Evaluation of carbon export from blue carbon ecosystems and allochthonous sequestration using eDNA techniques

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Abstract

Blue carbon ecosystems such as mangroves and seagrass meadows (coastal marine ecosystems dominated by halophytic vascular plants) are regarded as a global carbon dioxide (CO2) sink supported by high net community production. A part of the excess organic carbon (OC) production by these ecosystems is stored for a long term as persistent OC in underlying sediments, while the rest is exported to outside the system (open ocean) without being remineralized. In order to properly assess the role of blue carbon ecosystems in the global carbon cycle, the fate of exported OC must be elucidated. A part of the OC exported to the open ocean may be decomposed and remineralized quickly while in the ocean surface and return to the atmosphere as CO2. In such a case, the export production cannot be regarded as a long-term carbon sink. On the other hand, the exported OC may either be (1) stored for a long term in the offshore sediment as detrital OC, (2) stored as refractory dissolved organic carbon (RDOC) in seawater, or (3) settled down in the bathypelagic layer and subsequently remineralized into CO2 there. In these cases, carbon does not return to the atmosphere in the short term and can be included in net CO2 sequestration. It is obvious that carbon pools corresponding to these three processes exits in the ocean. However, it is technically extremely difficult to clarify whether and to what extent carbon derived from the blue carbon ecosystems is contained in these pools. The purpose of this study is to demonstrate by using environmental DNA techniques that OC derived from the blue carbon ecosystems can be transported to and stored in open ocean sediments. As a case study, coastal area off the west coast of Busuanga Island, Philippines, was set as study site, where natural coral reefs, seagrass beds, and mangroves are relatively well preserved. DNA probes for MatK sequences (part of chloroplast DNA) of two mangrove species (Rhizophora mucronata, Sonneratia alba) and two seagrass species (Enhalus acoroides, Thalassia hemprichii) as well as ITS sequence (part of nuclear DNA) of R. mucronata were designed. Then, the DNA copy numbers of respective sequences contained in extracts from surface sediment samples were quantified by the qPCR method. In addition, the organic and inorganic carbon concentrations and the specific surface area of the surface sediment samples were determined, and the origin of the sediment OC was assessed using a carbon stable isotope mixing model. During sample collection, seismic profiling with a sub-bottom profiler was also conducted to evaluate thickness of sediment accumulated in the studied area. In this presentation, we summarize the results of these surveys to evaluate the areal extent to which seagrass- and mangrove-derived OC is transported and stored in relatively intact state, and identify environmental conditions that influence the accumulation in open ocean sediments of OC derived from blue carbon ecosystems. Difficulties in conve

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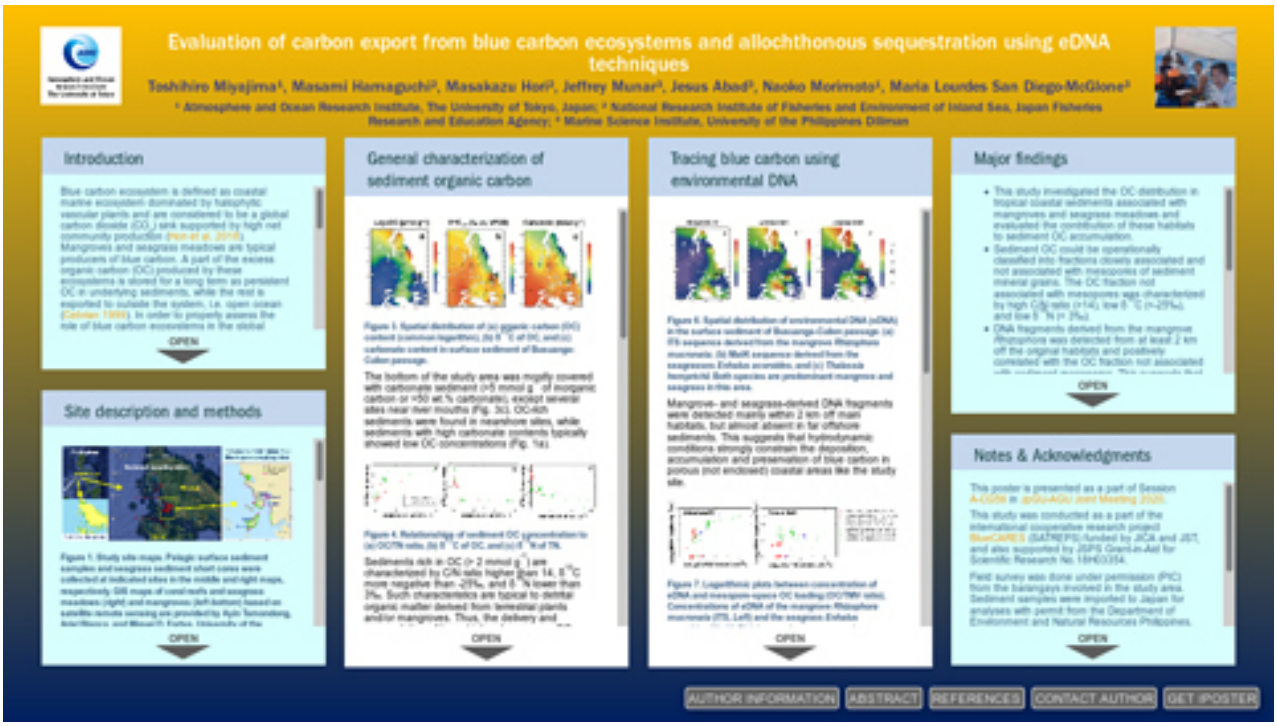

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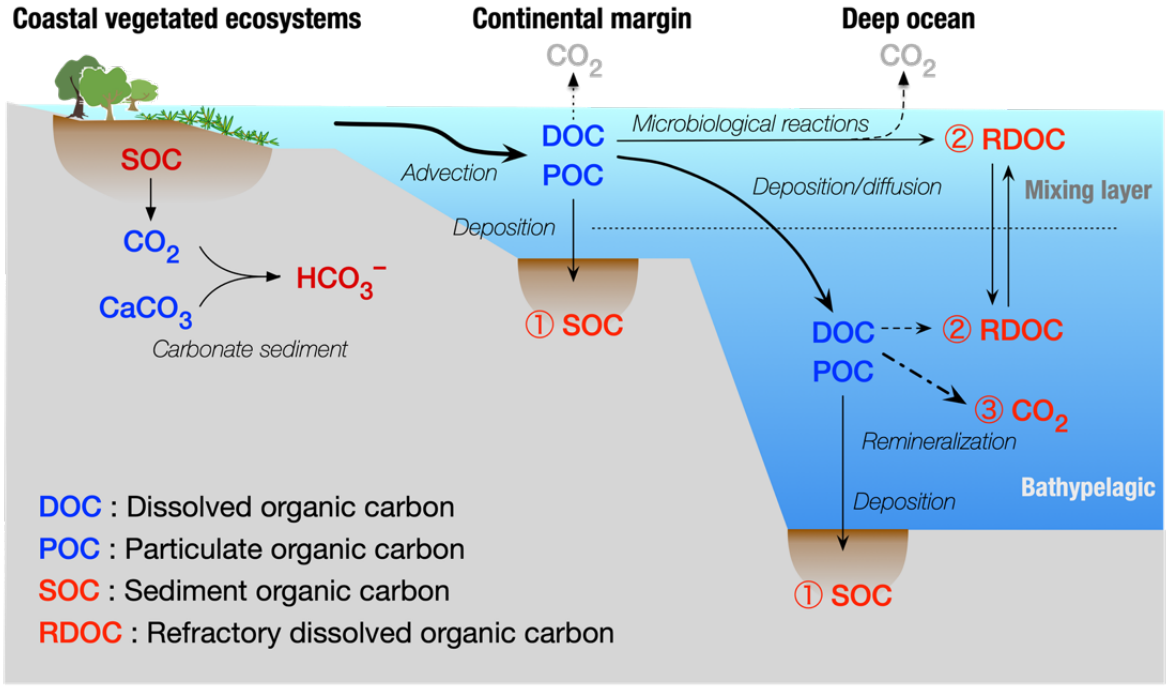
INTRODUCTION

Blue carbon ecosystem is defined as coastal marine ecosystem dominated by halophytic vascular plants and are considered to be a global carbon dioxide (CO_2) sink supported by high net community production (Hori et al. 2018 (https://doi.org/10.1007/978-981-13-1295-3_1)). Mangroves and seagrass meadows are typical producers of blue carbon. A part of the excess organic carbon (OC) produced by these ecosystems is stored for a long term as persistent OC in underlying sediments, while the rest is exported to outside the system, i.e. open ocean (Cebrian 1999 (http://dx.doi.org/10.1086/303244)). In order to properly assess the role of blue carbon ecosystems in the global carbon cycle, the fate of exported OC must be elucidated (Abo et al. 2018 (https://doi.org/10.1007/978-981-13-1295-3_9)).

A part of the OC exported to the open ocean may be decomposed and remineralized quickly and return to the atmosphere as CO_2. In such a case, the export production cannot be regarded as a long-term carbon sink. In contrast, the exported OC may be

1. stored for a long term in the offshore sediment as detrital OC;
2. stored as refractory dissolved organic carbon (RDOC) in seawater; or
3. settled down in the bathypelagic layer and subsequently remineralized into CO_2 there.

In these cases, carbon does not return to the atmosphere in the short term and can be included in the net CO_2 sequestration.



Although carbon pools corresponding to these three processes obviously exist in the ocean, it is technically extremely difficult to clarify whether and to what extent carbon derived from the blue carbon ecosystems is preserved in these pools (Miyajima and Hamaguchi 2018) (http://dx.doi.org/10.1007/978-981-13-1295-3_2). The fate of exported OC may be largely different between different blue carbon ecosystems depending on life forms, successional stage, and hydrodynamic conditions.

The purpose of this study is to demonstrate by using environmental DNA techniques that OC derived from mangroves and seagrass meadows can be transported to and stored outside the original habitats. For the case study, a shallow coastal area between Busuanga and Culion Islands of the Calamianes group of islands in Palawan, Philippines, where rich mangroves and seagrasses are preserved in relatively pristine state, was examined.

SITE DESCRIPTION AND METHODS

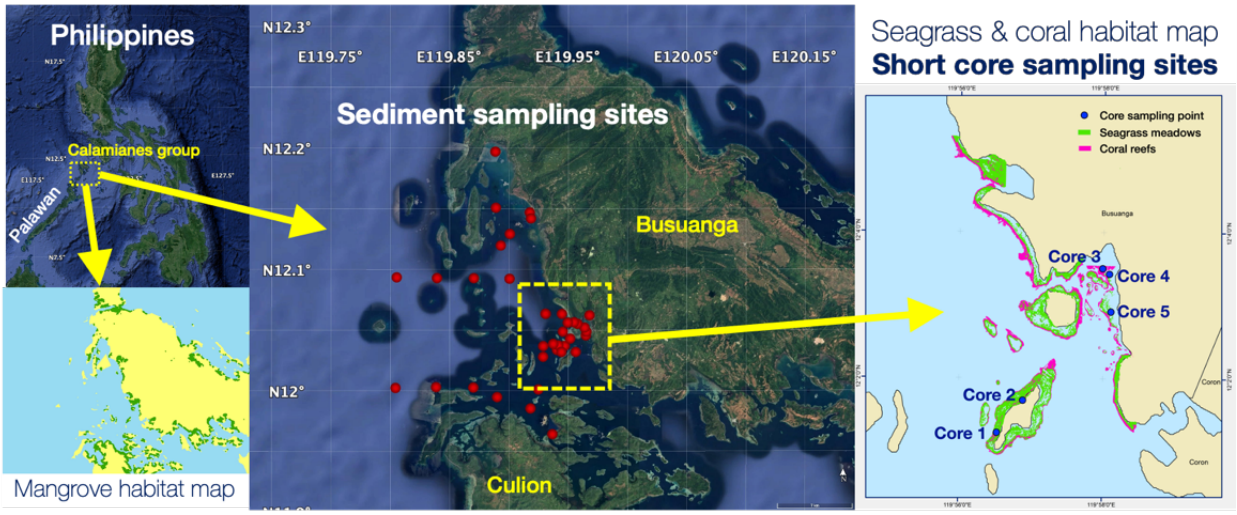


Figure 1. Study site maps. Pelagic surface sediment samples and seagrass sediment short cores were collected at indicated sites in the middle and right maps, respectively. GIS maps of coral reefs and seagrass meadows (*right*) and mangroves (*left bottom*) based on satellite remote sensing are provided by Ayin Tamondong, Ariel Blanco, and Miguel D. Fortes, University of the Philippines. The other maps are based on Google Earth.

Field survey was conducted in February 2018 (pelagic sediment sampling, sub-bottom profiling) and September 2018 (seagrass core sampling, water sampling) in coastal area between Busuanga and Culion Islands, Palawan, Philippines (Fig. 1). Surface sediments were collected using a Van-Veen grab from a boat. Seagrass sediment cores were collected by a handy acrylic corer.

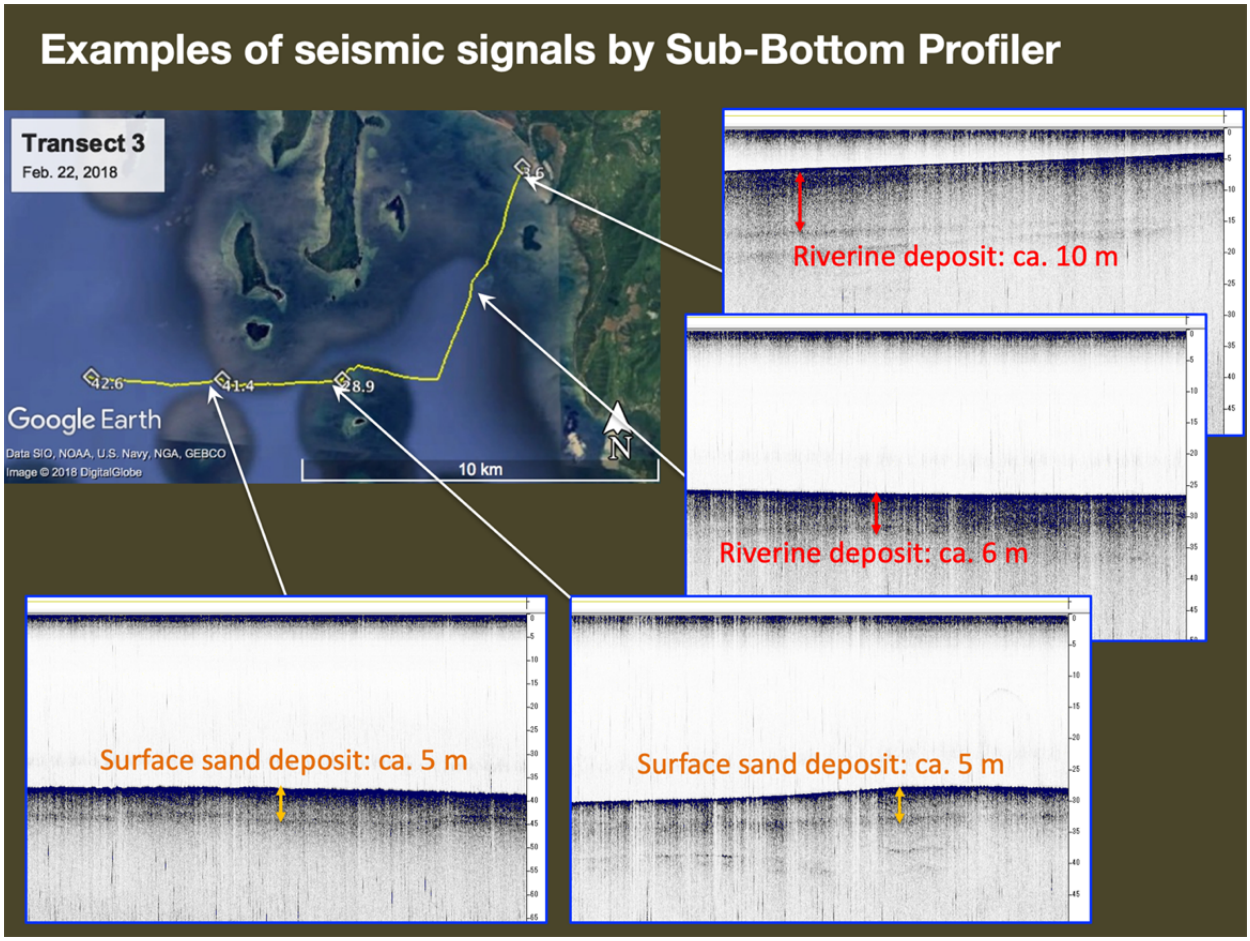


Figure 2. Examples of sub-bottom profiling data.

Sub-bottom profiler (Edgetech SB-216s 3100 series) detected riverine mud deposit of ca. 10 m thick at the Busuanga River mouth (NE-end point of Transect 3, Fig. 2), which was rapidly thinning with increasing distance from the mouth. Bottom of offshore area was mostly covered with carbonate sand or mud, with occasional emergence of living corals.

Sediment sample analyses:

Organic carbon (OC), total nitrogen (TN), and their isotopic compositions ($\delta^{13}C_{OC}$, $\delta^{15}N_{TN}$) were determined by EA-IRMS. For samples that contained >40% carbonate, those amounts were determined separately for acid-soluble and insoluble fractions. For methodological details, see Miyajima et al. (2015). (http://doi.org/10.1002/2014GB004979)

Carbonate concentration was determined by ion chromatography after dissolving carbonate in dilute HCl. Specific surface area and mesopore distribution were analyzed by the BET method based on N_2-adsorption isotherm. See Miyajima et al. (2017) (http://doi.org/10.1002/lno.10478) for details.

Environmental DNA of mangroves and seagrasses was determined by real-time PCR after Hamaguchi et al. (2018) (http://dx.doi.org/10.1002/lom3.10242). For technical reasons, we prepared and applied probes for the ITS sequence of the mangrove *Rhizophora mucronata*, and the MatK sequence of the mangroves *R. mucronata* and *Sonneratia alba*, as well as of the seagrasses *Thalassia hemprichii* and *Enhalus acoroides*. These species are two of the most abundant mangrove and seagrass species in the study site.

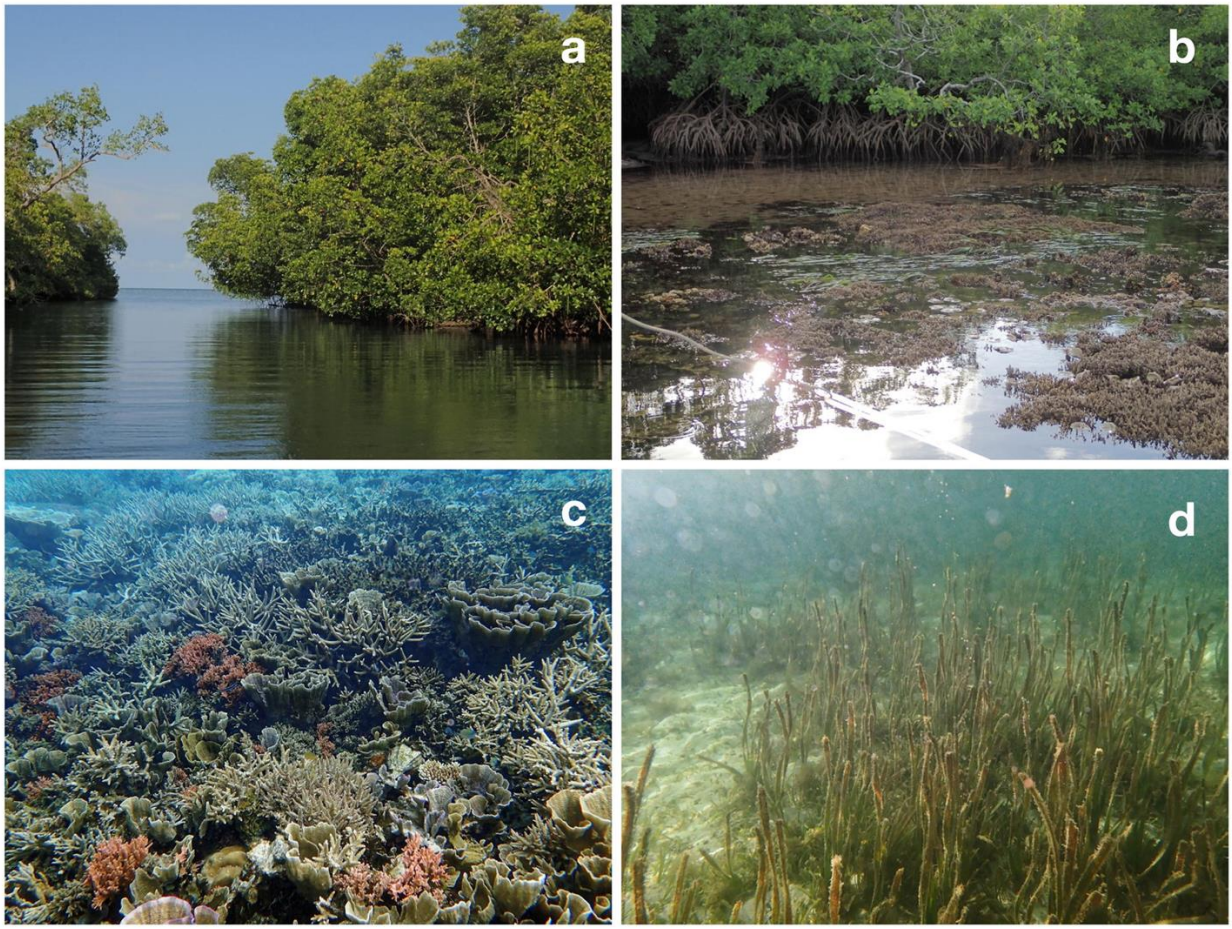


Plate 1. Typical coastal habitats around Busuanga Island, Philippines. (a) Mangrove (*Rhizophora* + *Avicennia*)-fringed channel in Calauit, Busuanga Island. (b) Emergent corals (*Porites cylindrica*) and seagrasses (*Enhalus acoroides*) just in front of mangrove stand (*Rhizophora* spp.). (c) High-diversity coral community near Concepcion, Busuanga Island. (d) Mixed-species seagrass meadow dominated by *Enhalus acoroides*.

GENERAL CHARACTERIZATION OF SEDIMENT ORGANIC CARBON

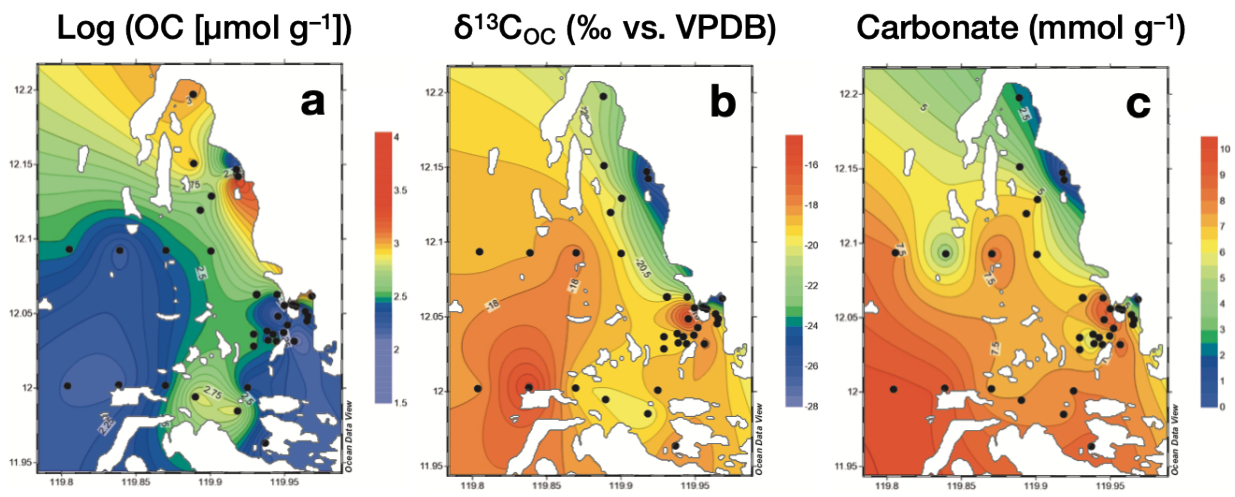


Figure 3. Spatial distribution of (a) organic carbon (OC) content (common logarithm), (b) $\delta^{13}C$ of OC, and (c) carbonate content in surface sediment of Busuanga-Culion passage.

The bottom of the study area was mostly covered with carbonate sediment (>5 mmol g^{-1} of inorganic carbon or >50 wt.% carbonate), except several sites near river mouths (Fig. 3c). OC-rich sediments were found in nearshore sites, while sediments with high carbonate contents typically showed low OC concentrations (Fig. 1a).

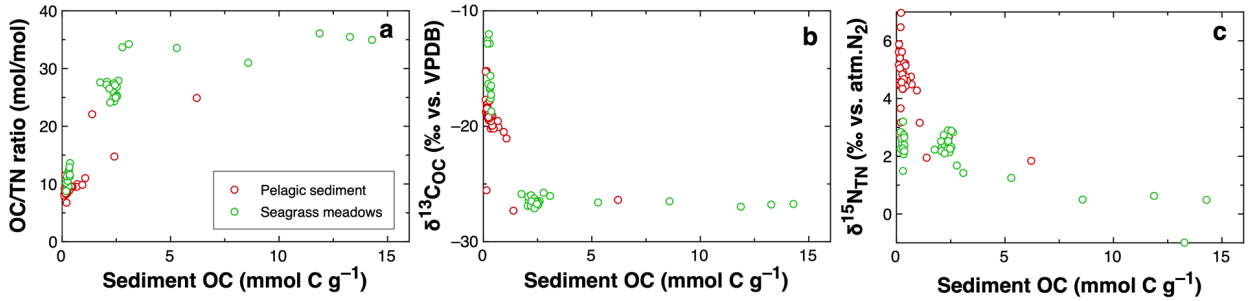


Figure 4. Relationships of sediment OC concentration to (a) OC/TN ratio, (b) $\delta^{13}C$ of OC, and (c) $\delta^{15}N$ of TN.

Sediments rich in OC (> 2 mmol g^{-1}) are characterized by C/N ratio higher than 14, $\delta^{13}C$ more negative than -25‰, and $\delta^{15}N$ lower than 3‰. Such characteristics are typical to detrital organic matter derived from terrestrial plants and/or mangroves. Thus, the delivery and accumulation of terrestrial and mangrove OC seem to be strong determinants of sediment OC distribution in this area.

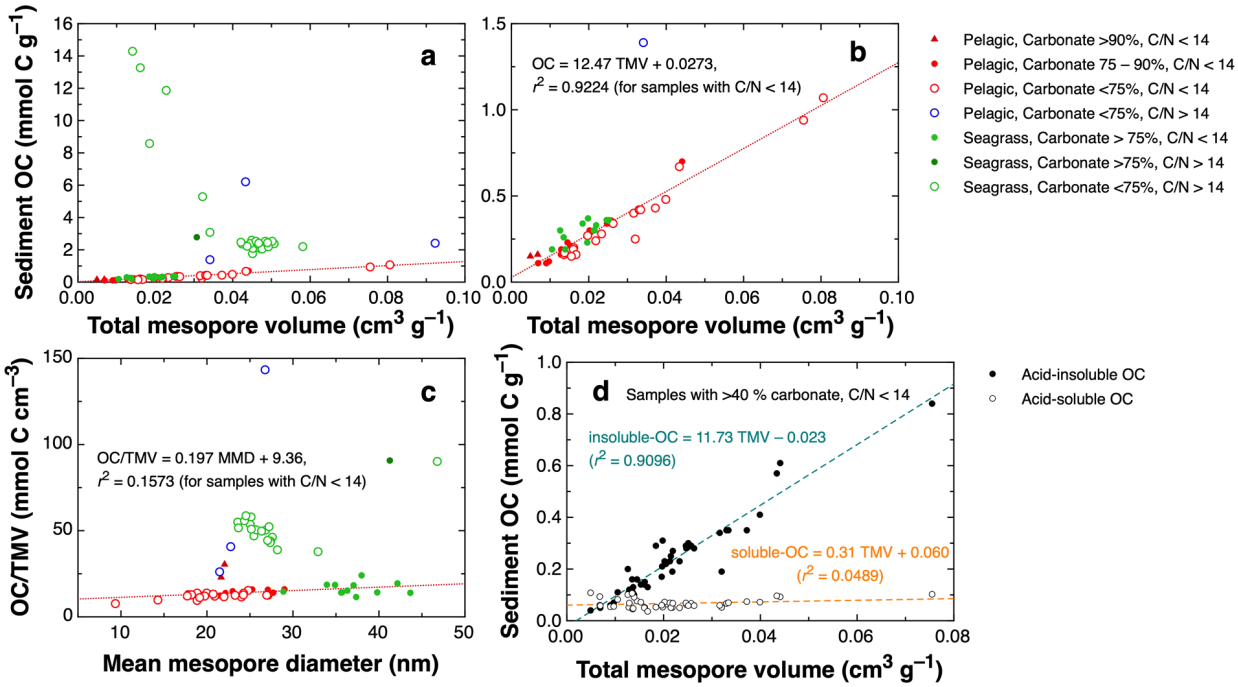


Figure 5. (a) Linear correlation of sediment OC content to total mesopore volume (TMV) for the samples with C/N < 14. (b) Magnified view of (a). (c) Variability of mesopore OC loading (OC/TMV ratio) as a function of mean mesopore diameter. (d) OC-TMV correlation evaluated separately for acid-soluble and insoluble fractions of carbonate sediments.

Sediment OC in sediment samples with C/N ratio <14 strongly correlated with total mesopore volume (TMV) determined by the BET method (r^2 = 0.9224; Fig. 5a,b). Mean mesopore diameter varied from 9.3 to 46.8 nm, but did not significantly affect the OC-TMV relationship for the same samples (Fig. 5c). These results suggest that OC was principally preserved in mesopore spaces on the surface of carbonate sediment grains. The slope of regression indicates that mesopore spaces could retain ca. 150 mg of OC per 1 cm^{-3}.

Sediment with higher OC/TN ratios (>14) showed large positive deviation from the above correlation (Fig. 5a).

In carbonate sediments, OC of the acid-insoluble fraction was strongly constrained by the TMV, but OC of the acid-soluble fraction showed relatively stable concentration irrespective of TMV (Fig. 5d).

From these observations, we conclude that OC preserved in carbonate-dominated sediment can be operationally separated into three fractions.

1. Fraction-1 is OC included in carbonate minerals and recovered as soluble OC upon acid dissolution of carbonate. The $\delta^{13}C$ of this fraction ranges between -22‰ and -14‰, but may be as high as -10‰ in seagrass meadow sediments. The concentration of this fraction seems relatively invariable (Fig. 5d).
2. Fraction-2 type is OC packed within mesopore spaces on the surface of carbonate minerals (typically 20 – 40 nm in diameter). This OC is recovered as insoluble material upon acid dissolution of carbonate. The $\delta^{13}C$ of this fraction is typically between -22‰ and -16‰. The concentration of this fraction is variable and apparently dependent on bottom geomorphology, being relatively higher in the center of basins.
3. Fraction-3 is detrital organic particles not strongly associated with sediment minerals. The $\delta^{13}C$ of this fraction seems more negative than -25‰. The concentration of this fraction is sometimes quite high (Fig. 5a) depending on hydrodynamics that allows accumulation of organic detritus.

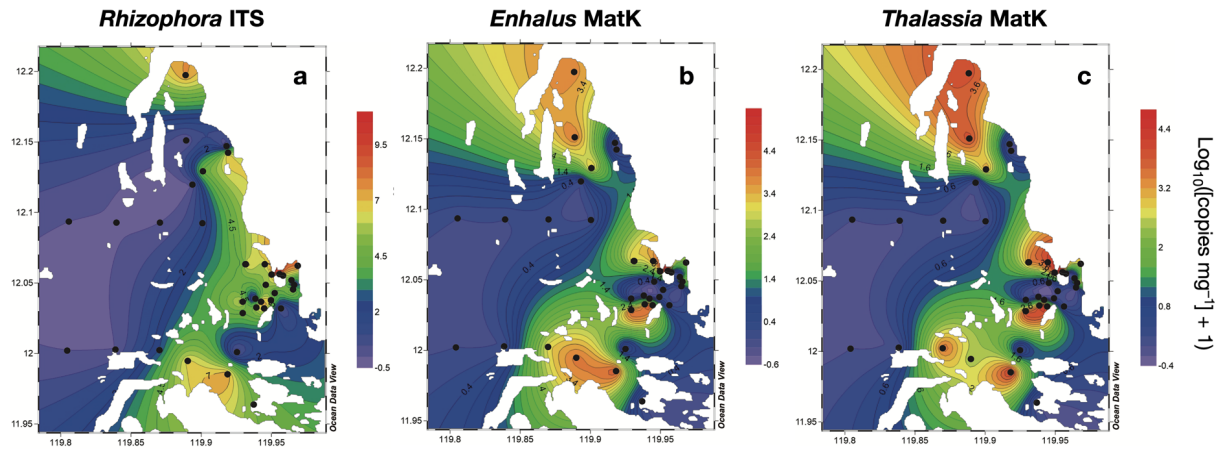


Figure 6. Spatial distribution of environmental DNA (eDNA) in the surface sediment of Busuanga-Culion passage. (a) ITS sequence derived from the mangrove *Rhizophora mucronata*; (b) MatK sequence derived from the seagrasses *Enhalus acoroides*, and (c) *Thalassia hemprichii*. Both species are predominant mangrove and seagrass in this area.

Mangrove- and seagrass-derived DNA fragments were detected mainly within 2 km off main habitats, but almost absent in far offshore sediments. This suggests that hydrodynamic conditions strongly constrain the deposition, accumulation and preservation of blue carbon in porous (not enclosed) coastal areas like the study site.

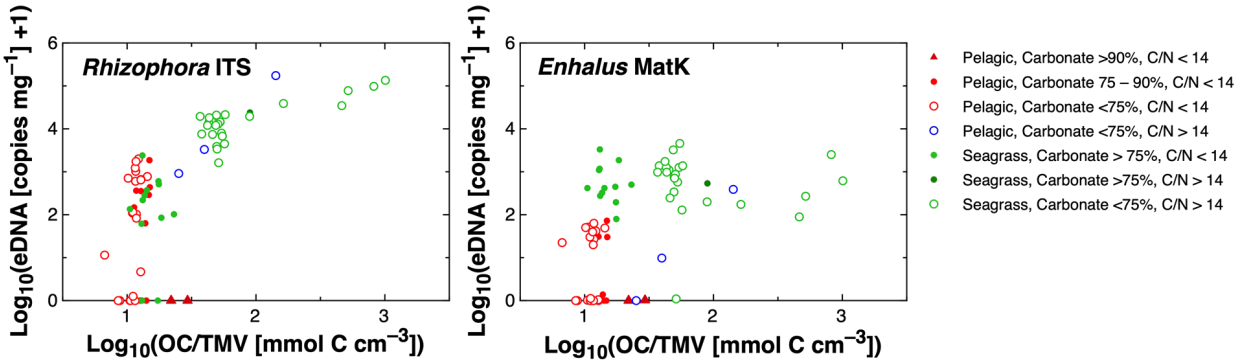


Figure 7. Logarithmic plots between concentration of eDNA and mesopore-space OC loading (OC/TMV ratio). Concentrations of eDNA of the mangrove *Rhizophora mucronata* (ITS, *Left*) and the seagrass *Enhalus acoroides* (MatK, *Right*) were shown as examples.

Concentration of the *Rhizophora* eDNA was positively correlated with the OC/TMV ratio, suggesting that *Rhizophora*-derived OC stored in sediment is not strongly associated with sediment minerals but exists as detrital organic particles buried in sediment (i.e. Fraction-3).

Concentration of seagrass eDNA showed no such correlation with OC/TMV, but was typically higher in seagrass meadow sediments than in offshore sediments. This fact suggests that seagrass-derived OC is mainly stored within sediment of the same habitats and not effectively preserved outside the original habitats. Seagrass eDNA stored in seagrass meadow sediments is considered to be originated mainly from the belowground parts (rhizomes and fine roots) of seagrasses.

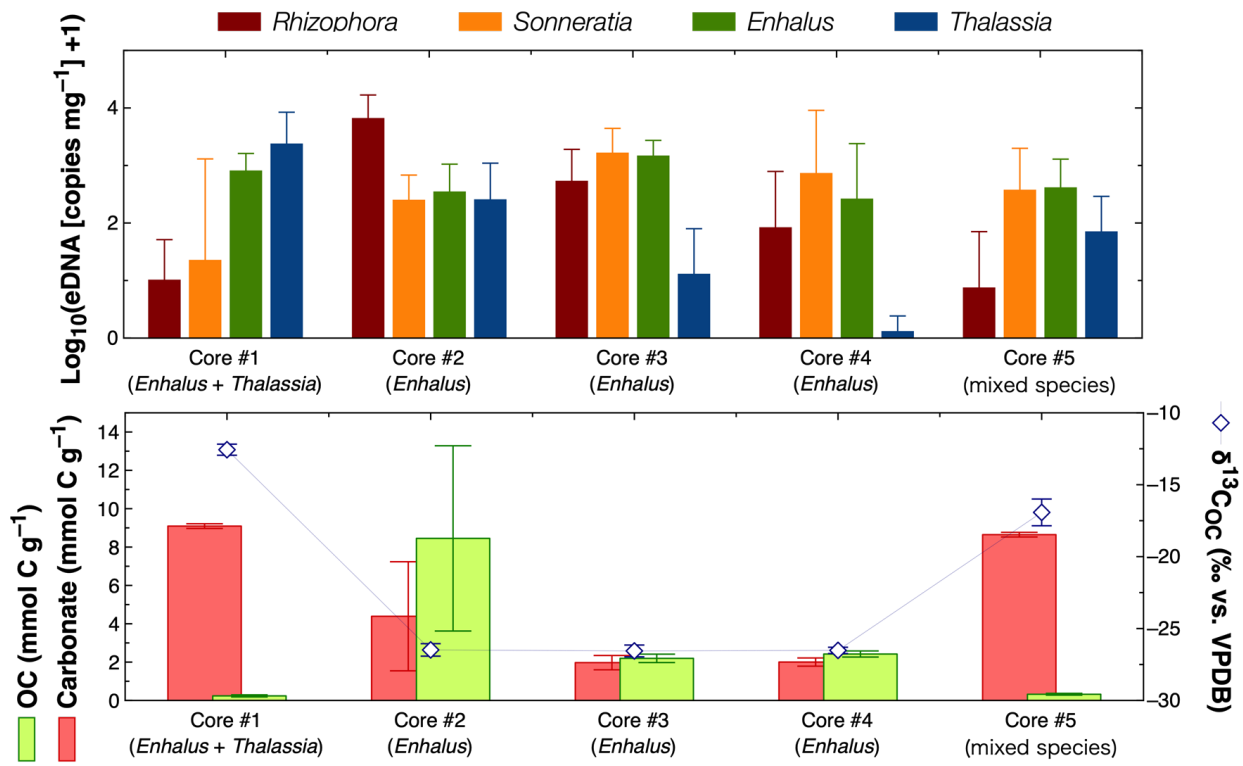


Figure 8. Environmental DNA (MatK sequences) detected from five seagrass bed sediment cores (see Fig. 1, depth average and SD; *top*) compared with average concentrations of OC and carbonate and the $\delta^{13}C$ of OC (*bottom*). Dominant seagrass species are shown in parenthesis.

Concentration of mangrove eDNA detected in these seagrass meadow sediments (especially cores #2–4) was generally higher than that in pelagic sediments (see also Fig. 7), which showed evidence of efficient accumulation by seagrass meadows of allochthonous OC exported from adjacent mangrove habitats. Relative abundance of eDNA of *Enhalus* vs. *Thalassia* reflects relative abundance of respective seagrass species in each habitat.

MAJOR FINDINGS

- This study investigated the OC distribution in tropical coastal sediments associated with mangroves and seagrass meadows and evaluated the contribution of these habitats to sediment OC accumulation.
- Sediment OC could be operationally classified into fractions closely associated and not associated with mesopores of sediment mineral grains. The OC fraction not associated with mesopores was characterized by high C/N ratio (>14), low $\delta^{13}C$ (<-25‰), and low $\delta^{15}N$ (< 3‰).
- DNA fragments derived from the mangrove *Rhizophora* was detected from at least 2 km off the original habitats and positively correlated with the OC fraction not associated with sediment mesopores. This suggests that mangrove-derived detrital OC can be exported and preserved as refractory detrital organic particles buried in offshore sediments.
- DNA fragments of seagrasses could also be detected in sediment distant from the original habitats, but the concentration was relatively low. Most of seagrass-derived OC seems to be stored in sediments of the original habitats.
- eDNA results also showed evidence of efficient accumulation and preservation of allochthonous mangrove-derived OC in seagrass meadows.

NOTES & ACKNOWLEDGMENTS

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Field survey was done under permission (PIC) from the barangays involved in the study area. Sediment samples were imported to Japan for analyses with permit from the Department of Environment and Natural Resources Philippines. Tsuyoshi Kanda kindly arranged for obtaining the permits. Sediment core sampling in seagrass meadows was assisted by Yoshiyuki Tanaka, Mikko Garcia, and Green Ann Cruz. GIS data were provided courtesy of Ariel C. Blanco.

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ABSTRACT

Blue carbon ecosystems such as mangroves and seagrass meadows (coastal marine ecosystems dominated by halophytic vascular plants) are regarded as a global carbon dioxide (CO_2) sink supported by high net community production. A part of the excess organic carbon (OC) production by these ecosystems is stored for a long term as persistent OC in underlying sediments, while the rest is exported to outside the system (open ocean) without being remineralized. In order to properly assess the role of blue carbon ecosystems in the global carbon cycle, the fate of exported OC must be elucidated. A part of the OC exported to the open ocean may be decomposed and remineralized quickly while in the ocean surface and return to the atmosphere as CO_2. In such a case, the export production cannot be regarded as a long-term carbon sink. On the other hand, the exported OC may either be (1) stored for a long term in the offshore sediment as detrital OC, (2) stored as refractory dissolved organic carbon (RDOC) in seawater, or (3) settled down in the bathypelagic layer and subsequently remineralized into CO_2 there. In these cases, carbon does not return to the atmosphere in the short term and can be included in net CO_2 sequestration. It is obvious that carbon pools corresponding to these three processes exits in the ocean. However, it is technically extremely difficult to clarify whether and to what extent carbon derived from the blue carbon ecosystems is contained in these pools.
The purpose of this study is to demonstrate by using environmental DNA techniques that OC derived from the blue carbon ecosystems can be transported to and stored in open ocean sediments. As a case study, coastal area off the west coast of Busuanga Island, Philippines, was set as study site, where natural coral reefs, seagrass beds, and mangroves are relatively well preserved. DNA probes for MatK sequences (part of chloroplast DNA) of two mangrove species (*Rhizophora mucronata*, *Sonneratia alba*) and two seagrass species (*Enhalus acoroides*, *Thalassia hemprichii*) as well as ITS sequence (part of nuclear DNA) of *R. mucronata* were designed. Then, the DNA copy numbers of respective sequences contained in extracts from surface sediment samples were quantified by the qPCR method. In addition, the organic and inorganic carbon concentrations and the specific surface area of the surface sediment samples were determined, and the origin of the sediment OC was assessed using a carbon stable isotope mixing model. During sample collection, seismic profiling with a sub-bottom profiler was also conducted to evaluate thickness of sediment accumulated in the studied area.
In this presentation, we summarize the results of these surveys to evaluate the areal extent to which seagrass- and mangrove-derived OC is transported and stored in relatively intact state, and identify environmental conditions that influence the accumulation in open ocean sediments of OC derived from blue carbon ecosystems. Difficulties in converting the data of DNA copy numbers into the amount of OC derived from specific plant species in the sediment will be also discussed.

[In Japanese]

マングローブや海草藻場等のブルーカーボン生態系（塩生維管束植物が優占する極浅海域生態系）は高い群集純生産に支えられた二酸化炭素（CO2）吸収源となっている。群集純生産に対応する過剰有機炭素生産量のうち、一部は生態系内の堆積物中に難分解性有機炭素として長期貯留されるが、残りは有機物の状態のままで系外（外洋）に移出されている。地球環境に対するブルーカーボン生態系の役割を正当に評価するためには、この系外移出された有機炭素の行方を解明する必要がある。一般に、有機炭素が外洋に移出されたのち、海洋表層にあるうちに速やかに分解無機化が進むと、再びCO2として大気に回帰するため、正味のCO2吸収源として算入されない。それに対して移出有機炭素が、①有機炭素のままで外洋堆積物中に長期貯留されるか、②難分解性溶存有機炭素となって海水中に長期貯留されるか、もしくは③深海に沈降したのちに分解無機化を受けて海洋深層水中にCO2としてとどまるならば、短期的には大気に回帰しないので、正味のCO2隔離に算入することができる。これら①②③に対応する炭素プールが存在することは明らかだが、そのプールの中にブルーカーボン生態系由来の炭素がどのくらい含まれているのかを明らかにすることは技術的に極めて難しい。このことがブルーカーボン生態系のCO2吸収源評価を正確に行うための障壁となっている。

本研究ではこのうち①のプロセス、すなわちブルーカーボン生態系から移出された有機炭素が外洋域の堆積物中に長期貯留されることを実証することを目的として、環境DNAをベースとした研究技術の開発を行った。事例研究として、今回は天然の海草藻場やマングローブが比較的良好に保存されているフィリピン・ブスアンガ島と、その西側約20 kmの範囲の海域を対象とした。この地域に優占するマングローブ２種（Rhizophora mucronata, Sonneratia alba）と海草２種（Enhalus acoroides, Thalassia hemprichii）のMatK配列（葉緑体DNAの一部）、およびR. mucronataのITS配列（核DNAの一部）に対するプローブを設計し、この海域の多点採取した表層堆積物からの抽出物に含まれるDNAコピー数をqPCR法によって定量した。また表層堆積物試料の有機・無機炭素濃度と比表面積を定量するとともに、炭素安定同位体比混合モデルによる起源推定法を適用した。採集時には船上からサブボトムプロファイラーによる観測を行い、堆積物の蓄積状況を合わせて調査した。今回の発表ではこうした調査結果の概要を報告するとともに、海草・マングローブ由来有機炭素の分布域、それらの有機炭素が貯留されやすい外洋堆積物の条件、種ごとの残留率の違いを支配する要因、堆積物中のDNAの定量結果から特定植物由来の有機炭素量を推定する際の問題点等について考察を加える。

REFERENCES

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