Do *Undaria pinnatifida* **Seaweed Farms Have Potential to Sequester Carbon?**

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ABSTRACT

Recently, seaweed farms have been attracting attention as they are expected to contribute to carbon sequestration. In order to evaluate the carbon sequestration function of seaweed farms, it is necessary to clarify whether the organic matter derived from cultivated seaweed is sequestered in the sediments of these farms. In this study, we focused on the sediments of an *Undaria pinnatifida* farm in Matsushima Bay, Miyagi Prefecture, Japan and evaluated the organic carbon contained in the sediments.Sediments were collected with an acrylic pipe with a diameter of 30 mm and a length of 1 m. Samples were subdivided into 10 cm segments and frozen at -18 °C or below. Total organic carbon (TOC) was analyzed with a TOC analyzer, and a digital PCR was used to identify the presence of *U. pinnatifida* eDNA. The eDNA of *U. pinnatifida* was detected from sediment layers between 0 cm to 28 cm from sites with a history of *U. pinnatifida* farming. However, eDNA was detected only between 0 cm to 8 cm in sediments with no farming history. TOC from farm sediments (mean±standard error) was 2.58±0.063%, whereas TOC taken from sites with no farming history was 0.669±0.023%. We hypothesize that seaweed farming can enhance TOC content in sediments below the farms and contribute to carbon sequestration.

Keywords: Climate change/ eDNA/ Macroalga/ Seaweed farm/ Sediments/ TOC

1. INTRODUCTION

The IPCC Sixth Assessment Report states that climate change is a consequence of greenhouse gas emissions from human activities and that mitigation and adaptation measures to reduce emissions are urgently needed [1]. In this context, one of the measures to mitigate climate change is to Human activities since the Industrial Revolution, including fossil fuel consumption, deforestation, and industrialization, have led to a rapid increase in atmospheric greenhouse gas concentrations from 280 parts per million (ppm) in the 19th century to over 410 ppm today [2,3]. Maintain and expand carbon sinks [4,5].

The ocean is an important carbon pool and is estimated to absorb about one-third of the carbon emitted by human activities [6]. Of this, the carbon sequestered and stored by the marine environment is known as "blue carbon" [7]. Historically, coastal mangroves, salt marshes, and seagrass beds have been considered carbon sinks. However, this view has recently been challenged, and seaweed beds have also been suggested to play an important role as blue carbon [8,9,10], drawing attention to seaweeds.

Persistent organic matter secreted by seaweeds can contribute to long-term carbon sequestration [11], with fucoidan secreted by brown algae being an example [1]. Therefore, seaweed farms are also being promoted worldwide for their potential to effectively capture atmospheric carbon dioxide [13,14,15]. However, our knowledge of the carbon sequestration process and the amount of carbon sequestered by seaweeds is still limited, and although some studies have assessed the amount of carbon sequestered by seaweed farms, these were often estimated from seaweed biomass and primary production [16,17,18]. Therefore, future studies should measure the amount of organic carbon in sediments to determine whether seaweed farms act as blue carbon. In this study, I focused on wakame

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seaweed (*Undaria pinnatifida*), which is cultivated nationwide and has been shown to release fucoidan, a persistent organic matter [19]. I then examined organic carbon in the sediment, hypothesizing that sediments from sites with a history of seaweed farming may sequester more organic carbon, and that genes derived from cultivated seaweed may be more abundant at sites with a history of seaweed farming.

2. METHODOLOGY

2.1 Location

This study was conducted in Matsushima Bay, Miyagi Prefecture (Figure 1). The study area is in the inner part of Sendai Bay, where the water depth is mostly less than 5 m [20]. The study area, Matsushima Bay, is included in the 88 closed marine areas defined by the Ministry of the Environment.

Figure 1. Study sites were in Matsushima Bay, Miyagi, Japan. "Study" refers to sites with a history of seaweed farming, and 'Control' refers to sites with no farming history.

2.2 Sediment Core Survey

Sediment coring was conducted on September 14, 2023. Core collection sites were selected based on visual confirmation of sea conditions on the day of the survey. Cores were collected by divers. An acrylic pipe (1 m in length, 30 mm in diameter) was driven vertically into the seafloor with a rubber hammer to a depth of 60 cm. The acrylic pipe was capped with a silicone stopper, extracted from the sediment, and then a second stopper was placed at the bottom of the core to prevent sediment from spilling out of the pipe. Location information was recorded using a handheld GPS (GPSMAP66i, Garmin). The sediment cores were transported to Yuriage Factory, Riken Foods, Inc. (Natori City, Miyagi Prefecture). Sediment cores were extruded at 10 cm intervals from the bottom and extruded samples were collected in sealable plastic bags. Samples were frozen in a -20°C freezer.

2.3 Sediment Organic Carbon Determination

The organic carbon content of the sediments was analyzed with a total organic carbon analyzer (soilTOC, Elementar Japan). In this study, frozen samples were dried in an oven at 60°C for at least 48 hours and then ground to homogeneity using a mortar and pestle.

2.4 Measurement of the genetic content of wakame seaweed (Undaria pinnatifida) in sediments

Species-specific environmental DNA (eDNA) analysis was conducted using digital PCR to measure the amount of wakame (*Undaria pinnatifida*) genes in the sediments. The analysis was performed on two core samples, one from a sediment sample at a site with a history of wakame farming and one from a sediment sample at a site with no wakame farming history. Samples were taken from

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each extruded sample from one representative core from each site. EDNA analysis was done by a contracted agency (Bioengineering Lab Co. Ltd.).

2.5 Data analysis

Data analysis was performed using R (R version 4.3.2: R Development Core and Team). Data results were expressed as mean ± one standard error. A t-test was performed to test for differences in organic carbon content between the study and control plots. The significance level was set at 0.05. For sediment core data, three core samples for which insufficient sediment samples could be collected were excluded from the data.

3. RESULTS AND DISCUSSION

3.1 Organic carbon content in sediment

13 sediment core samples were collected: 8 core samples from sites with a history of wakame (*U. pinnatifida*) seaweed farming and 5 core samples from sites with no wakame farming history. The organic carbon content of the sediments was $2.58\pm0.063\%$ for samples from sites with a history of wakame farming and $0.669 \pm 0.023\%$ for samples from sites with no wakame farming history. The differences in the organic carbon content among the sites were statistically significant (Welch t-test: t5.0199=32.088, p-value<0.0001; Figure 2). Our analysis suggests that sites with a history of seaweed farming may store more organic carbon derived from wakame seaweed in their sediments. We plan to evaluate the export of organic carbon farms into the seafloor by collecting organic matter settling into sediment.

Figure 2. Box-and-whisker plots of organic carbon content in sediments from sites with no history of wakame (*Undaria pinnatifida*) farming (Control) and sites with a history of wakame farming (Study). The dots plotted in this box-and-whisker plot represent values 1.5 times greater than the interquartile range. The minimum, first quartile, second quartile, third quartile, and maximum values for sites with no history of wakame farming were 0.5415 %, 0.6140 %, 0.6575 %, 0.7110 %, and 0.8555 %, respectively. The minimum, first quartile, second quartile, third quartile, and maximum values were 2.140 %, 2.386 %, 2.522 %, 2.759 %, and 3.025 %, respectively, for sites with a history of wakame seaweed farming.

3.2 Genetic content of wakame (Undaria pinnatifida) in sediment

Species-specific eDNA analysis using digital PCR was used to measure the amount of wakamederived genes in the sediments. EDNA analysis revealed that wakame genes were detected in sediment samples from sites with a history of wakame farming at depths ranging from 0 cm to -28 cm and from -48 cm to -58 cm. Sediment samples from sites with no wakame farming history had wakame genes detected at depths ranging from 0 cm to -8 cm (Figure 3).

These results suggest that some of the persistent dissolved organic carbon (DOC) and runoff biomass released by wakame seaweed was deposited in the sediments within the wakame farming site in large quantities. Another possible cause for the detection of wakame genes in the sediments within the wakame farming site at depths of -48 cm to -58 cm could be due to agitation of the sediments by the Great East Japan Earthquake Tsunami [20] or contamination of sediment samples from other layers in the process of collecting the sediment samples.

On the other hand, a few genes were detected in sediments at depths of 0 cm to - 8 cm, even from sediments at sites with no history of wakame farming. These results suggest that some of the persistent DOC released by wakame farming, as well as some of the biomass from runoff, may have been transported away from the farm and deposited in the sediment. In previous studies, genes derived from seaweed have been detected in sediments at sites distant from natural seaweed beds [21].

The number of genes detected in samples from sites with a history of wakame farming decreased with the depth of the sediments. This could be attributed to the fact that wakame farming production in Matsushima Bay began in earnest in 1988, with production increasing as the years passed [22]. However, since sediment agitation was confirmed by Ota et al. (2017) [20], it is necessary to determine the age of the sediments, clarify to which sediment layer agitation occurred, and clarify the relationship between the age of the sediments and the reason why wakame genetic material decreased with the depth of the sediments.

The results of these studies suggest that wakame farming sites have the potential to store a large amount of organic carbon in the sediments within their farms and play an important role in providing organic carbon in the sediments. In order to accurately determine the origin and contribution rate of carbon to carbon sequestration in seaweed farm sediments in the future, it will be necessary to increase the number of survey sites and data, and to compare the results with those of other farm waters.

Figure 3. Wakame (*Undaria pinnatifida*) DNA concentrations in sediment per sample from sites with no history of wakame (*Undaria pinnatifida*) farming (Control) and sites with a history of wakame (*Undaria pinnatifida*) farming (Study).

4. CONCLUSIONS

Sediment organic carbon content was found to be higher in sediments from sites with a history of wakame farming than in sediments from sites with no wakame farming history. Wakame genes were also identified in sediments from sites with no wakame farming history, suggesting that some of the wakame-derived carbon produced in wakame farms is leaching out of the farms. The results of this study support the carbon sequestration capacity of wakame farms and provide fundamental knowledge for blue carbon crediting. Future work is needed to determine the amount of organic carbon derived from farmed seaweed species in sediments and to quantify the amount of carbon sequestered by seaweed farms.

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