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Dual indicators link geochemistry to microbiota in blue carbon soils

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ABSTRACT

In blue carbon ecosystems, biomarkers and indicators have been developed to assess soil biogeochemical processes and history. In this study, we investigate blue carbon soils to determine if geochemical characteristics can predict soil microbial characteristics, and secondly to investigate if these two indicators can reveal novel information about processes about soil formation and alteration. First, phospholipid fatty acids (PLFAs) identified soil microbial functional groups and abundances. Microbes varied strongly among the three blue carbon ecosystems – seagrass, mangrove and saltmarsh – and to a lesser extent with soil age/depth. Then, x-ray fluorescence using a core scanner (ITRAX) was used to identify geochemical traits of the soil. Lastly, statistical procedures were carried out to test the ability of ITRAX-derived geochemical characteristics to explain the variation in PLFA composition. The soil geochemical indicators for organic matter (OM) source and turnover explained most of the variability in PLFAs, followed by indicators for redox and grain size. For both indicators the saltmarsh soils were associated with relatively higher OM availability and reactivity and microbial abundance. High OM samples also supported a range of microbial functional groups, including fungi and the co-existence of prokaryotic aerobic and anaerobic metabolisms. In contrast, increased depths representing older soil ages showed decreases in OM reactivity and shifts in OM processing through reduced active microbial abundance. We suggest that x-ray fluorescence data used as an indicator in conjunction with other biomarkers can be used to assess multiple aspects of blue carbon soils (biology, provenance, physicochemistry), while also possessing opportunity for future development as rapid field technique.

1. Introduction

In coastal vegetated, or blue carbon, ecosystems, biomarkers are used to indicate the biogeochemical processes occurring within the soil profile. The most common types of indicators used in coastal vegetated soils are those that identify the source of organic matter or organic carbon (Geraldi et al., 2019). In its earliest application, biochemical compounds found in plants, such as n-alkanes and lignin phenols, were used as taxonomic fingerprints of coastal organic carbon (Wilson et al., 1985; Benner et al., 1987; Wang et al., 2003). Stable isotope signatures of bulk soils are also commonly used for indicating organic carbon sources from broad primary producer groups, e.g. terrestrial or marine (Geraldi et al., 2019), and this provenance information can be used to quantify autochthonous versus allochthonous sources for management, modelling and accounting of organic carbon in coastal vegetated ecosystems (Geraldi et al., 2019). Yet, there is currently limited or underutilised capacity for indicators to inform on the current conditions or processes that could influence the preservation or transformation of blue carbon.

Biomarkers can provide information on the biological processes occurring within the soil, including post-depositional transformation and the identification of organisms involved in those processes. Phospholipid fatty acids (PLFAs) can be used to identify the metabolic pathways and functional groups of active prokaryotic, fungal and algal microorganisms important to coastal soil carbon cycling (Boschker and Middelburg, 2002). For example, PLFA biomarkers have been used to identify microbial community shifts with wetland restoration and to assess soil organic matter (OM) chemistry and availability (Bossio et al., 2006; Fanin et al., 2019). When combined with stable isotope analyses, either natural or with enriched labelling, PLFAs and other biomarker compounds can reveal the source and rate of OM utilisation by microorganisms (Boschker and Middelburg, 2002; Bouillon and Boschker, 2006; Spivak and Ossolinski, 2016; Geraldi et al., 2019). While PLFAs have limited taxonomic resolution and are dependent on the availability

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of reference standards for quantitative outputs, multi-indicator approaches are being used to overcome some of the limitations of using a single biomarker. For example, combining PLFA biomarkers with respiratory quinone biomarkers has provided greater resolution of microbial community structure (Kunihiro et al., 2014), while combining biomass estimates from PLFAs with eDNA produced absolute abundances of taxonomic groups to help sequencing-based microbial datasets be comparable across different sample types (Lewe et al., 2021).

Geochemical indicators to describe changes in sediment characteristics or environmental change in sedimentary profiles have been developed from elemental profiles using X-ray fluorescence (XRF). The ITRAX core scanner from Cox Analytical Systems, for example, has been used for the paleo-reconstruction of coastal and marine soil and sediment conditions (Gadd et al., 2015) and the development of indicators based on single element intensities or elemental ratios (Rothwell and Croudace, 2015). For example, the indicators from ITRAX analyses have been used in saltmarsh and mangrove ecosystems to identify changes in the soil profile related to land-use changes, including metals that indicate anthropogenic (Cu, Zn and Pb) and terrestrial (Ti) inputs (Gadd et al., 2015; Ewers Lewis et al., 2019). The ITRAX indicators also may be used to infer changes in water flow (e.g. tidal inundation, precipitation) that would affect grain size (Zr:Rb), and the OM source and redox chemistry as shown through elements common to anoxic marine conditions (Cl, Ti:Ca, Fe, S; Kelleway et al., 2017; Dale et al., 2019; Hapsari et al., 2020). Recently, the elemental indicators obtained from XRF have been used to identify drivers of soil formation and composition in seagrass ecosystems (Piñeiro-Juncal et al., 2020; Pineiro-Juncal et al., 2021). While ITRAX/XRF data is often interpreted in the context of the down-core profile, here, a multivariate approach was applied to identify specific indicators that are driving the variation among cores, through depth or time (Piñeiro-Juncal et al., 2020; Pineiro-Juncal et al., 2021). The indicators that had similar loadings in a principal component were then linked to factors, such as OM source (e.g., Rb, Ti, Al, Si, Mn and Pb for silty lithogenic materials), content (Sr and Ca for biogenic sediment) and humification (S and Br) (Pineiro-Juncal et al., 2021). Used in this way, ITRAX/XRF data may provide new or additional insight into changes in blue carbon availability, composition and transformation over time and depth.

In this study, we used PLFA biomarkers to identify how the microbial functional groups differ with ecosystem (saltmarsh, mangrove, seagrass) and depth (top 30 cm), both in abundance and diversity. We will then statistically identify the geochemical indicators obtained from ITRAX that are important in explaining the variation in microbial profiles. Our objective is to explore the overlap between microbiological and geochemical features, in addition to how the dual indicators may reveal novel information about soil condition, formation and alteration in blue carbon soils.

2. Materials and methods

2.1. Sample sites, collection and processing

Soil cores (30 cm deep) from two sites, each with seagrass, mangrove and saltmarsh habitats, were sampled within Western Port Bay, Victoria, Australia in March 2016: Rhyll (−38.45858, 145.28903) and Warneet (−38.22501, 145.30554). Both sites contained *Zostera muelleri* seagrass meadows, *Avicennia marina*, and saltmarsh dominated by *Salicornia quinqueflora*. Soils for fatty acid analysis were sampled at each site with a 5 cm diameter PVC push core (n = 4), and refrigerated at 4 °C before extruding within 72 h of sampling. Compaction was roughly estimated by measuring the inner core length and outer core length during the coring process. The correction factor was then applied throughout the core prior to sectioning, following to depth intervals used in Ewers Lewis et al. (2018), plus a subsurface layer: 0–2 cm, 2–4 cm (subsurface), 14–16 cm and 28–30 cm. Two separate cores of the same dimensions were taken for age-dating and X-ray Fluorescence (XRF) core scanning by ITRAX. The cores for age-dating were sectioned every 1 cm and dried at 50 °C to obtain dry bulk density values. The soil sections were then sieved at 63 μm before sending for analysis. ITRAX cores were sent intact for analysis.

2.2. PLFA core processing, extraction and analysis

Each depth interval for the fatty acid cores were subsampled in the centre of the slice leaving 0.5 cm outer buffer zone in order to avoid soil contamination along the edges of the core during the sampling process. Live plant biomass is abundant in fatty acids and thus could potentially mask the microbial fatty acid signatures in the soils, therefore after freeze drying plant biomass was removed via sieving. Fine plant biomass was abundant for mangrove and saltmarsh soils and so were sieved at 125 or 63 μm. For coarser grained samples with little to no plant matter present, the soils were sieved at 300 μm or plant matter was removed by hand. A maximum of 6 g of soil (~4–5 mL in 11 mL glass tubes) was used for fatty acid extractions.

All solvents used were LC/GC-MS-Grade, and glassware was solvent-washed prior to use to remove any lipid contaminants. Bulk lipid extraction was performed by adding 5 mL 2:1 chloroform:methanol (MeOH) to the soil samples. After shaking for 2 h, 1.05 mL of ultrapure water was added (final ratio 2:1:0.8 chloroform:MeOH:H2O), and the samples were shaken for an additional 30 min. After centrifugation at 780 RCF (x g) for 5 min, the bottom chloroform layer was transferred to a new tube. A second extraction was performed on the soil by adding fresh chloroform, shaking overnight and re-isolation of the chloroform fraction. The combined chloroform fraction, or total fatty acid fraction (tFA), was dried down in a speed-vacuum and stored at −80 °C until the next fractionation step.

The tFA fraction was reconstituted in 2:1 chloroform:MeOH and half the sample was taken for solid phase extraction (SPE) (Olmstead et al., 2013) to isolate phosophlipids. The silica columns (normal phase, 45 μm, 6 mL, 500 mg; SampliQ, Agilent Technologies, Santa Clara, CA, USA) were conditioned with 5 mL MeOH followed by two rounds of 4 mL chloroform in 1% acetic acid. The tFA fractions were reconstituted in the chloroform/acetic acid solution before loading the sample onto the column. Neutral lipids were first eluted with 5 mL chloroform in 1% acetic acid followed by glycolipids in two rounds of 4 mL 9:1 acetone: MeOH. Phospholipid fatty acids (PLFAs) were eluted with 5 mL MeOH.

Samples were randomised into six groups across site, habitat and depth treatments to minimise batch effects during extraction and analysis. First, total fatty acids were extracted using a chloroform-methanol-water solution, then phospholipids were isolated via solid phase extraction (SPE) (Olmstead et al., 2013). An internal standard (Myristic acid, d_{27}; Fluka, Sigma Aldrich, Darmstadt, Germany) was added to the PLFA fractions when re-dissolving in 2:1 chloroform:MeOH. Transesterification was performed using MethPrep in a 9:1 ratio (Grace Alltech, Columbia, MD, USA). The internal standard was also added to the bacterial methyl esters and 10Me-16:0 methyl ester standards (Matreya LLC, State College, PA, USA). Samples were analysed using gas chromatography coupled with a single quadrupole mass spectrometer (GC-MS; Trace DSQ, Thermo) with a TR-FAME column (100 m, 0.25 mm ID, 0.2 μm film; TRACE, Thermo Fisher, Victoria, Australia). Dilution factors were included in the final PLFA concentrations then normalised to μg g^{-1} soil.

2.3. Age-dating & ITRAX

Age-dating and ITRAX analyses were performed at the Australian Nuclear Science and Technology Organisation (NSW, Australia). Sediments for ^{210}Pb age dating were sliced in 1 cm intervals up to 21 cm, sieved at 63 μm and dried prior to analysis (Trevathan-Tackett et al., 2018). ^{210}Pb age dating were analysed according to Atahan et al. (2015), whereby ^{210}Po and ^{226}Ra activities were measured by alpha spectrometry. Briefly, total ^{210}Pb activity was measured indirectly from its

progeny ^{210}Po, supported ^{210}Pb was measured indirectly from its grandparent radioisotope ^{226}Ra, then unsupported ^{210}Pb was estimated by subtracting the activity of supported ^{210}Pb activity from total ^{210}Pb activity. Sedimentation rates and soil age estimates were calculated using both Constant Initial Concentration (CIC) and Constant Rate of Supply (CRS) models (Arias-Ortiz et al., 2018). Due to limited resources, age dating was performed only on Rhyll soils. ITRAX analyses (Cox Analytical Systems, Mölndal, Sweden) was performed at both sites on the top 30 cm of the cores after bisecting longitudinally with the core cutter (Ewers Lewis et al., 2018). Specifically, the cores were set in a molybdenum tube and scanned at 55 mA and 30 kV with an exposure time of 10 s. Intensity data were obtained at 1 mm intervals down-core (Rothwell and Croudace, 2015).

2.4. Biomarkers and statistical analyses

PLFAs and ITRAX element intensities or elemental ratios were used as microbial community and geochemical indicators, respectively (Tables 1 and 2, and references therein). The PLFA biomarkers had varying degrees of specificity including general fatty acids found in all (micro) organisms, fatty acids specific to eukaryotic microorganisms (fungi, protists), and prokaryotic-specific fatty acids. Within the gram-positive and gram-negative prokaryote groups, several fatty acids have been previously used to indicate anaerobic functional groups, including methanotrophs and sulphate-reducing bacteria (Table 1 and references therein). For the ITRAX indicators, we included elements or elemental ratios that are linked to organic matter characteristics and soil formation, including terrestrial versus marine sources, grain size and redox conditions (Table 2 and references therein).

Variation in PLFA concentrations and diversity was analysed with a 3-way PERMANOVA, with *site* (Rhyll, Warneet), *ecosystem* (mangrove, saltmarsh, seagrass) and *depth* (0–2 cm, 2–4 cm, 14–16 cm, 28–30 cm) as fixed factors. Data were square-root transformation before calculating the Euclidean distance resemblance matrix for analysis. A Monte Carlo correction (P(MC)) was applied in cases where permutations were <200. SIMPER analyses were used to identify PLFAs driving the differences for significant pairwise tests. A Principal Components Analyses was performed on the resemblance matrix.

The potential for the environmental ITRAX soil indicators to explain the PLFA biomarkers was tested with Distance-based linear modelling (DistLM), using BEST selection procedure and AIC criterion, and visualised with dbRDA. To match the sampling depths between the datasets, ITRAX data were averaged across 0–20, 20–40, 140–160 and 280–300 mm depths. A draftsman plot indicated any co-correlations and skewness, resulting in the log transformation of Cu:Ti, Mn:Fe, Fe:S, Ca:TI, Si:Ti and Fe, and log(X^2) transformation of magnetic susceptibility. Only axes that explained >1% of variation of the fitted model were considered (Piñeiro-Juncal et al., 2020). Rhyll seagrass soils at 28–30 cm were excluded due to a short ITRAX core. Software PRIMER + Permanova (v7; Anderson et al., 2008) was used for statistical analyses.

3. Results and discussion

3.1. PLFA biomarkers within blue carbon soils

Compositionally, the soil microbial communities were dominated by prokaryotes, which were approximately 10-fold higher in abundance than fungi (Figs. S1–S2). The saltmarsh soils had 2-10-fold higher PLFA concentrations than the other ecosystems, and had a subsurface maximum of PLFAs at 2–4 cm (Fig. S2). Ecosystem type and soil depth had the greatest influence on the soil PLFA concentrations and composition (2-way interaction, Pseudo-$F_{12,101}$ = 5.0354, P-perm <0.001; Table S1). Furthermore, ecosystem type accounted for 87.9% of the total variation in the PC1 axis, while depth accounted for 4.4% of the variation in PC2 (Fig. 1, representing concentration and composition). A weak 3-way interaction was also detected, with differences in PLFA composition between the sites within the saltmarsh soils at both surface and deeper depths resulting in a significant 3-way interaction (Rhyll > Warneet for select PLFAs; Pseudo-$F_{12,101}$ = 2.3362, P-perm = 0.036). The highest loadings in PC1, included the general PLFA 16:0, followed by cy19:0, an anaerobe biomarker (Fig. 1). SIMPER analyses also showed that these two biomarkers were also driving the differences between the saltmarsh PLFA profile and those of the mangrove and seagrass soils (P(MC) ≤ 0.027 for most pairwise comparisons, Table S1). The negative PC2 loadings (surface) were attributed to methanotroph biomarkers (16:1c, 18:1c), while positive PC2 loadings (deeper) were attributed to cy19:0 and cy17:0 (general anaerobes) and anaerobic biomarker 10Me16:0 (sulphate reducing bacteria). The biomarkers along the positive PC2 axis were typically correlated to the two deeper soil depths, particularly for the saltmarsh samples (Fig. 1), and likely reflected anaerobic conditions of deeper soils. The 28–30 cm depth below the rhizosphere also was consistently the lower in PLFA concentrations across all samples (Fig. S2, Table S1), with SIMPER analysis identifying differences attributed to reductions in 16:0, 18:1c, and a15:0 biomarkers.

The PLFA biomarkers indicated that blue carbon ecosystem type and depth strongly influenced microbial abundance and diversity. The relatively high concentration of active microbes in the saltmarsh soils

Table 1
List of indicator groups for phospholipid fatty acid (PLFA) analyses used in this study. SRB = sulphate reducing bacteria. PLFA notation X:YnZ, where X = number of carbons, Y = number of double bonds, and Z = double bond location. PLFA prefixes: cy = cyclopropyl X, i = iso-, a = anteiso-, OH = hydroxy, Me = methyl.

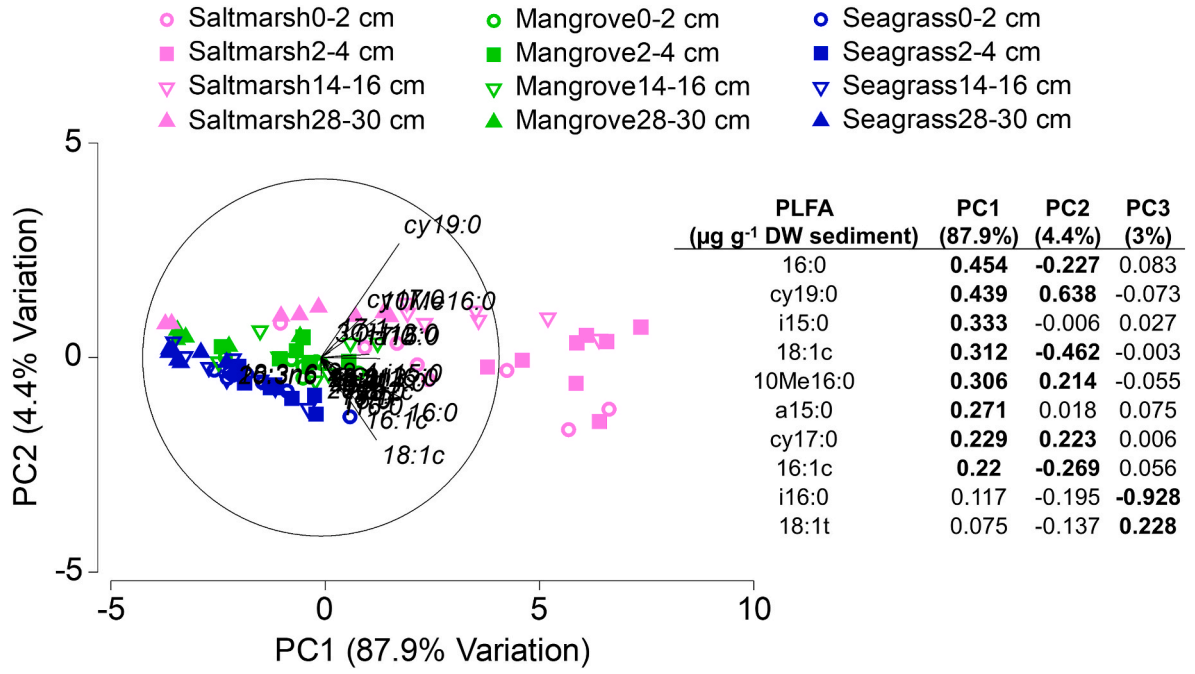
General Microbial Fatty Acids[b,c]	Eukaryotes		Prokaryotes			
15:0	Fungi[d,e]	18:1t	Gram Negative[g]	3OH12:0	Gram Positive[g]	i15:0
15:1		18:2c		OH14:0		a15:0
16:0		18:3n3		2OH16:0		i16:0
17:0		18:3n6	*Anaerobes*[e]	cy17:0	*SRB*[d,h]	10Me16:0
18:0	Possible protists[e,f]	20:3n6		cy19:0		(i17:1[a])
		20:4	*Methanotrophs*[d]	16:1c		17:1
		20:5		18:1c		
		22:4				
		22:5				
		22:6				

[a] Fatty acid not available as a standard, so SRB quantification will be underestimated.
[b] Findlay et al., 1990.
[c] Perry et al., 1979.
[d] Boschker et al., 2002.
[e] Vestal et al., 1989.
[f] Raghukumar et al., 2008.
[g] Piotrowska-Seget and Mrozik 2003.
[h] Vasquez et al., 2016.

Table 2
List of indicator groups for X-ray fluorescence (XRF) analyses using an ITRAX core scanner. Mag. Sus. = magnetic susceptibility.

Organic Matter		Grain Size		Redox	
Input, Concentration	inc:coh ratio[a,b]	Coarse Substrate	Sr[c]	Change in redox and oxygen availability	Mn:Fe[a]
OM Breakdown	Cu:Ti[d]	Coarse:Clay ratio	Zr:Rb[a,b]	Reducing conditions, anoxic environment	Fe:S[d]
Terrestrial Source	Mag. Sus[1-3]				S[1,d]
	Al:Si[d]				
	Ti[a,e]				
	Fe[a,e]				
Marine Source	Cl[a,e,f]				
	S[1,d]				
	K[13]				
	Br[e]				
Marine:Terrestrial ratio	Ca:Ti[b]				
	Si:Ti[b]				

[a] Ewers Lewis et al., 2019.
[b] Kelleway et al., 2017.
[c] Pineiro-Juncal et al., 2021.
[d] Rothwell and Croudace, 2015.
[e] Piñeiro-Juncal et al., 2020.
[f] Hapsari et al., 2020.



PLFA (μg g^{-1} DW sediment)	PC1 (87.9%)	PC2 (4.4%)	PC3 (3%)
16:0	**0.454**	**-0.227**	0.083
cy19:0	**0.439**	**0.638**	-0.073
i15:0	**0.333**	-0.006	0.027
18:1c	**0.312**	**-0.462**	-0.003
10Me16:0	**0.306**	**0.214**	-0.055
a15:0	**0.271**	0.018	0.075
cy17:0	**0.229**	**0.223**	0.006
16:1c	**0.22**	**-0.269**	0.056
i16:0	0.117	-0.195	**-0.928**
18:1t	0.075	-0.137	**0.228**

Fig. 1. Principal components analysis of all PLFA biomarkers. PC1 predominantly represents variation in PLFAs between saltmarsh soils and soil from mangrove and seagrass ecosystems. PC2 likely represents the variation between deeper soil layers (14–16 and 28–30 cm), the subsurface layer and the surface layer. Table shows PC loadings >0.2 in either direction in bold. See Table 1 for the functional groups to which the PLFAs belong.

likely reflect higher amount of organic carbon in the top 30 cm compared to the other ecosystems (Ewers Lewis et al., 2018). The subsurface maximum at 2–4 cm in the saltmarsh soils, comprising aerobic and anaerobic groups, is likely capturing the surface and parts of the rhizosphere, and suggestive of potential microniches that allow both metabolic pathways to co-exist (Brodersen et al., 2018; Kolton et al., 2020). The availability or reactivity of the OM could also be greater in the saltmarsh soils, as indicated by the relatively lower ratio of gram positive to gram negative bacteria (GP:GN ratio, Fig. S3). Gram negative bacteria have been linked to simple compounds in organic soils, while gram positive bacteria have been correlated with more complex compounds like carbonyls (Fanin et al., 2019). The soils in this study with lower ratios and thereby greater carbon availability for microbial consumption (i.e. saltmarsh soils and surface depths, Fig. S3) are also the soils with greater PLFA concentrations (Fig. S2). Conversely, the reduction in PLFA concentrations and increase in GP:GN ratios with depth for all three ecosystems likely coincides with reduced carbon resources below the rhizosphere for the microbes to utilise.

3.2. Geochemical characteristics explaining microbial biomarkers

Sedimentation rates and age estimates were obtained for the three ecosystems at Rhyll (Fig. 2). The unsupported ^{210}Pb activity did not reach background at the deeper depths for the mangrove soils, possibly due to mixing (Fig. 2b), so all mangrove estimates are for the top 10 cm. The CRS model estimated similar sedimentation rates in the top 15 cm for the seagrass and saltmarsh ecosystems (CRS model 1.4 and 1.5 mm y^{-1}, respectively). The CIC model estimated a similar rate for saltmarsh, but for seagrasses, there were two distinct rates obtained down-core (Fig. 2c), leading to CIC sedimentation rates of 8.7 mm y^{-1} in the top 10 cm, and 1.1 mm y^{-1} in the bottom 20 cm. The high surface sedimentation rate may be due to the sheltered, depositional environment

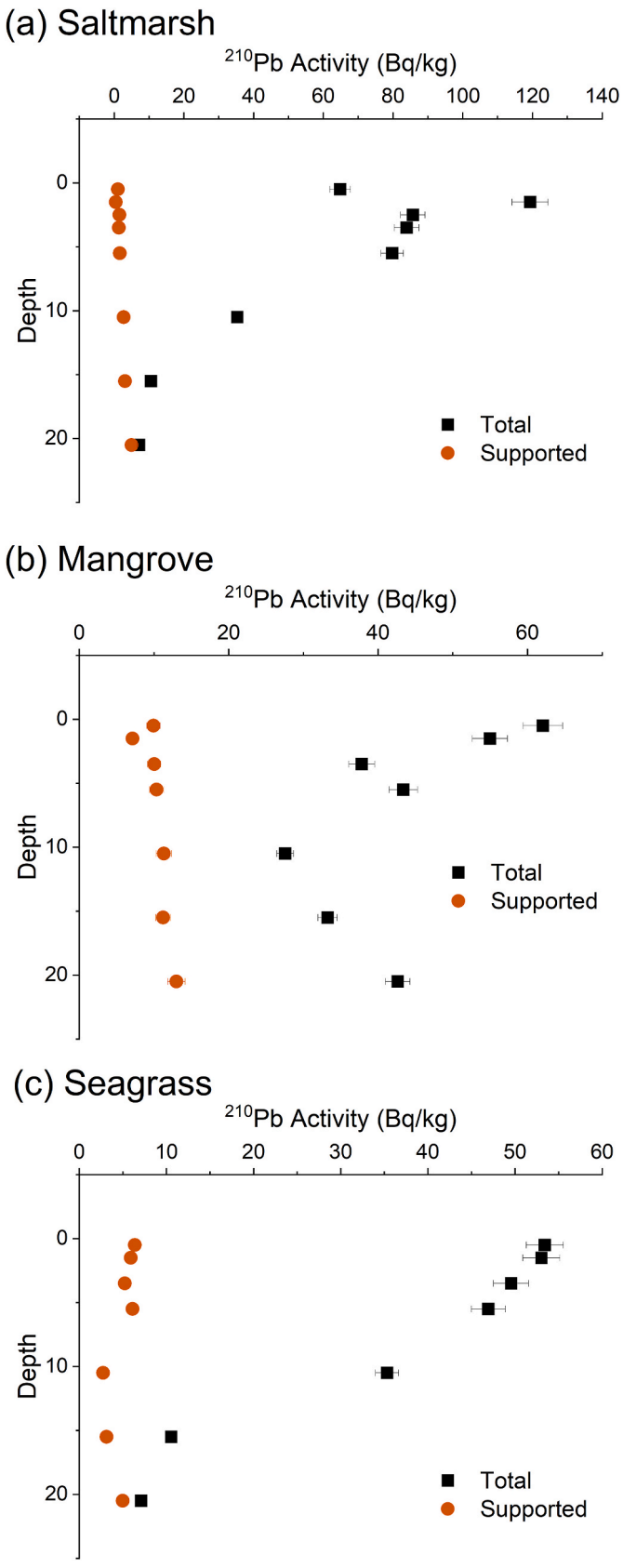


Fig. 2. Total and supported ^{210}Pb activity profiles from the Rhyll site.

(Marsden et al., 1979). The mangrove sedimentation rate was 2.8 mm y^{-1} (top 10 cm, CIC model). Using the CIC models, the ages estimated for the top 20 cm was 100 ± 5 y for seagrass soils and 135 ± 6 y for saltmarsh soils, whereas the top 10 cm of mangrove soils were dated at 36 ± 8 y.

Three co-correlations (>0.8) were identified within the ITRAX indicators, including Cu:Ti and K, Cu:Ti and inc:coh (incoherent/coherent ratio), and Ti and K. The positive correlation between Cu:Ti (OM breakdown) and inc:coh (OM concentration) suggests that soils with high OM are also experiencing post-deposition oxidation. This may be particularly important for marine sources of OM, as shown by the positive co-correlations between Cu:Ti and K. There was a strong positive correlation (0.919) between Ti and K, which represent terrestrial and marine sources of OM, respectively. It is possible that these sites are receiving inputs of both indicators simultaneously, rather than dominance of one over the other.

When tested individually in the DistLM, 10 of the 16 ITRAX indicators explained significant variation in the PLFA signatures (see Fig. S4 for down-core variation of each indicator). Here, the highest explained variances were attributed to marine OM (Br, 63%), OM concentration (inc:coh, 61%) and OM breakdown (Cu:Ti, 52%) indicators (Fig. 3). When all indicators were tested in combination using the BEST test, Br was still the most important geochemical parameter for PLFA composition (AIC = 97.02), followed by similar scores for Br + inc:coh (AIC = 90.68) and Br + Mn:Fe + Fe (90.56). Br has been shown to be a good indicator of marine OM in *Posidonia oceanica* seagrass soils, as bromine binds to OM during humification (Pineiro-Juncal et al., 2021). However, for this study, the higher Br signature was predominant for the saltmarsh soils above 16 cm (dbRDA, Fig. 4), the latter also separating along dbRDA axis 1 (Fig. 4). Furthermore, the relatively higher concentration of marine-sourced OM (Br + inc:coh), could be linked to a greater concentration of fresh OM, as allochthonous marine input and/or exudates and fresh root detritus deposits are within the upper layers. These results suggest that soil OM formation in the last 100 years has been from saltmarsh and/or marine sources rather than terrestrial/silt sources. Together these data suggest that the OM concentration, source and quality, also indicated by GP:GN, supports the concentration and beta diversity of microbial functional groups represented in the PLFA biomarkers (Fig. 4, S2-S3).

Depth differences explained little variation along dbRDA axis 2 (Fig. 4). The positive PC2 loadings associated with the top 6 cm were correlated with grain size (Sr), anoxic conditions (Fe:S), OM degradation (Cu:Ti) and marine OM (Ca:Ti), indicative of anaerobic breakdown of marine OM across the three ecosystem types. In contrast, Fe and magnetic susceptibility (MagSus) had strong PC2 loadings. It is possible that the depths below 14 cm may be capturing a transition between the rhizosphere and a deeper horizon or indicates past deposition of terrestrial runoff (Piñeiro-Juncal et al., 2020; Pineiro-Juncal et al., 2021). During this transition, it is likely that redox conditions changed as a result of a lack of oxygen below the rhizosphere, but also a higher proportion of deposited allochthonous OM survived the remineralisation and accumulation processes.

3.3. Assessment of dual microbial and geochemical indicators in blue carbon soils

By utilising both (micro)biological and geochemical indicators, we were able to identify how historical soil formation and characteristics influence the abundance and diversity of living soil microbiota. We found that higher OM concentrations and humified OM indicative of denser root matte soils, such as the saltmarsh in this study and *Posidonia* in Piñeiro-Juncal et al. (2021), supported high microbial abundances and metabolic diversity. The deposition of fresh OM and exudate production at or near the surface resulted in OM-rich soil comprised of readily available or reactive types of carbon as indicated by the microbial community itself (GP:GN ratio) and OM breakdown and source

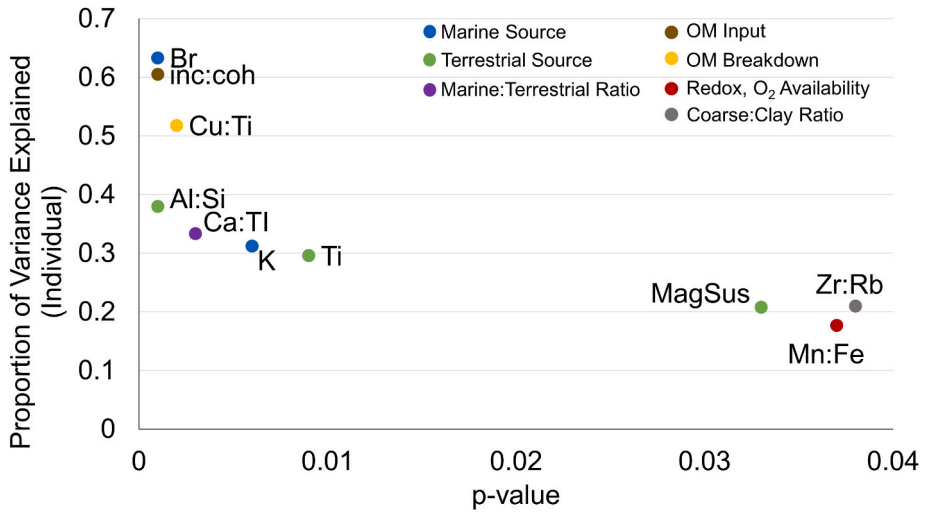
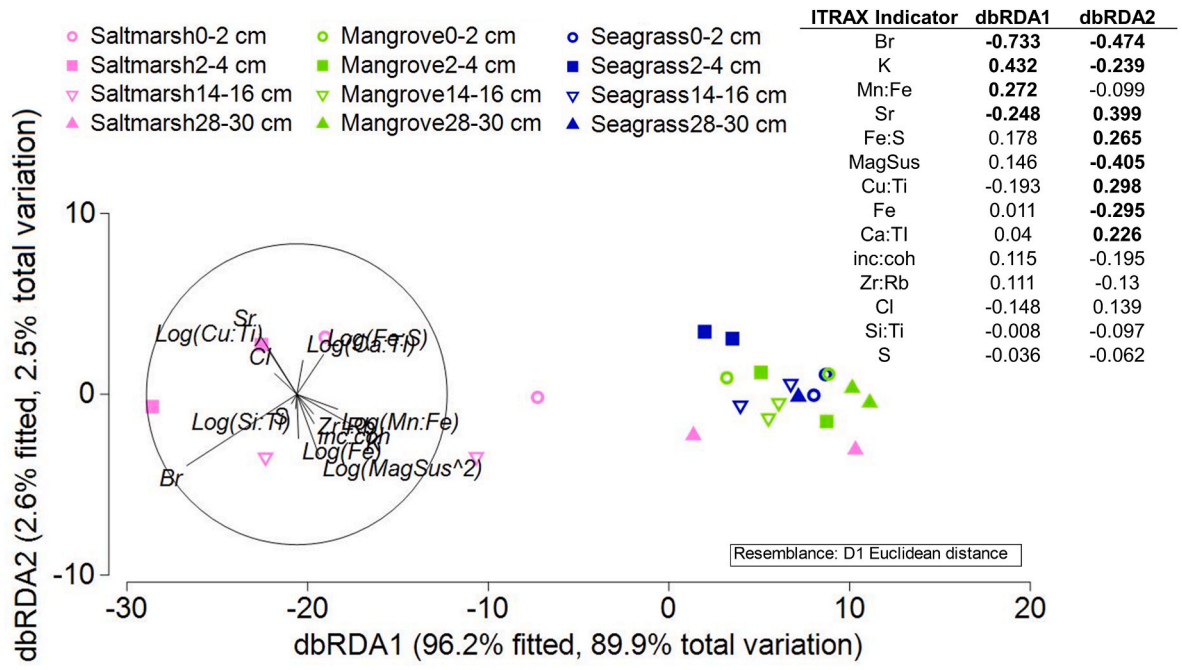


Fig. 3. DIST-LM marginal test results. ITRAX variables shown were significant when explaining the proportion of variance in the PLFA data for each indicator individually. Legend indicates to which the group each indicator belongs.



ITRAX Indicator	dbRDA1	dbRDA2
Br	**-0.733**	**-0.474**
K	**0.432**	**-0.239**
Mn:Fe	**0.272**	-0.099
Sr	**-0.248**	**0.399**
Fe:S	0.178	**0.265**
MagSus	0.146	**-0.405**
Cu:Ti	-0.193	**0.298**
Fe	0.011	**-0.295**
Ca:Tl	0.04	**0.226**
inc:coh	0.115	-0.195
Zr:Rb	0.111	-0.13
Cl	-0.148	0.139
Si:Ti	-0.008	-0.097
S	-0.036	-0.062

Fig. 4. Distance-based redundancy analysis following distance-based linear modelling to identify potential relationships between geochemical parameters and the soil microbial communities via PLFA biomarkers. PLFA points represent the entire PLFA biomarker community per sample. Table shows relationships between dbRDA coordinate axes and ITRAX variables, with values > 0.2 in either direction in bold. PLFA and ITRAX values represent means. See Table 2 for the indicator group to which the ITRAX variables belong.

information provided by ITRAX. Further, in addition to anaerobic functional groups, the surface soils and rhizosphere showed evidence of methanotrophy, a process shown to be increasingly important in blue carbon cycling (Jones et al., 2003; Lee et al., 2017; Shiau et al., 2018). We also noted a minor presence of fungal communities that could represent mycorrhizal fungi in the roots (Wilde et al., 2009) or saprobic fungi breaking down detritus near the soil surface (Raghukumar 2017). The OM from the two deeper depths, representing soils >50 years in age, were lower in concentration and less reactive, resulting in a depletion of microbiota, especially at 28–30 cm depths. These soils supported significantly less microbiota potentially from post-depositional OM processing (limited resources) and depletion or the selective preservation of more recalcitrant terrestrial OM over time (e.g. inaccessibility; Brodersen et al., 2019; Macreadie et al., 2019; Spivak et al., 2019).

4. Conclusion and future directions

While our approach was a correlative snapshot on a small range of samples, our results suggest that these biogeochemical indicators could be a tool to link soil history with contemporary process and conditions. To our knowledge this study is the first to use the geochemical indicators in a multivariate approach to describe soil characteristics from multiple blue carbon ecosystem types. In addition to the core-scanner approach we used here (ITRAX), XRF data can be obtained with benchtop and handheld instruments, with the latter option providing opportunity to develop in-house lab or field measurements (e.g., Markey et al., 2008;

Mejía-Piña et al., 2016) for rapid characterisation of blue carbon soils. Further, the non-destructive nature of XRF is conducive to sampling multiple indicators from the same core or core slice, e.g. PLFA, stable isotopes. In our study, the relatively broad resolution of both microbial functional groups and geochemical parameters seemed to facilitate our dual indicator approach. To further assess the capacity of XRF as a robust indicator, alone and in conjunction with other biomarkers, samples representing a diversity of OM and soil types (sand, carbonate, clay) within each ecosystem type and across soil ages is recommended for future XRF indicator studies. In summary, x-ray fluorescence as an indicator complements existing biomarkers and is a promising, novel tool for interrogating biogeochemical processes in blue carbon soils.

CRediT authorship contribution statement

Stacey M. Trevathan-Tackett: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Damien L. Callahan:** Writing – review & editing, Methodology, Formal analysis. **Rod M. Connolly:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Peter I. Macreadie:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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