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Article

# Export and dispersal of coastal macrophyte-derived organic matter to deep offshore sediment around the Tokara and Yaeyama Islands, southwest Japan: Evaluation using quantitative DNA probing techniques

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MIYAJIMA Toshihiro, HAMAGUCHI Masami, NAKAMURA Takashi, KATAYAMA Hajime and HORI Masakazu (2022) Export and dispersal of coastal macrophyte-derived organic matter to deep offshore sediment around the Tokara and Yaeyama Islands, southwest Japan: Evaluation using quantitative DNA probing techniques. *Bulletin of the Geological Survey of Japan*, vol. 73(5/6), p. 313–321, 3 figs and 1 table.

**Abstract:** Vegetated coastal habitats such as seagrass meadows, macroalgal beds, and mangroves export large amounts of organic carbon (OC) to offshore regions. The exported OC may be consumed as a food source by various pelagic and benthic organisms, enhancing secondary production, or may settle and be buried in offshore sediment, contributing to carbon sequestration. Hence, OC export is an important ecosystem service of coastal wetlands supporting connectivity from coastal to offshore habitats. We studied the dispersal of detrital organic matter derived from coastal macrophytes to offshore sediment around the Tokara and Yaeyama Islands (subtropical western North Pacific) using DNA probing techniques and compared the results with the bulk OC concentration and granulometric properties of the sediment. The results showed that dispersal of macrophyte detritus was constrained hydrographically by the strong Kuroshio current flowing near the study areas. Mangrove- and seagrass-derived organic matter exported from coastal habitats of the Yaeyama Islands was detected around the Yaeyama Islands and accumulated in deep-basin sediments (>1000 m) of the southern Okinawa Trough; however, its dispersal seemed to be confined by the Kuroshio. By contrast, sediments around the Tokara Islands often contained macroalgal materials that were rarely found in those around Yaeyama Islands. Most of the macroalgal organic matter found in the sediments of the Tokara area likely originated in coastal habitats of continental China and was transported by the Kuroshio across the shelf of the East China Sea to the northern Okinawa Trough, where it was trapped within sediment. The bulk OC concentrations in the sediment of both areas were constrained by the granulometric properties of the sediment, such as specific surface area. However, the abundance of macrophyte-derived organic matter did not correlate with the concentration or stable isotope ratio of the bulk-sediment OC, implying that macrophyte OC represents a minor fraction of the bulk OC stored in the sediment of the study areas.

**Keywords:** Blue carbon, Carbon sequestration, Coastal wetland, Environmental DNA, Kuroshio, Macroalga, Mangrove, Outwelling, Seagrass

## 1. Introduction

Macrophyte-dominated coastal ecosystems such as seagrass meadows, macroalgal beds, mangroves, and tidal marshes play quantitatively important roles in the oceanic carbon cycle due to their prominent capacity to capture and sequester organic carbon (OC). It has been estimated that mangroves and seagrass meadows, which occupy only about 0.2 % of the world's ocean area, sequester OC in their sediments at an average rate of

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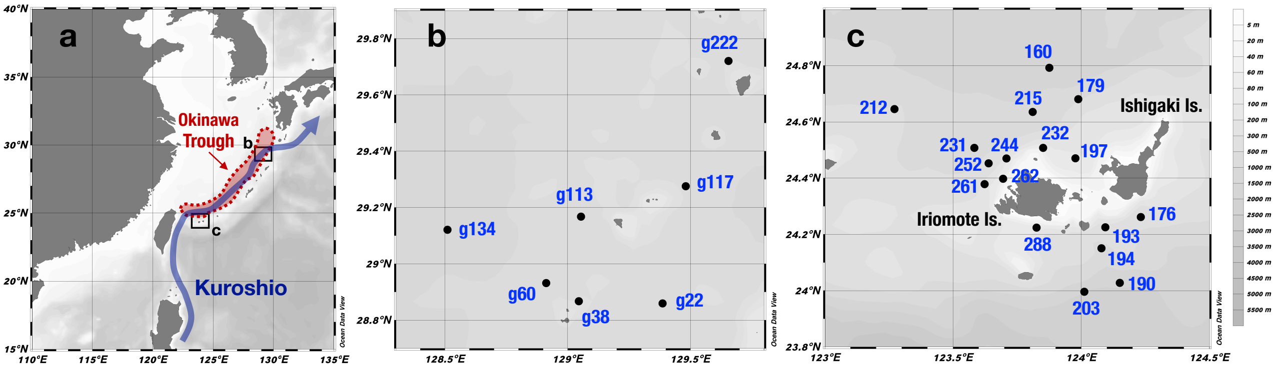


Fig. 1 Regional map (a) indicating the locations of the two study areas and the Okinawa Trough, together with the flow path of the Kuroshio current. Sediment sampling sites are shown in local maps of the area around the Tokara Islands (b) and Yaeyama Islands (c). The maps were generated using Ocean Data View ver. 5.5.2.

more than 100 g C m$^{-2}$ y$^{-1}$ (Alongi, 2018), that is, two or three orders of magnitude higher than the average OC burial rate in open ocean sediment, which illustrates the disproportionally large contribution of these two habitats to the entire oceanic carbon budget. Furthermore, because net primary production in macrophyte-dominated ecosystems largely exceeds consumption and respiration within the ecosystems (Cebrian, 1999), the export flux of OC from these ecosystems to the outer ocean can be quite large and represent an important pathway of energy and carbon from the coastal region to pelagic realms. The export of macrophyte-derived OC may influence the pelagic marine ecosystem in two mutually exclusive ways. First, exported OC may be consumed as a food source by heterotrophic animals and microorganisms, thereby enhancing secondary production and the complexity of both pelagic and benthic food webs in the open ocean (Heck *et al.*, 2008; Queirós *et al.*, 2019). Second, a portion of the exported OC may be stored for a long time as buried OC in deep-sea sediment or refractory dissolved OC in the water column and thereby contribute to increasing carbon sequestration in the ocean (Reichardt, 1987; Jennerjahn and Ittekkot, 2002; Santos *et al.*, 2021). The recent advent of molecular biological tools such as deoxyribonucleic acid (DNA) metabarcoding and quantitative polymerase chain reaction (qPCR) has enabled researchers to trace organic matter derived from specific primary producers and exported outside their original habitats (Reef *et al.*, 2017; Queirós *et al.*, 2019; Ortega *et al.*, 2020). However, available data are still insufficient to constrain the spatial extent of export of macrophyte-derived OC to the deep ocean and evaluate the importance of these two roles quantitatively.

In this study, we compared the spatial extent of dispersal of macrophyte-derived materials to offshore sediments in two different subtropical waters with contrasting hydrographic settings. To detect and quantify macrophyte-derived materials in the offshore sediment, we used specific DNA-probing techniques based on qPCR (Hamaguchi *et al.*, 2022). One study area surrounding the subtropical Yaeyama Islands, southwest Japan, is characterized by abundant mangroves and seagrass meadows in shallow nearshore habitats (Nakasuga *et al.*, 1974; Tanaka and Kayanne, 2007) that are supposed to export a large amount of OC to the surrounding offshore area. The other study area, the Tokara Islands, is located to the northeast of the Yaeyama Islands, where coastal macrophytes are relatively sparse (Kawano *et al.*, 2012; Terada and Watanabe, 2017) and the oceanic environment is directly influenced by the Kuroshio (Chen *et al.*, 1992), one of the world's strongest ocean currents, which might have a strong influence on the dispersal and accumulation of macrophyte OC. We compared the abundance and composition of macrophyte-derived materials between these two areas. We also examined the relationships among macrophyte-derived materials and bulk sediment characteristics, such as OC and granulometric properties, to infer the quantitative role of the exported OC in carbon burial in the offshore sediment.

## 2. Materials and Methods

### 2. 1 Sample collection and pretreatment

Seven surface sediment samples were collected around the Tokara Islands (28.85–29.72° N, 128.51–129.66° E; Fig. 1b) during cruise GB21–1 in March 2021 using a grab sediment sampler (40 × 40 × 20 cm). Portions of the collected samples were packed in prewashed plastic containers and immediately frozen. The samples were kept in a freezer below −20°C until they were freeze-dried. Additional 17 surface sediment samples collected around the Yaeyama Islands (23.99–24.80° N, 123.27–124.24° E; Fig. 1c) during cruise GK19 in June and July 2019 were used for comparison. However, the latter group of samples had been stored at room temperature for 5 months prior to freeze-drying, which might have resulted in partial degradation of delicate organic matter, including DNA, and possible underestimation of OC concentrations and

DNA copy numbers.

After freeze-drying, the dried sediment was gently crushed and passed through a 1-mm-mesh stainless-steel sieve to remove pebbles and shells. The fraction of the sediment < 1 mm was homogenized using an agate mortar and pestle, and then stored in precleaned and tightly capped 30-mL glass vials in a desiccated room for subsequent analyses.

**2. 2 Analytical methods**

The concentrations and stable isotope ratios of bulk OC and total nitrogen (TN) in non-carbonate sediments (< 40 wt.% carbonate) were determined using an elemental analyzer–isotope ratio mass spectrometer (EA–IRMS; Flash 2000/Conflo IV/DELTA V Advantage, Thermo, Germany) after acid decarbonation. A 20–100-mg portion of each homogenized sediment sample was weighed into a silver cup for elemental analysis, and 2 M HCl was added dropwise to remove carbonate as $CO_2$. Then the silver cup was dried at 60°C and packed into a tin capsule for elemental analysis before being subjected to EA–IRMS. For carbonate sediments, OC and TN were analyzed for acid (4 M HCl)-soluble and insoluble fractions separately, according to the method described in Miyajima *et al.* (2015). Concentrations and isotope ratios of bulk OC and TN were calculated as the sum and the weighted average of the values for the acid-soluble and insoluble fractions, respectively.

To determine the carbonate concentration in sediment, a preweighed (ca. 100-mg) portion of homogenized sediment was dissolved in 1 M HCl. After appropriate dilution with ultrapure water, insoluble materials were removed by centrifugation (2000 *g*, 20 min, 15°C), and concentrations of major cations in the supernatant were determined by ion chromatography (881 Compact IC pro, Metrohm, Switzerland). The content of $(Ca, Mg)CO_3$ in the original sample was calculated from the measured concentrations of $Ca^{2+}$ and $Mg^{2+}$ after correction for porewater-derived Ca and Mg, assuming that the measured $Na^+$ was derived solely from porewater.

Analyses of sediment granulometric properties, such as specific surface area (BET method) and mesopore distribution, were performed via $N_2$ adsorption measurement (BELSORP mini II, MicrotracBEL, Japan) following the preparation protocol described in Miyajima *et al.* (2017).

The concentrations of macrophyte-derived DNA fragments were determined in clean laboratories at the National Research Institute of Fisheries Technology (Hatsukaichi Station) using qPCR techniques applied to the freeze-dried sediment samples (Hamaguchi *et al.*, 2018). The specific primers and probes were designed based on the deposited or originally determined internal transcribed spacer (ITS) region sequences for detecting and quantifying DNA fragments of three dominant mangrove species found in the Yaeyama Islands (i.e., *Rhizophora stylosa*, *Sonneratia alba*, *Bruguiera gymnorrhiza*), three subtropical (*Thalassia hemprichii*, *Enhalus acoroides*, *Cymodocea rotundata*), and one temperate (*Zostera marina*) species of seagrass commonly found in Japan, as well as six groups of macroalgae that commonly occur or are cultivated around Japan (see Table 1). Among the macroalgae groups, subtropical and temperate *Sargassum* are represented by the subgenera *Sargassum* and *Bactrophycus*, respectively (Stiger *et al.*, 2003), and the *Ulva* group, cultivated *Porphyra*, temperate kelps, and *Saccharina* group were defined based on published or originally determined ITS-region sequences for *Ulva* spp., *Porphyra yezoensis*, *Ecklonia* spp. and *Eisenia bicyclis*, and *Saccharina* spp., respectively. Details of the design of probes and primers can be found elsewhere (Hamaguchi *et al.*, 2022). Triplicate qPCR runs were executed for each combination of samples and probes. The detection ($3\sigma$) and quantitation ($10\sigma$) limits of DNA fragments were tentatively defined based on the standard deviation ($\sigma$) of the log-transformed copy number of *E. acoroides* gene fragments determined for three representative samples with eight repetitive qPCR runs, and these were 190 and 890 copies $g^{-1}$, respectively. It should be noted that the detectability of specific DNA fragments from sediment depends, to some extent, on the choice of probe sequence. In our experience, the detectability of tropical seagrass DNA using probes specific to the ITS region of the nucleus DNA is somewhat lower than the detectability with probes specific to the region coding maturase K (MatK) of the plastid DNA of the same species. Therefore, our abundance data of tropical seagrass DNA may be considered conservative estimates.

## 3. Results and Discussion

**3. 1 Spatial distribution of bulk sediment properties**

The concentration of bulk OC in the surface sediment did not exceed 1.0 mmol C (g dry weight)$^{-1}$ in either the Tokara or Yaeyama areas (Fig. 2a). OC was depleted at sites shallower than 700 m but increased with increasing depth ($r = 0.653$, $p = 0.006$). By contrast, concentrations of inorganic carbon (IC; calcium + magnesium carbonate) were quite high (> 70 wt.%) at sites shallower than 700 m and decreased with depth in the Yaeyama area ($r = -0.858$, $p = 0.005$; Fig. 2b). In the Tokara area, carbonate concentrations were always lower than 50 wt.%, and did not correlate with depth. TN concentrations were closely and positively correlated with OC concentrations in both the Tokara ($r = 0.999$) and Yaeyama ($r = 0.965$) areas. The OC/TN atomic ratio varied between 7.1 and 11.4, and it was negatively correlated with depth ($r = -0.677$, $p = 0.019$) in the Yaeyama area.

The bulk carbon stable isotope ratio ($\delta^{13}C$) of OC in the surface sediment ranged from −23 ‰ to −19 ‰, with most samples falling between −22 ‰ and −20 ‰ (Fig. 2c). This range closely coincides with the range of $\delta^{13}C$ of suspended particulate organic matter (POM) collected from Sekisei Lagoon between Ishigaki and Iriomote Islands (Fig. 1c; unpublished data). The nitrogen stable

Table 1 Concentrations of DNA fragments detected in surface-sediment samples collected in coastal waters of the Yaeyama and Tokara areas (arranged from shallow to deep sites). Symbols: +++++, > 30,000; ++++, 10,000–30,000; +++, 3,000–10,000; ++, 890–3,000; +, 190–890; ±, 1–190; –, < 1 (unit: copies per gram dry weight of sediment). See text for a detailed description of the macroalgal categories. The tentatively defined detection and quantitation limits are 190 and 890 copies per gram (see text), respectively.

| | | | **Mangroves** | | | **Seagrasses** | | | | **Macroalgae** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Area | Site ID | Depth (m) | *Rhizophora stylosa* | *Sonneratia alba* | *Bruguiera gymnorrhiza* | *Thalassia hemprichii* | *Enhalus acoroides* | *Cymodocea rotundata* | *Zostera marina* | *Sargassum* (subtropical) | *Sargassum* (temperate) | *Ulva* group | Cultivated *Porphyra* | Temperate kelps (*Ecklonia*, etc.) | *Saccharina* group |
| **Yaeyama coastal waters (cruise GK19)** | *288* | 75 | +++++ | - | - | - | - | - | - | - | ± | - | - | - | - |
| | *262* | 110 | - | - | - | - | - | - | - | - | + | - | - | - | - |
| | *197* | 235 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | *232* | 253 | ++++ | - | - | + | - | - | - | - | ++ | - | - | - | ± |
| | *193* | 327 | ++++ | - | - | - | ++ | ++ | - | - | - | - | - | - | - |
| | *261* | 444 | - | - | - | - | ++ | - | - | - | - | - | - | - | - |
| | *244* | 610 | - | - | - | - | - | +++ | - | - | - | - | - | - | - |
| | *176* | 676 | - | - | - | - | +++ | - | - | - | + | - | - | +++ | - |
| | *212* | 832 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | *252* | 952 | ++++ | - | - | - | +++ | - | - | - | - | - | - | - | - |
| | *194* | 987 | - | - | - | - | - | +++ | - | - | - | - | - | - | - |
| | *231* | 1242 | +++++ | - | - | - | ++ | +++ | - | - | ± | - | + | - | - |
| | *215* | 1665 | ++++ | - | - | - | +++ | ++++ | - | - | - | ++ | - | - | - |
| | *179* | 1759 | ++++ | - | +++++ | - | +++ | ++ | - | - | - | - | - | - | - |
| | *203* | 1839 | +++++ | - | - | - | +++ | ++++ | - | - | - | - | - | - | - |
| | *160* | 1887 | +++++ | - | +++ | - | - | ++++ | - | - | - | - | - | - | - |
| | *190* | 1985 | ++++ | ± | ++++ | - | - | - | - | ± | ++++ | - | - | - | - |
| **Tokara coastal waters (cruise GB21-1)** | *g38* | 489 | - | - | - | - | - | - | - | - | +++++ | ++ | - | ± | + |
| | *g222* | 509 | - | - | - | - | - | - | - | - | ++++ | +++ | - | ± | + |
| | *g117* | 576 | - | - | - | - | - | - | - | - | +++ | - | ± | - | - |
| | *g22* | 823 | - | - | - | - | - | - | - | - | +++++ | - | - | - | + |
| | *g113* | 828 | - | - | - | - | - | - | - | - | +++ | - | ++ | + | - |
| | *g60* | 841 | - | - | - | - | - | - | +++ | + | + | - | - | ± | + |
| | *g134* | 1151 | - | - | - | - | - | - | - | ++ | +++ | +++++ | - | ± | - |

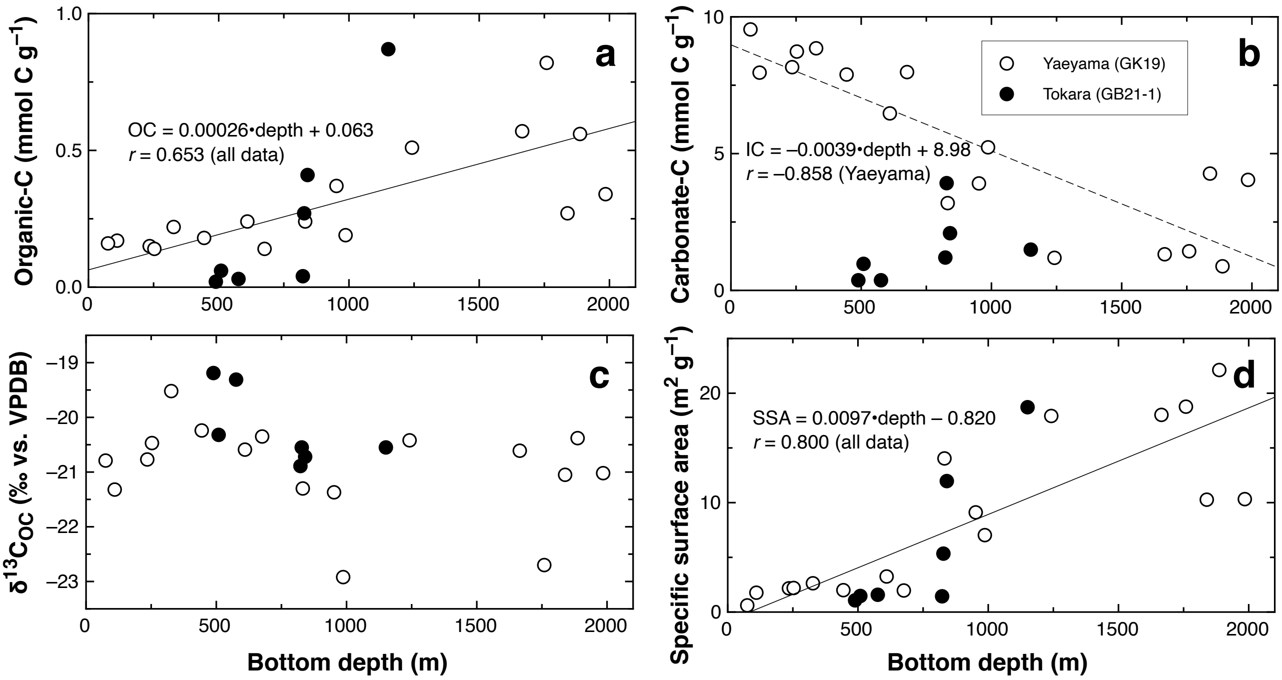


Fig. 2 Concentrations of bulk organic (a) and inorganic (b) carbon in sediment, carbon stable isotope ratios of sediment bulk organic carbon ($\delta^{13}C_{OC}$; c), and specific surface areas of sediment (d) plotted against bottom depths of the sampling sites. Circle, Yaeyama area; dot, Tokara area.

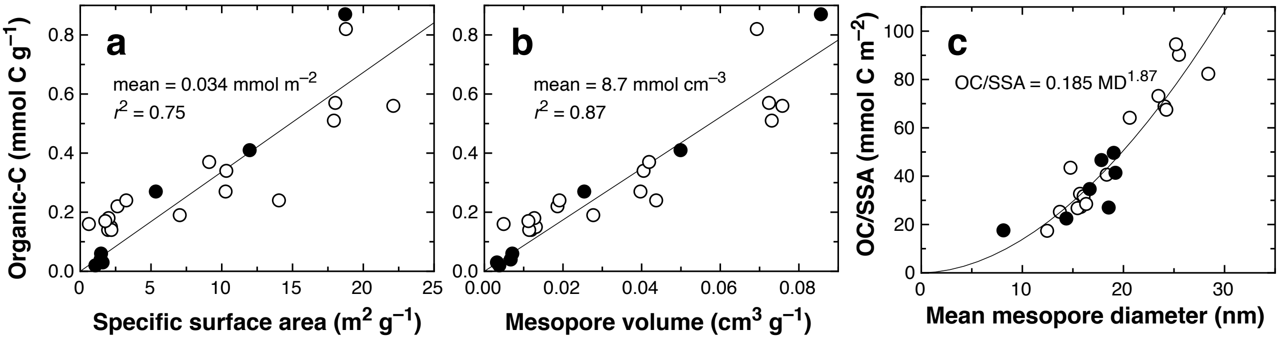


Fig. 3 Relationships between bulk organic carbon concentrations (OC) and granulometric properties of the sediment. (a) Correlation between OC and specific surface area (SSA); (b) correlation between OC and total mesopore volume; and (c) curvilinear relationship between the OC/SSA ratio and mean mesopore diameter. Circle, Yaeyama area; dot, Tokara area.

isotope ratio ($\delta^{15}N$) of TN ranged from +4.8 ‰ to +6.8 ‰, with three outliers between +7.2 ‰ and +9.3 ‰. The $\delta^{15}N$ values of sediment TN were higher than those of the POM collected from Sekisei Lagoon (0 ‰ to +3 ‰; unpublished data). The isotope ratios did not show a significant depth trend and collectively imply that the majority of the sediment organic matter in these areas originated from autochthonous suspended POM (i.e., phytoplankton), and was affected to some extent by post-depositional bacterial reworking (early diagenesis), as indicated by the increase in $\delta^{15}N$ values.

The specific surface area (SSA; Fig. 2d) and total mesopore volume (TMV) of the sediment, as determined by $N_2$ adsorption analysis, were commonly low for the samples collected from shallow (< 700 m) sites (< 5 $m^2\ g^{-1}$ and < 0.02 $cm^3\ g^{-1}$, respectively). Both SSA and TMV were positively correlated with depth ($r$ = 0.800, $p$ = 0.001 and $r$ = 0.761, $p$ = 0.002, respectively). Mean mesopore diameters (MMDs) were generally higher for carbonate than non-carbonate sediments and therefore correlated positively with carbonate concentration ($r$ = 0.832, $p$ = 0.001) and negatively with depth ($r$ = −0.602, $p$ = 0.010). These results show that sediment granulometry is constrained by water depth, with the average sediment grain size becoming finer with increasing depth.

The concentrations of bulk OC in the sediments depended closely on the SSA ($r$ = 0.888, $p$ = 0.0004; Fig. 3a). Such a relationship has frequently been reported for continental shelf sediments and has been interpreted to indicate a close association between OC and mineral surfaces as a principal mechanism for stabilization of OC in sediment (Keil and Mayer, 2014). The ratio of OC to SSA is usually close to 0.07 mmol C $m^{-2}$ for non-carbonate shelf sediments (Mayer, 1994) and a bit higher for shallow-water carbonate sediments (Suess, 1973). However, the average OC/SSA ratio for our samples (0.034 mmol C $m^{-2}$) was only half the typical value of shelf sediments. We also found that the bulk OC concentration of our sediment samples depended even more closely on TMV ($r$ = 0.936, $p$ = 0.0002; Fig. 3b). The OC/SSA ratio was not always constant and clearly depended on MMD (Fig. 3c), which implies that the bulk OC is not only associated with mineral surfaces but is also embedded in mesopore spaces present on mineral surfaces. Assuming that the bulk OC in sediments has the same density as glucose (1.58 g $cm^{-3}$), the average OC/TMV ratio of 8.7 mmol C $cm^{-3}$ (Fig. 3b) indicates that about one-sixth of the available mesopore spaces in our sediment samples were filled with OC. These findings imply that the sediment OC in the study areas is preserved in close association with the steric surface structure of sediment minerals, although the exact mechanism of the association might be somewhat different from the well-known cases of shelf non-carbonate sediment and shallow-water carbonate sediment.

### 3. 2 Detection of macrophyte-derived DNA from sediments

The existence of macrophyte-derived materials in the offshore sediments was successfully demonstrated by detecting DNA sequences specific to macrophyte species in both the Tokara and Yaeyama areas (Table 1). The original samples from the Yaeyama area (GK19) were stored at room temperature for several months before freeze-drying, which might have resulted in DNA degradation by bacteria and fungi. However, the abundance of total macrophyte DNA detected from Yaeyama samples was similar to or even higher than that from the Tokara samples (GB21–1), suggesting that the storage temperature did not seriously influence the detectability of DNA in stored sediments. Based on the correlation of OC with SSA and TMV, the bulk OC in the analyzed samples was preserved in sediment through interactions with mineral surfaces and mesopores (Fig. 3). It is similarly presumed that DNA molecules contained in these samples were also effectively protected from microbiological enzymatic hydrolysis by sorptive interactions with sediment minerals (Cai *et al.*, 2006).

Mangrove and subtropical seagrass species were detected in the surface sediments of the Yaeyama area but not in those from the Tokara area. The mangrove species *Rhizophora stylosa* and *Bruguiera gymnorrhiza* and the seagrasses *Enhalus acoroides* and *Cymodocea rotundata* were often detected in relatively high abundance in sediment samples from deeper than 1,000 m in the Yaeyama area (Table 1). This implies that the deep basin of the Okinawa Trough to the north of the Yaeyama Islands represents an important offshore sink of OC derived from the coastal vegetated habitats that are abundant around these islands. By contrast, DNA fragments of macroalgae were frequently detected in sediments from the Tokara area but were relatively scarce in sediments from the Yaeyama area. The abundance of macroalgal DNA fragments did not correlate with depth. DNA fragments of *Bactrophycus*, a temperate subgenus of brown algae, were most abundant around the Tokara Islands. Notably, traces of cultivated *Porphyra* and *Saccharina* were also detected at some sites.

The abundances of DNA fragments of the groups of macrophytes listed in Table 1 did not show significant correlations with the bulk OC concentrations or SSA values of the sediments. Although the abundances of DNA fragments of the mangrove species *R. stylosa* and the seagrass *C. rotundata* were marginally correlated ($p = 0.04$), no significant correlation was detected for any other combination of macrophyte groups. The ranges of $\delta^{13}C$ values typical of seagrasses and mangroves are considerably higher and lower, respectively, than the typical range of pelagic phytoplankton. Therefore, the contribution of these macrophytes to the sediment OC pool might be evidenced by the variation in $\delta^{13}C$. However, the $\delta^{13}C$ values of the sediment bulk OC did not correlate with the abundance of DNA fragments derived from any group of the macrophytes examined. This implies that the fraction of macrophyte-derived OC in the bulk OC pool in most of the offshore sediments is consistently small.

The sediment concentration of DNA fragments derived from a specific plant species or group should be correlated with the concentration of OC derived from the same species or group. For example, Hamaguchi *et al.* (2018) found that the concentration of *Zostera marina* DNA fragments in an eelgrass bed sediment core showed a significant positive correlation with the concentration of seagrass-derived OC estimated by carbon isotope mass balancing, being approximated by the following relationship:

$$[\text{DNA concentration (copies (g dry sediment)}^{-1})] = 4.7401\ [\text{seagrass-OC } (\mu\text{mol (g dry sediment)}^{-1})]^{2.072}.$$

Assuming that the same relationship can be applied to the subtropical seagrass DNA–OC relationship in offshore sediments, it follows that 0–34 % (average, 9.4 %) of OC detected in sediments of the Yaeyama area originated from detritus derived from seagrasses (*Thalassia hemprichii* + *E. acoroides* + *C. rotundata*). However, this estimation is still at a very preliminary stage. More reliable and extensive datasets of relationships between macrophyte-derived OC and DNA preserved in sediments are needed to quantify the contribution of macrophyte-derived OC in the sedimentary OC stock.

### 3. 3 Factors constraining dispersion of macrophyte-derived organic matter

The contrasting distribution of macrophyte-derived DNA fragments between the Yaeyama and Tokara areas evidences strong control by the Kuroshio current of the dispersal of macrophyte-derived detritus to the open ocean. The Kuroshio current originates from the tropical Philippine Sea (Gordon *et al.*, 2014), flows northward between the Yaeyama Islands and Taiwan, enters the East China Sea (ECS), and then turns eastward to the north of the Yaeyama Islands (Fig. 1a). It then flows northeastward, passes through the Tokara Islands leaving the ECS, and enters the Pacific Ocean, hydrographically isolating the Yaeyama as well as the Miyako, Okinawa, and Amami islands from Taiwan, continental China, the Korean Peninsula, and the main islands of Japan (Ichikawa and Beardsley, 1993). Therefore, most of our sampling sites in the Yaeyama area were to the south of the Kuroshio, and many sites in the Tokara area were just below it.

Our DNA data (Table 1) imply that mangrove- and seagrass-derived material dispersed from vegetated coastal habitats around the Yaeyama Islands is not extensively transported by the Kuroshio current but accumulates mainly within a confined region to the south of the Kuroshio, likely due to hydrodynamic forcing typical of this region (Hasunuma and Yoshida, 1978; Hsin *et al.*, 2008). Because mangrove and seagrass detritus are typically relatively dense and fibrous, it can settle rapidly to deeper layers and accumulate in sediment before being transported long distances by ocean currents.

By contrast, DNA fragments derived from macroalgae were found mainly in the area around the Tokara Islands and were relatively rare in the Yaeyama area. This implies that the source of macrophyte-derived materials in the Tokara area is in the vicinity of the Tokara Islands or somewhere upstream of the Kuroshio current. In fact, small patches of subtropical *Sargassum* spp. are present around the Tokara Islands (Terada and Watanabe, 2017), and these may have been sources of the DNA fragments of this group detected in the sediments of this area. However, temperate *Sargassum* and *Ulva* groups rarely occur in this area (Terada and Watanabe, 2017). The other macrophyte groups for which DNA fragments were detected from Tokara samples, such as *Porphyra*, *Saccharina*, and *Z. marina*, are absent in the Ryukyu Archipelago, including the Tokara Islands. *Porphyra* is an edible seaweed that is cultivated extensively near the mouth of the Changjiang River, as well as on Penghu Island to the west of Taiwan. Therefore, it is possible that detritus and DNA fragments of *Porphyra* were exported by the Taiwan warm current from these aquaculture areas (Zhu *et al.*, 2004), transported by the Kuroshio across the shallow ECS, and eventually trapped within the deep Okinawa Trough near the Tokara Islands. Direct hydrographic connectivity from the coast of Taiwan to the area surrounding the Tokara Islands has also been supported by model simulations of particle dispersion (Horoiwa *et al.*, 2022). Similarly, *Saccharina* is an industrially important seaweed that is cultivated extensively along the northern Yellow Sea (Bo Hai) coast (Yang *et al.*, 2009; Hu *et al.*, 2021). It is possible that detritus derived from cultivated *Saccharina* was transported by a surface current flowing southward to the east of the Shandong Peninsula toward the area near the Changjiang River mouth (Beardsley *et al.*, 1985), and then carried northeastward by the Kuroshio and eventually trapped in sediment of the deep basin near the Tokara Islands (Hu *et al.*, 2021). A similar pathway could also be assumed for transportation of detritus of *Z. marina*.

In fact, naturally occurring brown algae such as *Sargassum* species are much more abundant along the coast of continental China than in the Ryukyu Archipelago. Thus, it is likely that most of the DNA fragments of temperate *Sargassum* that were abundant in sediments of the Tokara area also originated in continental China and were transported as drifting algae by the Kuroshio (Mizuno *et al.*, 2014; Xiao *et al.*, 2019; Yuan *et al.*, 2022). Thus, it is evident that the Kuroshio current is the principal agent determining the dispersal and accumulation in sediments of macrophyte-derived organic matter, at least in the northern part of the Okinawa Trough near the Tokara Islands (Fig. 1a).

Although our carbon isotope results suggested that macrophyte-derived organic matter represents a relatively minor fraction of the bulk sediment OC in our study areas, it is still possible that the offshore sediment is a quantitatively important sink of the excess OC produced in coastal macrophyte-dominated habitats. Macrophyte detritus exported from these habitats and accumulated in offshore sediment may be an important source of carbon and energy for benthic organisms living in the sediment. The origin and composition of such macrophyte detritus clearly differ between the southern and northern regions of the Okinawa Trough due to the dominant hydrographic influence of the Kuroshio. Such a difference may not only influence sediment organic chemistry but also affect the abundance, composition, and metabolic activity of benthic animals and microorganisms in deep-sea sediment. This is an interesting problem raised by this study that should be addressed in the future.

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## 定量的 DNA 検出技術を利用したトカラ列島・八重山諸島近海堆積物における沿岸植生帯由来有機物の分散・貯留状況の解析

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### 要　旨

沿岸海浜部に発達する海草藻場・大型藻類群落・マングローブ等の植生帯からは，その一次生産物が外洋域に多量に流出しており，深海への炭素貯留や深海生物生産の駆動に貢献することを通して沿岸植生帯による生態系サービスの重要な媒体となっていると考えられるが，その実態は断片的にしか分かっていない．本研究では，新たに開発した定量的 PCR 技術を応用して，八重山諸島近海及びトカラ列島近海の外洋表層堆積物における植物由来 DNA 分子の面的分布を定量的に評価することにより，沿岸植生帯に由来する有機物の深海底への供給を植物種別に立証し，起源推定を行うとともに，その分散過程を支配する要因としての黒潮の役割について考察した．また，比較のために底質中の全有機炭素，炭素・窒素安定同位体比，炭酸塩，比表面積等の分布を調査し，外洋底質への有機炭素貯留とそれに対する沿岸植生帯の潜在的寄与に関して検討を行った．