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Blue Carbon in the Persian Gulf: Evidence of Phytoplankton Contribution to Carbon in Sediments

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Abstract

Blue carbon ecosystems, such as mangroves, seagrasses, and tidal marshes, are critical for carbon sequestration and climate change mitigation to ensure environmental sustainability. This study provides a review of the limited inventories of blue carbon habitats in the Persian/Arabian Gulf, highlighting limited spatial and temporal coverage as well as the uncertainties in estimates that are quantified using inconsistent methodologies and satellite resolution limitations. The main focus of this paper is a discussion on the consideration of phytoplankton in blue carbon dynamics, which remains understudied, in the Gulf. To underpin the evidence of phytoplankton permanent burial in marine sediments, shotgun metagenomic sequencing was used and 26 phytoplankton species were identified in sediment cores, showing the dominance of *Aureococcus anophagefferens* and *Thalassiosira pseudonana*, and underscoring their potential role in carbon sequestration in the northern Gulf, though their inclusion in blue carbon frameworks is complicated by taxonomic diversity and uncertain sequestration pathways. The permanent burial of phytoplankton in these shallow marine and coastal areas brings an important discussion on their inclusion in blue carbon estimates. The use of remotely sensed data for blue carbon habitat mapping needs standardisation and the use of high spatial and spectral resolution remote sensing to improve blue carbon assessments in the region. This study provides firm evidence of phytoplankton presence using eDNA calls for refining the carbon accounting frameworks in the Gulf and beyond, underscoring the importance of refining blue carbon assessments to support evidence-based environmental sustainability and climate action. By integrating phytoplankton contributions into carbon sequestration, more realistic and inclusive frameworks can be developed, enhancing regional strategies for climate change mitigation and coastal ecosystem conservation.

Keywords: blue carbon; Persian Gulf; phytoplankton; metagenomics; carbon sequestration; remote sensing

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1. Introduction

Coastal vegetation, such as mangroves, tidal marshes, and seagrass meadows, is highly effective at absorbing and retaining organic carbon [1–3]. This ability makes these blue carbon ecosystems, possibly including macroalgae [4–10], critically important for long-term carbon storage. The per-unit-area rate of carbon capture in mangrove forests, tidal marshes, and seagrass meadows can exceed that of terrestrial forests by 10 to 100 times [1,11]. This remarkable efficiency results in these systems accounting for approximately 50% of the total carbon stored within the world's marine sediments. Building on the reports of carbon

cycling potential of seagrasses [12] and phytoplankton, the "blue carbon" concept emerged in 2009 [13] to account for the contribution of marine vegetation as a carbon sink. With the years of research, the estimation of carbon sequestration and storage potential by the blue carbon habitat has improved, resulting in its consideration as an effective strategy for climate change mitigation and adaptation [3].

The intricate, multi-faceted character of blue carbon has driven the emergence of a dynamic research domain that necessarily bridges the biophysical sciences and the social sciences, fostering critical intersections with conservation practice, economic valuation, and legal-policy frameworks [14]. This multidisciplinary approach has catalysed collaborative research among researchers, institutions, and governments, focusing on the coastal ecosystem restoration and conservation to reduce greenhouse gas emissions, enhance climate resilience, and sustain critical ecosystem services. The intricacy and multidisciplinary nature of the blue carbon concept have also bred ambiguity and differing interpretations of its core definition. Although the study of marine carbon stocks and cycles is a critical scientific foundation, it is merely one piece of the puzzle. With the rise in blue carbon interest, the three seminal reports have firmly taken root in both research and policy circles [3,10,13]. This growing engagement has opened the door to a wider variety of perspectives, all of which are now actively steering the discourse forward.

Blue carbon ecosystems not only sequester carbon but also help improve water quality [15]. These habitats, especially mangroves and seagrass, act as buffers against flooding and extreme events [16,17], serve as nursery grounds for fish species [18], and prevent coastal erosion [19], making blue carbon ecosystems valuable for both climate change mitigation and adaptation efforts [20]. The role of blue carbon has gained prominence, especially following its incorporation into global climate policies such as Nationally Determined Contributions [20–22].

In the last twenty years, blue carbon science has expanded considerably. This growth has been fuelled by increased research focused on tackling key questions regarding how the conservation and restoration of coastal ecosystems—mangroves, tidal marshes, and seagrasses—can contribute to mitigating and adapting to climate change [20]. While recent analyses have begun mapping the global trajectory of blue carbon research [23,24], a significant gap persists: a comprehensive, longitudinal assessment of the field's development that explicitly includes macroalgae (seaweeds) and, more conspicuously, microalgae alongside the traditionally studied 'big three' ecosystems remains lacking. This gap is particularly relevant given the ongoing scientific debate and emerging evidence about macroalgae's potential role in the global carbon cycle, including sequestration pathways and quantification challenges [20,25–30].

Lovelock and Duarte [14] have eloquently summarised the criteria of different habitats to be included under blue carbon, along with the uncertainties in their efficacy in the removal of greenhouse gases, long-term storage of carbon dioxide, and capability to enhance carbon stocks (Table 1).

Table 1. The blue carbon ecosystems and their potential for GHG removal and carbon stock enhancement.

Habitat	GHG Removal Potential	Long-Term Storage of Fixed CO_2	Enhance Carbon Stocks
Mangroves	High [31]	High [32]	High [10,13]
Tidal Marsh	High [31,33]	High [33]	High [34,35]
Seagrass	High [36]	High [37]	High [11]
Sabkha	Inconclusive [14]	Inconclusive [14]	Inconclusive [14]
Macroalgae	High [38]	Inconclusive [38]	High [7]
Microalgae	High [39]	Inconclusive [6]	Inconclusive [14]

2. Materials and Methods

A multi-faceted methodological approach was utilised in this study that includes a review of the limited existing literature on blue carbon in the region, a critical examination of the techniques used by previous works that have often not highlighted the limitations, and original genomic laboratory work to underpin the fact that phytoplankton in general were observed in sediment cores, leading to a new perspective since their burial appears permanent, and therefore we question why they are not included in blue carbon estimates. A systematic review of scientific literature was conducted to compile an inventory of blue carbon habitats (mangroves, seagrasses, and salt marshes) within the Persian Gulf region. Peer-reviewed publications and technical reports were identified using academic databases and institutional resources. The primary focus was to collate reported spatial coverage in each habitat type under blue carbon. The evidence of phytoplankton sequestering carbon in Kuwait's marine sediments is underpinned by genomic data. Marine sediment cores up to 30 cm depth were collected from 20 sites (Figure 1), 3–40 km away from the coast of Kuwait, further details are provided in Supplementary Materials Table S1.

The method of extraction is described in detail elsewhere [40]. For ease of readership, we are putting in brief the DNA extraction procedure. Sediment samples were collected across a depth gradient of 5 to 30 m. For each site, total genomic DNA was isolated from 0.25 g of sediment obtained from the core bottom, utilising the DNeasy® Power Soil Pro® Kit (QIAGEN, Germantown, MD, USA). To ensure representativeness, six independent extractions were performed per site and subsequently combined into a single composite sample. DNA concentration was determined fluorometrically with a Broad Range dsDNA assay kit (Qubit, Thermo Fisher Scientific, Waltham, MA, USA). Integrity was verified via electrophoresis on a 0.8% agarose gel (45 min, 100 V; Bio-Rad, Neuried, Germany) [39]. Sample purity was evaluated spectrophotometrically (Nanodrop, Fisher Scientific, Waltham, MA, USA) by calculating the A260/A280 absorbance ratio, with values approximating 1.8 for all extracts. Purified DNA was archived at −80 °C for subsequent analysis. For metagenomic analysis, DNA samples were prepared for next-generation sequencing. Libraries were constructed from ~1 µg of input DNA through fragmentation by sonication, followed by end-repair, A-tailing, and indexing. These libraries were sequenced with 150-base-pair paired-end reads on an Illumina NovaSeq 6000 platform. The resulting raw reads, totalling roughly 12 gigabytes per library, were transferred to the online bioinformatics environment of the Chan Zuckerberg ID (CZ ID) platform. Quality checks were performed on the uploaded sequences through their in-built pipelines. First and foremost, the sequence format was verified; thereafter, adapter sequences and low-quality reads (Ns) were removed. This was followed by the removal of host sequences (human DNA) and duplicate reads using STAR [41].

A downstream computational pipeline was implemented, beginning with the random selection of two million read pairs for uniform analysis. For taxonomic characterisation, filtered reads were aligned to nucleotide (NCBI NT; Minimap2) [42] and protein (NCBI NR; DIAMOND 2.2.16) reference databases [43]. Independent de novo assembly was conducted with SPAdes v3.8.1 [44], and the quality and representativeness of assembled contigs were evaluated by mapping reads back to them via Bowtie2 to determine coverage depth [45]. Contigs were subsequently subjected to homology searches using BLASTN and BLASTX v 2.16.0 for precise taxonomic binning. To minimise false positives, detected microbial taxa were required to pass a triple-threshold filter: an abundance cut-off of 10 RPM, a minimum aligned read length of 250 bp, and a reference genome coverage greater than 5% [46]. The commands are available at https://github.com/arpcard/rgi (accessed on 12 July 2025).

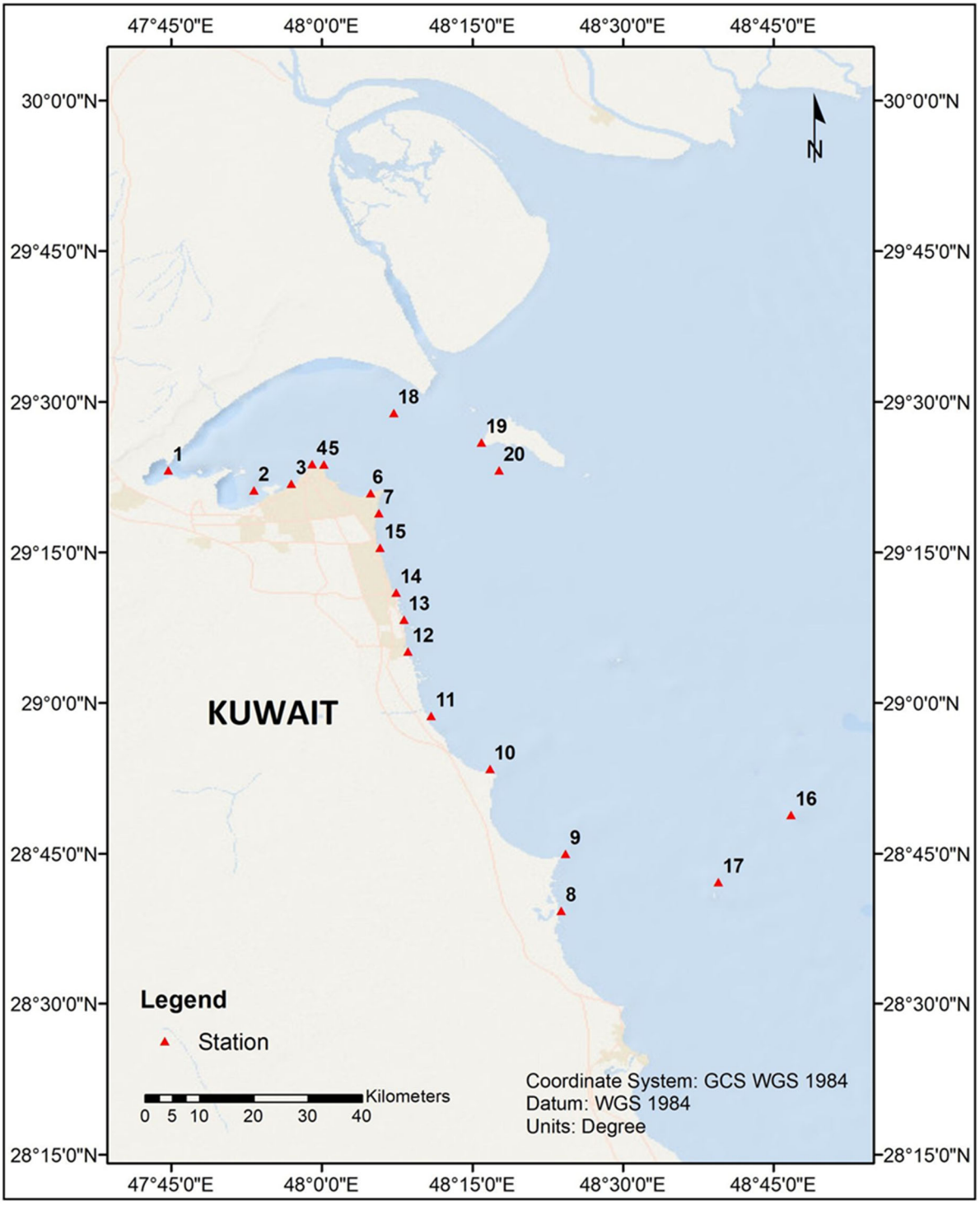


Figure 1. Sampling location in the Kuwait marine area.

3. Results

Review of the blue carbon habitats shows that the data is quite limited in the Gulf region. With patchy and temporally spaced assessments conducted in the region, the data are summarised in Table 2. This includes reports of mangroves, seagrass, and salt marshes. However, the time in between these surveys is quite large. Several of these surveys are based on the use of satellite-derived mapping of habitat, which has significant inaccuracies and spatial limitations, as 30–72 m resolution Landsat datasets have been used.

Table 2. Area in m^2.

	Bahrain	Iran	Iraq	Kuwait	Oman	Qatar	KSA	UAE	Total	Reference
Mangroves	580,000	65,240,000		580,000		9,970,000	10,360,000	78,760,000	165,500,000	[47]
	1,000,000	90,000,000	0	0	7,000,000	6,000,000	36,000,000	162,000,000	302,000,000	[48]
	4,000,000	89,000,000				9,000,000	204,000,000	40,000,000		[49]
		420500 (Qesham Island) [50]			11,000,000					[51]
Seagrass	920,000,000	2,120,000,000		55,000,000	363,000,000	883,000,000	789,000,000	1,630,000,000		[48]
								2,957,070,000 *		[52]
	500,000,000 to 1,000,000,000 [53]			2,000,000–3,000,000 + [54]	50,000,000 [55]	6,000,000–10,000,000 [56], 15,000,000–20,000,000 (Lusail) [57]	370,000,000 [58]	5,500,000,000 (Abu Dhabi) [58], 30,000,000 (Ruwais) [59], 500,000 (Das Island) [60], 70,000,000–80,000,000 (Mubarraz Island) [61], 11,100,000 (Umm Al Quwain) [62], 10,470,000 (Ras Al-Khaima) [62], 10,500,000 (Dubai) [62], 292,200,000 (Abu Dhabi) [62]	67,900,000,000–73,200,000,000 [63]	[63]
Salt marsh			262,000,000		21,000,000		2,000,000	51,000,000	336,000,000	[48]

* Sharjah = insignificant ~0; Ajman = insignificant ~0; Umm Al Quwain = 11,100,000 m^2; Ras Al Khaimah= 10,470,000 m^2; Dubai = 10,500,000 m^2; Abu Dhabi = 2,922,000,000 m^2. + = Found in three intertidal areas in Doha, Dbaiyyah, and Nuwaiseeb but not quantified.

Traditionally, the big three blue carbon ecosystems were mangroves, seagrasses, and salt marshes [11]. However, emerging research suggests that macroalgae may also play a significant, yet underappreciated, role in carbon sequestration [25].

A significant portion of the data reported in Table 2 has been mapped using satellite remote sensing, which offers a quick and cost-effective option to map and monitor blue carbon stocks in this region. Some of these estimates have used MODIS, Landsat, and Sentinel satellite data, which are significantly different in their spectral and spatial capabilities. Where MODIS provides data in a 250–1000 m resolution range, Sentinel provides data in 10–60 m, and Landsat usually is 30 m unless Landsat TM 5 or older data are used, which can be 72.5 m. On the other hand, new high-resolution satellites like WorldView-3/4 can map small mangrove and seagrass beds due to their 0.3 m spatial resolution. Hyperspectral satellites, like PRISMA and EnMAP, have not been used in the region, even though they have the capabilities to detect subtle spectral differences in vegetation health and sediment carbon. The most commonly used methodology in studies on the Gulf was the normalised difference vegetation index (NDVI), which is the ratio of red and infrared bands in satellite data. It is simple to use but still has serious limitations in mapping underwater seagrass beds, introducing remarkable uncertainties in estimates. On the other hand, the use of high-resolution satellite data like Sentinel-2 for mapping mangroves in Qatar reported an accuracy of >85%. In another study from Qesam, Iran's mangroves were mapped in the Hara protected area using Sentinel-1 and 2 images with reported accuracies of over 90% [64].

The Gulf region has utilised remote sensing technologies effectively to monitor mangrove ecosystems, evaluate forest- and tree-level characteristics, and analyse vegetation indices across varying spatial and temporal scales. There are 58 remote sensing-based studies published between 2010 and 2022 in the Gulf. Satellite imagery, particularly Landsat-derived NDVI (73.3%), was the predominant data source (75.2%), followed by IKONOS (15%), Sentinel (11.7%), WorldView (10%), QuickBird (8.3%), SPOT-5 (6.7%), MODIS (5%), and others like PlanetScope (5%). In contrast, aerial imagery (6.7%), LiDAR (5%), and UAV/drone data (3.3%) were less frequently utilised [65]. Based on these satellite-based

observations, it is observed that mangrove coverage declined in Saudi Arabia, Oman, Bahrain, and Kuwait, while a significant expansion was observed in Qatar between 1996 and 2020. The UAE maintained stable mangrove levels, largely due to government-led conservation efforts, including afforestation and restoration via seeding and planting initiatives. However, these datasets have high uncertainties even for mangrove quantification stemming from methodological inconsistencies, variations in satellite resolution, and classification techniques. Studies using advanced algorithms like Artificial Neural Network, Random Forest, Support Vector Machines (SVMs), and Deep Learning to classify blue carbon habitats from high-resolution satellite imagery have reported very high accuracies.

In this study, we have used eDNA-based approaches that have emerged as a powerful tool in the identification and characterisation of these ecosystems based on their genetic material. An added advantage of these techniques is that a physical specimen is not needed; rare and low-abundant species can be captured from below-water environments [66].

Diverse taxa belonging to phyla Cyanobacteria, Dinophyta, Dinoflagellates, Bacillariophyta, Haplophyta, Chlorophyta, Ochrophyta, Cryptophyta, and Euglenophyta are classified as phytoplankton. In the present study, the prevalence of Bacillariophyta and Chlorophyta was recorded. About 26 algae species were found. The reads ranged from 0 to 163 nucleotides per million (Figure 2). They are listed in Table 3 below with a brief description of their taxonomies. A total of 11 species were detected at all 20 locations, namely in order of abundance: *Aureococcus anophagefferens* > *Emilliania huxleyi* > *Clamydomonas reinhardtii* > *Micromonas commoda* > *Auxenochlorella protothecoides* > *Chlorella variabilis* > *Micromonas pusilla* > *Thalassiosira pseudonana* > *Chloropicon primus* > and *Phaeodactylum tricornutum*. The remaining were absent at some sites, with *Prolithon onkoides* found at only five locations. *Aureococcus anophagefferens* was the most dominant species with roles in the formation of harmful algal blooms.

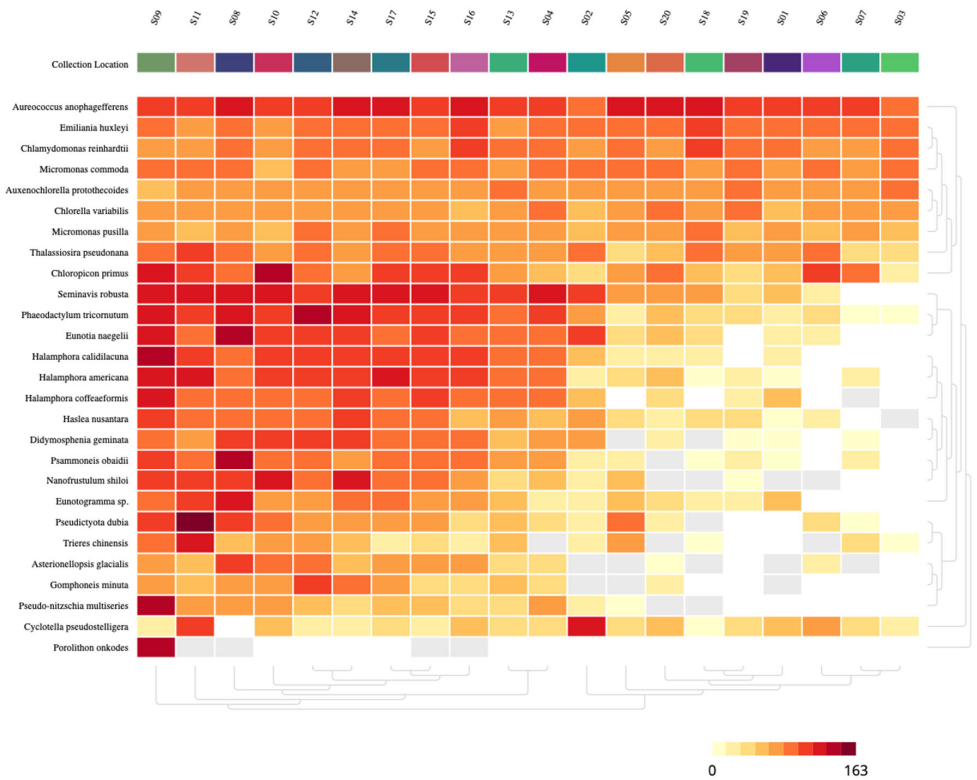


Figure 2. eDNA signatures of phytoplankton communities in marine sediments of Kuwait. Each coloured box marks the presence of the corresponding species listed on the left-hand side panel. The collection location is specified at the top of the heatmap. The colour coding is performed according to the number of reads per million, and a scale for this has been provided at the bottom right.

Table 3. Commonly found phytoplankton species in the sediments of Kuwait.

Species	Clade-Phylum	Possible Role/Cause
Aureococcus anophagefferens	Clade-Diaphoretickes; Phylum-Gyrista	Heterokont algae that cause algal blooms.
Emilliania huxleyi (now *Gephyrocapsa huxleyi*)	Clade- Diaphoretickes; Phylum-Haptista	Photosynthetic plankton that form algal blooms in nutrient-depleted waters. Release a group of chemicals known as alkenones.
Chlamydomonas reinhardtii	Clade- Viridiplantae; Division-Chlorophyta	Single-cell green phototrophic near surface. On oceanic floors, it grows in the presence of organic carbon.
Micromonas commode	Clade- Viridiplantae; Division-Chlorophyta	Green algae are a major contributor to picoplanktonic biomass in oceanic and coastal regions.
Auxenochlorella protothecoides (formerly known as *Chlorella protothecoides)*	Clade- Viridiplantae; Division-Chlorophyta	Facultative heterotrophic algae with a high lipid content and application in biofuel research and dietary supplements.
Chlorella variabilis	Clade- Viridiplantae; Division-Chlorophyta	Single-cell green algae are widely exploited as a source of food and energy.
Micromonas pusilla	Clade- Viridiplantae; Division-Chlorophyta	Green algae are and major contributor of picoplanktonic biomass in oceanic and coastal regions.
Thalassiosira pseudonana	Clade-Diaphoretickes; Phylum-Gyrista	Marine diatoms with high CO_2 absorption.
Chloropicon primus	Clade- Viridiplantae; Division-Chlorophyta	Green algae with key roles in phytoplanktonic communities.
Seminavis robusta	Clade- Stramenophiles; Phylum- Bacillariophyta	Marine biofilm-forming diatoms.
Phaeodactylum tricornutum	Clade-Diaphoretickes; Phylum-Gyrista	Marine diatoms capable of growing in absence of silicon.
Eunotia naegelii	Clade- Stramenophiles; Phylum- Bacillariophyta	Diatoms.
Halamphora calidilacuna	Phylum- Heterokontophyta	Diatoms.
Halamphora Americana	Phylum- Heterokontophyta	Diatoms.
Halamphora coffeaeformis	Phylum- Heterokontophyta	Diatom.
Haslea nusantara	Clade- Stramenophiles; Phylum-Bacillariophyta	Blue Diatoms.
Didymosphenia geminate	Clade-Diaphoretickes; Phylum-Gyrista; Class Bacillariophyceae	Invasive diatom species.
Psammoneis obaidii	Clade- Stramenophiles; Phylum- Bacillariophyta	Diatoms found in sediments or as epiphytes.
Nanofrustulum shiloi	Clade- Stramenophiles; Phylum- Bacillariophyta	Diatoms with high biotechnological potential.
Eunotogramma sp.	Clade- Stramenophiles; Phylum- Bacillariophyta	Diatoms.
Pseudodictyota dubia		Brown algae.
Trieres chinensis	Clade- Stramenophiles; Phylum- Bacillariophyta	Marine diatoms.
Asterionellopsis glacialis	Phylum- Heterokontophyta; Class- Bacillariophyceae	Diatoms.

Table 3. *Cont.*

Species	Clade-Phylum	Possible Role/Cause
Gomphoneis minuta	Clade- Stramenophiles; Phylum- Bacillariophyta	Diatoms.
Pseudo-nitzschia multiseries	Clade- Stramenophiles; Phylum- Bacillariophyta	Marine planktonic diatoms account for 4.5% of the pennate diatoms found across the world.
Cyclotella pseudostelligera (now *Discostella pseudostelligera*)	Phylum- Heterokontophyta; Class- Bacillariophyceae	Diatoms.
Porolithon onkodes	Phylum-Rhodophyta	Red algae.

4. Discussion

The assessment of blue carbon in the Gulf region is of paramount importance since the land vegetation cover in this extremely hot and arid region is limited, and the Gulf waters with mangroves, seagrass meadows, salt marshes, and high oceanic productivity take up a significant quantity of CO_2 and store carbon in the Gulf sediments. Despite the importance of the blue carbon in the region, not many systematic assessments have been carried out recently. The use of satellite data to map the spatio-temporal coverage of different blue carbon components has serious limitations for use in seagrass mapping. For mangrove mapping, a standard approach using high-resolution satellite data should be adopted to reduce uncertainty in mangrove mapping for blue carbon assessment in the Gulf.

A significant challenge in incorporating macroalgae into blue carbon frameworks lies with the perception that it does not lead to permanent burial of carbon, apart from their vast phylogenetic and ecological diversity, which contrasts sharply with the more uniform foundation species of salt marshes, seagrasses, and mangroves. Macroalgae are a polyphyletic group distributed across the planet, Chromista, and bacterial kingdoms, spanning four phyla (Rhodophyta, Phaeophyta, Chlorophyta, and Cyanophyta) and approximately 60 orders [67–70]. The divergence in the macroalgae also brings in substantial morphological and functional variability that influences carbon (C) dynamics [71].

Macroalgae display a continuum of life-history strategies, ranging from structurally complex, slow-growing K-selected forms such as kelps and Fucales, to fast-colonising, opportunistic r-selected species like *Ulva lactuca* [72]. The thick, resistant tissues of K-selected macroalgae, which are comparatively low in nutrients, decompose slowly, thereby promoting greater long-term carbon sequestration [25,73]. Kelp forests, for example, export approximately 82% of their primary production [74], at a rate notably higher than the ~43% average for macroalgae in general [75,76]. Similarly, crustose coralline algae (Rhodophyta), another K-selected group, exhibit high degradation resistance, though their net carbon storage requires consideration of the CO_2 released during calcification [77].

The carbon production and storage in blue carbon habitats are affected by the morphology of the thallus. The photosynthetic efficiency [78], growth rates [79], and even decomposition rates [80] are dependent on the thallus morphology. The type of algae is also different vertically, with dominance of green algae at the surface and red coralline algae typically dominating in deeper regions due to their lower light requirements than other macroalgae [81].

The likelihood of macroalgal carbon reaching the bottom sediments for long-term sequestration depends on their buoyancy, i.e., Fucales and Laminariales are buoyant species with extensive spatial coverage over vast oceans, enhancing the chances of deep-sea carbon burial [82,83]. In contrast, r-selected algae decompose rapidly, limiting their carbon sequestration potential [6,7]. Despite the evidence that macroalgae contribute to blue carbon stocks, their high taxonomic variability and diverse characteristics result in

high uncertainty in carbon storage estimates compared to vascular plants [23,84]. The mangroves and seagrasses store the carbon in situ, whereas macroalgae are transported to long distances, hence they are likely to sequester carbon in deep-sea sediments and offshore ecosystems [25]. It can also involve a short transport period and quick burial of microalgae in coastal and shallow areas, as we are witnessing in the case of the Gulf. The spatial coverage of macroalgae is vast; it is estimated to cover 3.4 million km^2 of coastal habitats globally, and contributes roughly 1.5 Pg C yr^{-1} to net primary production [25]. Some estimates suggest 11–56% of macroalgal carbon is buried in marine sediments, which makes it a significant quantity to be considered in blue carbon assessments [66].

The evidence of microalgae contributing up to 60% carbon in seagrass meadows and mangrove sediments has not validated its inclusion in the blue carbon assessment globally, except for China and South Korea, where seaweed aquaculture is part of national blue carbon programmes. All the scientific evidence of macroalgal burial in sediments proves beyond doubt that they are permanently capturing carbon in sediments. It is now up to the research community to devise mechanisms for how to incorporate it into carbon accounting and blue carbon frameworks. Due to the diversity of micro- and macroalgae, providing empirical value would be inappropriate, but leaving it unaccounted for would underestimate any blue carbon assessment. Their inclusion would aid in assessing the precarious balance in climate change mitigation and adaptation strategies. The fact that macroalgae, including kelps and brown seaweeds, cover vast coastal areas globally, with kelp forests alone spanning 20,000–400,000 km^2, their preservation by limiting bottom trawling and even developing seaweed farms in China spanning over 1250 km^2 offers enhancement of the macroalgae carbon de-sequestration potential while reducing pressure on wild stocks.

The reports on phytoplankton indicate that they are photoautotrophs transporting ~5–12 Pg C yr^{-1} to the deep ocean [76], which is significant when considering their role in oceanic carbon sequestration. Though not traditionally classified as blue carbon, their role in carbon export is critical for climate regulation [77]. Recent models suggest that phytoplankton contributions could double previous blue carbon estimates if their offshore sequestration pathways are included [78]. For instance, seaweed farming alone may sequester up to 0.17 Pg C yr^{-1} if managed for carbon removal [79].

5. Conclusions

While microalgae, including phytoplankton, benthic microalgae, and other unicellular algae, play a crucial role in global carbon cycling, their inclusion in blue carbon assessment is not accounted for, probably as their sequestration in bottom sediments is difficult to quantify, primarily due to large spatial dispersal [6,24]. Studies have shown microalgae's contribution to carbon in coastal sediments in Malaysia [74]. Prasad et al. [75] gave exhaustive evidence of CO_2 uptake by microalgae, and our findings of microalgae from benthic sediments provide evidence of their burial and contribution to the carbon stocks in the Gulf.

Recently, metagenomic analysis has been recognised as an effective tool to monitor marine organisms [85]. In this study, we have employed the shotgun method, which sequences the entire DNA, whereas targeted metabarcoding, which has been reported earlier, specifically picks phytoplankton species. The lineages of macroalgae—encompassing Rhodophyta (red algae), Chlorophyta (green algae), and Phaeophyceae (brown algae)—span a vast phylogenetic diversity [86] and exhibit a wide array of metabolic pathways for carbon fixation [87]. Metagenomics offers a precise method for identifying unique macroalgal sequences; some have used 18S-V7 sequencing to identify eDNA from macroalgae in coastal sediments [66], while we preferred to use the Shotgun method for its ability to

screen the entire DNA present. However, current limitations in barcode reference libraries pose significant challenges to accurate macroalgal DNA identification [88–90].

The targeted metabarcoding is likely to introduce PCR biases, and species-level taxonomic identification will be spurious. Shotgun metagenomics is more cost-intensive than targeted metabarcoding, but gives much more detailed information. However, a large dataset in shotgun sequencing will remain unidentified as not enough phytoplanktonic species have been discovered and are available in the published dataset [85]. The targeted metabarcoding will divert all unidentified phytoplankton into unclassified taxa. A study examined the diatom species composition in Sendai Bay through whole metagenome sequencing and compared it with morphological methods. The group discovered the former as a powerful tool for taxonomic identification and precisely picking changes in species composition that could not be captured by the latter [85,91,92].

Despite these constraints, our environmental DNA (eDNA) analyses—though restricted by the relatively limited reference database (8896 macrophyte taxa)—successfully detected 26 distinct species in blue carbon sediments, many of which were not observed in contemporary field surveys. This finding supports the growing literature emphasising the necessity of incorporating macroalgae into future blue carbon assessments and the usefulness of using NGS to assess carbon contribution from phytoplankton within the marine sediments. The information generated can be useful to underpin the evidence that phytoplankton contribute to the permanent burial of carbon in bottom sediments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su18021102/s1, Table S1: Sampling locations and physico-chemical parameters.

Author Contributions: Conceptualization, S.U.; Methodology, S.U.; Software, N.H.; Formal analysis, N.H.; Investigation, S.U., N.H., M.B., M.F., Y.A.-B., S.A.-R., M.A.-S. and G.A.-Q.; Resources, M.B., M.F., Y.A.-B., S.A.-R., M.A.-S. and G.A.-Q.; Data curation, M.B. and M.F.; Writing—original draft, S.U. and N.H.; Writing—review & editing, S.U.; Supervision, S.U. All authors have read and agreed to the published version of the manuscript.

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