



## Carbon sequestration in macroalgal mats of brackish-water habitats in Indian Sunderbans: Potential as renewable organic resource

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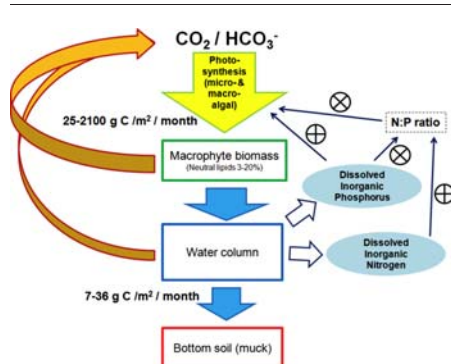
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### HIGHLIGHTS

- Coastal brackish-water sites in Sunderbans have high algal diversity seasonally.
- High P content and low N:P ratio in water positively affect biomass carbon content.
- Most sites were eutrophic for P.
- Very low proportions of biomass OC are buried in muck.
- Different algal taxa are suitable as biodiesel and nutraceutical feedstock.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Large influx of excess nutrients into sub-tropical brackish-water habitats is expected to radically affect the algal populations in the heavily populated Sunderbans brackish-water ecozone. Twelve selected brackish-water sites in the Indian Sunderbans were surveyed to investigate the growth performance of mat-forming dominant algal/cyanobacterial macrophytes and their potential for carbon (C) sequestration into hydrologic and pedologic pools. The mats were dominated by particular taxa at different seasons related to physico-chemical properties of the wetland habitats. Different environmental variables and biomass productivity parameters were measured on fortnightly basis to assess the carbon cycle related to dominant algal blooms of the study area. The dominating species at the twelve sites included seven genera (*Spirogyra*, *Rhizoclonium*, *Ulva*, *Cladophora*, *Pithophora*, *Chaetomorpha*) belonging to Chlorophyta, three genera (*Polysiphonia*, *Gracilaria*, *Catenella*) belonging to Rhodophyta and *Lyngbya majuscula* from cyanobacteria. Multivariate statistical methods indicated that nutrient availability, particularly dissolved P concentration and N:P ratio in the water column, along with salinity in the water column mainly affected biomass yield and C sequestration of mat-forming macrophytes and OC input into water column. However, OC contents of underlying muck proved to be very stable, though small influxes of OC occurred at each bloom. High biomass yields ( $34\text{--}3107\text{ g/m}^2$ ) of the dominant mat components accumulated enormous stocks of OC, very little of which reaches the pedologic pool. This transient biomass might be utilized as dietary supplements or biofuel feedstocks. Availability of important dietary fatty acids in *Spirogyra punctulata*, *Gracilaria* sp., *Polysiphonia mollis*, *Rhizoclonium riparium*, *R. tortuosum*, *Pithophora oedogonia* and *Ulva lactuca* was considered as suitability of these species as nutraceuticals. Fatty acid compositions of *L. majuscula*, *Catenella repens*, *R. tortuosum* and *Cladophora crystallina* were estimated to be applicable for producing biodiesel for usage in sub-tropical climates.

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## 1. Introduction

The world's oceans together form a global sink for atmospheric CO<sub>2</sub> – estimated to absorb  $2 \pm 0.8$  Pg carbon (C) annually (Behrenfeld et al., 2002; Lal, 2008). The chief mediators of this process are marine vegetation, especially algae, which are responsible for the storage of 71% of the oceanic C – termed “blue carbon” (Nellemann et al., 2009; Chung et al., 2011). Algal growth in marine habitats is subject to local and seasonal variations in nutrient availability and other physico-chemical factors, and in its turn regulates oceanic C sequestration through organic matter production and processing (Mukai et al., 1985; Pregnall and Rudy, 1985; Choudhury and Pal, 2010; Daines et al., 2014). High rates of C exchange take place at the air-water interface where algae grow rapidly to form blooms of floating and epiphytic mats (Chung et al., 2011). Algal mats absorb inorganic carbon (IC) present as either CO<sub>2</sub> in the atmosphere or HCO<sub>3</sub><sup>-</sup> in water, and convert it to organic carbon (OC) which is almost 42–80% of their biomass (Allen and Spence, 1981; Fogg, 1983). However, some of this fixed OC – mostly in the form of polysaccharides – is released by algal cells and form a large portion of dissolved organic carbon (DOC) in the water column (Engel et al., 2004). Eutrophic conditions encourage the exudation of a larger proportion of DOC than oligotrophic conditions would. At the end of growing period, algal biomass sinks as an amorphous aggregate of living and dead cells, excretory products and detritus matter forming a rich source of particulate organic carbon (POC) – a phenomenon known as “marine snow”, which enriches the bottom soil or muck. In deep water ecosystems, POC is ultimately transported from offshore to deeper waters, having a direct role in the biogeochemical cycling of C and other associated nutrients such as N and P (Wetz et al., 2008). The POC is gradually buried in the bottom sediments by a “biological pump”, while DOC is either degraded by the activity of heterotrophs in the water column or undergoes flocculation and deposition at the bottom of the water body along with POC (von Wachenfeldt and Tranvik, 2008). Subsequent upwelling of the buried C acts as a feedback system increasing the strength of primary productivity (Fowler and Knauer, 1986).

Compared to oceanic ecosystems, shallow lakes and peat-lands absorb even higher amounts of atmospheric carbon, almost the whole of which is subsequently deposited at the bottom in the form of POC, and after complete decomposition and transformation, is added to the bottom soil or the pedologic OC pool (Dean and Gorham, 1998). Fresh-water microalgae have been reported to exhibit almost 100% efficiency in fixing CO<sub>2</sub> from air (Ramaraj et al., 2014, 2015). Following from all evidence, tropical brackish-water systems at the coastal borders between land and oceans is inferred to exhibit high C sequestration rates due to seasonally variable algal/cyanobacterial (ACB) populations. Extensive mangrove swamps and tidal mudflats of the Indian Sunderbans (21° 31'N – 22° 53'N and 88° 37' – 89° 09'E) – a part of Ganga-Brahmaputra deltaic system – form an economically important ecosystem mainly used for fish production and sewage treatment. An enormous flux of nutrients, including nitrogen (0.49 mol/m<sup>2</sup> mangrove) and phosphorus (0.043 mol/m<sup>2</sup> mangrove), passes through this region annually due to flow of Hooghly river alone, which sustains very high levels of microbial population and diversity in the sediments of this region and yet causes such low water clarity in the deltaic rivulets as to maintain a predominantly heterotrophic ecosystem (Biswas et al., 2004; Mukhopadhyay et al., 2006; Ramanathan et al., 2008). However, phytoplankton populations here go through seasonal blooms which photosynthesize at high rates and thus enhance net absorption of atmospheric CO<sub>2</sub> by the aquatic ecosystem. Moreover, proximity of brackish-water bodies in this region to human habitations increases the chance of extensive eutrophication leading to vigorous ACB growth (Manna et al., 2010). Previous studies in mangrove communities have shown that local vegetation, microbiota and seasonality significantly influence OC contents and C fluxes in the water column and underlying sediments (Ray and Shahraki, 2016; Ouyang et al., 2017). Even the distribution of OC in ACB biomass components, particularly fatty acids (FAs), is subject to

seasonal changes (Sushchik et al., 2010; Venkatesalu et al., 2012; Polat and Ozogul, 2013). The present investigation was aimed at studying the environmental constraints on floating ACB macrophyte populations at several shallow brackish-water sites across the Indian Sunderbans and their role in C sequestration process. Also, the extensive growth of dominant macrophyte taxa provided an incentive to determine their potential as sustainably harvestable biodiesel or nutraceutical feedstock. Our data showed that utilizing the large quantities of macrophyte biomass as a renewable resource is a feasible alternative for carbon footprint mitigation.

## 2. Materials and methods

### 2.1. Geographical distribution of sampling sites

Twelve brackish-water sites were chosen across the Indian Sunderbans (21° 31'N – 22° 53'N, 88° 37'E – 89° 09'E) for sampling of ACB communities. All of these sites were located outside the protected regions of the Indian Sunderbans. The twelve sites lay between 22°01.131'N and 22°30.334'N latitudes, and between 88°39.647'E and 88°52.857'E longitudes. The twelve sites were assigned codes corresponding to relative distances in northerly and easterly directions, formatted as N[X]E[X] (Table 1). For example, the westernmost and easternmost sites would be coded E1 and E12 respectively, while the southernmost and northernmost sites would be coded N1 and N12 respectively.

### 2.2. Collection, preparation and identification algal samples

ACB samples were collected from twelve experimental sites in the brackish-water zone of the Indian Sunderbans and the locations were recorded (Table 1). Sampling was performed twice a month, at 15 days intervals, for two consecutive years (December 2013 – November 2015). No endangered species were involved in the sampling process. Collections were done with the permission from Office of the Directorate of Forests, Government of India. At each sampling site, one square meter area was demarcated and the biomass collected for the estimation of yield of algal biomass. Samples were collected in air-tight transparent plastic bags and kept on ice to prevent decomposition during transportation. In the laboratory, the samples were washed with excess water to get rid of epiphytes and sand particles. The algal samples were identified with proper monographs (Smith, 1950; Desikachary, 1959; Prescott, 1962; Krishnamurthy, 2000), voucher specimens were maintained and assigned to Calcutta University Herbarium (CUH).

### 2.3. Analysis of ACB biomass

Each ACB sample was oven-dried separately at 60 °C so as to obtain constant weight (Chinnasamy et al., 2010; Gorain et al., 2013), which was noted as dry weight of the sample. The different taxa in each sample were identified and their biomass proportions were determined as percentage dry weight (%dw) of total sample. The dried biomass of the dominant taxa from each site was then analyzed for oxidizable organic carbon (OC) content (Nelson and Sommers, 1982), total carbohydrate content (Johanson, 1953), total protein content (Lowry et al., 1951) and total lipid content on dry cell weight (%dcw) basis. Lipids were extracted and quantified according to gravimetric method described by Bligh and Dyer (1959). The FA components of the lipid fraction were identified and quantified after conversion to methyl esters by the method described by Barman et al. (2012).

### 2.4. Collection and analysis of soil samples

Soil samples from the bank and the muck below the ACB mat were collected in transparent plastic bags and sealed. These samples were

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