The study was considered a necessary step to address the broader motive of outlining the connectivity between macroalgal beds and carbon standing stocks of sedimentary systems in fjordic environments. To avoid misinterpretation and erroneous conclusions when tracing the origin of OM, spatial-specific isotopic values occurring within macroalgae must be explored and acknowledged.

2 Materials and methods

2.1 Study area

Over two days in September 2021, intertidal sampling was carried out across three sites located at Loch Creran, Western Scotland (Figure 1). Reaching 12.8 km in length, with deepened basins shaped by glacial erosion, Loch Creran exhibits geomorphological characteristics that are typical of fjordic sea lochs (Hunt et al. 2020). The loch is divided into four primary basins each divided by shallow underwater sills. The lower basin represents the deepest area (49 m) though, the average depth within Loch Creran is 13.4 m (Smeaton et al. 2017). Owing to its relatively small size which provides a direct link between land and sea, hydrodynamic patterns are closely coupled to those occurring outside of the loch (Gage 1972). The residence time of the water is 3 days and exchange with coastal waters occurs to the west, at the mouth of Loch Creran where it meets with Loch Linnhe. The main source of freshwater input is received by the River Creran located at the head of the loch, in the northeast corner. The River Creran supplies the loch with approximately 286.3×10^6 m^3 yr^{-1} of freshwater and governs salinity patterns in the main basin, ranging from 30 to 33 (Almroth-Rosell et al. 2012). Despite its limited exposure to wave action, tidal flushing combined with strong surface winds facilitates the ventilation of bottom water. This contributes to the mixing of saline and freshwater and helps prevent the depletion of oxygen in bottom waters. Loch Creran displays high sedimentation rates and is considered a net sink for OM derived from both terrestrial and marine origins (Hunt et al. 2020).

2.2 Sample collection

During spring tides, intertidal macroalgal samples were collected from the three sampling locations (Figure 1). Site 1 was located towards the mouth of Loch Creran at Shian Bay. Within the vicinity of the lower basin was Site 2, and north of the Creagan Narrows was Site 3. All three sampling locations were consistent in their geomorphology, each supporting dense beds of macroalgae. Sedimentation and accumulation of mud were noticeable in the lower regions of all sites.

With the exception of *Chorda filum* (Linnaeus) Stackhouse 1797 and *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl *et* G.W. Saunders 2006 that were obtained in the sublittoral, specimens were randomly sampled from low to highwater marks, where individuals were collected and stored within a Ziplock bag. This applied to individuals present in strandlines, however, note that individuals were selected on the basis of tissue being fresh, not visibly desiccated and likely capable of maintaining photosynthetic functions (Frontier et al. 2021). In April 2022, subtidal kelps were collected during SCUBA dives by volunteers from the University of Aberdeen Sub-Aqua Club (approximate coordinates: 56°18′45.122″N, 5°40′18.455″W; 56°22′54.4″N, 5°36′2.214″W; 56°32′39.547″N, 5°16′36.044″W). All samples were

stored in a cool box prior to their transportation to the laboratory where they were then sorted, identified to the lowest taxonomical level possible and frozen at −20 °C. Due to difficulties in identification, specimens belonging to Chlorophyta (green algae) were grouped together.

2.3 Sample processing and analysis

The continuation of sample processing first involved a preliminary test to determine whether cleaning methodology would distort the isotope values of the macroalgae. As such, a defrosted specimen of *Fucus vesiculosus* Linnaeus 1753 from Site 1 was subsampled and cleaned with one of two treatments: Milli-Q water or 5 % HCl. Apical tissue from the blades to the distal ends of fronds was preferentially selected, avoiding degraded material to ensure consistency throughout the replicates (Figure 2).

Sub-samples of *F. vesiculosus* (<5 cm) were swilled with seawater collected from Loch Creran. The material was then blotted dry to remove seawater and was randomly allocated to undergo one of the two treatments (Milli-Q $n = 5$, HCl $n = 5$). Cleaning with either treatment was performed by swilling the material and gently scrubbing the surface free of any contaminating matter such as inorganic and organic particles as well as epiphytic organisms. The clean sub-samples were then freeze-dried (−50 °C) and stored at −20 °C. Freeze-dried samples were further processed in a ball mill (RETSCH MM 400) and the homogenised material contained within pre-combusted (550 °C) glass vials and stored at −20 °C. 2 mg ± 0.05 aliquots of the homogenised material were weighed into tin cups and processed for SIA.

The analyses of carbon and nitrogen isotopic compositions were performed on a Sercon 20-20 isotope ratio mass spectrometer coupled with a Sercon ANCA gas solid liquid analyser (School of Biological Sciences, University of Aberdeen). Results are expressed in the δ(‰) notation as deviations from standards following the formula:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}}) - 1) \times 10^3$$

where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The corresponding standards were the international Vienna Peedee Belemnite (VPDB) and atmospheric nitrogen for ^{13}C and ^{15}N, respectively. During analysis, the samples were dispersed amongst replicates of a laboratory-specific

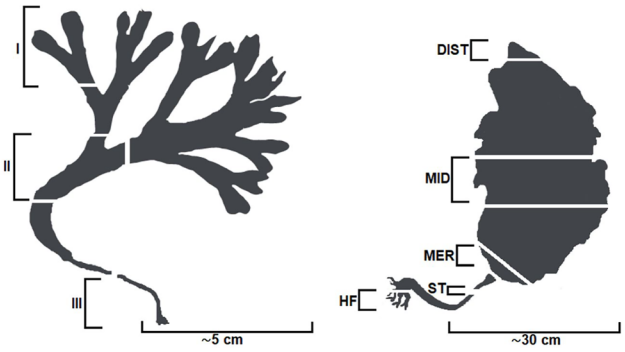

Figure 2: Diagrams showing the different sections of thalli subsampled to investigate intra-individual, interspecific and spatial variability in $\delta^{13}C$ and $\delta^{15}N$. (A) *Fucus vesiculosus* (applicable to other fucoid intra-individual sub-samples). (B) Regions of Laminariales where triplicate sub-samples were obtained. HF, holdfast; ST, stipe; MER, meristem; MID, middle section of lamina; DIST, distal end of lamina.

reference material (wheat flour) along with National Institute of Standards and Technology reference materials (glutamic acid USGS40 and USGS41a). To measure procedural and instrumental error, triplicate aliquots of a given sample and reference materials were analysed throughout the analysis period.

There were no significant differences between the mean $\delta^{15}N$ and $\delta^{13}C$ of *F. vesiculosus* subsamples cleaned with either 5 % HCl or Milli-Q water ($T_8 = -0.457$, $p = 0.660$ for $\delta^{15}N$ and $T_8 = -2.053$, $p = 0.074$ for $\delta^{13}C$). As such, processing of the remaining samples was performed in a manner as previously described using only Milli-Q water for cleaning. Except for the small epiphytic red alga, *Vertebrata lanosa* (Linnaeus) T.A. Christensen 1967, the green algae and *C. filum*, tissue occurring in the distal ends of fronds were used to investigate spatial variability occurring among sampling locations. Whole thalli were instead processed for *V. lanosa* and the Chlorophyta groups to obtain enough material for SIA. Because of the difficulties in collecting a whole individual, *C. filum* tissue was sampled based on it being consistent in appearance.

To investigate variability in isotopic values occurring within individuals of selected fucoid species, tissue samples were collected from the holdfasts (extending <5 cm into the stipe) and the middle

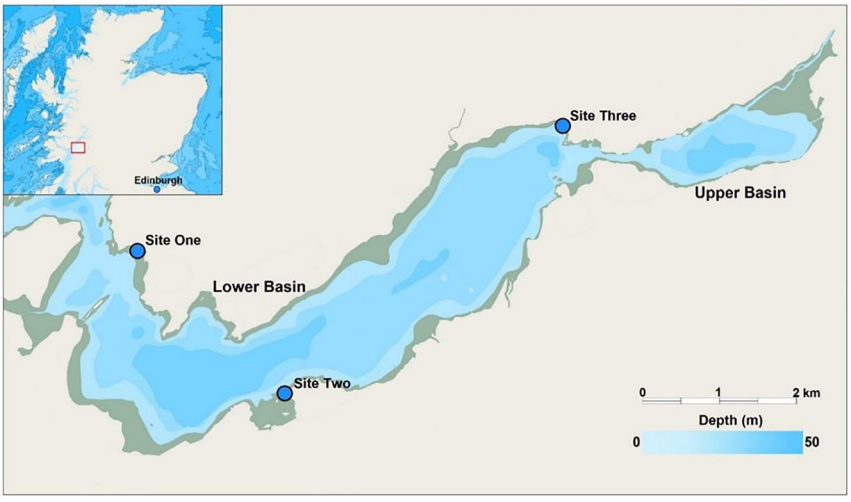

Figure 1: Location map of the study area at Loch Creran (56°32′14.947″N, 5°19′12.209″W) showing the three intertidal sampling locations and bathymetry (m). Map data: Digimap.

sections of thalli (Figure 2). With the larger kelp species, tissues samples were obtained from the holdfast, stipe, meristem (<5 cm from the transition between the stipe and the blade), and the basal to distal ends of the lamina. However, due to a reduced number of kelps collected from Loch Creran, triplicates could only be produced from the same individual rather than from three independent individuals. Cleaning and preparation for SIA were nonetheless performed in a consistent manner across all investigations.

2.4 Statistical analysis

All data exploration and subsequent statistical analyses were performed using Minitab 19. Triplicate samples obtained from the meristematic region of the single *S. latissima, Laminaria hyperborea* (Gunnerus) Foslie 1885 and *Laminaria digitata* (Hudson) J.V. Lamouroux 1813 individuals were pooled to generate a mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$. Meristematic tissue was chosen to represent these ratios due to the chronic erosion of the distal ends of Laminariales which differs from the apical growth more commonly displayed by the other species. Therefore, sampling tissue that was representative of new growth granted a level of consistency for interspecific comparisons.

Prior to the application of statistical tests, data were checked for outliers using Grubb's tests, fitted to probability plots to assess normality and Levene's test were used to determine homoscedasticity. Two-way ANOVAs were used to test for differences in the isotopic values corresponding to the intra-individual subsamples from the fucoid species, with species and thallus region as independent variables and $\delta^{13}C$ or $\delta^{15}N$ as the dependent variable. Upon failing to meet the assumptions of ANOVA, Kruskal-Wallis tests were used to compare the median $\delta^{13}C$ and $\delta^{15}N$ between the species. Because of the small sample size, data corresponding to the Laminariales were not included in the interspecific analyses. The results are reported at the $p < 0.05$ significance level.

3 Results

3.1 Macroalgal identification and distribution

Across the three intertidal sampling locations, a total of 9 species were confidently identified and processed for stable isotope analysis. Fucoid species were the most prevalent in terms of their presence throughout the sampling locations. Specifically, samples of *Ascophyllum nodosum* (Linnaeus) Le Jolis 1863, *Fucus serratus* Linnaeus 1753, *F. vesiculosus* and *Pelvetia canaliculata* (Linnaeus) Decaisne *et* Thuret 1845 were obtained and processed from all sampling localities. *Fucus spiralis* Linnaeus 1753 samples were collected from Site 1 and Site 3 whereas samples of *Fucus ceranoides* Linnaeus 1753 were collected only from Site 1. An ecad of the species *A. nodosum,* hereafter referred to as *A. nodosum* f. *mackayi* (Turner) A.C. Mathieson *et* Dawes 2017 was sampled from Sites 1 and 2.

In the intertidal, the fucoid species exhibited typical patterns of habitat zonation where *P. canaliculata, A. nodosum* f. *mackayi* and *F. spiralis* were consistently sampled in the higher reaches of the intertidal whereas, *F. serratus* was collected from the foot of the intertidal. *Ascophyllum nodosum* and *F. vesiculosus* were primarily collected from the middle regions of the sampling sites.

The kelp species *C. filum* was collected from all three sampling locations, whilst a single individual of *S. latissima* was obtained exclusively from Site 3 during the intertidal surveys. The epiphytic red alga (Rhodophyta) *V. lanosa,* present on individuals of *A. nodosum* was sampled from Sites 1 and 3. Single individuals of *L. digitata, L. hyperborea* and *S. latissima* were collected from the subtidal in 2022. As for macroalgae belonging to the taxon Chlorophyta, individuals were collected from Site 2 and Site 3 and were distributed throughout the upper shore or areas exposed to freshwater inflow. It was not attempted to identify the Chlorophyta to lower taxonomic levels.

3.2 Intra-individual variation of $\delta^{13}C$ and $\delta^{15}N$ values

A two-way ANOVA revealed a significant interaction between tissue subsamples $\delta^{13}C$ (I–III) and the fucoid species from which the samples were excised ($F_6 = 4.84$, $p = 0.002$). The triplicate tissue samples corresponding to the distal ends (I) of *A. nodosum, F. vesiculosus* and *P. canaliculata* mostly had higher $\delta^{13}C$ in comparison to the other regions where samples were excised (Figure 3). Excluding *A. nodosum*, $\delta^{13}C$ values of the distal ends for the fucoid species were the most consistent at the replicate level. The distal ends of *A. nodosum* $\delta^{13}C$ varied by 2.5 ‰ amongst the replicates. *Fucus spiralis* failed to display marked differences in $\delta^{13}C$ between the three regions of tissue subsamples where the values of all tissue types were dispersed within the range of –17 to –19 ‰. *Ascophyllum nodosum, F. spiralis* and *P. canaliculata* conformed to a similar pattern, where $\delta^{13}C$ decreased with distance from the distal ends. *Fucus vesiculosus* exhibited the greatest range in $\delta^{13}C$ (6 ‰).

A two-way ANOVA revealed a significant interaction between tissue subsamples $\delta^{15}N$ (I–III) and the fucoid species from which the samples were excised ($F_6 = 4.03$, $p = 0.006$). Nonetheless, there was an overlap between the three species and their associated thalli sampling regions (Figure 3). Despite the overlap in $\delta^{15}N$, *A. nodosum* subsamples became enriched in ^{15}N with distance from the distal ends. *Fucus spiralis* and *F. vesiculosus* displayed a dissimilar and less definitive pattern, with subsamples from the middle sections depleted in ^{15}N relative to other regions where subsamples were excised (I and III). Within the range of $\delta^{15}N$ values for *P. canaliculata* (5.50–7.80 ‰), the middle sections (II) had

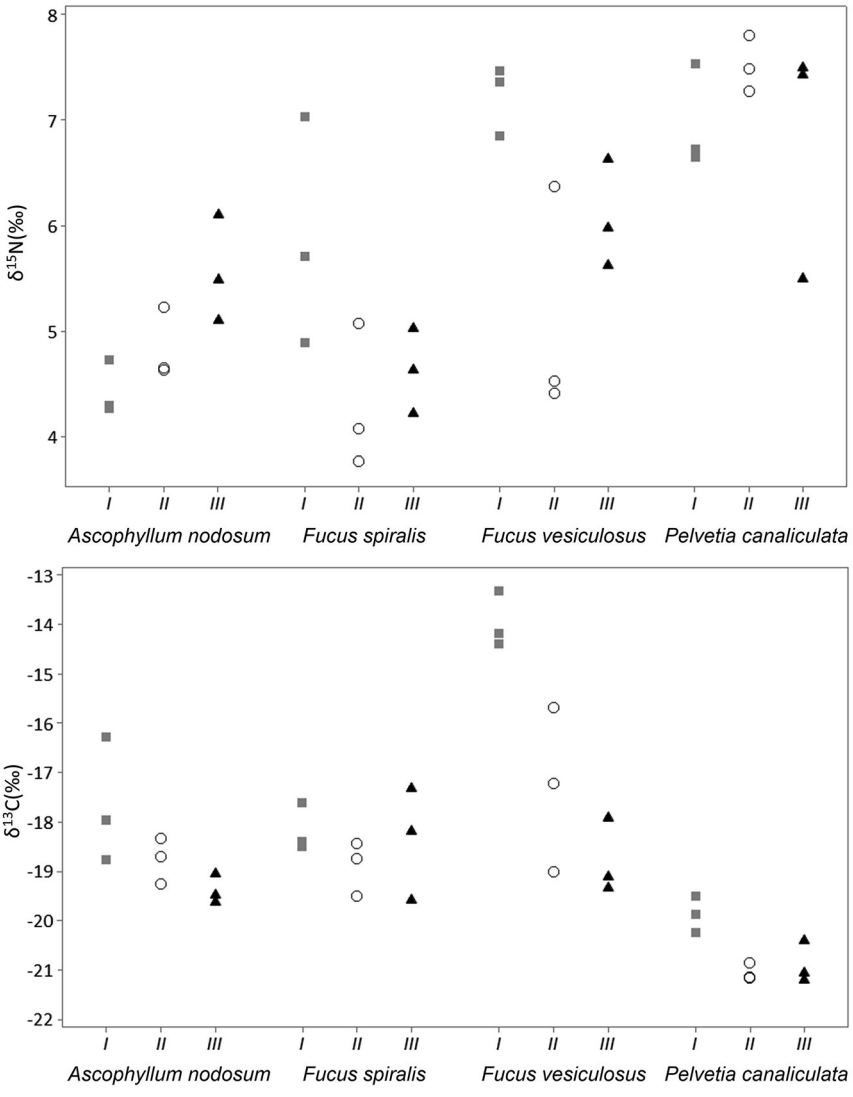

Figure 3: Individual isotope values corresponding to the thalli sections subsampled (see Figure 2) to investigate intra-individual variability in δ^{15}N (top) and δ^{13}C (bottom) for fucoid species.

high δ^{15}N values. The range of δ^{15}N in the distal regions was greatest in *F. spiralis* (2.96 ‰) and lowest in *F. vesiculosus* (0.61 ‰).

Within the lamina of *S. latissima* (sublittoral), tissue obtained from the middle section was the most enriched in ^{13}C whereas tissue from the meristematic region at the base was the most depleted (Figure 4). Tissue samples from the oldest part of the lamina displayed intermediate δ^{13}C values (distal ends). An outlying replicate from the distal ends was removed and therefore, the values from this region should be interpreted with caution. Nonetheless, the range in δ^{13}C from the replicate distal end subsamples was 1.29 ‰. In comparison to the *S. latissima* individual obtained from the sublittoral, the *S. latissima* individual collected from the subtidal in 2022 had lower δ^{13}C values. Only subsamples from the middle and meristematic region of lamina were comparable, whereas samples from the distal end and holdfast had substantially lower δ^{13}C values. The latter were consistent to the intra-individual subsamples of *L. digitata,* within the range of −21 to −22 ‰. The subsamples from *L. hyperborea* did not have markedly different δ^{13}C values and were in a range like that of *S. latissima* (sublittoral) collected from the initial sampling trip in 2021.

δ^{15}N of the meristematic tissue subsamples from *S. latissima* (sublittoral) were the most consistent, along with those excised from the middle section of the lamina (Figure 4). The most ^{15}N enriched tissue samples were from the holdfast however, this was a region exhibiting high variability at the replicate level. Following the removal of the outlying replicate, the distal end subsamples were amongst the most depleted in ^{15}N, with δ^{15}N values of 5.34 ‰ and 6.25 ‰. Despite this, there were no regular patterns or isotopically distinct groups between the thallus sampling regions and δ^{15}N for *S. latissima* (sublittoral). The tissue

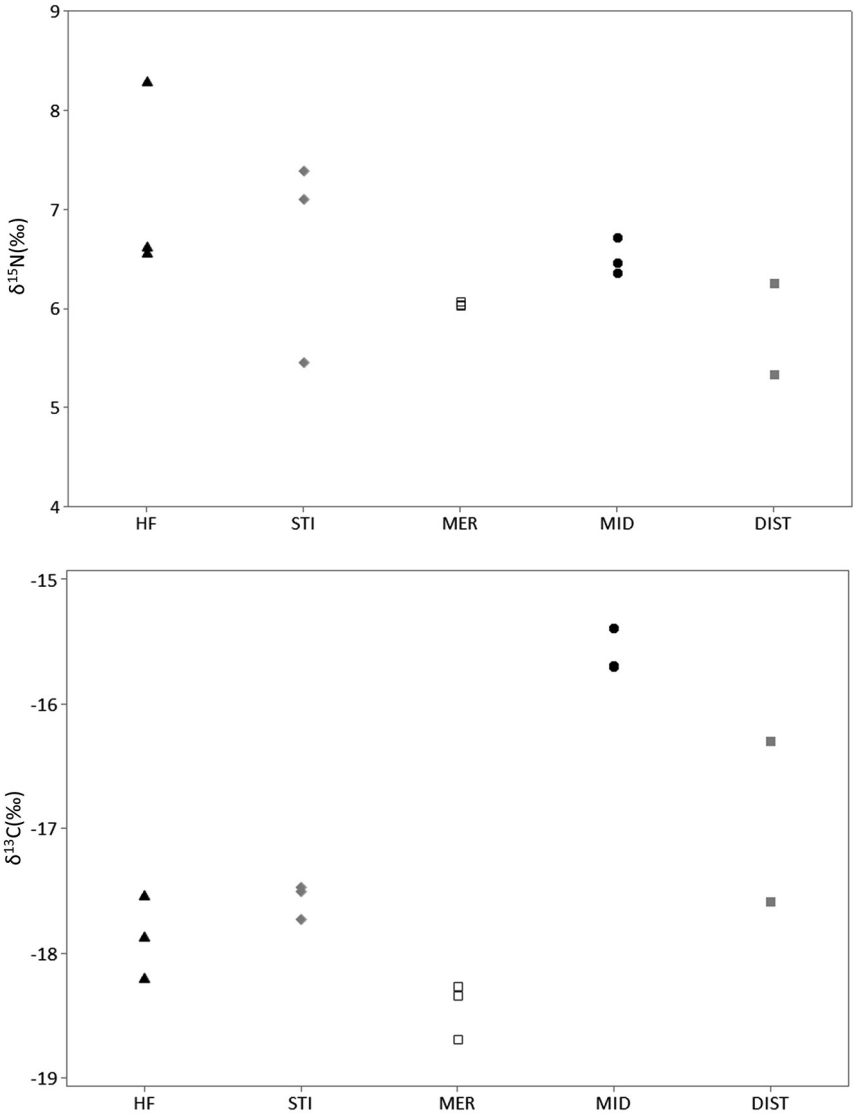

Figure 4: Individual isotope values corresponding to the thallus sections that were subsampled to investigate intra-individual variability in δ^{15}N (top) and δ^{13}C (bottom) for *Saccharina latissima* (sublittoral). HF, holdfast; ST, stipe; MER, meristem; MID, middle section of lamina; DIST, distal end of lamina.

samples from *L. hyperborea* and *S. latissima* collected in 2022 (subtidal) conformed to a defined pattern, where the younger tissues from the meristematic and middle section of the lamina had the lowest δ^{15}N values, followed by those excised from the distal ends. For both *L. digitata* and *L. hyperborea,* subsamples from the holdfast were the most enriched in ^{15}N.

3.3 Interspecific variation of δ^{13}C and δ^{15}N values

Large variability in the mean δ^{13}C values were found between the macroalgal taxa sampled at Loch Creran (Figure 5). *Fucus serratus* displayed the highest mean δ^{13}C whilst the lowest were observed in *V. lanosa* and *L. digitata*. The range between the mean δ^{13}C of *F. serratus* and *V. lanosa* was 9.16 ‰. Of the fucoid species sampled, *P. canaliculata* was the most isotopically depleted in ^{13}C and the least variable, followed by *F. spiralis* that displayed a mean consistent with that of *A. nodosum* f. *mackayi*. Despite this, *A. nodosum* f. *mackayi* was the most variable in δ^{13}C along with *F. vesiculosus* and the Chlorophyta. The mean δ^{13}C (±SD) of the Chlorophyta was −14.32 ± 2.10 ‰. Excluding *S. latissima, L. digitata* and *L. hyperborea*, a Kruskal-Wallis test confirmed significant differences in the median δ^{13}C values among taxa (H_7 = 110.44, p < 0.001). However, due to the stark variability and overlap between the species, the interspecific significance in the median δ^{13}C is likely attributable to *V. lanosa* and *P. canaliculata*.

When excluding *S. latissima, L. digitata* and *L. hyperborea*, the interspecific difference between the median δ^{15}N of the macroalgal taxa was significant (H_7 = 45.63., p < 0.001). In contrast to δ^{13}C, the range in δ^{15}N (mean ± SD) at

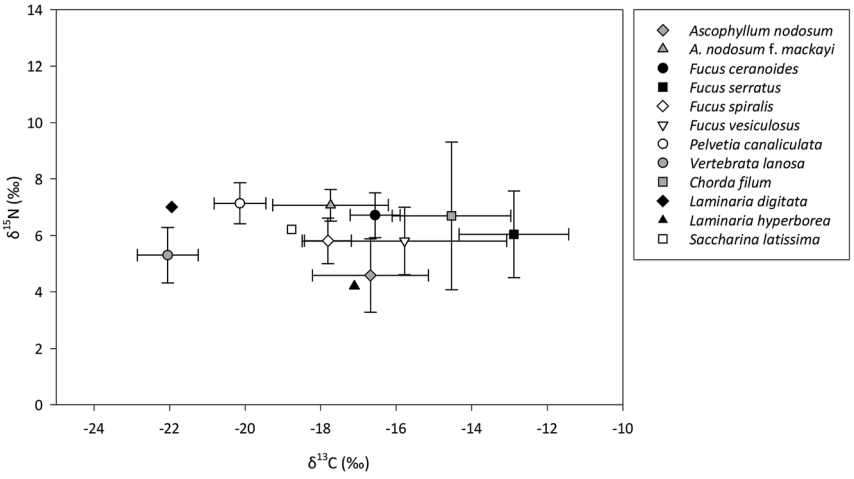

Figure 5: Mean (±SD when $n > 3$) δ^{15}N and δ^{13}C of the identified macroalgal species sampled at Loch Creran. Triplicate meristematic samples were obtained from the same individual for *Saccharina latissima*, *Laminaria hyperborea* and *Laminaria digitata*. Whole thalli were homogenised to obtain sufficient material for *Vertebrata lanosa*, whereas δ^{13}C and δ^{15}N represent samples from the distal ends of the Fucoids.

Loch Creran was reduced, though some taxa exhibited great variability (Figure 5). *Pelvetia canaliculata* displayed the highest mean δ^{15}N whereas, the lowest was observed in *L. hyperborea*. The range in the mean δ^{15}N between *L. hyperborea* and *P. canaliculata* was 2.94 ‰. Interestingly, *A. nodosum* f. *mackayi* was isotopically enriched (^{15}N) relative to *A. nodosum* and was consistent amongst its replicates. In line with the δ^{13}C signals corresponding to *F. ceranoides* and *F. spiralis*, their intraspecific variability in δ^{15}N was reduced in comparison to the other species and groups. Nevertheless, with reference to the scale of the study area, the few individuals sampled perhaps poorly represent the isotopic values of *F. ceranoides* and *F. spiralis* at Loch Creran, with $n = 3$ and 9, respectively. The mean δ^{15}N of *C. filum* was slightly higher relative to *S. latissima* but it also exhibited noticeable variability, second to the Chlorophyta. The mean δ^{15}N (±SD) of the Chlorophyta was 7.03 ± 4.64 ‰.

3.4 Spatial variation of δ^{13}C and δ^{15}N values

With reference to explorative bi-plots of δ^{13}C – δ^{15}N (Figure 6), few of the macroalgal taxa sampled at Loch Creran exhibited δ^{15}N variability in accordance with their sampling location of origin. For example, individuals of *V. lanosa* produced δ^{15}N values that were set apart into two clusters. Samples of this alga collected from Site 1 had higher δ^{15}N relative to those from Site 3. *Fucus vesiculosus* and *F. serratus* conformed to a pattern of δ^{15}N variability like that of *V. lanosa*. Whilst it was less pronounced, *F. vesiculosus* and *F. serratus* sampled from Site 3 were largely depleted in ^{15}N relative to Sites 1 and 2. The between site variability in δ^{15}N for *A. nodosum* was less marked, with there being overlap between each site. *Ascophyllum nodosum* f. *mackayi* showed no distinct differences in the δ^{15}N of individuals collected from either Site 1 or 2. This was consistent with *F. spiralis*, although there was a greater range in δ^{15}N at the replicate level (2.96 ‰). The triplicate *C. filum* samples collected from Site 1 showed more negative δ^{15}N values.

There were few instances, where macroalgal species displayed variability in δ^{13}C that could be attributed to their sampling locality. δ^{13}C values for *A. nodosum* f. *mackayi* sampled from Sites 1 and 2 were within the range of –20.37 to –15.14 ‰. Despite there being slight overlap and replicate variability, samples from Site 1 were situated towards the enriched end of this range (Figure 6). In contrast, *A. nodosum* f. *mackayi* from Site 2 was mostly depleted in ^{13}C, with low δ^{13}C values. The Chlorophyta from Sites 2 and 3 exhibited a similar pattern to *A. nodosum* f. *mackayi* where their δ^{13}C values were within a broad range (–19.66 to –10.91 ‰) with overlap between the two sites. Samples of *V. lanosa* from Site 1 had low δ^{13}C with respect to those from Site 3 however, the differences are perhaps indeterminate given the small sample size and replicate variability. *Chorda filum* δ^{13}C values were parted into two groups, with samples from Sites 2 and 3 occupying a range of –15.08 to –12.53 ‰. *Chorda filum* δ^{13}C values from Site 1 were more consistent and lower. Within the range of δ^{13}C for *A. nodosum* sampled at Loch Creran (6.18 ‰), there was no distinct trend representative of the individual sampling locations.

4 Discussion

Naturally occurring stable isotopes (^{13}C, ^{15}N) have been proposed as potential biomarkers to differentiate between carbon sources and their relative contributions to pools of OM within sedimentary systems. The successful application of paired SIA has helped to identify the importance of macroalgae in distal food webs (Renaud et al. 2015). Furthermore,

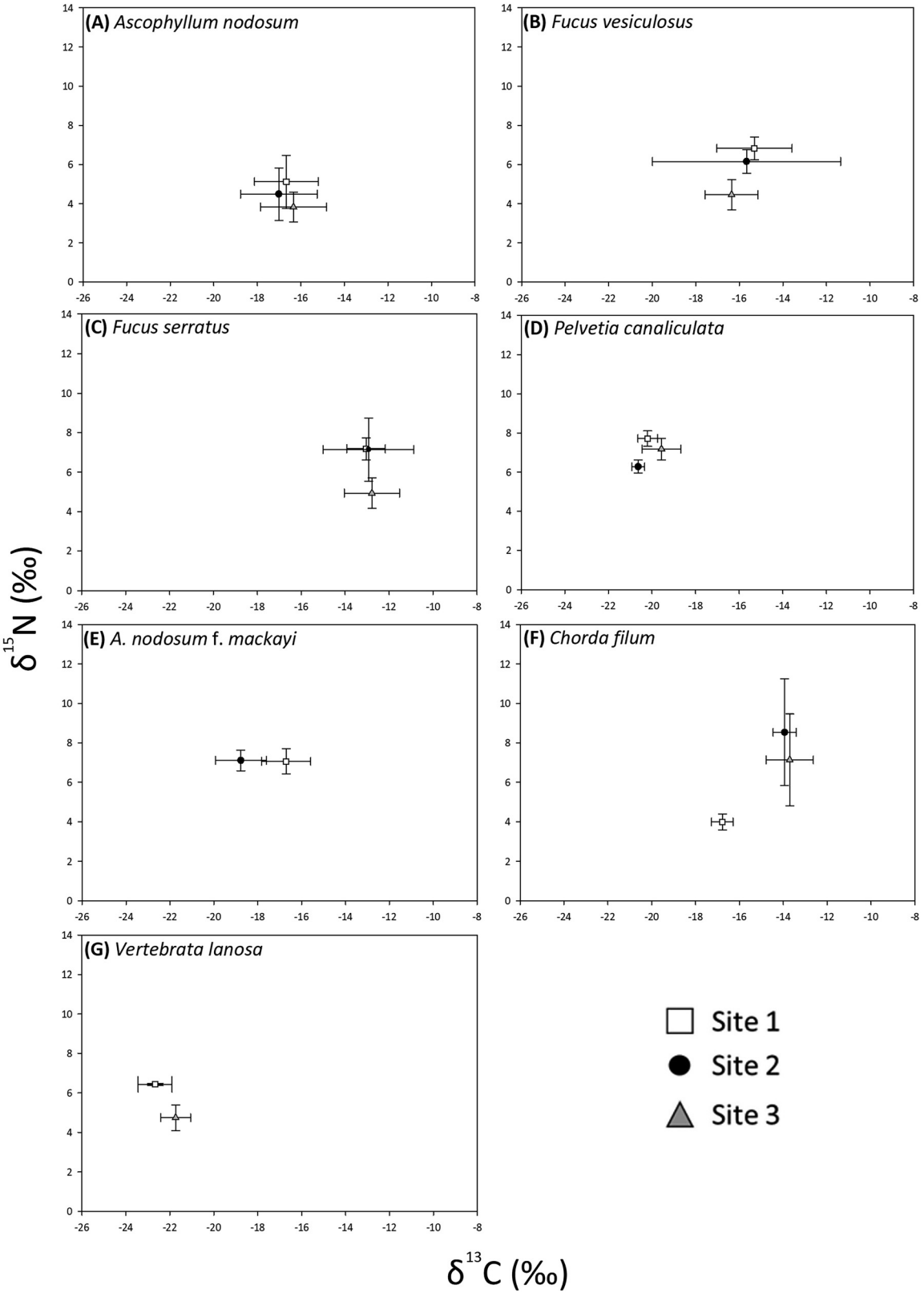

Figure 6: Mean (±SD) δ^{15}N and δ^{13}C of selected macroalgal species from different sampling sites at Loch Creran.

investigations of $\delta^{13}C_{org}$ and $\delta^{15}N$ in Arctic fjords have revealed the significance of macroalgal detritus in comprising sedimentary OM (Zaborska et al. 2018). Analysis of $\delta^{13}C$ and $\delta^{15}N$ may, therefore, be appropriate in determining the role of macroalgae as a BC source within a fjordic sea loch. However, there is a challenge when using stable isotopes to study pathways of OM based on macroalgae. The natural isotopic variation occurring in macroalgae can be substantial (both intraspecific and interspecific) and has the potential to confound attempts at differentiating between carbon sources. Hence, it is important to develop a sound understanding of the variation in the stable isotope composition of macroalgae if these data are to be used in future investigations.

4.1 Using $\delta^{13}C$ to determine terrestrial or marine sources of C_{org} in marine sediments and definition against other marine primary producers

The isotopic composition of primary producers is indicative of the bioavailable source used of $CO_{2(At)}$ (atmospheric), $CO_{2(Aq)}$ (aqueous) or HCO_3^-, as well as the mechanisms of its assimilation (C3, C4, CAM) (Diaz-Pulido et al. 2016). Despite increasing concentrations of $CO_{2(At)}$ in the terrestrial environment, the fractionated $\delta^{13}C$ measurements of terrestrial Plantae are relatively stable (Mook 1986; Tütken 2011). This provides reliable ranges of terrestrial $\delta^{13}C$ values relating to their metabolic process.

Collectively, the pool of marine DIC is less depleted in ^{13}C than $CO_{2(At)}$ ($\delta^{13}C \sim -7.5$ ‰), where $\delta^{13}C$ for HCO_3^-, CO_3^{2-} and $CO_{2(Aq)}$ are approximately −0.5 ‰, 2 ‰ and −10 ‰ respectively (Mook 1986; Mook et al. 1974). Therefore, it is possible to provide an indication of origin when comparing the $\delta^{13}C$ of marine primary producers to terrestrial ones. However, like terrestrial Plantae, marine macrophytes have several factors that impact the variation of the $\delta^{13}C$ values including taxonomy, metabolic pathways as well as age, season, location and exposure to sunlight. Because of these sources of variation, it may also be possible to differentiate between marine macrophytes by their $\delta^{13}C$ values and provide valuable insight for the identification of C_{org} sources in marine sediments.

Some species of red, brown and green macroalgae have been observed to selectively follow C3/C4 'like' pathways depending on the availability and form of DIC (Brechignac and Andre 1984; Johnson and Raven 1986; Kremer 1984; Liu et al. 2020; Reiskind and Bowes 1991; Valiela et al. 2018). Macroalgae can passively take up CO_2 however, to compensate for the low concentrations of CO_2 (~1 %) in seawater, many employ carbon concentration mechanisms (CCMs), allowing the utilization of HCO_3^- (Raven et al. 2002). Hence, the uptake of one source over the other will be reflected in the isotopic values of tissues which is a result of the difference in the isotopic weights of HCO_3^- (relatively ^{13}C-enriched) and CO_2 (relatively ^{13}C-depleted). Naturally occurring stable isotopes of carbon in macroalgae can, therefore, be indicative of the presence or absence of CCMs, or more generally, reflect the primary source of DIC utilized (Stepien 2015). Specifically, macroalgae with tissues exhibiting $\delta^{13}C$ lower than −30 ‰ are classed as non-CCM species, relying on the diffusive uptake of CO_2 alone. $\delta^{13}C$ greater than −10 ‰ are instead associated with the sole use of HCO_3^-, and intermediate values between the former and the latter are indicative of facultative HCO_3^- and CO_2 uptake (Diaz-Pulido et al. 2016; Raven et al. 2002).

The isotopic values of the entire macroalgal inventory sampled from Loch Creran fell within the brackets of both HCO_3^- and CO_2 users ($\delta^{13}C$ = −30 to −10 ‰). Towards the CO_2 end of this range (^{13}C-depleted) was *V. lanosa* whereas the high $\delta^{13}C$ (^{13}C-enriched) recorded in the Chlorophyta groups is suggestive of frequent HCO_3^- uptake. The absence of species recording low $\delta^{13}C$, indicative of obligate CO_2 uptake, is likely related to the reduced diversity of macroalgal species incorporated in this study. Collectively, the $\delta^{13}C$ means of the macroalgae sampled, fell within the gap between $\delta^{13}C$ values of C3 and C4 terrestrial Plantae. They were sufficiently outside the expected ranges of marine Plantae such as seagrasses and salt marshes: however, the data obtained was not separate from the expected range of POM which includes phytoplankton (see Figure 7). It must be noted that the $\delta^{13}C$ range for the Laminariales and Rhodophyta is represented by a small number of sample replications collected from Loch Creran and therefore, it is not statistically reliable. It is recommended that further samples be analysed to achieve a reliable data set on the mean ranges. It is also important to note that the ranges depicted in (Figure 7) are a synthesis of many studies but that they too are subject to variation. As such, this identification by $\delta^{13}C$ data alone is not absolute and should be considered indicative of the presence of macroalgae in the sediment and identification supported by other relevant analyses.

4.2 Using $\delta^{13}C$ as a biomarker to differentiate between red, brown and green macroalgae and potential differentiation between Fucoids and Laminariales

High interspecific variation in $\delta^{13}C$ was revealed in the study however, with few exceptions, sources of variation could not

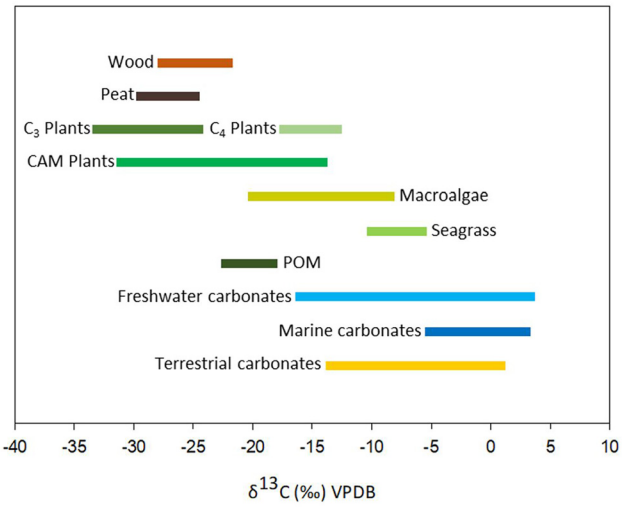

Figure 7: Expected $\delta^{13}C$ ranges of inorganic carbon sources and primary producers, as well as terrestrial and marine sources of organic matter (modified from Wagner et al. 2018).

be attributed to the species, order, and genus levels. The lack of clearly defined groups concerning the higher taxonomic levels is not surprising given the low diversity of macroalgal species collected from Loch Creran; where only one species (*V. lanosa*) represented the phylum Rhodophyta and four ambiguously grouped growth forms represented the phylum Chlorophyta. Even within the brown algae (Ochrophyta), species were largely of the order Fucales. Unfortunately, few individuals of *S. latissima, L. digitata* and *L. hyperborea* were sampled and therefore poorly represent these species.

Vertebrata lanosa and *L. digitata* formed an independent assemblage that could be distinguished from the whole macroalgal inventory however, as mentioned above, the small sample sizes affect the reliability of this observation. Relative to the other macroalgae collected from Loch Creran, *V. lanosa* and *L. digitata* were notably depleted in ^{13}C, followed by *P. canaliculata*. The lower $\delta^{13}C$ values of both *V. lanosa* and *P. canaliculata* concurred with prior expectations and values previously recorded (Raven et al. 2002; Schaal and Grall 2015; Surif and Raven 1990). The other species and groups instead fell within the range ($\delta^{13}C$) of −20.37 to −9.27 ‰, with the Chlorophyta and samples of *F. serratus* constituting the enriched position in this range (high $\delta^{13}C$). One might be inclined to conclude that *V. lanosa* and perhaps *L. digitata* could be distinguished from the larger pool of macroalgal sources by using $\delta^{13}C$ alone. However, in a scenario where there is a greater number of macroalgal sources that better represent the broad macroalgal phyla present at Loch Creran, the values in the present study may very well become masked and lose their recognizable identity.

When plotting macroalgae $\delta^{15}N$ against $\delta^{13}C$ (Figure 5), there were no strong groupings by order. The Laminariales were distributed amongst the mean $\delta^{13}C$ of the Fucoids but as mentioned previously, *L. digitata* was more ^{13}C-depleted than the Fucoids and other Laminariales (*L. hyperborea* and *S. latissima*). Given the small sample size and the variables of location, depth and season that the samples were obtained, it is not possible to conclude that these variations are the result of any one particular driver. However, this observation is worthy of further investigation to determine if the variation observed is an artefact or is due to depth and specific form of DIC uptake (Cornwall et al. 2015; Diaz-Pulido et al. 2016; Raven et al. 2002).

Overall, the high interspecific variation in $\delta^{13}C$ illustrates the difficulties in discriminating between the macroalgal species as well as between higher taxonomic groups (e.g. order and genus). Differences in higher taxa may become apparent when incorporating larger sample sizes of a broader range of macroalgal species from different phyla which have been demonstrated previously (Dethier et al. 2013). Nevertheless, a notable overlap between major algal assemblages can be expected which will again confound inferences concerning contributions to pools of sedimentary OM (Hanson et al. 2010). The variability in the isotopic composition of macroalgae at Loch Creran may also be greater than what has been recorded here, as variation is likely to occur with species that inhabit the subtidal region which was not extensively sampled in this study (Drobnitch et al. 2018). Furthermore, variability may be attributed to temporal differences which were beyond the scope of the present study (Dethier et al. 2013). As a result, comparing isotopic values of macroalgae collected from different months could be deceiving.

Authors studying the isotopic composition of carbon in macroalgae, and similarly reporting high isotopic variability, often suggest taxonomy to be a source of this variation. For example, an analysis of $\delta^{13}C$ from a large stock of macroalgal specimens from the Gulf of California attributed 46 % of the variation to genera, 57 % to the species level and 35 % to morphofunctional groups (Velázquez-Ochoa et al. 2022). This was in line with the results of a similar, large-scale assessment of macroalgae on the east coast of Australia, revealing a strong taxonomic foundation for $\delta^{13}C$ variability (66 %) (Lovelock et al. 2020). Investigations exploring the isotopic composition of macroalgae typically attribute this taxon-specific variability in $\delta^{13}C$ to processes of photosynthetic dissolved organic carbon (DIC) acquisition as described above (Diaz-Pulido et al. 2016; Lovelock et al. 2020; Marconi et al. 2011; Raven et al. 2002; Stepien 2015; Velázquez-Ochoa et al. 2022).

By contrast, the authors of a detailed review, found no clear taxonomic patterns in macroalgae $\delta^{15}N$ data (Marconi et al. 2011). This was apparent here, where the variability in $\delta^{15}N$ among the taxa was substantial such that there were no isotopically distinct groups that could be differentiated from the whole macroalgae inventory.

4.3 Interspecific variation and the implications with site and zonation

As the majority of the macroalgae sampled in this study inhabit the intertidal region, they are subject to different periods of emersion due to tidal changes. The community structure of macroalgal species throughout the intertidal region is dependent upon physical and biotic factors and consequently dictates the periods of exposure to air (Ingólfsson 2005). Species occurring higher up the shore are regularly exposed to the atmosphere for prolonged periods relative to those occurring at the foot of the intertidal region. During emersion, a thin film of seawater may encapsulate the thallus of an individual however, the content of DIC within, will not be sufficient to support photosynthesis (Maberly et al. 1992). As such, intertidal macroalgae can assimilate atmospheric CO_2, which constitutes a large proportion of carbon required for photosynthesis when emersed (Raven and Hurd 2012). The difference between $\delta^{13}C$ of atmospheric and dissolved CO_2 is negligible however, both are depleted in ^{13}C relative to HCO_3^-. Increased atmospheric CO_2 assimilation due to increased emersion time can be expected and reflected in the isotopic composition of macroalgae that are capable of HCO_3^- uptake (lower $\delta^{13}C$) (Cooper and McRoy 1988).

The $\delta^{13}C$ of the fucoid species sampled here were in accordance with the findings from a previous study (Maberly et al. 1992). This provided results where more negative $\delta^{13}C$ values were recorded in *P. canaliculata* and the opposite for *F. serratus* (Figure 5). This suggests that increased exposure to the atmosphere is associated with more negative $\delta^{13}C$ signals. Another important factor is that the intertidal is characterised by high exposure to irradiance. This is recognised to alleviate the high-energetic demands required for the functioning of CCMs in intertidal species (Diaz-Pulido et al. 2016). As such, the prevalence of macroalgae with CCMs is thought to be consistent in environments receiving sufficient light to supply the energy required by CCMs. Alternatively, the primitive, yet low energetic nature of diffusive CO_2 uptake means that the reliance on CCMs in macroalgae is reduced in habitats where light is limiting (e.g. deeper, subtidal habitats) (Velázquez-Ochoa et al. 2022). With increasing depth, the decrease in photosynthetically active radiation is reflected in a reduced photosynthetic capacity, hence lower carbon demands and ultimately minimal investment in CCMs (Diaz-Pulido et al. 2016; Lovelock et al. 2020). Macroalgae dependent on CO_2 uptake ($\delta^{13}C < -30$ ‰), notably the Rhodophytes (35 % are CO_2 dependent) are often observed to inhabit deeper, subtidal regions (Cornwall et al. 2015; Diaz-Pulido et al. 2016; Raven et al. 2002). This may help to explain the lack of species representing the Rhodophytes sampled in the intertidal at Loch Creran.

Growth rates and associated photosynthetic activity will strongly influence the $\delta^{13}C$ signals in macroalgae (Carvalho et al. 2009; Wiencke and Fischer 1990). An example with relevance to the present study is shown by Chlorophyta groups. The morphological characteristics of the Chlorophyta groups sampled in the study show resemblance to species of the genus *Ulva* (Bunker et al. 2017). As opportunistic algae, *Ulva* exhibit fast growth rates which are supported by their ability to use a combination of C3 and C4 pathways (Liu et al. 2020; Valiela et al. 2018). Liu et al. (2020) demonstrated that carbon fixation by *Ulva prolifera* involves CO_2 assimilation via the C3 pathway, whilst carbonic anhydrase mechanisms are utilized for HCO_3^- when CO_2 is limiting, and under high light conditions, the C4 pathway is used. This versatility in carbon fixation allows for the rapid proliferation of their populations when necessary nutrients are abundant (Baweja et al. 2016; Lovelock et al. 2020). Assuming higher growth rates relative to the other species, the more positive $\delta^{13}C$ values recorded by the Chlorophyta group may be a result of higher carbon requirements to meet such photosynthetic demands (Velázquez-Ochoa et al. 2022). The higher uptake of DIC will eventually lead to the exhaustion of available DIC, such that enzymes cannot preferentially discriminate against ^{13}C over its lighter counterpart, ^{12}C (Drobnitch et al. 2018).

Because of the complexity associated with $\delta^{13}C$ values in macroalgae, taxon-specific variability may not necessarily be an exclusive function of the presence or absence of CCMs alone (Lovelock et al. 2020). Other intrinsic (taxonomic and functional group) factors such as morphology and physiology and environmental factors have the potential to influence carbon acquisition and ultimately $\delta^{13}C$ signals in macroalgae. The macroalgal $\delta^{13}C$ values will depend on the discrimination of isotopes (^{13}C, ^{12}C) during processes of carbon assimilation in photosynthesis (Carvalho et al. 2009). Factors related to life forms have the potential to influence such processes and consequently isotopic discrimination. For example, the thallus morphology of an individual, will in part, influence the diffusion boundary layer which can regulate the supply rate and usage of DIC to macroalgae (Finlay et al. 1999; Hurd 2000). Other influential taxon-specific properties

include photosynthetic intensity and respiration rates as well as the leakage of DIC during its uptake (Carvalho and Eyre 2011; Maberly et al. 1992).

When focussing specifically on the δ^{15}N data for each taxon, some species exhibited δ^{15}N values that were correlated with their sampling location of origin. This was most notable for *F. vesiculosus, F. serratus* and *V. lanosa*. For all three of these species, δ^{15}N values were lowest in samples collected from Site 3; the sampling location furthest inland. When considering the variability that has been recorded within and between the taxa, the few spatial correlates of δ^{15}N observed here do not appear to be substantial. As such, spatial variability in the isotope values recorded at Loch Creran (δ^{15}N) may not prove problematic when considering the application of paired SIA in this context.

In comparison to δ^{13}C, variation in macroalgal δ^{15}N is more likely a result of environmental conditions rather than physiological and phylogenetic factors. One explanation of this is the reduced discrimination of ^{15}N by macroalgae in comparison to that of ^{13}C (Marconi et al. 2011). As a result, it is widely accepted that macroalgae assimilate ^{15}N in proportion to its availability such that there is a close association between bioavailable nitrogen in aquatic ecosystems to the nitrogen incorporated into these macrophyte's tissue (McClelland and Valiela 1998). Coastal waters will receive both terrestrial and oceanic inputs of NO_3^- and NH_4^+, contributing to the pool of dissolved inorganic nitrogen (DIN). Like the distinct isotopic values of DIC utilized by macroalgae, sources of DIN also vary in their isotopic weights (Sigman et al. 2000). For example, nitrogen sources derived from nitrogen fixation, or the decomposition of terrestrial OM tend to be lower in ^{15}N than sources containing upwelled NO_3^-. δ^{15}N values of the macroalgae sampled in this study will, therefore, be influenced by the prevalence and availability of nitrogen sources received by Loch Creran (Ochoa-Izaguirre and Soto-Jimenez 2014).

For reasons outlined above, previous studies using δ^{13}C and δ^{15}N of standing stock for identification of sedimentary carbon have observed variation in ranges in both isotopes. Given that variation, the common practice has been to take multiple growing site fingerprints of the macroalgae when identifying C_{org} in sediments. Research on shelf macroalgal pathways in Plymouth (Moura Queirós et al. 2019) has indicated that the location of the donating stand of macroalgae has a significant impact on isotope values (δ^{13}C and δ^{15}N) and must be treated as separate sources. It follows that it, therefore, may be possible to use these pairs to provide an indication of the source of bulk macroalgae detritus. This has also been observed along a North/South gradient of fjords in Norway and Svalbard where each fjord has a significant enough variation in these values to be treated independently (Buchholz et al. 2019). However, in this research, though there was considerable between site variation in both δ^{13}C and δ^{15}N, it was not significant, with close groupings for each intertidal species (Figure 6). This similarity between the three sites is unexplained and may be an artefact of small sample size or may be indicative that microenvironments such as small fjords may not produce the variation seen when sites are more spatially diverse. Hence, spatial patterns in isotopic values may not be pronounced in a fjordic sea loch such as Loch Creran, unless they display sharp gradients in their environmental parameters (e.g. salinity, nutrients and solar irradiance) (Cornelisen et al. 2007; Hunt et al. 2020). The literature providing data on multiple site fingerprinting and variability by location is sparse and therefore, should be an avenue for further research.

4.4 Variation by sample position on specimen and its implications in identifying detritus and material in sediment

There was significant intra-individual variation in δ^{13}C evident among select fucoid and kelp species. *Ascophyllum nodosum*, *F. vesiculosus* and *P. canaliculata* subsamples, excised from the distal end of the frond were isotopically enriched in δ^{13}C relative to basal sections of the frond and the stipe. *Fucus vesiculosus* displayed the greatest variation for δ^{13}C in the intra-individual investigations, up to 6 ‰ within the same individual. *Saccharina latissima* displayed a similar pattern, where the proximal and distal sections of the lamina had the highest δ^{13}C values. These findings are consistent with previous investigations that demonstrated variability of δ^{13}C within the lamina of a single individual of *S. latissima* (as *Laminaria longicruris*) (Buchholz et al. 2019; Stephenson et al. 1984). Nevertheless, it must be stressed that in this study, replicates for *S. latissima* (sublittoral and subtidal) as well as *L. digitata* and *L. hyperborea* (subtidal) were obtained from single individuals that could be sampled during this project; better replication would be needed to reinforce this observation.

A source of isotopic variation within a single macroalgal individual may be attributed to the differential storage of isotopically distinct biochemical components (Stephenson et al. 1984). For example, the holdfast and stipe serve functional roles that differ from those of the photosynthetically active lamina. The lamina of *S. latissima* can be recognised as an area of intense carbon metabolism which can be associated with the synthesis of storage products like laminarin

and mannitol (Chapman and Craigie 1978). The age of tissue may also explain variation in stable isotope ratios of macroalgae. In kelps, the distal ends of the lamina represent the oldest tissue whereas the area above the meristem is the youngest. Patterns of increasing $\delta^{13}C$ from meristematic to younger, proximal tissues are sometimes followed by the depletion of $\delta^{13}C$ in senescent material occurring in the distal ends (Buchholz et al. 2019). This pattern was apparent in the present study, where the proximal sections of the lamina were slightly enriched in $\delta^{13}C$ in comparison to the distal ends. Fucoid species, instead, generally exhibit apical growth, where the distal end of the frond represents the youngest tissue (Viana et al. 2015). The older material within these individuals occurs lower down the frond, which is referred to as the middle sections of the thallus here (II). The degradation of older material appears to be a likely cause of intra-individual variation in the isotopic composition of macroalgae (Hill and McQuaid 2009). Hence, this might help to explain the observation of intermediate $\delta^{13}C$ values in the middle section of the thalli of fucoid species and lower $\delta^{13}C$ in the distal ends of *S. latissima*.

In this study, two patterns of intra-individual $\delta^{15}N$ variability were observed. *Ascophyllum nodosum* subsamples became enriched in ^{15}N with distance from the distal ends. *Fucus spiralis* and *F. vesiculosus* displayed a dissimilar and less definitive pattern, with subsamples from the middle sections depleted in ^{15}N relative to other regions where subsamples were excised. Perennial macroalgae like the ones sampled for intra-individual investigations here can be regarded as retroactive indicators of DIN inputs (Savage and Elmgren 2004). Differences in $\delta^{15}N$ could therefore indicate temporal differences in DIN inputs received by Loch Creran.

This broad range in isotope values within the frond of a single individual provides a confounding factor that could again prevent the development of reliable investigations using stable isotopes to determine the contribution of macroalgal-derived C_{org} to sediments. It is important to acknowledge the role of senescing and decomposition processes in altering the isotopic values of macroalgal tissues (Hill and McQuaid 2009). This extends to mechanisms of breakdown to better understand the material that most likely reaches adjacent depositional areas; to either enter the food chain or be stored in marine sediments. For example, a large proportion of kelp production is released as detritus which will likely have a different isotopic composition than fresh proximal tissue (Smale et al. 2022; Stephenson et al. 1984). Some of that release may continue to photosynthesise for several months during the degradation process (Frontier et al. 2021.) Both degradation and bacterial activity associated with the breakdown of macroalgal biomass are suggested to change isotopic signals (Braeckman et al. 2019; Hill and McQuaid 2009; Miranda et al. 2022). Decomposition related changes are typically linked to fractionation during microbial degradation and differing rates of decomposition between macroalgae tissues (Bouillon et al. 2011). Alternatively, if a whole individual is displaced from the substrate by a storm event, it too could enter adjacent sedimentary systems (Krause-Jensen and Duarte 2016).

Without recognising the potential effect of degradation on isotope values, it would be inappropriate to assume that detrital material reaching depositional areas is identical to the tissue sampled from living individuals (Hill and McQuaid 2009). The importance of this has been illustrated by the range in $\delta^{13}C$ along the thalli of independent macroalgal individuals. This is especially important given that one of the assumptions of using SIA in tracing studies, is that isotope signals of OM sources are not significantly altered by decomposition (Geraldi et al. 2019). Developing isotope values of material presumed to be detritus was beyond the time frame of this project but should be considered for future studies.

4.5 Multi-tracer or complementary approaches

Previous research has demonstrated the effectiveness of paired SIA of ^{13}C and ^{15}N as a method for identifying sources of macroalgal OM in sediments. For example, an investigation using SIA in the English Channel highlighted the transport of macroalgal detritus into deep coastal sediments (Moura Queirós et al. 2019). The isotopic separation between primary sources of OM investigated (e.g. planktonic and macroalgal communities) was sufficient, that they could serve as distinct sources within mixing models. Additionally, the two macroalgal communities sampled were isotopically distinct that they too could be treated as individual sources of OM. Moreover, a study in two Arctic fjords has revealed the significance of macroalgal detritus in comprising sedimentary OM using SIA (Zaborska et al. 2018). Fresh macroalgae were notably enriched in ^{13}C relative to macroalgal detritus which allowed for a separation between fresh and detrital sources in mixing models based on $\delta^{13}C$. Like the results presented here, these studies reveal the capacity of paired SIA to differentiate between marine macrophytes, providing valuable insight for the identification of C_{org} sources in marine sediments. Despite the success in the examples described, the macroalgal sources were not differentiated to lower taxonomic levels using stable isotope data alone. For example, Moura Queirós et al. (2019) used environmental DNA (eDNA) in combination with paired SIA to

separate the sources of C_{org}. Additionally, these investigations were confined to focal species, sampling a lower diversity of species, unlike the wider macroalgal inventory developed here. The variation of macroalgal isotope values recorded in this study reveal the challenges that will likely arise when using these data to distinguish between overlapping sources. Potential drivers and sources of variation will further complicate attempts at differentiating between macroalgal C_{org} sources to a higher taxonomic resolution.

Evidently, variation in macroalgal isotopic values could distort species-level and potentially phylum-level resolution, resulting in erroneous interpretations regarding contributions to a pool of OM. Overcoming this challenge would require an integrated approach, involving multiple biomarkers or techniques to facilitate the differentiation of C_{org} sources within the marine environment (Geraldi et al. 2019). For example, fatty acid (FA) profiles have proven to be superior in distinguishing between macroalgal species in comparison to SIA (Dethier et al. 2013). By incorporating both approaches into food web mixing models, the contribution of carbon sources to higher trophic levels has been more accurately determined. When combined with $\delta^{13}C$, the analysis of deuterium (^{2}H) has also proven to be a valuable tool to differentiate seagrasses from macroalgae (Duarte et al. 2018). Whilst this is not the resolution sought after in the present study, this technique could be useful when there is great overlap between the carbon stable isotope values of macrophyte lineages. Additionally, there is an emerging body of literature pointing toward the use of eDNA as a novel approach to determining sources of C_{org} within sediments (Moura Queirós et al. 2019; Ortega et al. 2020). The use of eDNA has been suggested to outperform traditional techniques such as SIA and could overcome limitations that have hindered accurate understandings of the contribution of macroalgal-derived C_{org} to marine sediments (d'Auriac et al. 2021; Ortega et al. 2020). There are, however, some inconsistencies with eDNA techniques that must be resolved so that they can be used to accurately estimate the species abundance within OM pools of interest, rather than the presence/absence alone (Ortega et al. 2020). In future investigations, novel approaches like those involving eDNA could help to reduce the scope of variation specific to a particular area of study. For example, in Loch Creran, identifying species that are present in C_{org} sequestration hotspots through eDNA analysis could help to eliminate species and taxa from mixing models that rely on stable isotope data. Informing mixing models with prior information, such as linking seasonality of detritus export to specific species could also help to recognise the likelihood of different outcomes occurring. Unfortunately, due to financial constraints, these techniques could not be applied here but, they are nevertheless a promising approach to facilitate species-specific identification of macroalgal-derived C_{org} sources.

4.6 Conclusions

The results of this study demonstrate the inherent variability occurring in macroalgal stable isotope values ($\delta^{13}C$, $\delta^{15}N$). Variation in $\delta^{15}N$ and $\delta^{13}C$ occurred both within and between taxa sampled at Loch Creran and became evident when studying different scales. As such, variability in the isotopic values explored here was evident within individual thalli of selected Fucoids and Laminariales, at the replicate level as well as between sampling localities. Despite variability acting at a range of scales, there were few consistent or predictable patterns regarding differences in the isotopic values of macroalgae. $\delta^{13}C$ provided some insight for the identification of C_{org} sources in marine sediments (e.g. terrestrial vs. marine) and in some instances, could be used to discriminate among taxa. For example, the red alga *V. lanosa* and the kelp *L. digitata* had distinct isotope values, though in a more comprehensive investigation, extending to the subtidal, their distinct values might be masked. Consequently, if these data are used alone to discriminate between macroalgal C_{org} sources and their relative contribution to a pool of OM within sedimentary systems, the development of accurate conclusions will be challenging. Ultimately, the signal to noise ratio within these data demonstrates the need for complementary techniques or multi-tracer approaches to aid in the differentiation between macroalgal carbon sources rather than relying on stable isotopes as a biomarker alone.

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