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Seagrass contributes substantially to the sedimentary lignin pool in an estuarine seagrass meadow

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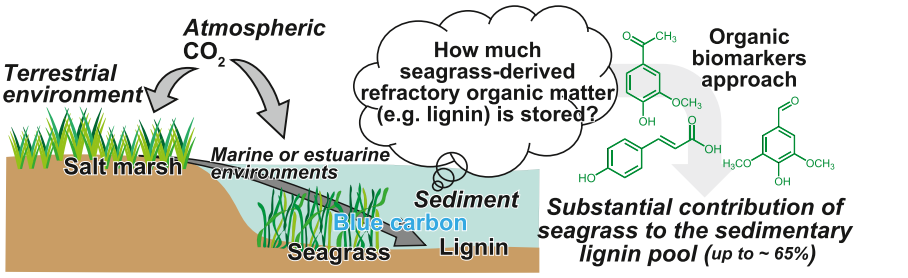
HIGHLIGHTS

- We used biomarkers to determine the origins of sedimentary organic C in a lagoon.
- The terrestrial contribution to organic matter was high near river mouths.
- Seagrass-derived lignin was up to ~65% stored in surface sediments.
- Lignin was relatively persistence with depth in sediments.
- Persistence of biomarkers in sediments differed at the millimeter scale.

GRAPHICAL ABSTRACT



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ABSTRACT

Shallow coastal ecosystems are reservoirs of carbon derived from allochthonous organic matter and autochthonous organic matter produced by microalgae and macrophytes. Carbon stored in vegetated coastal ecosystems has attracted broad attention as an important component of carbon sinks. Characterizing the source of carbon in sediments is essential for quantifying the carbon-sequestration function of shallow coastal ecosystems. In this study, we investigated the origins of organic matter using organic biomarkers (lignin phenols, fatty acids, cutin acids, diacids, and ω-hydroxy acids) in surface sediments in a seagrass-dominated lagoon (Furen Lagoon, Japan). Biomarkers derived from allochthonous vascular plants, such as long-chain fatty acids, showed higher concentrations near river mouths. Furthermore, biomarker signals indicated that sedimentary organic carbon originated in large part from degraded allochthonous vascular plants including roots. A Bayesian mixing model using the ratios of syringyl phenols to vanillyl phenols and cinnamyl phenols to vanillyl phenols indicated that up to about 65% of lignin in the sediments was derived from seagrass. This result indicates a substantial contribution of seagrass to the sedimentary lignin pool in an estuarine seagrass meadow. However, the percent contribution of seagrass to the lignin pool varied, with higher values near a tidal inlet and relatively low values near river mouths. Vertical profiles of organic biomarkers varied with the differences in degradability of organic compounds. Specifically, long-chain fatty acids decreased with increasing depth more than the other compounds, suggesting that they degraded more easily. Conversely, we observed a tendency for lignin phenols to be selectively preserved in the vertical sediment profiles. Our results show that sediment organic biomarkers can provide diverse information such as the composition and origins of organic carbon, the contribution of seagrass derived lignin, and the varying degrees of decomposition. This approach should bring new insights to the estimation of carbon in future blue carbon studies.

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1. Introduction

The ocean is an important resource for sequestering atmospheric CO_2. Blue carbon—carbon captured by marine organisms—plays an important role in global carbon sequestration. Blue carbon stored in sediments can be sequestered from the atmosphere at geological timescales (Fourqurean et al., 2012; Macreadie et al., 2012; Mateo et al., 1997; McLeod et al., 2011). The burial rates of organic carbon (OC) are estimated to be higher in shallow coastal ecosystems such as estuaries and vegetated coastal ecosystems (238 Tg C y^{-1}) than in the open ocean (6 Tg C y^{-1}) (Nellemann et al., 2009). In addition, shallow coastal ecosystems store a large amount of terrestrially-derived OC because a substantial amount of OC (0.9 Pg C y^{-1}) is imported from terrestrial areas (Regnier et al., 2013). Seagrass meadows occupy only a relatively small portion of coastal waters (around 0.1%), yet they are an important component of oceanic carbon reservoirs (10–18%) with an annual carbon accumulation rate of 138 ± 38 g C m^{-2} y^{-1} (mean ± SE) (Costanza et al., 1997; Duarte et al., 2005; McLeod et al., 2011) although a more recent study proposed a lower estimate of 29 ± 41 g C m^{-2} y^{-1} (Miyajima and Hamaguchi, 2019). Previous studies investigating carbon sequestration processes in seagrass meadows suggest that terrestrial and seagrass-derived OC are the major contributors to OC stored in the sediments (Bianchi et al., 1999; Bianchi et al., 2002; Kennedy et al., 2010; Watanabe and Kuwae, 2015a).

Estimating the origin of sedimentary OC in vegetated coastal ecosystems is helpful for quantifying the values of these ecosystems for sequestering blue carbon (Macreadie et al., 2019). The quantification of the carbon sequestration capacity also makes it possible to calculate an economic value based on the world market carbon price, and thereby advocate for the restoration of coastal ecosystems from an economic perspective (Murray et al., 2011).

Organic carbon derived from allochthonous terrestrial plants and autochthonous primary producers such as seagrass and algae forms a complex mixture in coastal ecosystems, so biogeochemical proxies have been used to determine the sources of OC. The stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have frequently been used to estimate the sources of OC in coastal ecosystems (Garcias-Bonet et al., 2019; Kennedy et al., 2010; Ricart et al., 2020; Watanabe and Kuwae, 2015a). In these attempts, the percentage contribution (hereafter, "%contribution") from sources such as terrestrial plants, seagrass, and algae to OC in a sediment is estimated by using mixing models with the isotopic signatures of the contributors as end members. This isotopic approach has enhanced our understanding of the origins, cycling, and distribution of carbon in coastal ecosystems. However, estimating potential sources using such a mixing model with bulk isotopic compositions does not always provide a suitable value for %contribution. For example, the overlap of isotopic signatures among OC sources makes it difficult to separate these contributions accurately (Garcias-Bonet et al., 2019). Mixing models should provide more accurate %contribution estimates by using signatures that do not overlap.

Seagrasses are angiosperms that live in seawater and have lost their stomatal and ultraviolet protection functions, which are necessary for terrestrial plants, at the genetic level in order to adapt to seawater environments (Olsen et al., 2016). However, lignin, which is the main component of vascular plants, has been identified in seagrass (Kaal et al., 2018; Klap et al., 2000), where it appears to protect the lacunal system from water pressure and offer protection from microorganisms (Klap et al., 2000). In the evolutionary sense that terrestrial plants evolved from marine algae, lignin played a key role. It has been proposed that the evolution of vascular plants began with the earliest plants to acquire lignin, at an earlier evolutionary stage than vascular plants, and thus, lignin biosynthesis is absent in charophytes, chlorophytes, and rhodophytes, but is present in bryophytes and tracheophytes (Vanholme et al., 2010; Weng and Chapple, 2010; Xu et al., 2009). Therefore, determining the %contribution of potential sources using a mixing model with lignin signatures is expected to provide estimates of carbon sources without considering the contributions of algae. In addition, lignin has been regarded as a substantial component of blue carbon because it is less susceptible to degradation and is thus selectively stored as sedimentary OC (Cragg et al., 2020). Therefore, estimating the origin of lignin itself in the sediments is also an important aspect of assessing the carbon sequestration capacity of shallow coastal ecosystems.

One analytical method for analyzing lignin content uses thermally-assisted hydrolysis and methylation with tetramethylammonium hydroxide (the TMAH method). With this method, hydrolysis and derivatization are carried out simultaneously with heat and chemical assistance, allowing for a relatively quick pretreatment for analysis by gas chromatography–mass spectrometry (GC–MS) (Challinor, 2001). Using this method, lignin is analyzed as three phenylpropanoid monomers known as lignin phenols: syringyl phenols (S), vanillyl phenols (V), and cinnamyl phenols (C). These monomer units have different abundance ratios among plant types: angiosperms have a high S:V ratio, whereas gymnosperms are characterized by a low S:V ratio because they have almost no syringyl phenols, and herbaceous plants have a high C:V ratio (Hedges and Mann, 1979; Kristensen et al., 2009; Thevenot et al., 2010). Furthermore, lignin phenols can be used as an indicator of the degree of degradation of plant fragments using the acid-to-aldehyde ratios (Ad:Al) of vanillyl and syringyl phenols (Ertel and Hedges, 1985; Hedges et al., 1988). Additionally, the TMAH method can be used to analyze not only lignin, but also a wide variety of other organic compounds such as cutin acids, which constitute the cuticular layer of leaves, ω-hydroxy carboxylic acids (ω-OH acids) and α,ω-dicarboxylic acids (diacids), which are components of suberin in roots, and fatty acids (Challinor, 2001; Grasset and Ambles, 1998; del Rio and Hatcher, 1998). Thus, the organic matter compositions obtained from the TMAH method can serve not only as simple biomarkers of plants, but also to estimate the part-specific sources (e.g., leaves or roots) of plant-derived OC contributions to sediments and the degree of degradation. They are also expected to provide much information for the qualitative assessment of carbon distribution in coastal areas.

In this study, we, therefore, used the TMAH method to investigate the composition of organic matter in surface sediments of Furen Lagoon, Japan. Our aims were to determine distribution trends and characteristics of carbon and to quantitatively assess the lignin derived from seagrasses and allochthonous vascular plants using a mixing model and the obtained lignin biomarkers. Furen Lagoon is a semi-enclosed brackish lagoon with a large seagrass meadow surrounded by terrestrial environments, with contributions to OC from both autochthonous and allochthonous sources (mainly from salt marshes). Such a simple geological feature as this lagoon allows us to estimate carbon sources using a mixing model, and therefore this is a suitable model site to investigate blue carbon. In fact, several studies of blue carbon have been conducted at Furen Lagoon (Tokoro et al., 2014; Watanabe and Kuwae, 2015a, 2015b; Watanabe et al., 2019).

2. Materials and methods

2.1. Sampling location

Furen Lagoon is located in the eastern part of Hokkaido, Japan (43°19′46.5″N, 145°15′27.8″E) (Fig. 1) and has a wide salinity range of 1–30 (Tokoro et al., 2014). The total area of the lagoon is 57.4 km^2, and the maximum width and depth are 0.6 km and 11 m, respectively. Overall, however, the lagoon is very shallow, with an average depth of approximately 1 m. There are three major inflowing rivers: Pon-Yausubetsu River, Yausubetsu River, and Furen River (Fig. 1). Salt-marsh plants such as sedges prevail around the lagoon (Fig. 1), and their allochthonous OC flows into the lagoon through the rivers. The seagrass *Zostera marina* is dominant in Furen Lagoon and occupies 67% of the total area of the lagoon (Tokoro et al., 2014; Watanabe and Kuwae, 2015a) (Fig. 1). There are many ranches surrounding the

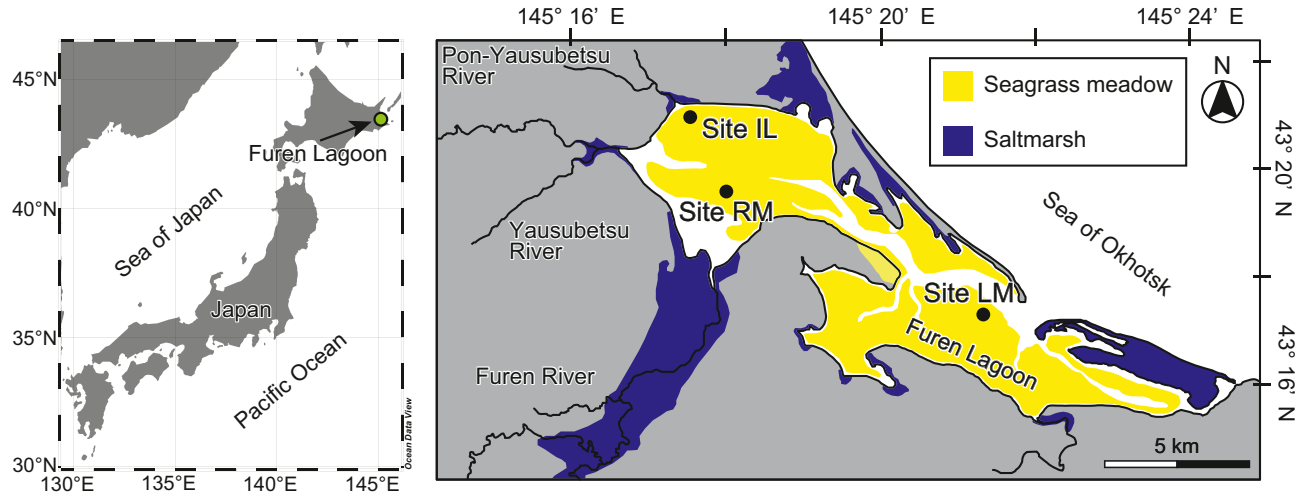

Fig. 1. Maps of Japan and Furen Lagoon showing the locations of sampling sites.

lagoon, and these are one of the causes of eutrophication in the lagoon (Montani et al., 2011).

2.2. Sampling of sediment and biomass

We collected surface sediment samples from three sites in Furen Lagoon using a small boat. All sediment samples were collected from inside the *Z. marina* meadow, but were collected from three different sites: inner lagoon (Site IL), river mouth (Site RM), and lagoon mouth (Site LM) (Fig. 1). The samples were collected using an acrylic piston corer. Although sediment samples were taken within the seagrass meadow, sampling was conducted to avoid including living seagrass or debris. The acrylic cores containing the sampled sediment were covered at both ends with rubber caps and brought onboard. The overlying water was then carefully drained out with a tube.

The cores obtained were cut into sections from 0 to 2, 2–5, 5–10, 10–15, and 15–20 mm, and three sub-samples were collected from each section for triplicate analysis. The sediment cores were cut at millimeter intervals by slowly pushing the sediment up with a screw-type rod from the bottom of the core. After cutting, large visible debris in the sediment such as leaves was removed. The core sections were freeze-dried immediately after the cut, powdered using a mortar and pestle, and then stored in a freezer (−20 °C) until they were analyzed. Carbon and nitrogen in these sediments and their isotopic compositions have been reported in previous studies (Watanabe and Kuwae, 2015a; Watanabe et al., 2019). For example, $\delta^{13}C$ values in surface sediment at Sites IL, RM, and LM were approximately −26‰, −25‰, and −17‰, respectively.

We collected allochthonous vascular plant and seagrass samples from Furen Lagoon and the surrounding allochthonous environments. Three *Z. marina* samples were randomly collected from the lagoon, and we used a sample from leaves as a representative part of the plant. We collected leaf samples from three species of allochthonous vascular plants (*Schoenoplectus* sp., *Acorus* sp., and *Moliniopsis* sp.) from the environments surrounding Furen Lagoon, including a salt marsh (supplementary Table S1). Salt-marsh plants are not typically considered terrestrial because they are coastal/estuarine and blue-carbon plants. However, in this study, we defined the salt-marsh and terrestrial plants as "allochthonous vascular plants". As with the sediment samples, these seagrass and allochthonous vascular plant samples were lyophilized and powdered, and then stored in a freezer until they were analyzed.

2.3. Thermochemolysis with TMAH reagent

The TMAH method followed the procedure used in previous studies (Nakakuni et al., 2017, 2020). Briefly, a sample (approx. 3 mg for plant samples, approx. 100 mg for sediment samples) was placed in a glass ampoule that had been previously heated at 500 °C for 3 h, and then an internal standard (nonadecanoic acid d_{50}) for quantitative analysis and 100 μL TMAH reagent (25% w:v in methanol) were added to the ampule. The ampoule was placed in a desiccator for 30 min to evaporate the solvent and then subjected to nitrogen reflux on a hot plate at 40 °C for further drying. After drying, the ampoule was sealed under vacuum and heated in an oven at 300 °C for 30 min. The heated ampoule was cooled to room temperature and the products were extracted with ethyl acetate.

2.4. GC–MS conditions

The GC–MS was performed on an Agilent 5973 MS/Agilent 6890 GC system with a DB-5 ms column (0.25 mm inner diameter, 30 m length, 0.25 μm film thickness) and helium as the carrier gas at a flow rate of 1 mL min^{-1}. The injection mode was splitless, and the injection-port temperature was 300 °C. The GC oven was initially set at 60 °C (2 min), and then the temperature was increased to 310 °C at a rate of 6 °C min^{-1} and held at 310 °C for 20 min. The temperature of the MSD transfer-line heater was 290 °C. Quadrupole and ion source temperatures were 150 °C and 230 °C, respectively. Ionization was performed in EI mode, and mass data in the 50–600 Da range were obtained in full scanning mode. Semi-concentrations were calculated by comparison with the internal standard.

2.5. Data analysis

We calculated the %contribution of the seagrass *Z. marina* and allochthonous vascular plants to lignin in the sediments at each site by using a Bayesian mixing model (SIAR) (Parnell et al., 2010; Phillips et al., 2014) with S:V and C:V values. The S:V and C:V ratios have been widely used to estimate the origin of lignin because of the different ranges of values among plants (Dittmar and Lara, 2001; Goñi et al., 1993; Ishiwatari et al., 2006; Pondell and Canuel, 2020; Thevenot et al., 2010). Such compositional features should be useful in this study for determining the origin of lignin in the sediments. The allochthonous vascular plants from around the lagoon (S:V = 1.24 ± 0.118, C:V = 6.99 ± 0.991) and the seagrass (S:V = 0.0660 ± 0.0206, C:V = 0.853 ± 0.153) from Furen Lagoon were selected as potential sources of lignin in the sediments (Table 1). A scatter plot revealed low C:V ratios in the sediments that could not be explained by the values in the seagrass and the allochthonous vascular plants (Fig. 2); therefore, we assumed that this resulted from selective degradation of cinnamyl phenols. So added as potential source values the C:V ratio of degraded allochthonous vascular plants

with degradation of cinnamyl phenols of 25%, 50%, and 75% (5.24 ± 0.743, 3.49 ± 0.496, and 1.75 ± 0.248, respectively). Opsahl and Benner (1995) examined the effects from the early diagenesis of lignin over a 4-year period using litter bags. The C:V ratio showed a marked decrease with time, but the S:V ratio remained constant. Therefore, we did not consider the selective degradation of syringyl phenols in our assumed values. In the mixing model, the different %contributions of lignin in the sediments were calculated from the relationship among the seagrass, the allochthonous vascular plants, and the material assumed to be degraded allochthonous vascular plants; the sum of these contributions was 100%.

We also estimated the allochthonous %contribution to sediment OC by using fatty acids as a tracer. Long-chain fatty acids (LCFA; C_{28}–C_{32}) are characteristic biomarkers for allochthonous vascular plants including salt-marsh plants, primarily found in the wax components (Cranwell, 1974; Meyers and Ishiwatari, 1993; Zimmerman and Canuel, 2001). Therefore, the ratio of LCFA to short-chain fatty acids (SCFA: C_{14}–C_{18}) can be used as a tracer for estimating the allochthonous contribution to sediment OC. The allochthonous %contribution as determined by fatty-acid content was calculated by the following equation:

$$\%\text{alloch by fatty acid} = \frac{(LCFA:SCFA)_{\text{sample}} - (LCFA:SCFA)_{\text{non-alloch}}}{(LCFA:SCFA)_{\text{alloch}} - (LCFA:SCFA)_{\text{non-alloch}}} \times 100,$$

where "non-alloch" and "alloch" refer to non-allochthonous vascular plants and allochthonous vascular plants, respectively. The ratio $(LCFA:SCFA)_{\text{non-alloch}}$ is included in the equation for completeness, but because LFCA are not detected from contributors other than allochthonous vascular plants (such as phytoplankton or seagrass), this ratio can be replaced with a value of zero. Therefore, the actual calculation was carried out using the ratio of the LCFA:SCFA ratio of the sample ($[LCFA:SCFA]_{\text{sample}}$) to the LCFA:SCFA ratio of the allochthonous vascular plants examined in this study ($[LCFA:SCFA]_{\text{alloch}}$). The latter ratio was 0.06 ± 0.02 (Table 1). The %contributions to OC from allochthonous sources to sediments at depths of 2 mm, 5 mm, and 15 mm at Site IL calculated by this formula were 111%, 105%, and 130%, respectively (supplementary Table S2). Therefore, because these calculated values exceeded 100%, they were considered to be 100% and this value was used to calculate the mean %contributions at Site IL. The fact that the %contributions at this site exceeded 100% could be due to the fact that we did not capture the plant end-member candidates with higher LCFA content, or that the selective degradation of short-chain fatty acids resulted in a higher percentage of LCFA in the samples. Therefore, the values for the %contribution as determined by fatty-acid content at Site IL should be interpreted with caution.

We used the Tukey–Kramer test for multiple comparisons of data from each site; differences were considered significant at $P < 0.05$. We also compared a surface section (0–5 mm) and a bottom section (10–20 mm) at each site using Student's *t*-test, which was also considered significant at $P < 0.05$. All statistical processing in this study, including the Bayesian mixing model, was conducted using the R statistical software (R Core Team, 2020).

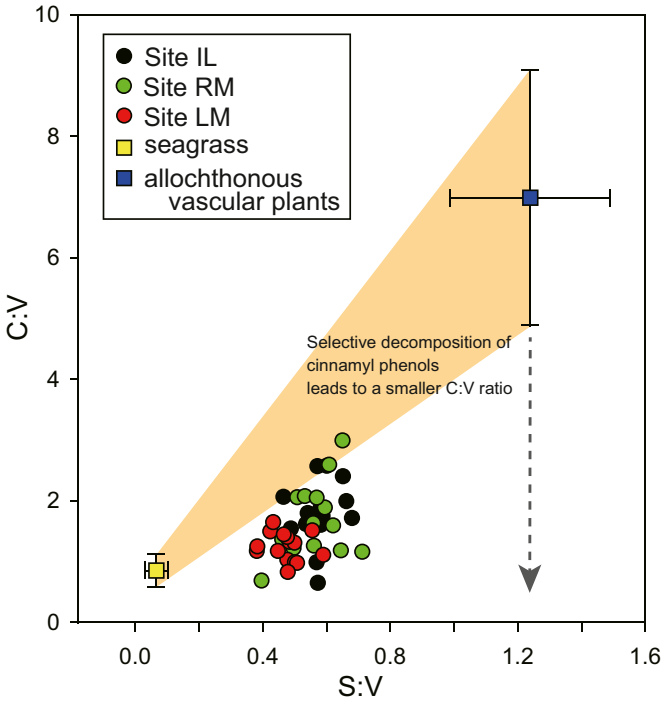

Fig. 2. Scatter plot showing cinnamyl phenol:vanillyl phenol (C:V) and syringyl phenol: vanillyl phenol (S:V) ratios in the surface sediments (0–20 mm) at each site in Furen Lagoon, Japan, in potential sources of organic carbon. Black, Site IL; green, Site RM; red, Site LM; yellow, seagrass; blue, allochthonous vascular plants. Error bars indicate standard deviations. The data for each site should fit within the orange-shaded area if they are explained by simple mixing of the values for allochthonous vascular plants and seagrass. The selective degradation of cinnamyl phenols works toward decreasing the C:V ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results

3.1. Interpretation of organic biomarkers

Lignin phenols, fatty acids, cutin acids, diacids, and ω-OH acids were detected from the sediments of a seagrass meadow in Furen Lagoon, Japan. These compounds were detected as methyl derivatives by reaction with TMAH reagent (Challinor, 2001); however, it should be noted that the names of the structures described below are their original structures. For the lignin phenols, we detected syringyl phenols (syringic acid, syringaldehyde, and acetosyringone), vanillyl phenols (vanillic acid, vanillin, and acetovanillone), and cinnamyl phenols (*p*-coumaric and ferulic acid). The sum of these three lignin groups

Table 1
Lignin-phenol compositions and LCFA:SCFA ratios in allochthonous vascular plants and seagrass (*Zostera marina*).

	Σ8[a] (μg g^{-1} dry wt)	S:V[b,d]	C:V[c,d]	LCFA:SCFA[e]
Allochthonous vascular plant leaves ($n = 3$)[f]	8200 ± 990	1.23 ± 0.252	7.00 ± 2.12	0.06 ± 0.02
Zostera marina leaves ($n = 3$)	911 ± 230	0.0660 ± 0.0438	0.853 ± 0.325	0

All values are mean ± standard deviation.
[a] Σ8 = lignin-phenol concentrations (cinnamyl + vanillyl + syringyl phenols).
[b] The ratio of syringyl phenols (S) to vanillyl phenols (V).
[c] The ratio of cinnamyl phenols (C) to vanillyl phenols (V).
[d] *t*-tests confirmed that there were significant differences in S:V and C:V between *Zostera marina* and the allochthonous vascular plants ($P < 0.01$).
[e] The ratio of long-chain fatty acids (LCFA, C_{28}–C_{32}) to short-chain fatty acids (SCFA, C_{14}–C_{18}).
[f] Lignin-phenol compositions at the species level for allochthonous vascular plants are presented in the Supplemental Material (supplementary Table S1).

(syringyl + vanillyl + cinnamyl phenols) is treated as the total lignin content (Σ8) (Hedges and Mann, 1979; Pondell and Canuel, 2020).

Fatty acids were found in a range of chain lengths from C_{14} to C_{32}. They are categorized by their chain length: SCFA ranging from C_{14} to C_{18} are derived from aquatic organisms such as phytoplankton (Canuel and Martens, 1993; Meyers, 2003; Meyers and Ishiwatari, 1993; Rieley et al., 1991), whereas LCFA ranging from C_{24} to C_{32} are derived from allochthonous vascular plants as mentioned above. In this study, the fatty acids were divided into SCFA and LCFA according to this categorization. However, C_{24} and C_{26} fatty acids were excluded from the LCFA as allochthonous biomarkers because they were also found in seagrass in this study (supplementary Fig. S1).

For cutin acids, we detected 8,16-, 9,16-, and 10,16-dihydroxyhexadecanoic acid, 7- and 8-hydroxyhexadecane-1,16-dioic acid, and 9,10,18-trihydroxyoctadecanoic acid. We present the total of these concentrations as the concentration of cutin acids. The total ω-OH acids in this study include ω-OH acids above C_{20} because these acids are found in abundance in suberin (Franke et al., 2005; Matzke and Riederer, 1991), a high-molecular-weight compound that forms in the roots of plants. Diacids were found ranging from C_{16} to C_{26}. These diacids are also a compound characteristically found in suberin (Mendez-Millan et al., 2011), but in this study we only included diacids ≥C_{20} because short-chain diacids (C_{16} and C_{18}) are sometimes found in leaf tissue (Franke et al., 2005).

3.2. Lignin composition in allochthonous vascular plant and seagrass

The lignin-phenol compositions of the allochthonous vascular plants and the seagrass are summarized in Table 1. The lignin-phenol concentration (Σ8) in the allochthonous vascular plants was 8200 ± 990 μg g^{-1} dry weight (dry wt). The S:V and C:V ratios of the allochthonous vascular plants were 1.23 ± 0.252 and 7.00 ± 2.12, respectively. On the other hand, Σ8 in the seagrass was 911 ± 230 μg g^{-1} dry wt, or about one-tenth that in the allochthonous vascular plants. The S:V and C:V ratios of the seagrass were 0.0660 ± 0.0438 and 0.853 ± 0.325, respectively, which are smaller than those for the allochthonous vascular plants.

3.3. Comparison between total organic carbon and biomarkers

We prepared scatter plots to visualize the relationships between the concentrations of the measured biomarkers and total organic carbon (TOC) content (Fig. 3). Values for Σ8 in the sediment samples were the highest among the biomarkers, ranging from 6.28 to 231 μg g^{-1} dry wt (70.0 ± 61.5 μg g^{-1} dry wt [mean ± SD]). The SCFA had the highest concentration following Σ8 and varied from 13.1 to 121 μg g^{-1} dry wt (32.8 ± 28.2 μg g^{-1} dry wt). Other compounds varied in a similar concentration range, with LCFA ranging from 0.00 to 6.54 μg g^{-1} dry wt (1.11 ± 1.64 μg g^{-1} dry wt), cutin acids from 0.992 to 19.2 μg g^{-1} dry wt (6.26 ± 4.64 μg g^{-1} dry wt), diacids (≥C_{20}) from 0.360 to 10.9 μg g^{-1} dry wt (1.94 ± 2.45 μg g^{-1} dry wt), and ω-OH acids (>C_{20}) from 0.280 to 22.5 μg g^{-1} dry wt (5.15 ± 3.92 μg g^{-1} dry wt). Among these compounds, lignin-phenol concentrations, SCFA, and LCFA showed significant, strong correlations with TOC ($r^2 > 0.6$, $P < 0.001$), and the concentration of diacids was moderately correlated with TOC ($r^2 = 0.42$, $P < 0.001$). On the other hand, there were no significant correlations between cutin acids and TOC or ω-OH acids and TOC ($r^2 = 0.017$ and 0.12, respectively).

The Ad:Al ratio in the sediments was calculated using the ratio of vanillyl phenols (the ratio of vanillic acid to vanillin) as an indicator of the oxidative degradation of lignin. The values of the Ad:Al ratio in the Furen Lagoon sediments varied from 0.585 to 2.13 (1.28 ± 0.389) (Fig. 3b). We found a significant correlation between the Ad:Al ratio and TOC ($r^2 = 0.547$, $P < 0.001$). The ratio of combined diacids and ω-OH acids to cutin acids ranged from 0.253 to 5.24 (average, 1.14 ± 0.978) and was significantly correlated with TOC ($r^2 = 0.588$, $P < 0.001$) (Fig. 3h).

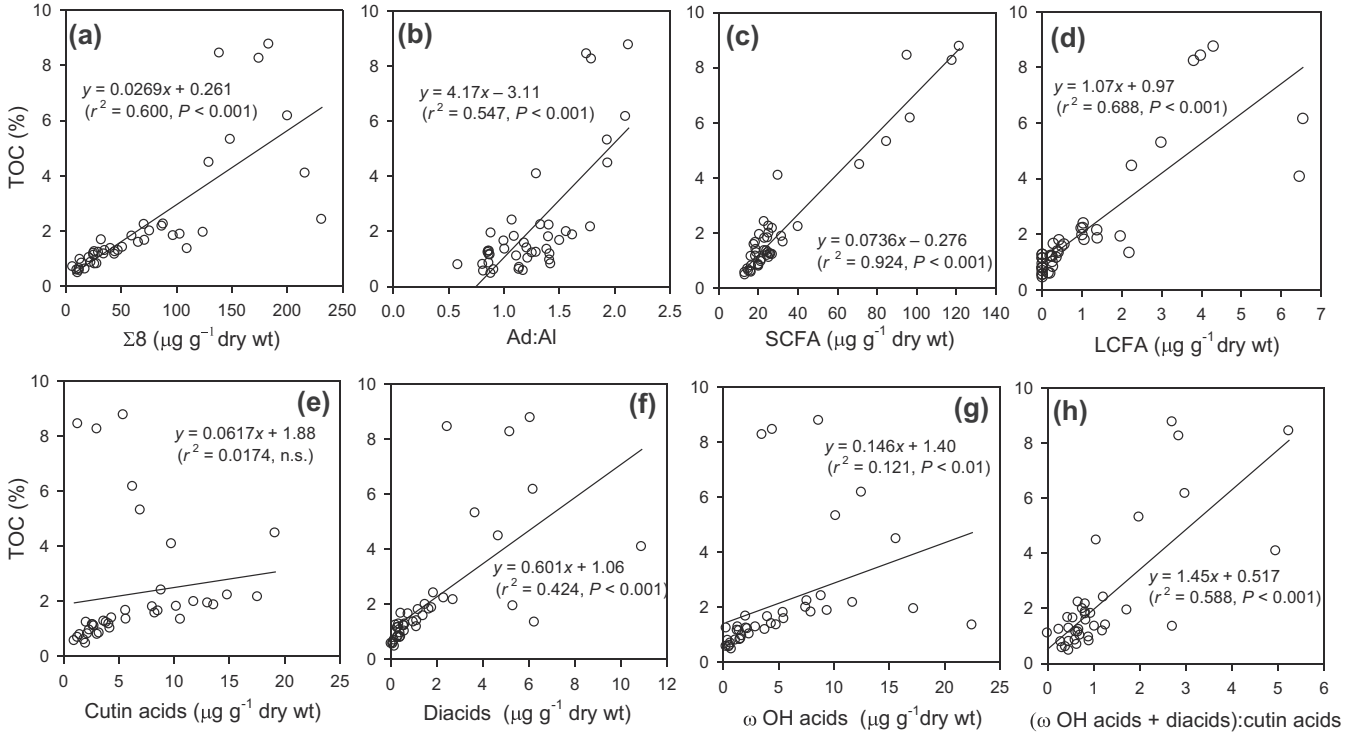

Fig. 3. Relationship between total organic carbon (TOC) content in the surface sediments (0–20 mm) of Furen Lagoon, Japan, and lignin-phenol concentrations (Σ8) (a), acid to aldehyde ratios of vanillyl phenols (Ad:Al) (b), short-chain fatty acids (SCFA) (c), long-chain fatty acids (LCFA) (d), cutin acids (e), diacids (f), ω-OH acids (g), and the ratio of (ω-OH acids + diacids) to cutin acids (h). The TOC data are from Watanabe and Kuwae (2015a) and Watanabe et al. (2019).

3.4. Spatial distributions of biomarkers

The Σ8 values in the sediments were significantly lower at Site LM (18.6 ± 2.26 μg g^{-1} dry wt) than at Sites IL and RM ($P < 0.05$; Tukey–Kramer test), and the concentrations at Site IL (102 ± 14.5 μg g^{-1} dry wt) and Site RM (93.2 ± 44.7 μg g^{-1} dry wt) were comparable (Fig. 4a). The S:V and C:V ratios at Sites IL (S:V, 0.581 ± 0.0156; C:V, 1.77 ± 0.139) and RM (S:V, 0.569 ± 0.0566; C:V, 1.68 ± 0.424) had a similar range (Fig. 4b and c). On the other hand, these values were significantly lower at Site LM (S:V, 0.477 ± 0.0172; C:V, 1.22 ± 0.0491) than at Sites IL and RM ($P < 0.05$). Ad:Al ratios were determined not to be different among the sites (Site IL, 1.32 ± 0.0805; Site RM, 1.35 ± 0.385; Site LM, 1.17 ± 0.0585) ($P > 0.05$) (Fig. 4d).

The concentrations of SCFA were significantly higher at Site RM (55.5 ± 27.5 μg g^{-1} dry wt) than at Sites IL (23.3 ± 1.17 μg g^{-1} dry wt) and LM (20.5 ± 1.40 μg g^{-1} dry wt) ($P < 0.01$) (Fig. 4e). On the other hand, there was no significant difference between Sites IL and LM ($P > 0.05$). The concentrations of LCFA differed markedly between Sites RM (2.04 ± 1.88 μg g^{-1} dry wt) and LM (0.13 ± 0.10 μg g^{-1} dry wt), with significantly higher values at Site RM ($P < 0.05$) (Fig. 4f), but the concentrations were not significantly different ($P > 0.05$) between Site IL (1.41 ± 0.60 μg g^{-1} dry wt) and Sites RM and LM.

The concentrations of cutin acids were significantly higher at Site IL (9.81 ± 1.03 μg g^{-1} dry wt) than at Sites RM and LM ($P < 0.05$ and $P < 0.01$, respectively), with no difference found between Sites LM (2.74 ± 0.273 μg g^{-1} dry wt) and RM (5.96 ± 5.06 μg g^{-1} dry wt) (Fig. 4h). Diacids showed significantly lower concentrations at Site LM (0.375 ± 0.0960 μg g^{-1} dry wt) than at the other sites ($P < 0.05$), and there was no difference in concentration between Sites IL (2.84 ± 0.793 μg g^{-1}) and RM (2.41 ± 1.57 μg g^{-1}) ($P > 0.05$) (Fig. 4i). ω-OH acids showed a significantly lower value ($P < 0.01$) at Site LM (1.42 ± 0.324 μg g^{-1} dry wt) than at Site IL (8.84 ± 1.64 μg g^{-1} dry wt). Concentrations at Sites IL and RM (5.47 ± 3.23 μg g^{-1} dry wt) were equivalent ($P > 0.05$), and there was no significant difference between Sites LM and RM ($P = 0.055$) (Fig. 4j). There was no significant difference in the ratio of (diacid + ω-OH acids) to cutin acids between Site IL (1.10 ± 0.153) and Site LM (0.569 ± 0.107) or between Sites IL and RM (1.62 ± 1.41) ($P > 0.05$). There was, however, a significant difference between Sites LM and RM, with higher values at Site RM ($P < 0.05$) (Fig. 4k).

3.5. Contributions of allochthonous vascular plant and seagrass sources to sedimentary lignin phenols

The S:V ratios of the allochthonous vascular plants were relatively high (1.23 ± 0.25) and the C:V ratio was 7.00 ± 2.12 (Fig. 2). On the other hand, the seagrass was characterized by a low S:V ratio (0.0660 ± 0.0438) and C:V ratio (0.853 ± 0.325). The ratios in sediments were different among sites, with the values at Sites IL and RM plotting closer to the allochthonous vascular plant values and those at Site LM closest to the seagrass values. However, the C:V ratios in sediments tended to be lower than the range that could be explained by a mixture of the allochthonous vascular plants and the seagrass.

The lignin biomarker mixing model showed different percentage contributions from the seagrass, fresh allochthonous vascular plants, and the assumed degraded allochthonous vascular plants at each site (Fig. 5). Sites IL and RM had a higher %contribution from the fresh allochthonous vascular plants than Site LM at 4.2% ± 3.1% and 3.8% ± 3.0%, respectively. However, at both sites, the degraded allochthonous vascular plant material predominated over the fresh allochthonous vascular plants, with Site IL showing 6.6% ± 4.5%, 12.0% ± 7.2%, and 20.8% ± 6.0% of the 25%, 50%, and 75% degraded material, respectively, and Site RM showing 5.6% ± 4.3% at 25% degradation, 11.1% ± 7.8% at 50% degradation, and 22.4% ± 7.1% at 75% degradation. The sum of the %contributions from the fresh and the degraded allochthonous vascular plants at Sites IL and RM were 43.6% and 42.9%, respectively. On the

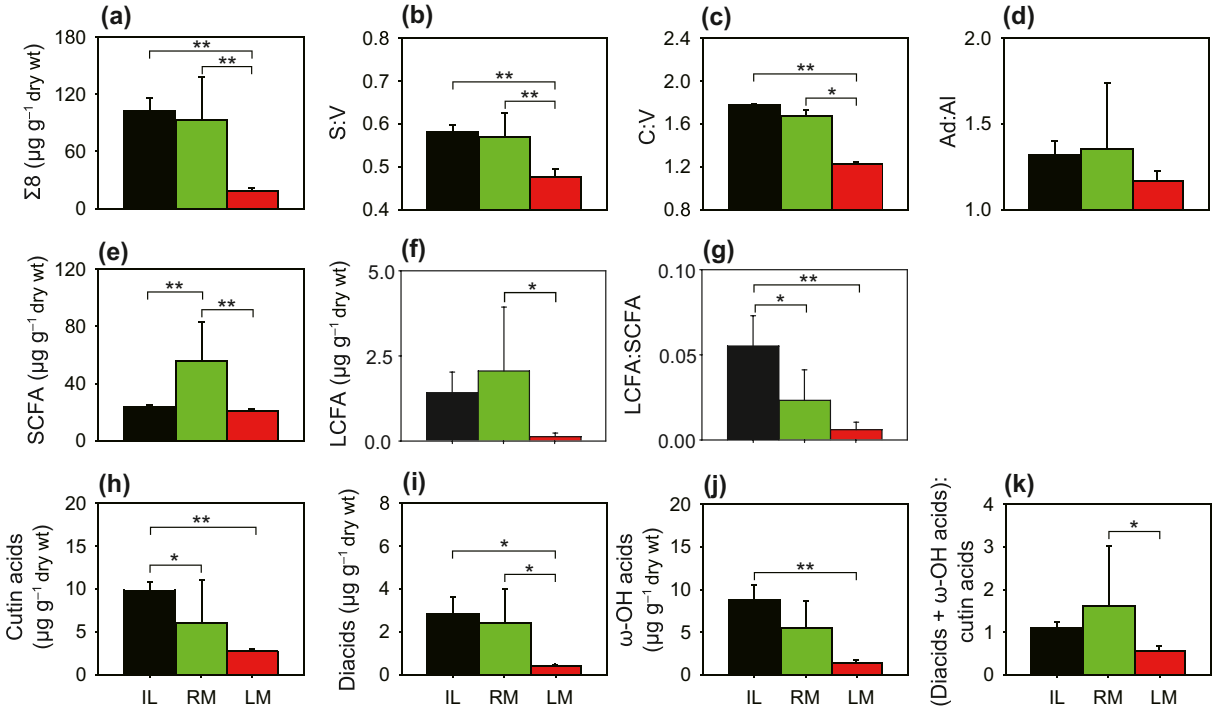

Fig. 4. Comparison of the mean concentrations of lignin phenols (Σ8) (a), syringyl:vanillyl phenol ratios (S:V) (b), cinnamyl:vanillyl phenol ratios (C:V) (c), acid to aldehyde ratios of vanillyl phenols (Ad:Al) (d), short-chain fatty acids (SCFA) (e), long-chain fatty acids (LCFA) (f), LCFA:SCFA ratios (g), cutin acids (h), diacids (i), ω-OH acids (j), and (diacids + ω-OH acids):cutin acids ratios (k) in the surface sediment (0–20 mm) at each site in Furen Lagoon, Japan. Black, Site IL; green, Site RM; red, Site LM. Asterisks (*) and double asterisks (**) indicate a significant difference at $P < 0.05$ and $P < 0.01$, respectively (Tukey–Kramer test). The absence of an asterisk indicates no significant difference. Error bars denote the standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

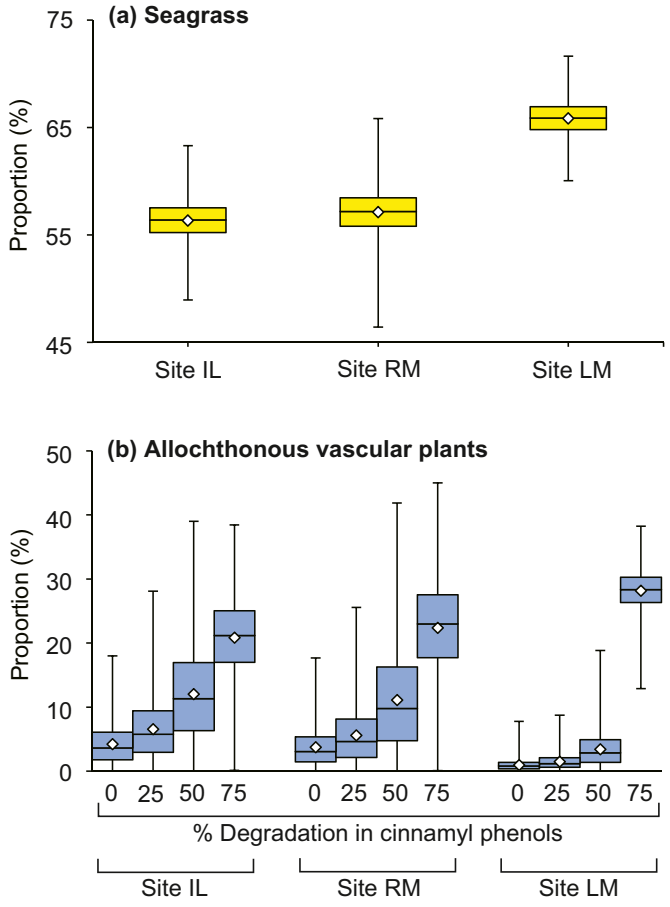

Fig. 5. The percentage contribution of the seagrass *Zostera marina* (a) and allochthonous vascular plants (b) to the sediment lignin pool as estimated by a mixing model using cinnamyl:vanillyl phenol ratios (C:V) and syringyl:vanillyl phenol ratios (S:V). The percent contributions in (b) include those from cinnamyl phenols from allochthonous vascular plants at three stages of decomposition. The box and the center line indicate the interquartile range and the median, respectively. Vertical lines denote the maximum and minimum values. The diamond symbol represents the mean value.

other hand, the %contributions of the seagrass at these sites were 56.3% ± 1.7% (Site IL) and 57.1% ± 2.1% (Site RM). At Site LM, the %contribution of the seagrass was higher than at the other sites, at 65.8% ± 1.6%. On the other hand, the contribution of the allochthonous vascular plants was the lowest, at only 1.0% ± 0.8%. In addition, the contributions of the 25% and 50% degraded allochthonous vascular plant material were also small at 1.5% ± 1.2% and 3.4% ± 2.6%, respectively. However, the contribution of the 75% degraded material was relatively high at 28.2% ± 3.9%.

The allochthonous %contributions as determined by fatty-acid contents also showed different values depending on the site. The highest %contribution was at Site IL, at 82.5% ± 21.5%. The lowest %contribution was at Site LM, at 9.7% ± 7.4%. For Site RM, the %contribution was 38.6% ± 30.0%, which is higher than that at LM.

3.6. Vertical distributions of biomarkers

The distribution of Σ8 values did not show any clear trend with depth at Site IL or LM (Fig. 6a). On the other hand, the Σ8 concentrations at Site RM showed a sharp decrease with increasing depth. Also, both SCFA and LCFA showed a tendency to decrease rapidly with depth at Site RM (Fig. 6b and c), as did lignin. Neither group of fatty acids showed any clear change with depth at Sites IL or LM. There were no consistent trends in the depth distributions of cutin acids at any site (Fig. 6d), whereas diacids and ω-OH acids at Sites IL and RM showed gradual decreases from the surface (Fig. 6e and f).

Organic biomarkers normalized to TOC showed different trends at each site (Fig. 7). The total concentration of lignin phenols relative to TOC is often expressed in units of milligrams per 100 mg of organic carbon [mg (100 mg OC)$^{-1}$] (Λ8). For consistency, we used these units to present the normalized concentrations of other biomarkers as well. At Sites RM and LM, *t*-tests confirmed that there were no significant differences in cutin acids, diacids or ω-OH acids between a surface section (0–5 mm) and a bottom section (10–20 mm) from the core samples from these sites ($P > 0.05$, supplementary Table S3). We therefore concluded that SCFA, cutin acids, diacids, and ω-OH acids were stable with depth (Fig. 7b, d–f). On the other hand, Λ8 increased slightly with depth at Site LM (Fig. 7a) with values significantly higher than those at the surface at Site LM ($P < 0.05$; supplementary Table S3), and it was stable at Site RM ($P = 0.09$; supplementary Table S3). In contrast, LCFA tended to decrease gradually with increasing depth at Site RM (Fig. 7c), and the bottom section had significantly lower LCFA when compared with the surface section (*t*-test, $P < 0.01$; supplementary Table S3). At Site IL, all compounds seemed to decrease with depth. However, *t*-tests showed that only Λ8, cutin acids, and ω-OH acids were significantly higher in the surface section compared to the bottom section (*t*-test, $P < 0.05$; supplementary Table S3). The proportional decrease with depth differed among the compounds. ω-OH acids had the highest proportional decrease, with concentrations in the deepest section (20 mm) about 20% of the concentrations in the surface sediments (Fig. 7f). In contrast, cutin acid concentrations in the deepest section were about 50% of the surface concentrations (Fig. 7d). Λ8 concentrations at depth were only reduced to around 60% of the surface concentrations (Fig. 7a).

We also tested for differences in biomarker compositional ratios at each site (S:V, C:V, and (diacids + ω-OH acids):cutin acids) between surface and bottom-layer values (*t*-test). We found no significant differences between the layers ($P > 0.05$), except for C:V at Site IL and (diacids + ω-OH acids):cutin acids at Site RM ($P < 0.05$) (Table S4 and Fig. S2).

4. Discussion

4.1. Characteristics of organic matter composition

The significant correlation between TOC and allochthonous vascular plant biomarkers indicates that allochthonous vascular plant-derived OC substantially contributes to TOC in the surface sediments of Furen Lagoon (Fig. 3). In particular, this is strongly supported by the relationship between LCFA and TOC (Fig. 3d). LCFA are derived from the wax components of allochthonous vascular plants, and seagrass is not considered a major source of LCFA in sediments (Volkman et al., 1980). In fact, the seagrass did not contain C_{28}–C_{30} LCFA although these LCFA were in the allochthonous vascular plants (supplementary Fig. S1).

The highest TOC concentrations in Furen Lagoon were found at Sites IL and RM, which are close to river mouths (Watanabe and Kuwae, 2015a). Similarly, allochthonous biomarkers were higher at Sites IL and RM (Fig. 4). Site IL is located at the mouths of Pon-Yausubetsu and Yausubetsu Rivers, and Site RM is close to the mouth of Furen River (Fig. 1). Previous analyses of allochthonous biomarkers in marine surface sediments and particulate OC have tended to show higher allochthonous biomarker concentrations in the vicinity of large inflowing rivers (Kuzyk et al., 2008; Onstad et al., 2000; Seki et al., 2014). This means that rivers play an important role in transporting allochthonous OC (Burdige, 2005; Cai, 2011; Hedges, 1992). Moreover, Kennedy et al. (2010) estimated the seagrass-derived OC in seagrass-meadow sediments by compiling measurements from various studies and found that allochthonous vascular plants were a major contributor to OC in the sediments, although seagrasses were potential contributors. Also, in Furen Lagoon, both elemental concentrations and their isotopic signatures (i.e., N:C ratios and $\delta^{13}C$ and $\delta^{15}N$ values) showed that allochthonous vascular plants made large contributions to sedimentary OC in both surface sediments and over vertical profiles (Watanabe and

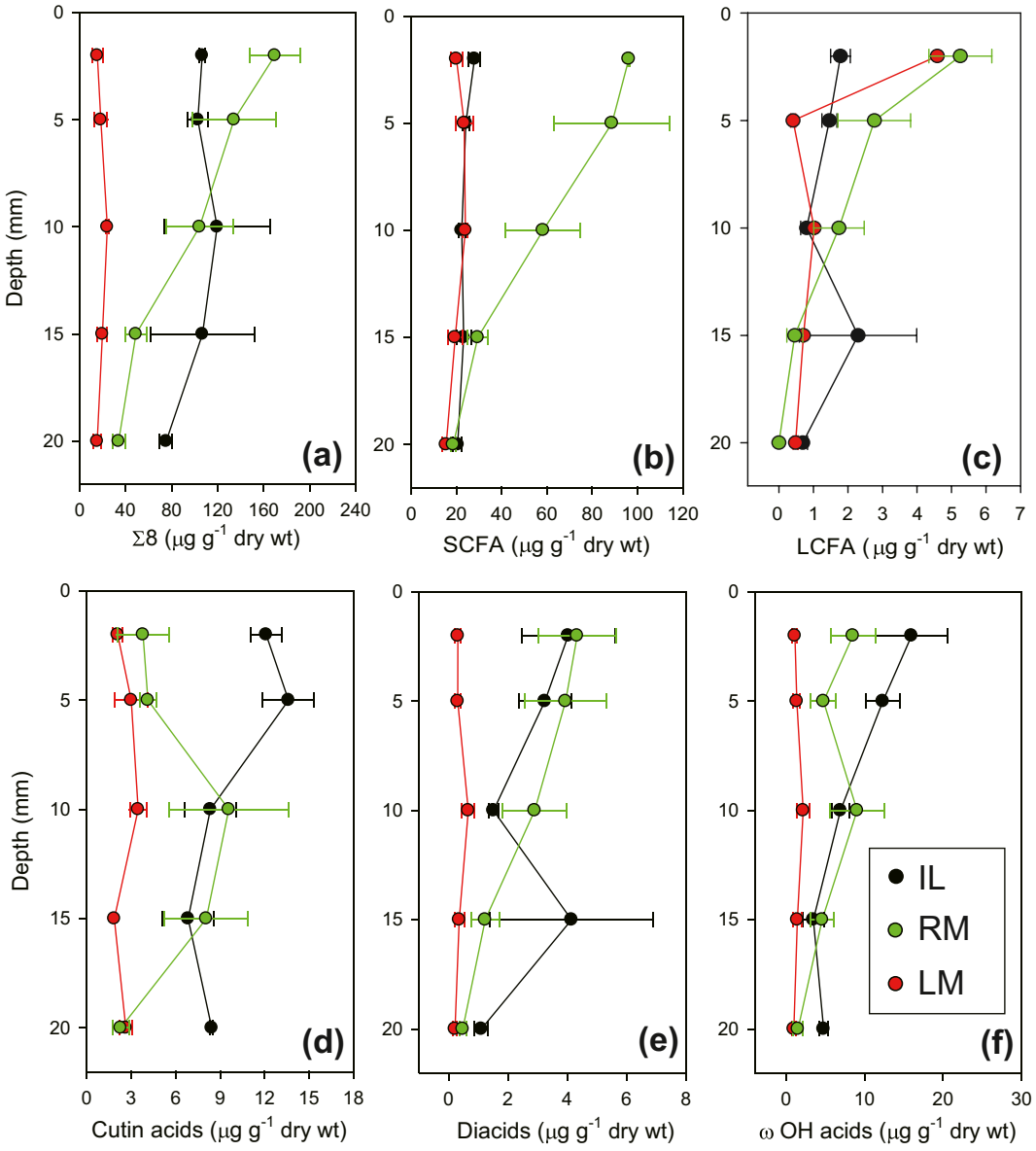

Fig. 6. Vertical distributions of lignin-phenol concentrations (Σ8) (a), short-chain fatty acids (SCFA) (b), long-chain fatty acids (LCFA) (c), cutin acids (d), diacids (e), and ω-OH acids (f) at each site in Furen Lagoon, Japan. Black, Site IL; green, Site RM; red, Site LM. Error bars show standard deviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Kuwae, 2015a; Watanabe et al., 2019). Our results reinforce these previous studies from an organic biomarker perspective, and we agree that allochthonous sources are a major contributor to OC in estuaries and vegetated coastal ecosystems.

Concentrations of SCFA in sediments were highly correlated with TOC (Fig. 3c), but they have multiple sources (Canuel and Martens, 1993; Meyers, 2003; Meyers and Ishiwatari, 1993; Rieley et al., 1991), coming from both phytoplankton and allochthonous vascular plants. On the other hand, there were no significant correlations between cutin acids, diacids, or ω-OH acids and TOC (Fig. 3e–g). However, the ratio of (ω-OH acids + diacids) to cutin acids was significantly correlated with TOC (Fig. 3h). ω-OH acids and diacids, which are compounds found in abundance mainly in roots, are constituents of suberin (Zeier et al., 1999), whereas cutin acids are found in the leaf cuticular layer (Holloway, 1982; Kolattukudy, 1980). Thus, a high (ω-OH acids + diacids):cutin acids ratio indicates a high contribution of root-derived OC. Based on this interpretation, the good correlation between this ratio and TOC can be interpreted as indicating that sediment with high TOC content (mainly of allochthonous origin) includes a high proportion of root-derived matter. Note that this result is presumed to indicate a significant contribution of plant debris that includes roots rather than a selective contribution of roots.

Our finding of a significant correlation between Ad:Al and TOC (Fig. 3b) suggests that the TOC sources contain a large amount of degraded plant products. Lignin changes from an aldehyde type to an acid type by oxidative degradation; therefore, the Ad:Al ratio is used as an indicator of the degree of lignin degradation (Hatcher and Minard, 1995; Hedges et al., 1988). Our results indicate that OC from allochthonous ecosystems is a major controlling factor for the OC in the surface sediments in Furen Lagoon, with degraded plant debris being the main source. A large proportion of allochthonous-derived OC transported to the oceans is reported to be highly degraded during transport through soils and rivers, and therefore it is relatively refractory and can be selectively stored in marine sediments (Hedges et al., 1994; Watanabe and Kuwae, 2015a; Zonneveld et al., 2010).

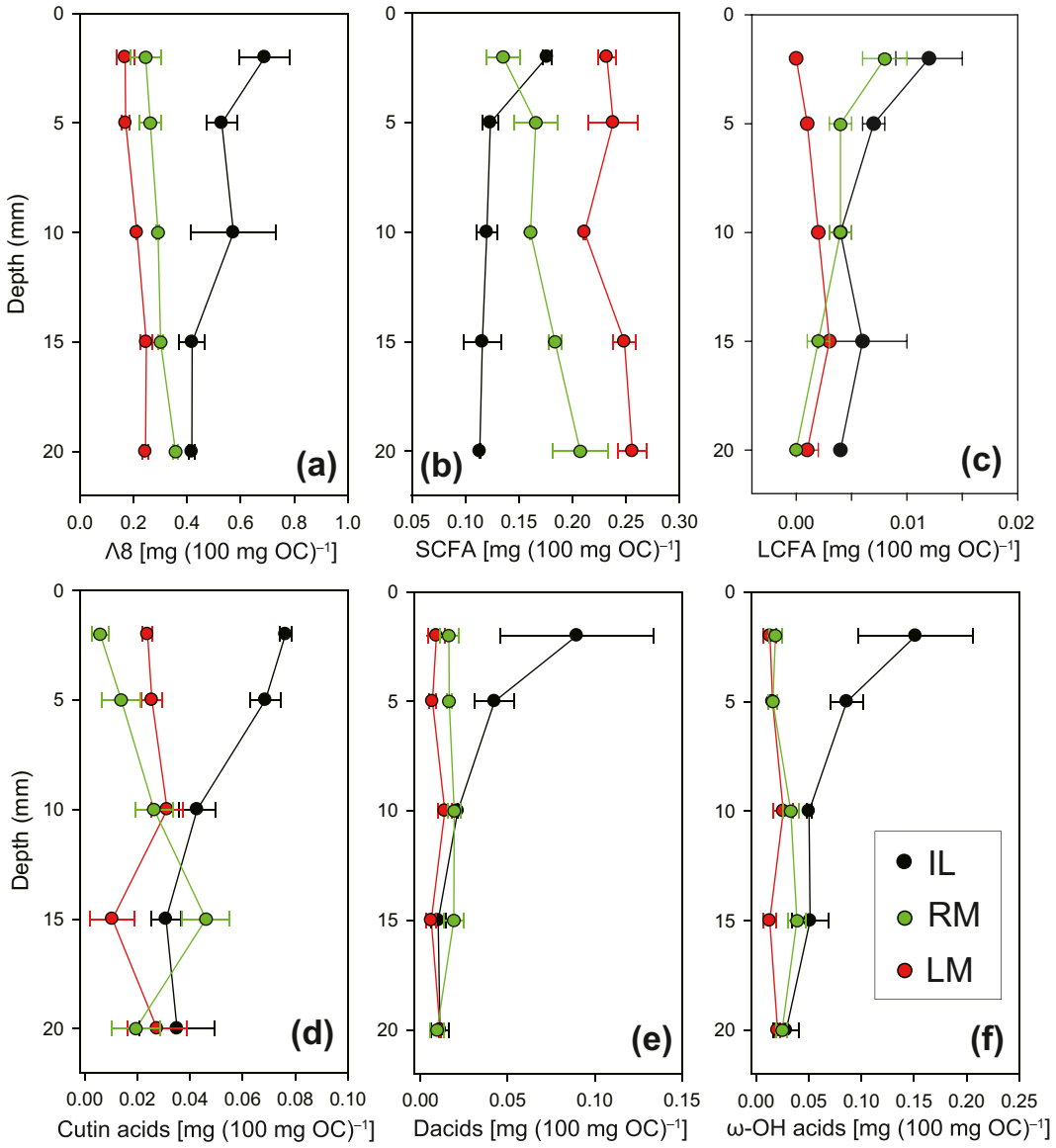

Fig. 7. Vertical carbon-normalized distributions [mg (100 mg OC)$^{-1}$] of lignin phenols (Λ8) (a), short-chain fatty acids (SCFA) (b), long-chain fatty acids (LCFA) (c), cutin acids (d), diacids (e), and ω-OH acids (f) at each site in Furen Lagoon, Japan. Black, Site IL; green, Site RM; red, Site LM. Error bars indicate standard deviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2. Contributions of allochthonous vascular plants and seagrass to sedimentary lignin

In this study, we established a mixing model using only lignin biomarkers. A mixing model using $\delta^{13}C$ and $\delta^{15}N$ values derives the %contributions from different sources in the bulk OC. On the other hand, our mixing model provides the %contribution of potential contributors in the lignin pool. Because lignin is an important component of blue carbon (Cragg et al., 2020), deriving the %contributions of potential contributors in lignin is important for discussing more substantial blue carbon sources. Furthermore, because this model is based on two variables, the S:V and C:V ratios, it is possible to calculate the %contributions of more than just two contributors. We determined the %contributions of a total of five contributors: seagrass (*Z. marina*) and allochthonous vascular plants separated into four levels of decomposition.

Our mixing model showed a higher contribution from allochthonous vascular plants (including fresh and degraded plants) at Sites IL and RM than at Site LM (Fig. 5). This result is consistent with the higher concentrations of allochthonous biomarkers at both of these sites. Additionally, most of the allochthonous contributions consisted of degraded products (Fig. 5). This suggests that some of the allochthonous-derived lignin was contributed as the decomposed form. Regarding the %contribution of allochthonous vascular plants at Site LM, the most predominant were the highly degraded allochthonous vascular plants (75% degradation) (Fig. 5). This may indicate that the degradation of allochthonous OC proceeds during the process of transport from riverine sites (i.e., Sites IL and RM) to the lagoon inlet (Site LM).

The differences in %contributions from allochthonous vascular plants and seagrass among the sites may result from changes in the relative contribution from seagrass with the higher contributions from allochthonous vascular plants at Sites IL and RM, which are closer to the influence of the rivers, and the lower allochthonous vascular plant contribution at Site LM, which is farther away from the rivers. Previous studies estimated OC sources in Furen Lagoon by using elemental and isotopic compositions (i.e., the N:C ratio and $\delta^{13}C$ and $\delta^{15}N$ values) of surface sediments and found that the contribution of allochthonous vascular plant-derived carbon decreased with an increase in salinity (decrease in river-water effect) (Watanabe and Kuwae, 2015a; Watanabe et al., 2019). Our results are largely consistent with this trend. Additionally, the high leaf density of seagrass meadows catches

allochthonous OC (Dahl et al., 2016; Gacia et al., 1999), which is another probable reason for the high proportion of OC with allochthonous vascular plant origins at our sites. Indeed, the proportional contributions of allochthonous vascular plants and seagrass to TOC at Site LM determined in our previous study were 23% and 77%, respectively (Watanabe et al., 2019), which are similar to our estimated values for lignin. However, the %contributions of allochthonous vascular plants and seagrass to TOC were 92% and 8%, respectively, at Site IL, and 87% and 13%, respectively, at Site RM (Watanabe et al., 2019), which differ greatly from the values that we estimated from lignin content.

One reason for these differences might be related to a difference in sedimentary processes. Whereas allochthonous vascular plant materials are input through soils and rivers, seagrass accumulates directly to sediments (Tanaya et al., 2018). Therefore, the lignin from seagrass should be less subject to degradation than that of allochthonous vascular plants. This could lead to lignin from seagrass being analyzed with greater amounts of intact lignin phenols with less degradation, whereas lignin from allochthonous vascular plants might be highly degraded during sedimentary processes and lose its lignin-phenolic structures, becoming unrecognizable as lignin phenols. As a result, it is possible that the %contribution of lignin from allochthonous vascular plants is underestimated. Another possible reason for differences between studies is the contribution from gymnosperms to the lignin pool. The values for the S:V and C:V ratios of gymnosperms are relatively low (Hedges and Mann, 1979; Kristensen et al., 2009; Thevenot et al., 2010), similar to those of the seagrass. Therefore, the end members for the C:V and S:V ratios of allochthonous vascular plants will be lower if the contribution from gymnosperms is high. However, in Furen Lagoon, the S:V and C:V ratios in the sediments near river mouths were significantly higher than those near the tidal inlet (Fig. 4), indicating that the major contribution from allochthonous vascular plants coming from the nearby river mouths would not be from gymnosperms. However, it is also possible that the contribution of seagrass to sedimentary OC may be overestimated because of the assumed values chosen for the degradation of lignin phenols, which will be discussed below.

Lignin is recalcitrant organic matter and therefore plays an important role in the long-term sequestration of carbon in sediments (Cragg et al., 2020). In mixing models calculated from only the carbon and nitrogen stable isotope ratios, it is only possible to calculate the %contributions to bulk OC. On the other hand, the calculation of %contributions using lignin biomarkers allows for more accurate determination of the %contribution for vascular plants including seagrass. In a previous study estimating blue carbon sources using lignin biomarkers, Chen et al. (2016) attempted to characterize the spatial distribution of sedimentary OC in a salt marsh using the bulk composition of nitrogen and carbon and their isotopic ratios and Λ8. Also, Barry et al. (2018) attempted to estimate the blue carbon stock in sediments in a seagrass meadow of *Thalassia testudinum* using lignin biomarkers. They estimated that approximately 70% of lignin in the sediments was derived from *T. testudinum*, and they determined that *T. testudinum*-derived OC accounted for approximately 40% of TOC in the sediments using lignin and $\delta^{13}C$ records. Our results are similar to this estimate and support the utility of approaches using lignin biomarkers. These studies strongly suggest that lignin can be an important source of TOC in sediments.

Additionally, using the Σ8 values obtained and the %contribution of seagrass, we determined the percentage of lignin-phenol carbon in the TOC in the sediment at Site LM, where the contribution of seagrass was the highest. The carbon content attributable to lignin phenols was determined by multiplying the percentage of carbon atoms in the structural formulae of the lignin-phenols by their concentrations. The results showed that lignin phenols comprised 0.10–0.14% of the carbon in TOC, and the carbon content from seagrass-derived lignin phenols was only 0.07–0.09% of TOC, although lignin is considered a substantial contributor to blue carbon. However, the freshly deposited OC in the surface sediments from various sources has not been subjected to selective degradation, and the proportion of lignin in this is still considered to be low. In addition, it should be noted that the lignin phenols (i.e., Σ8) are only a part of the lignin structure. Therefore, the amount of lignin calculated from Σ8 is very small. Thus, we speculate that the actual concentration of lignin is much higher than our measurements at Site LM.

When the compositional ratio of lignin phenols is used to estimate the %contribution, it is necessary to consider differences in degradation rates among the compounds. In this study, we attempted to address this bias by introducing assumed values in a stepwise degradation of cinnamyl phenols, but more complex systems will be required for an accurate simulation of the degradation rate. For example, this study does not take into account the fact that syringyl phenols are more easily degraded than vanillyl phenols (Hedges et al., 1985). In fact, at Sites IL and RM, we estimated that around 55% of the lignin in the sediment was derived from the seagrass (Fig. 5), despite the high concentrations of LCFA at these sites (Fig. 4). Such a discrepancy can also be inferred from the large difference between the %contribution from allochthonous vascular plants based on fatty acids and that from lignin at Sites IL and RM (supplementary Table S2). This could be due to overestimation of seagrass-derived lignin by not considering the degradation of syringyl phenols. Thus, it will be necessary to better understand the differences in the degradation of syringyl and cinnamyl phenols relative to vanillyl phenols during early diagenesis if the compositional ratios are to be used to calculate the %contribution of OC in the sediments.

Another approach to solving such a problem may be by using the annual average of lignin composition in particulate OC flowing through rivers as an end member. However, incubation experiments on lignin degradation confirmed that the C:V ratio decreases despite the S:V ratio remaining constant (He et al., 2019; Opsahl and Benner, 1995), which indicates that the rate of change through early diagenetic effects in the S:V ratio is not as great as in the C:V ratio. For example, Opsahl and Benner (1995) reported that the C:V ratio obtained from a 4-year decomposition experiment was approximately 75% lower than the initial value, whereas the S:V ratio remained at the initial value. Therefore, it is not clear whether syringyl phenols undergo degradation even though cinnamyl phenols were still present at high levels in our samples.

It should be also mentioned that the lignin composition in seagrass may vary between species. Specifically, the S:V ratio in *Posidonia* species (*P. australis*, 1.0 ± 0.4; *P. oceanica*, 0.4 ± 0.0; Kaal et al., 2018) is higher than that of *Z. marina* measured in this study. The end-member values for lignin compositions should be determined using in situ species.

Furthermore, the methods used in this study would overlook the direct lignin input from seagrass roots because we measured surface sediments. Seagrasses has a large network of underground roots (Duarte, 2002), which are already buried without having to pass through the oxidizing layer of the sediment. Therefore, root-derived carbon would be sequestered more efficiently (Trevathan-Tackett et al., 2020). For example, seagrass fragments in sediment from a 17-cm-deep box core were shown to be approximately 90% derived from below-ground detritus (i.e., the sheath, rhizome, and root) (Tanaya et al., 2018). Future studies should estimate the contribution of seagrass to the lignin pool in deeper sediments to assess the long-term dynamics of buried OC. On the other hand, the vertical distribution of the (ω-OH acids + diacids):cutin acids ratio in this study showed no difference between the surface and bottom layers at Site LM (*t*-test, $P = 0.38$; supplementary Table S3). Previous studies have reported a gradual increase in root biomass below 2 cm in the surface layer (Enríquez et al., 2001; McGlathery et al., 1998; Vichkovitten and Holmer, 2005), but at this site, no such trend was found between in the upper 2 cm of the surface layer. Therefore, we infer that seagrass roots did not contribute significantly at Site LM. Similarly, we found no significant differences in S:V and C:V ratios between the surface and bottom layers at Site LM (*t*-test, $P > 0.05$; supplementary Table S3), suggesting that there was no substantial change in the contributors to the lignin composition from the surface to the bottom layer. On the other hand, relatively high contributions from roots at Sites IL and RM, but we consider that most of these were derived

from allochthonous vascular plants because $\delta^{13}C$ values in the sediments at these sites were close to those of C3 plants (approx. −26‰; Watanabe and Kuwae, 2015a; Watanabe et al., 2019).

4.3. Trends in the depth distribution of organic matter

The vertical distributions of biomarker concentrations normalized by TOC show that the degradability is variable among biomarkers. Λ8 had significantly higher values in the bottom section of the sediment core from Site LM and was stable with depth at Site RM (Fig. 7a), which indicates that lignin would be selectively preserved. Kaal et al. (2019) investigated millennial-scale changes in biomarkers derived from *P. australis* in sediments and indicated that lignin is selectively preserved with increasing age. This property of high preservation in lignin is a factor in the high stock of blue carbon in sediments. Although our data are only for the surface sediments at a millimeter scale, we detected this trend even in such short-term deposition.

The LCFA showed a decreasing trend with depth at Site RM (Fig. 7c) even though other compounds did not show lower values in the bottom section of the profile, suggesting that LCFA are more degradable than the other compounds. However, a study investigating the continuous alteration of OM during transport through the water column to the surface sediment showed a trend toward selective preservation of allochthonous-derived compounds including LCFA (Wakeham et al., 1997). Therefore, it is necessary to study more cases to draw conclusions about the preservation of individual compounds. As for other compounds, we did not find significant differences between the surface and the bottom of our cores, although there did seem to be increasing or decreasing trends with depth. These results may indicate that the stabilities of the compounds that we measured were not significantly different at the millimeter scale. However, decomposition rates for each compound would be different because their proportional decreases were different at Site IL.

5. Conclusion

We detected lignin phenols, fatty acids, cutin acids, ω-OH acids, and diacids in the sediments of Furen Lagoon, Japan. Concentrations of lignin phenols and LCFA were significantly correlated with TOC. Because LCFA are organic compounds characteristic of allochthonous vascular plants, the main source of OC in the sediments (especially near river mouths) was likely allochthonous vascular plants. Such an interpretation concurs with previous studies using elemental and isotopic compositions. Furthermore, the Ad:Al and (ω-OH acids + diacids):cutin acids ratios were also highly correlated with TOC, which suggests that the allochthonous vascular plant-derived OC was from plant debris that contained degraded products. The results of a mixing model using the C:V and S:V ratios of lignin phenols showed that lignin derived from allochthonous vascular plants was rich in degradants and that the OC in sediments from river mouth sites was composed more from degraded allochthonous vascular plant material and less from fresh allochthonous vascular plant material.

The highest seagrass contributions were found near the lagoon inlet (Site LM), with about 65% of lignin derived from seagrass. However, at Sites IL and RM, where allochthonous vascular plant biomarkers were abundantly detected, the contribution of lignin from seagrass was likely overestimated. To improve this estimate, it will be necessary to specifically consider differences in the degree and rate of degradation among lignin phenols. The depth distributions of lignin phenols tended to show a slight rise with increasing depth and lignin appeared to be selectively stored. In contrast, LCFA normalized to TOC showed a decrease below the surface layer, suggesting that differences in preservation were a factor in determining the dynamics of organic biomarker tracers.

Quantifying the OC derived from potential sources of blue carbon and distinguishing the sources remains a challenge. Previous estimates of the %contribution of blue carbon sources have been based on environmental DNA and $\delta^{13}C$ and $\delta^{15}N$ signatures. In this study, we gained new insights into the composition of blue carbon sources by using multiple organic biomarkers, which allows us to discuss OC sources from a new perspective. Recent studies on blue carbon sources have revealed that contributions are primarily from specific parts (e.g., roots) and compounds (e.g., lignin). The analysis of multiple organic biomarkers using the TMAH method can be applied to a variety of sample categories, ranging from plants to suspended particles, and from surface sediments to sediments at geological time scales, and is expected to reveal specific information about contributors from the diversity of organic matter that can be detected.

CRediT authorship contribution statement

Masatoshi Nakakuni: Conceptualization, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **Kenta Watanabe:** Resources, Writing – original draft, Writing – review & editing, Funding acquisition. **Khoki Kaminaka:** Investigation, Data curation. **Yukiko Mizuno:** Investigation, Data curation. **Keiko Takehara:** Investigation, Data curation. **Tomohiro Kuwae:** Resources, Supervision, Project administration, Funding acquisition. **Shuichi Yamamoto:** Supervision, Project administration.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148488.

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Supplementary material

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Seagrass contributes substantially to the sedimentary lignin pool in an estuarine seagrass meadow

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Table. S1

Lignin-phenol composition in allochthonous vascular plants.

	Σ8[a] (μg g^{-1} dry wt)	S:V[b]	C:V[c]
Schoenoplectus sp.	6011	1.2	6.2
Acorus sp.	8390	1.5	9.4
Moliniopsis sp.	10202	1.0	5.4

[a]Σ8 = lignin-phenol concentrations (cinnamyl + vanillyl + syringyl phenols)

[b]The ratio of syringyl phenols (S) to vanillyl phenols (V)

[c]The ratio of cinnamyl phenols (C) to vanillyl phenols (V)

Table. S2

Percent contribution of allochthonous vascular plants to sediment organic carbon as determined by fatty-acid content.

	Depth in sediment (mm)				
Site	0–2	2–5	5–10	10–15	15–20
IL	100 (111)[a]	100 (105)[a]	58	100 (130)[a]	54
RM	91	42	37	23	0
LM	0	5	19	17	7

[a]Contributions with values exceeding 100% were considered to be 100%. The numbers in parentheses indicate the actual calculated value.

Table. S3

P-values for *t*-tests comparing measurements from a surface section (0–5 mm) and a bottom section (15–20 mm) of a core for each group of compounds at each site. Values in bold denote a significant value ($P < 0.05$).

Site	Λ8[a]	SCFA[b]	LCFA[c]	Cutin acids	Diacids	ω-OH acids
IL	**0.03**	0.17	0.92	**0.04**	0.12	**0.02**
RM	0.09	0.10	**<0.01**	0.08	0.70	0.09
LM	**0.01**	0.32	0.27	0.55	0.85	0.69

[a]Λ8 = total concentration of lignin phenols normalized to total organic carbon content

[b]SCFA = short-chain fatty acids (C_{14}–C_{18})

[c]LCFA = long-chain fatty acids (C_{28}–C_{32})

Table. S4

P-values for *t*-tests comparing measurements from a surface section (0–5 mm) and a bottom section (15–20 mm) of a core for compositional ratio biomarkers at each site. Values in bold denote a significant value ($P < 0.05$).

Site	S:V[a]	C:V[b]	(diacids + ω-OH acids):cutin acids
IL	0.66	**0.01**	0.18
RM	0.05	0.14	**0.03**
LM	0.07	0.11	0.38

[a]S:V = syringyl:vanillyl phenol ratios

[b]C:V = cinnamyl:vanillyl phenol ratios

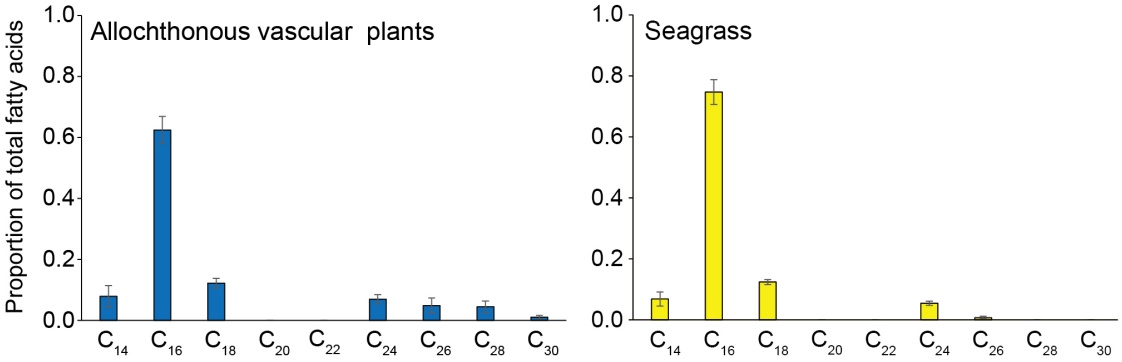

Fig. S1

Ratios of individual fatty acids to total fatty acid contents (by concentration) for allochthonous vascular plants (n = 3) and the seagrass *Zostera marina* (n = 3). Error bars indicate standard deviations.

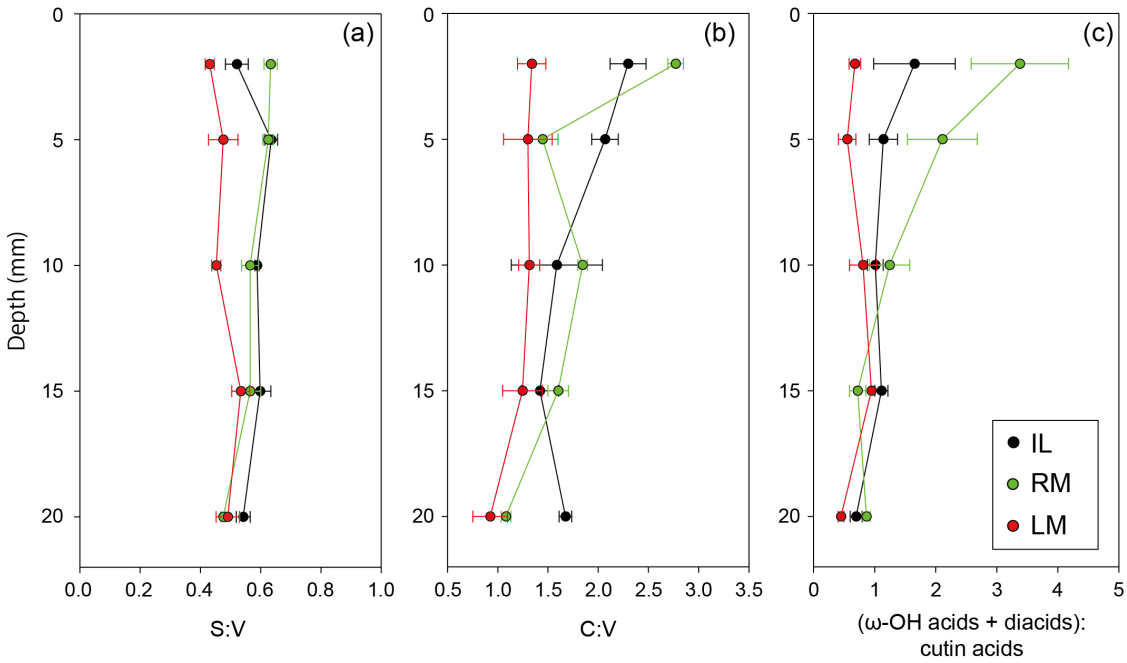

Fig. S2

Vertical distributions of syringyl:vanillyl phenol ratios (S:V) (a), cinnamyl:vanillyl phenol ratios (C:V) (b), and (diacids + ω-OH acids):cutin acids ratios (c) at each site in Furen Lagoon, Japan. Black, Site IL; green, Site RM; red, Site LM. Error bars show standard deviations.