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# Assessing the Carbon Sequestration Potential of Spirogyra Algae in Achieving Sustainable Development Goals (SDGs): Biomass Estimation and Microbial Interactions in the Chi River Basin, Thailand

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## ABSTRACT

This study examined the carbon sequestration potential of Spirogyra algae in the Chi River Basin, Thailand, focusing on biomass, chemical composition, and interactions with sediment microorganisms. The average moisture and carbon content by dry weight were 97.3% and 36.8%, respectively, consistent with previous studies. Among the sampling sites, Spirogyra at Site 3 showed the highest carbon content, while Site 4 samples contained significantly higher nitrogen levels. Statistical analysis indicated a positive correlation between Spirogyra biomass, sediment organic carbon (SOC), and total organic matter (SOM). Principal component analysis confirmed carbon, biomass, and SOC/SOM as the key drivers of system variability. Microbial communities, particularly Rheinheimera and Flavobacterium sasangense, were found to play crucial roles in organic matter decomposition and nutrient cycling, enhancing carbon storage stability in freshwater ecosystems. Together, Spirogyra and sediment microorganisms act as an integrated carbon storage system that supports greenhouse gas reduction, ecosystem balance, and progress toward the Sustainable Development Goals (SDGs), especially SDG 13, SDG 14, and SDG 15.



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## 1. INTRODUCTION

Climate change poses a major threat to the survival of organisms [1-3]. The IPCC predicts that a global temperature rise of 1.5 °C will result in substantial losses of plant, animal, and microbial species, along with negative consequences for human well-being. Greenhouse gases account for 75% of global carbon emissions, the primary driver of global warming [1-3]. Algae, a diverse group of eukaryotic organisms, exhibit rapid growth and high photosynthetic efficiency, enabling them to sequester carbon dioxide ($CO_2$) more effectively than terrestrial plants. Certain algal species demonstrate $CO_2$ uptake efficiencies 10–50 times greater than those of land plants [5-7].

Technologies to reduce $CO_2$ emissions are under active development [8,9], with many focusing on $CO_2$ reuse by photosynthetic algae. Algae offer an environmentally friendly biological pathway for $CO_2$ utilization, capturing light energy and using $CO_2$ as a carbon source for photosynthesis [10-12]. As $CO_2$ is the main nutrient for growth and biomass production, algal uptake helps reduce atmospheric concentrations [13,14]. Supplementing microalgae cultures with $CO_2$ can further enhance biomass yields [15,16]. In addition, algae can precipitate $CO_2$ as calcium carbonate, providing a long-term carbon storage mechanism.

Spirogyra is a genus of freshwater microalgae, locally known as "Tao" in the Chi River Basin of Northeast Thailand, where it is traditionally consumed as food [17-19]. This filamentous green alga engages in diverse interactions with microbial populations, ranging from symbiotic to competitive or pathogenic [20-22]. It can serve as a nutrient source for certain microorganisms while releasing antimicrobial compounds that suppress others [23]. Moreover, Spirogyra contributes to nutrient cycling and bioremediation, indirectly shaping microbial communities [24,25].

This study investigated the carbon sequestration potential of Spirogyra in the Chi River Basin by measuring biomass, analyzing carbon stocks, and exploring its interactions with sediment microbial communities. The findings provide valuable insights into the ecological functions of freshwater algae, support resource conservation, and inform potential industrial applications. Additionally, this research advances progress toward the United Nations Sustainable Development Goals (SDGs), particularly SDG 13 (Climate Action) and SDG 15 (Life on Land).

## 2. METHODS

### 2.1. Sample Collection

The Chi River Basin spans 14 provinces in Northeast Thailand. Sampling was conducted at four sites: (i) Site 1 (47P 809992.92E 1728486.64N, UTM), located at Lahan Lake in Nongbuayai Subdistrict, Jaturat District, Chaiyaphum Province; (ii) Site 2 (47Q 818007.66E 1836584.98N, UTM), a natural canal connected to the Sern River, a branch of the Chi River in Chum Phae District, Khon Kaen Province; (ii) Site 3 (48Q 290553.34E 1809533.71N, UTM), situated in Nong Bak Pond in Nong Bak Subdistrict, Ching Yuen District, Maha Sarakham Province; and (iv) Site 4 (48P 420152.17E 1715600.94N, UTM), a natural canal connected to the Chi River in Phayard Subdistrict, Maha Chana Chai District, Yasothon Province. Spirogyra and sediment samples were collected from all sites between January and March 2025.

### 2.2. Biomass and Total Organic Carbon Content of Spirogyra

The biomass percentage was calculated using Equation (1).

$$iomass(g/m^2) = \frac{m_{dry-}m_{blak}}{A_{sample}} \quad (1)$$

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where $m_{dry}$ is dry weight of algae, $m_{blank}$ is weight of the blank sample, and $A_{sample}$ is the area of the sample collection site ($m^2$).

### 2.3. Soil Organic Carbon (SOC) and Soil Organic Matter (SOM) in The Sediment

The carbon content of the soil was measured using a CHN-628 series LECO analyzer and expressed as soil organic matter (SOM) through Equation (2).

$$\%SOC = \%C_{orgnaic} \quad (2)$$

where SOM is the percentage of soil organic matter, and C represents the value obtained from the CHN analyzer. The %SOM was calculated using Equation (3).

$$\%SOM = \%SOC \times 1.724 \quad (3)$$

where %SOM is the rate obtained from the first equation, and 1.742 is the van Bemmelen conversion factor (58% of carbon in soil organic matter) [26,27].

### 2.4. Microflora Diversity Analysis

Total genomic DNA was extracted from whole Spirogyra tissues using the DNeasy Plant Kit (Qiagen, Germany), following the manufacturer's protocol. PCR mixtures for amplicon generation were prepared with the sparQ HiFi PCR Master Mix (Quantabio, USA) for Illumina next-generation sequencing (NGS) platforms, employing universal primers targeting the V3–V4 hypervariable region of the bacterial 16S rRNA gene. PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen, Germany) and quantified with the Qubit dsDNA HS Assay Kit. Sequencing libraries were constructed using the Metavx Library Preparation Kit, purified with magnetic beads, and assessed using an Infinite 200 PRO microplate reader. NGS was performed on the Illumina HiSeq 2500 platform. Sequence data were clustered into operational taxonomic units (OTUs) using VSEARCH (v. 1.9.6) against the 16S rRNA reference database, with a 97% sequence identity threshold. Venn diagrams were generated using the corresponding function in R (v. 3.1.1).

### 2.5. Characterization of The Surface Morphology of Spirogyra

The surface morphology of Spirogyra was analyzed using a scanning electron microscope (SEM; JSM-6610, JEOL Ltd., Japan). Prior to imaging, the samples were gold-coated and examined at an accelerating voltage of 15 kV.

### 2.6. Data Analysis

The data were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test. A p-value of $< 0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 22). (24)

## 3. RESULTS AND DISCUSSION

### 3.1. Quantitative Measures of Biomass and Percentage of Carbon in Spirogyra

The average moisture content of Spirogyra in the Chi River basin was 97.3% (± 0.346%), while the average carbon content on a dry-weight basis was 36.8% (± 1.13%). Spirogyra collected from Site 3 exhibited a significantly higher carbon percentage than those from Sites 1 and 2 (**Table 1**; **Figure 1(a)**). The carbon content of the algae ranged from 35.6% to 37%, which is consistent with previous reports indicating an average carbon content of Spirogyra between 34.5% and 39.26% [28,29].

The average nitrogen content of Spirogyra was 6.9% (± 0.432%) by dry weight. Samples from Site 4 had a significantly higher nitrogen content compared to those from Sites 1, 2, and

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3 (**Table 1**; **Figure 1(b)**). Typically, nitrogen content in Spirogyra ranges from 2% to 4% [30,31], while other algal species generally contain 1% to 14% nitrogen by dry weight [32-34]. Nitrogen plays a vital role in algal growth and metabolism, particularly in the synthesis of proteins and nucleic acids. The nitrogen accumulation in algae is influenced by species type, habitat, and interactions with the microbial community [35-37].

The average biomass of Spirogyra was 7.19% (± 1.19%) by dry weight. Biomass at Site 4 was markedly lower than at Sites 1, 2, and 3 (**Table 1**; **Figure 1(c)**). Reported values for Spirogyra biomass generally range between 4% and 15% of dry weight [19,38].

**Table 1.** Biomass quantity and carbon and nitrogen percentages in Spirogyra within the Chi River basin, Northeast Thailand.

| Location | %Carbon | %Nitrogen | % Moisture | Biomass (g m$^2$) |
|---|---|---|---|---|
| Site1 | 35.6 ± 0.896$^a$ | 6.90 ± 0.077$^a$ | 96.9 ± 0.319$^a$ | 26.5 ± 3.38$^a$ |
| Site2 | 36.4 ± 0.692$^a$ | 6.65 ± 0.201$^a$ | 97.2 ± 0.083$^a$ | 7.87 ± 1.35$^b$ |
| Site3 | 38.3 ± 0.162$^b$ | 6.53 ± 0.037$^a$ | 97.6 ± 0.248$^a$ | 22.5 ± 4.17$^a$ |
| Site4 | 37.0 ± 0.095$^{ab}$ | 7.52 ± 0.301$^b$ | 97.4 ± 0.342$^a$ | 12.3 ± 1.71$^b$ |
| **Average** | 36.8 ± 1.13 | 6.90 ± 0.432 | 97.3 ± 0.346 | 17.3 ± 8.21 |

Note: $^{a,b,c,d}$ indicate significant differences according to Tukey's HSD test ($p < 0.05$).

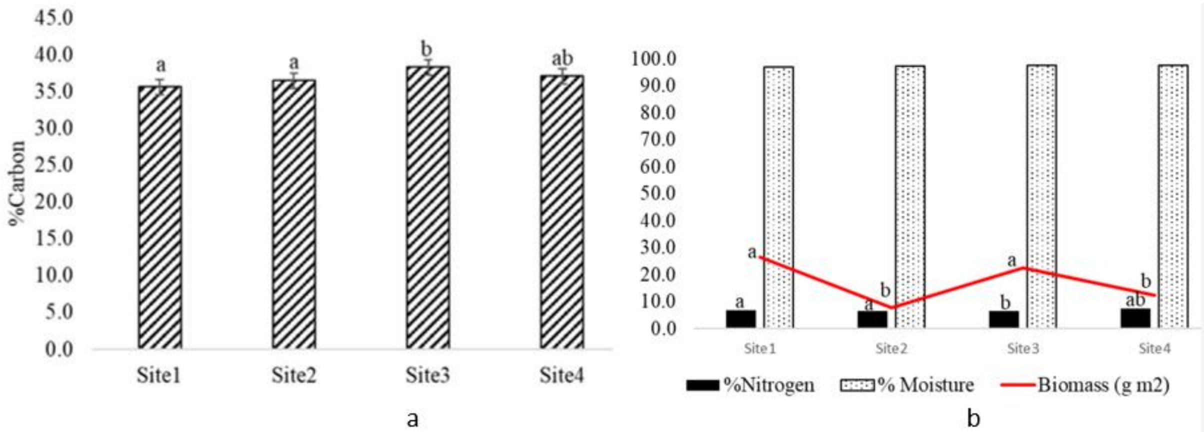


**Figure 1.** (a) Carbon (%), (b) nitrogen (mg kg-1), and (c) biomass (mg kg-1 of dry weight) content of Spirogyra in the Chi River basin, Northeast Thailand.

### 3.2. Sediment Properties

The sediment properties indicated that the average soil organic matter (SOM) content was 4.25% (± 2.04%). SOM at Site 2 was significantly lower than at Sites 1, 3, and 4 (**Table 2**; **Figure 2**). Variations in SOM content across sites were attributed to differences in organic matter sources, physicochemical conditions, microbial activity, and anthropogenic influences. The average soil organic carbon (SOC) content was 2.47% (± 1.18%), with Site 2 again showing markedly reduced SOC compared to Sites 1, 3, and 4 (**Table 2**). The consistency between SOM and SOC values reflects that SOM is derived directly from sediment organic carbon [39,40].

The mean sediment pH was 5.02 (± 0.715), with Site 2 recording a significantly lower pH than Sites 1, 3, and 4 ($p < 0.05$). Electrical conductivity (EC) was also significantly lower at Sites 2 and 4 compared with Sites 1 and 3 ($p < 0.05$; **Table 2**; **Figure 2**).

Munsell color analysis of dried sediments further characterized site conditions. Site 1 (5YR 2.5/1, black) indicated high organic matter and reducing conditions typical of anaerobic aquatic environments. Site 2 (7.5Y 8/2, pinkish white) showed a pale, low-chroma color, reflecting very low organic content dominated by carbonates or quartz, typical of well-

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drained, oxidizing environments. Site 3 (7.5Y 6/3, light brown) indicated low organic matter with some iron oxides, suggesting accumulation under moderately to well-drained oxidizing conditions. Site 4 (7.5Y 3/3, dark brown) reflected moderate to high organic matter and iron oxides, consistent with partially oxidized conditions and incomplete drainage. These results are summarized in **Table 2**.

**Table 2.** Sediments properties of the Chi River basin, Northeast Thailand.

| Site | %Carbon | %SOM | Soil pH | EC | Monsell Color* |
|---|---|---|---|---|---|
| Site 1 | 3.06 ± 0.149[ac] | 5.28 ± 0.257[ac] | 4.86 ± 0.011[abc] | 2.51 ± 0.162[a] | Black |
| Site 2 | 0.525 ± 0.034[b] | 0.906 ± 0.060[b] | 4.28 ± 0.020[b] | 614 ± 29.7[b] | Pinkish white |
| Site 3 | 3.66 ± 0.159[c] | 5.80 ± 0.275[c] | 5.00 ± 0.831[abc] | 2.46 ± 0.084[a] | Light brown |
| Site 4 | 2.92 ± 0.077[ac] | 5.04 ± 0.132[ac] | 5.94 ± 0.028[c] | 916 ± 47.0[c] | Dark brown |
| Average | 2.47 ± 1.18 | 4.25 ± 2.04 | 5.02 ± 0.715 | 384 ± 414 | |

Note: [a,b,c,d] indicate significant differences according to Tukey's HSD test ($p < 0.05$). SOM is the soil organic matter (mg kg-1), SOC is the soil organic carbon (mg kg-1), and EC is electrical conductivity.

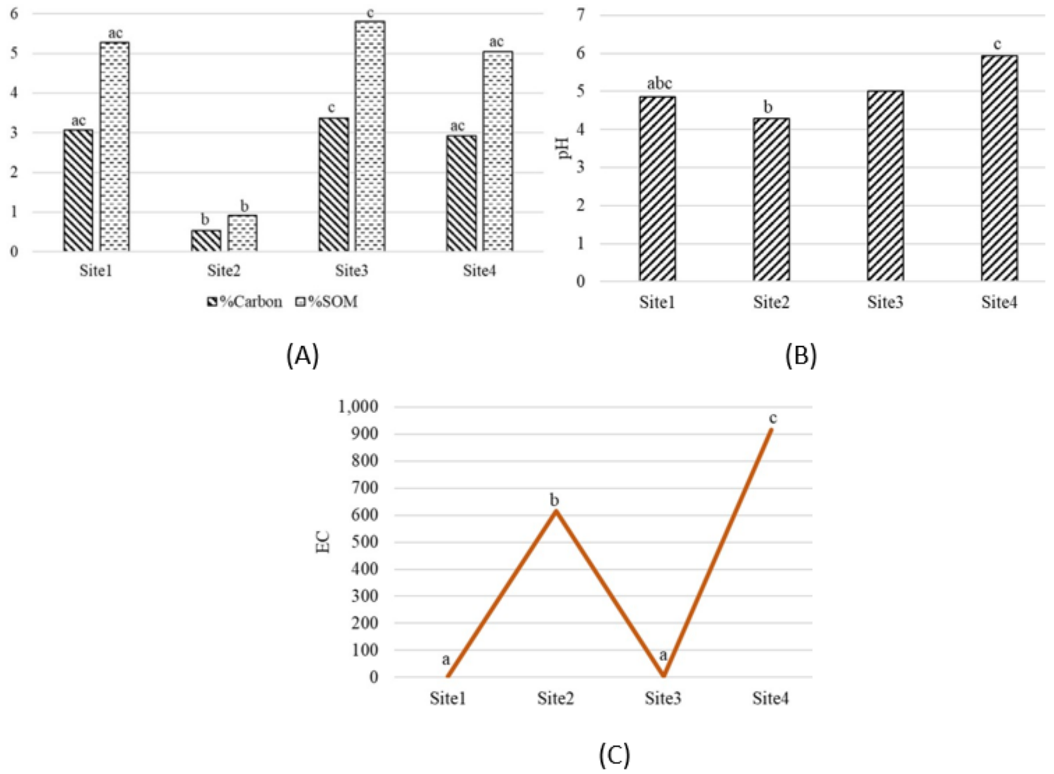


**Figure 2.** SOM and SOC percentages (A), pH (B), and EC (C) in the sediment of water resources in the Chi River basin, Northeast Thailand.

### 3.3. Relation and PCA Analysis of Spirogyra Biomass and Carbon In Sediment

The relationship analysis between the biocomposition of Spirogyra and sediment properties revealed several notable statistical associations (**Table 3**). The carbon content of Spirogyra (%C_Alg) was significantly and positively correlated with its moisture content (%Mo_Alg) ($r = 0.741$, $p < 0.01$), indicating that carbon storage in the alga may depend on cellular moisture status. Similarly, the nitrogen content (%N_Alg) was positively correlated with soil pH ($r = 0.688$, $p < 0.05$), suggesting that slightly alkaline soil conditions may enhance nitrogen accumulation in Spirogyra.

The relationship between Spirogyra biomass (Alg_Bi) and sediment properties showed a strong positive correlation with soil organic carbon (%SOC) and soil organic matter (%SOM) (r

= 0.727, $p < 0.01$). This finding highlights the role of Spirogyra in enhancing sediment carbon and organic matter content, supporting the hypothesis that freshwater algae are key drivers of carbon accumulation in aquatic ecosystems. As expected, %SOC and %SOM displayed a perfect correlation (r = 1.000, $p < 0.01$) because SOC is the principal component of SOM.

In contrast, a strong negative correlation was observed between electrical conductivity (EC) and Spirogyra biomass (r = −0.823, $p < 0.01$), indicating that elevated soil salinity suppresses Spirogyra growth and limits its contribution to sediment carbon sequestration. Overall, these results demonstrate that Spirogyra positively contributes to sediment organic carbon and matter accumulation [41,42]. However, this relationship is strongly mediated by environmental factors such as moisture, pH, and EC, which act as key regulators of algal biomass dynamics and carbon sequestration in freshwater ecosystems [43-45].

**Table 3.** Correlations between Spirogyra biomass and carbon in sediment.

| | %C_Alg | %N_Alg | %Mo_Alg | Alg_Bi | %SOC | %SOM | Soil pH | EC |
|---|---|---|---|---|---|---|---|---|
| %C_Alg | 1 | | | | | | | |
| %N_Alg | −0.184 | 1 | | | | | | |
| %Mo_Alg | 0.741** | −0.102 | 1 | | | | | |
| Alg_Bi | −0.098 | −0.181 | −0.270 | 1 | | | | |
| %SOC | 0.295 | 0.245 | 0.211 | 0.727** | 1 | | | |
| %SOM | 0.295 | 0.245 | 0.211 | 0.727** | 1.000** | 1 | | |
| Soil pH | 0.253 | .688* | 0.149 | 0.056 | .585* | .585* | 1 | |
| EC | -0.023 | .654* | 0.121 | −0.823** | −0.427 | −0.427 | 0.359 | 1 |

Note: *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). %C_Alg = percentage of carbon content in Spirogyra; %N_Alg = percentage of nitrogen content in Spirogyra; %Mo_Alg = percentage of moisture content of Spirogyra; SOM = soil organic matter; SOC = soil organic carbon; EC = electrical conductivity.

The results of the principal component analysis (PCA) of Spirogyra biomass and sediment carbon are presented in **Table 4** and **Figure 3**. Three principal components with eigenvalues greater than 1 were extracted, accounting for 93.84% of the total variance. PC1 explained 41.12% of the variance, PC2 explained 30.23%, and PC3 explained 22.49% (Figure 3a). For PC1, high positive loadings were observed for SOC (%SOC = 0.988), SOM (%SOM = 0.988), and Spirogyra biomass (Alg_Bi = 0.781), highlighting the strong association between algal biomass and sediment carbon accumulation. PC2 showed high positive loadings for EC (0.835), soil pH (0.745), and algal nitrogen content (%N_Alg = 0.785), reflecting the influence of soil chemical properties on algal composition. In contrast, PC3 was primarily associated with the internal properties of Spirogyra, with high negative loadings for %C_Alg (−0.819) and %Mo_Alg (−0.829), and a positive loading for %N_Alg (0.552).

The loading plot (**Figure 3(b)**) illustrates that %SOC, %SOM, and Spirogyra biomass cluster within PC1, emphasizing the role of algae in sediment carbon accumulation. In PC2, pH, EC, and %N_Alg are grouped, underscoring the importance of soil chemical factors. PC3 separates %C_Alg and %Mo_Alg from %N_Alg, indicating differences in the physiological characteristics of Spirogyra.

Overall, the PCA results confirm that more than 90% of the data variance can be explained by three principal components. PC1 represents carbon and organic matter accumulation processes, consistent with the strong correlations between algal biomass, SOC, and SOM. PC2 describes soil chemical properties, particularly the effect of pH on algal nitrogen content and the inhibitory influence of EC on algal biomass. PC3 captures internal physiological traits, reflecting variation across growth stages. Collectively, these findings demonstrate that

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Spirogyra biomass is a major driver of carbon sequestration, while environmental conditions and intrinsic algal traits jointly shape ecosystem dynamics [12,46-48].

**Table 4.** PCA of Spirogyra biomass and carbon in sediment.

| | PC1 | PC2 | PC3 |
|---|---|---|---|
| Eigenvalue | 41.118 | 30.233 | 22.491 |
| Cumulative (%) | 41.118 | 71.352 | 93.842 |
| %C_Alg | 0.34 | 0.295 | -0.819 |
| %N_Alg | 0.122 | 0.785 | 0.552 |
| %Mo_Alg | 0.202 | 0.381 | -0.829 |
| Alg_Bi | 0.781 | −0.546 | 0.262 |
| %SOC | 0.988 | 0.0996 | 0.0596 |
| %SOM | 0.988 | 0.0996 | 0.0596 |
| Soil pH | 0.53 | 0.745 | 0.217 |
| EC | −0.525 | 0.835 | 0.113 |

Note: %C_Alg = percentage of carbon content in Spirogyra; %N_Alg = percentage of nitrogen content in Spirogyra; %Mo_Alg = percentage of moisture content of Spirogyra; SOM = soil organic matter; SOC = soil organic carbon; EC = electrical conductivity.

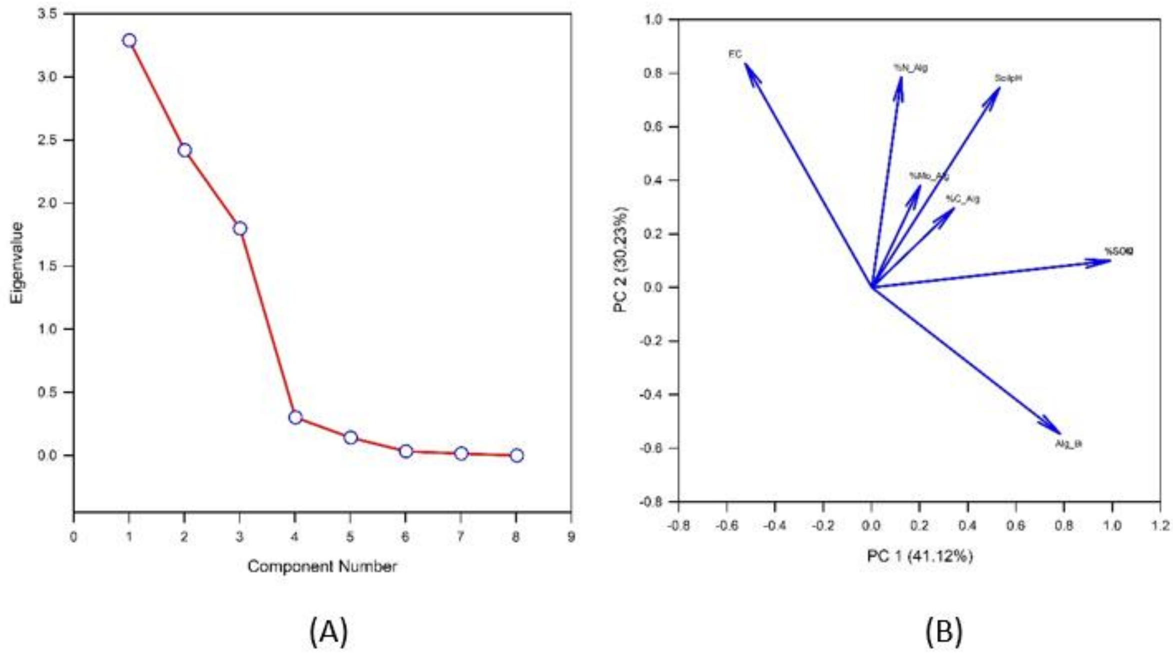


**Figure 3.** PCA of Spirogyra biomass and carbon in sediment in the Chi River basin: (a) Eigenvalues of the PCA components; and (b) PC Loadings.

### 3.4. Surface Morphology of Spirogyra

**Figure 4** (Sites 1A–4B) presents SEM micrographs of Spirogyra at 8000× magnification. The surface structure appears as overlapping, sheet-like layers, likely reflecting matrix collapse and rearrangement during the drying process. These sheets display a rough texture with crystal-like residues on the surface, which may represent mineral or salt crystals formed during or after drying.

The SEM analysis revealed that Spirogyra exhibits thin, overlapping, and rough surface layers with mineral or salt-like crystallization, most likely resulting from mineral deposition and crystallization during or after desiccation. These morphological characteristics align with the PCA findings, which highlighted the influence of soil chemistry—particularly pH and EC—

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on algal growth and composition. EC, in particular, is closely associated with salt or mineral crystallization on the algal surface [49,50]. In contrast, the collapse and rearrangement of the surface structure reflect internal physiological traits of the alga, such as carbon, moisture, and nitrogen content [51], which were captured in PC3.

Together, the SEM and PCA analyses provide complementary insights, illustrating how Spirogyra contributes to both mineralization processes and carbon sequestration in freshwater ecosystems.

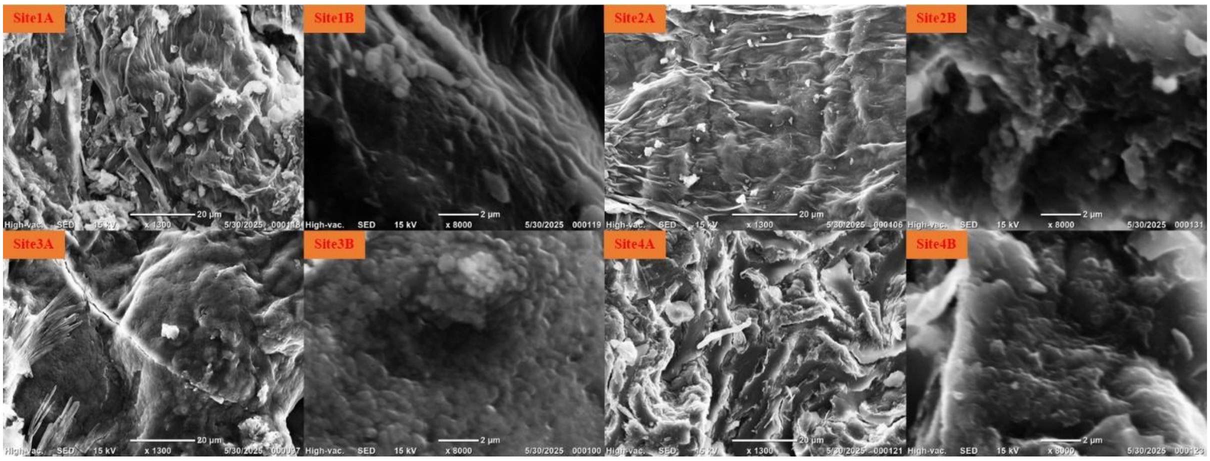


**Figure 4.** Surface morphology of Spirogyra in the Chi River basin, Northeast Thailand.

### 3.5. Microbial Communities Associated with Spirogyra in the Chi River Basin

**Figure 5** shows a heatmap illustrating the relative abundance of operational taxonomic units (OTUs) in Spirogyra sampled from different areas of the Chi River basin. The intensity of the blue shading reflects OTU abundance, with darker tones indicating higher abundance and lighter tones representing lower abundance or absence. Site 4 displayed dark blue signals (OTUs 4 and 5), while the other sites were lighter, indicating that Site 4 harbored a higher abundance of OTUs.

The Venn diagram comparing OTU distributions across the four sampling groups revealed 78 OTUs shared among all groups, suggesting the presence of a potential core microbiota consistently associated with Spirogyra. Site 2 exhibited the highest number of unique OTUs (37), implying a distinct microbial community potentially shaped by specific environmental factors or stressors. In contrast, Site 4 contained the fewest unique OTUs (7), indicating greater similarity to other groups (**Figure 5(b)**). The overlapping OTUs between certain site pairs (e.g., Sites 1 and 3) suggest similarities in environmental conditions or ecological niches.

**Figures 5(c)** and 5**(d)** present the relative abundances of bacterial genera across the four sites. Each color represents a genus, as indicated by the legend. Dominant genera included Rheinheimera and Nordella at Site 1; Acinetobacter and Nordella at Site 2; Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium and Nordella at Site 3; and Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium and Fluviicola at Site 4.

Analysis of microbial species composition (**Figure 5(d)**) further identified dominant taxa at each site. Site 1 was characterized by Rheinheimera and Flavobacterium sasangense. Site 2 was dominated by Corynebacterium and unclassified taxa. Site 3 showed high abundances of unclassified Flavobacterium and Oscillatoria sp. PCC-10802. Site 4 was dominated by unclassified groups and metagenomic taxa. These findings highlight the diversity and variability of microbial communities across sites, influenced by environmental conditions.

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Alpha diversity indices further demonstrated differences among sites (**Table 5**). Site 3 exhibited the highest diversity, with a Shannon index of 6.217 and a Simpson index of 0.965, suggesting a rich and evenly distributed microbial community. Conversely, Site 4 showed substantially lower diversity (Shannon = 1.716, Simpson = 0.581), potentially reflecting environmental stress or limited resource availability. Richness estimators (ACE and Chao1) supported this trend, indicating reduced microbial richness at Site 4. Good's coverage values were consistently high (>0.99), confirming sufficient sequencing depth.

These findings align with previous studies. [52] reported Rheinheimera, Acinetobacter, Sphingopyxis, Sphingomonas, Spirosoma, and Flavobacterium in freshwater algae, depending on environmental conditions and source communities. Similarly, our study identified Acinetobacter, Rheinheimera, and Flavobacterium as predominant genera in Spirogyra. Given that bacterial communities are strongly shaped by environmental factors [53,54], such factors likely contributed to the observed site-specific differences in microbial composition.

**Table 5.** Biodiversity indices of Spirogyra microbial communities in water sources in the Chi River basin.

| Location | ACE | Chao1 | Shannon | Simpson | Good's coverage |
|---|---|---|---|---|---|
| Site 1 | 371.351 | 392.364 | 5.667 | 0.959 | 0.993 |
| Site 2 | 344.496 | 357.029 | 4.488 | 0.912 | 0.992 |
| Site 3 | 410.973 | 403.06 | 6.217 | 0.965 | 0.997 |
| Sote 4 | 128.368 | 119.789 | 1.716 | 0.581 | 0.997 |

### 3.6. Carbon Sequestration Efficiency of Spirogyra in the Chi River Basin

Our findings indicate that Spirogyra in the Chi River Basin has considerable potential for carbon sequestration. On average, the samples contained 97.3% moisture and 36.8% carbon by dry weight, values that fall within the ranges reported in previous studies [55,56]. The mean nitrogen content was 6.9%, which is higher than typical values and reflects the alga's ability to accumulate nutrients for growth [57,58].

Sediment analysis revealed variations in soil organic matter (SOM) and soil organic carbon (SOC) across sites, with Site 2 showing the lowest values. This trend was consistent with pH, electrical conductivity (EC), and Munsell soil color, which collectively indicate organic matter content. Principal component analysis (PCA) confirmed the associations between algal biomass and SOC/SOM (PC1), the influence of pH and EC (PC2), and physiological differences among algae (PC3).

Scanning electron microscopy (SEM) further showed that Spirogyra possesses a plate-like surface structure with crystal-like mineral deposits, features that correlate with environmental chemistry [59,60]. Microbial community analysis identified 78 common OTUs, although diversity varied geographically. Key taxa such as Rheinheimera, Acinetobacter, and Flavobacterium play significant roles in organic matter decomposition and carbon cycling [61-63].

Overall, Spirogyra acts as a biomass producer, an organic matter enhancer, and a microbial habitat, supporting a complex carbon sequestration mechanism that is closely linked to environmental and ecological conditions. These findings underscore the ecological significance of freshwater algae in mitigating climate change.

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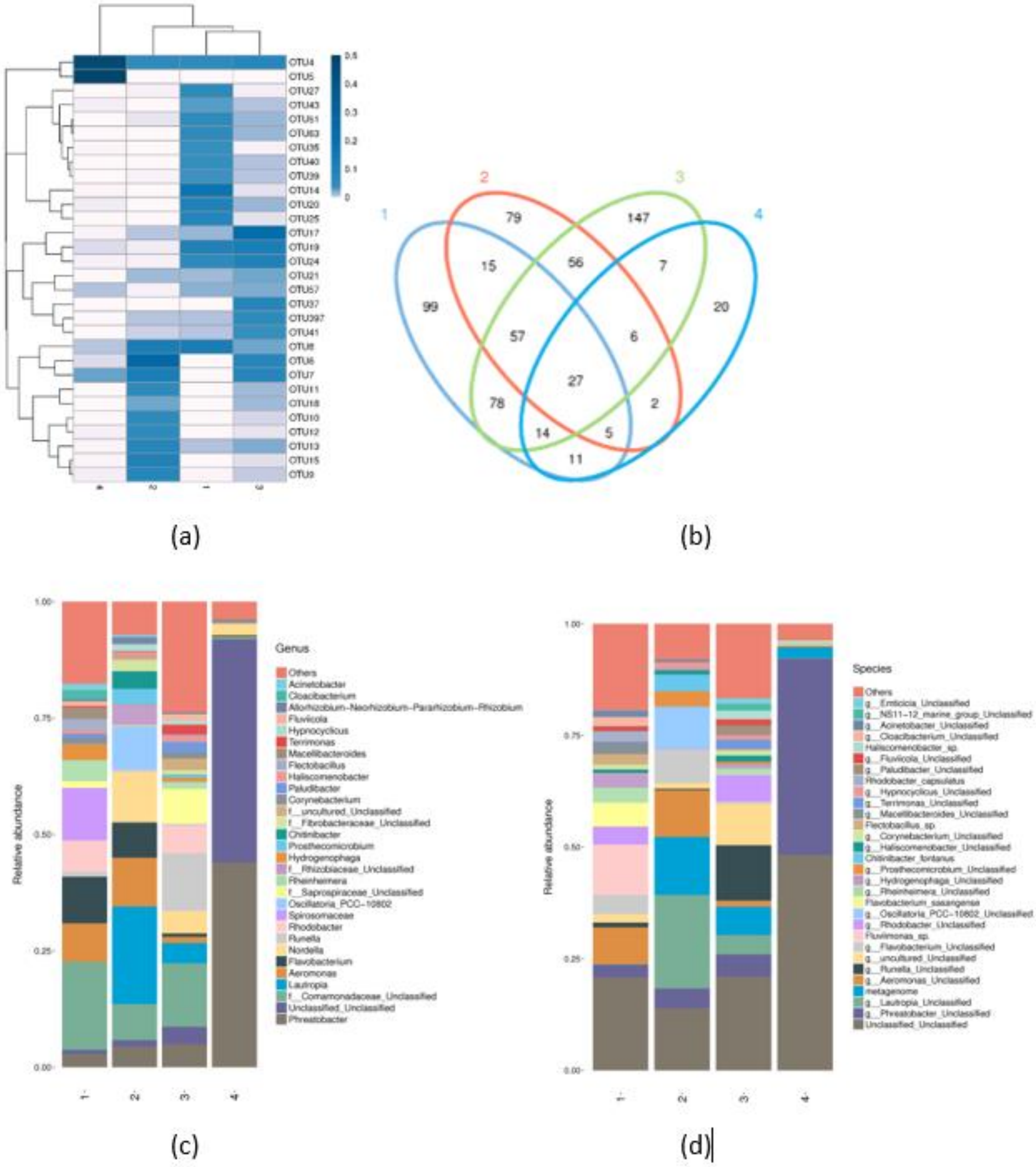

**Figure 4.** Diversity of the microbial community of Spirogyra in the Chi River basin. (a) OTU abundance clustering heatmap; (b) OTU Venn diagram; (c) stacked bar plot of the distribution of genera; (d) stacked bar plot of the genus and species distributions.

### 3.7. Environmental Drivers and Mechanistic Interpretation

The findings of this study demonstrate that environmental factors strongly influence the carbon sequestration potential of Spirogyra in the Chi River Basin. Biomass carbon values varied significantly among the sites, with Site 3 exhibiting the highest carbon content, whereas Site 4 showed the highest nitrogen content. These differences highlight the effects of water and sediment quality, as well as nutrient availability, on algal growth and chemical composition [64-67]. Correlation analysis further revealed a positive relationship between Spirogyra biomass and SOC/SOM, underscoring the role of algae in organic matter accumulation and carbon retention within the ecosystem [68].

Mechanistically, carbon sequestration in Spirogyra is driven by efficient photosynthesis, which promotes biomass production and tissue carbon storage. Following algal senescence, organic materials are deposited in sediments, enhancing SOC/SOM content. Concurrently,

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sediment microbial communities facilitate the decomposition and transformation of carbon and nitrogen, maintaining nutrient cycling [69]. PCA results align with this interpretation, showing that carbon, biomass, and SOC/SOM are the primary drivers of variability and represent the core of the sequestration system in the Chi River.

In conclusion, water and sediment quality are key determinants of Spirogyra's carbon sequestration potential, while the mechanistic process relies on the synergy between algal biomass production and microbial activity in sediments. Together, these factors create an integrated carbon storage system essential for the sustainable management of freshwater ecosystems [70].

### 3.8. The Relationship between Spirogyra and the SDGs

Our findings demonstrate that freshwater Spirogyra from the Chi River Basin possesses a high potential for carbon sequestration, with an average carbon content of 36.8% (dry weight). This value falls within the range reported in international literature (34.5–39.26%), indicating that this alga can serve as an important biological carbon sink in freshwater ecosystems [37,56]. Environmental conditions exert a clear influence; for instance, algae at Site 3 exhibited higher carbon content compared to the other sites, reflecting its sensitivity to site-specific water and sediment quality.

The detected nitrogen content (6.9% on average) highlights Spirogyra's capacity to accumulate nutrients and contribute to elemental cycling within the ecosystem, particularly through its interactions with sediment microbial communities that facilitate organic matter retention and enhance ecological stability. Correlation analysis revealed a significant positive relationship between Spirogyra biomass and SOC/SOM [71], consistent with PCA results showing that carbon and biomass are key variables explaining system variability [32].

In relation to the Sustainable Development Goals (SDGs), this study directly addresses SDG 13 (Climate Action) by emphasizing the role of Spirogyra in carbon sequestration and greenhouse gas mitigation, as well as SDG 14 (Life Below Water) and SDG 15 (Life on Land), by demonstrating the importance of algae–microbe interactions in maintaining freshwater ecosystem balance and conserving biodiversity. These findings suggest that Spirogyra can be developed as a nature-based solution for future environmental management.

## 4. CONCLUSION

The carbon sequestration potential of Spirogyra phytoplankton in the Chi River Basin was evaluated in this study. The chemical composition of the algae was influenced by environmental factors, as reflected by the higher carbon content at Site 3 and the higher nitrogen content at Site 4. On average, Spirogyra exhibited 97.3% moisture content and 36.8% carbon content by dry weight. Correlation analysis demonstrated a positive relationship between Spirogyra biomass and SOC/SOM. Sedimentary microorganisms, particularly Rheinheimera and Flavobacterium sasangense, served as key mediators in biomass degradation and the transformation of organic matter, thereby sustaining the system. The interactions between phytoplankton and microorganisms facilitated efficient carbon sequestration and cycling in freshwater ecosystems. PCA analysis further confirmed that the primary variables driving system variability were carbon, biomass, SOC, and SOM, consistent with the mechanism whereby Spirogyra produces biomass, which is later decomposed and accumulated as sedimentary carbon. Subsequently, microbial groups such as Proteobacteria and Bacteroidetes metabolize this carbon, recycling it within the system. Overall, these results suggest that Spirogyra and microbial communities such as Rheinheimera and Flavobacterium sasangense function as an integrated carbon storage system in freshwater habitats. This

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finding has substantial implications for achieving the Sustainable Development Goals, particularly SDG 13 (Climate Action), SDG 14 (Life Below Water), and SDG 15 (Life on Land), by enhancing our understanding of ecological carbon sequestration mechanisms.

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## 6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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