Heterogeneity of water quality signature and feedbacks to carbon sequestration in wetlands across some districts of West Bengal, India

ABSTRACT
Water quality measurements can indicate carbon status or algal biomass. Microalgae have an excellent ability to utilize all forms of dissolved inorganic carbon at different pH conditions. Water quality signature (WQS) using three different expressions with (i) pH; (ii) total alkalinity, hardness and total dissolved solids; and (iii) nitrate and conductivity of water was assessed in 32 wetlands distributed across 5 districts of West Bengal, India. Two zones were clearly discernible: coldwater (15–23 °C) high-altitude lakes in Darjeeling, and tropical (31–32 °C) low-altitude wetlands. Multivariate analysis of the Akaike information criterion (ACI) model revealed location-specific variability of agro-climatic and biogeochemical interactions. Dissolved inorganic carbon and inorganic nitrogen appeared to be important in regulating the phytocarbon content of microalgae. The wetlands located in the Gangetic alluvial tropical or semi-coastal areas (Hooghly, 24-Parganas, Nadia, Midnapore) were alkaline (pH = 7.52–7.97) where half-bound carbon dioxide comprised the major component (18–26%) of total inorganic carbon, with moderate to eutrophic (PO4-P: 0.16–0.23 mg/l) states which have a negative feedback to global warming. The heterogeneity of measured water quality signature consolidated the sanative nature of wetlands for their complex functional attributes with agro-climatic, biogeochemical and soil-water-biological interactions.

Key words | climate change, trophic state, water quality signature, wetlands

INTRODUCTION
Wetlands, covering nearly 5–8% of the terrestrial landscape, form a heterogeneous spectrum of aquatic habitats, water resources, hot spots of biodiversity, mitigation of flood, pollution and global warming, and coastal protection as well as providing a wide range of ecosystem services especially for the livelihood of millions in the tropical world. As a result of well defined ecological integrity among the component members of the ecosystem, wetlands have excellent self purification ability and a resilience to climate change. Though there are different suggested measures for challenging carbon sequestration and global warming, biological carbon sequestration or photosynthesis has become an alternative low cost, eco-friendly and sustainable tool to arrest carbon dioxide (CO2) from the atmosphere in a challenge to global warming. Algae have adopted two primary strategies in photosynthetic CO2 fixation by maximizing their performance through ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) which includes either the development of a CO2-concentrating mechanism (CCM) based at the level of the chloroplast, or the evolution of the kinetic properties of Rubisco. In general, Rubisco enzymes from algae have evolved a higher affinity for CO2.
when the algae have adopted a strategy for CO\textsubscript{2} fixation that does not utilize a CCM (Badger \textit{et al.} 2002).

Simultaneous carbon evasion/emission and sequestration in a water body is an enigma of nature. Wetlands are of special interest in the global cycling of carbon due to their vital role as a sink or source of carbon to the atmosphere. The net sinks for carbon in different wetlands have been estimated to be at rates up to 3 g C/m\textsuperscript{2}/d (Zhou \textit{et al.} 2009). The Antarctic peat lands in Siberia have been a major sink for atmospheric carbon since the last deglaciation (Kirpotin \textit{et al.} 2009). Hence, the world’s wetlands are significant sinks of carbon on the order of 830 Tg/year (Mitsch \textit{et al.} 2015). However, high rates of anaerobic microbial respiration of sedimentary pools of organic carbon under reduced and saline conditions have negative feedback to climate change by emitting CO\textsubscript{2} to the atmosphere (IPCC 2014). So, the differences between community assimilation and respiration as well as high rates of anaerobic microbial respiration in micro-oxic niches within the rhizosphere determine the net balance between opposite CO\textsubscript{2} fluxes (Zhou \textit{et al.} 2009). Notwithstanding, the manifold positive impacts of wetlands on the environment have led to arguments that wetland should not be destroyed or restored because of greenhouse gas (GHG) emissions under limited conditions (Mitsch \textit{et al.} 2015).

The feedback mechanisms regulating the stability or homeostasis of the wetland ecosystem are attributed to their primary, secondary and microbial subsystems that are interconnected and work in a coordinated network system. Anthropogenic climate change would bring out structural and functional changes in the hierarchical ecosystem, hydrology, physico-chemical milieu, and biotic components (Duran-Encalada \textit{et al.} 2017). Water quality of Lake Tana (Ethiopia) was evaluated using measurements and Landsat Images, and both linear and non-linear regression models between water quality parameters and reflectance of Landsat 7 ETM+ images were developed (Moges \textit{et al.} 2017).

A wide range of modelling techniques, such as knowledge-driven, data-driven, artificial intelligence approaches, artificial neural network (ANN)-based rainfall-runoff (R-R) models, WANN (Olyaie \textit{et al.} 2015), satellite images from Landsat 5, 7, 8 MODIS, and AVHRR, etc. (Friedl \textit{et al.} 2010), have been deployed over the past few decades for predicting R-R in hydrology (Wu & Chau 2011) or estimating suspended solid load (Alizadeh \textit{et al.} 2017) or characterization of persistent organic pollutant (Chau 2005). However, hardly any model has focused on water quality to predict the climate change scenario on wetlands.

Altitude and temperature dependent solubility of atmospheric carbon dioxide plays a key role in the buffering mechanism of water bodies (Wetzel 2005). Carbon dioxide reacts with water; the carbon atom of CO\textsubscript{2} is electron poor with an oxidation state of IV. The electron rich oxygen of water donates an electron pair to the carbon. After proton transfer from water to oxygen of the CO\textsubscript{2} unit, carbonic acid is formed. The chemical transformations of dissolved atmospheric carbon dioxide involves the formation of carbonic acid and then into bicarbonate and hydrogen, accompanied by lowering of pH, a situation which occurs when CO\textsubscript{2} is dissolved in water.

It is relevant to conceive that the changes in the physico-chemical properties of water are contemplated with the impact of climate change on water bodies. Again, tropical wetlands differ from temperate large and deep lakes in the framework of ecological characteristics; the former are more sensitive and reactive than the latter which encounter serious hypolimnetic oxygen depletion due to insufficient overturning, a result of global warming (Livingstone 1997) rather than an increase in organic matter related to eutrophication (Kumagai \textit{et al.} 2005).

In wetlands, water quality, nutrient and temperature regulated microalgal productivity often varies in different geographical locations, and would impact on carbon turnover from atmosphere to water bodies. As microalgae have an excellent ability to utilize all forms of inorganic carbon, such as free CO\textsubscript{2}, CO\textsubscript{3} and HCO\textsubscript{3} from water bodies of different pH conditions, assessment of water quality parameters would be of considerable use as a signature molecule of carbon sequestration. The CO\textsubscript{2}, CO\textsubscript{3}, HCO\textsubscript{3} system acts as a buffer and as a source of carbon for the photosynthetic microalgae at different pH conditions. The process of photosynthesis by algae and plants uses hydrogen, thus increasing pH levels (USGS 2013); respiration and decomposition, on the other hand, can lower pH levels. Most bodies of water are able to buffer these changes due to their alkalinity (Murphy 2007) and pH and alkalinity.
are directly related when water is at 100% air saturation. Though the measures for carbon sequestration of alkaline residues discharged from industrial wastes have been suggested to recover metals such as vanadium, cobalt, lithium and rare earths (Gomes et al. 2016), the long-term solution to high pH problems in ponds is to alter pond biology so that the net daily carbon dioxide uptake is near zero.

Climate-change driven ecosystem functioning, carbon sequestration and carbon sinks in sediments of temperate large and deep lakes are increasingly apparent (Mitsch et al. 2010), while the insights into the ecological effects of climate change have hardly been addressed in tropical small and shallow wetlands (Pal et al. 2017).

The state of West Bengal has a large number of wetlands concomitant with its unique geography and diverse agrogeo-climatic features. This provides an excellent opportunity to assess the carbon status and major nutrient-dependent chlorophyll and phyto-carbon content of microalgae in different wetlands under high altitude and cold, and low altitude and tropical conditions across the state of West Bengal, India. This research stemmed from the stimulus of the clear gap in understanding on the use of water quality as a signature molecule for the carbon status of wetlands. The purpose of the study was, therefore, to survey the water quality and chlorophyll content of microalgae in wetlands distributed in different agro-climatic zones under different geographical locations across different districts of West Bengal and to focus on water quality signature of different agro-climatic-nutrient dependent wetlands of West Bengal. In situ enclosure experiments were also performed in two wetlands differing in their trophic state and microalgal population to validate the impact of simulated green house conditions on chlorophyll content of microalgae and vice versa. This would help predict the potential to challenge the current global warming scenario and management required thereof. Thus, measurement of water quality, especially inorganic carbon, would indicate the carbon status or algal biomass. In other words, water quality monitoring is more pertinent for assessing a vast number of water bodies for their inorganic or organic carbon status, because the nutrient load-dependent chlorophyll content of microalgae and carbon footprint of wetlands imitate the overall functioning of wetlands.

**MATERIALS AND METHODS**

India (lat. 20.5937°N and 78.9629°E) is a vast country experiencing three major climatic groups, namely tropical rainforest, dry and subtropical humid climate. It is characterized by relatively low seasonal variations in atmospheric temperature and day length except in high altitude areas in the Himalayas (Jana 1998). Situated at its eastern side adjacent to Bangladesh, West Bengal is the only state in India with higher peaks of the Himalayas in the northern extremes and sea or coastal regions at the south, with both plains and plateaus and the Gangetic Delta intervening in between. Depending on soil and climate variations, West Bengal can be divided into six broad divisions: the hill region in the north, the Terai and Teesta alluvial region of North Bengal, the lateritic, red and gravelly undulating region in the west, the coastal alluvial region in the south and the Gangetic alluvial region in the west.

The climate of West Bengal varies from tropical savannah in the southern portions to humid subtropical in the north. With regard to temperature, the hill region in the north is distinctly cooler (Table 1) than the remaining plain areas. The main seasons are summer, rainy season, a short autumn, and winter. The average rainfall ranged from 8.94 to 562.22 mm in different districts of West Bengal (Table 2). While summer in the delta region is

**Table 1** Average monthly air temperature (°C) for 2011–2014 in different geographical locations of West Bengal, India

<table>
<thead>
<tr>
<th>Months</th>
<th>Darjeeling</th>
<th>Nadia</th>
<th>Hooghly</th>
<th>Midnapore</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>11.75</td>
<td>22</td>
<td>23</td>
<td>23.25</td>
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<tr>
<td>February</td>
<td>15</td>
<td>27.25</td>
<td>26.75</td>
<td>27</td>
</tr>
<tr>
<td>March</td>
<td>19.25</td>
<td>31.5</td>
<td>31.75</td>
<td>32.5</td>
</tr>
<tr>
<td>April</td>
<td>22.5</td>
<td>35</td>
<td>35</td>
<td>36.75</td>
</tr>
<tr>
<td>May</td>
<td>24</td>
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<td>36.75</td>
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<td>24.5</td>
<td>34</td>
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<td>36</td>
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<td>July</td>
<td>23.5</td>
<td>31.25</td>
<td>32</td>
<td>32.25</td>
</tr>
<tr>
<td>August</td>
<td>23.5</td>
<td>30.75</td>
<td>30.75</td>
<td>30.75</td>
</tr>
<tr>
<td>September</td>
<td>23.25</td>
<td>30.5</td>
<td>30.75</td>
<td>30.5</td>
</tr>
<tr>
<td>October</td>
<td>20.25</td>
<td>29.25</td>
<td>29.5</td>
<td>29</td>
</tr>
<tr>
<td>November</td>
<td>16.25</td>
<td>27</td>
<td>27</td>
<td>26.5</td>
</tr>
<tr>
<td>December</td>
<td>13.25</td>
<td>24.25</td>
<td>24.75</td>
<td>23.5</td>
</tr>
</tbody>
</table>
noted for its excessive humidity, the western highlands experience a dry summer with the highest day temperature ranging from 38 to 45 °C. The average minimum temperature of 15 °C is common over the plains. However, the Darjeeling Himalayan Hill region experiences a harsh winter, with occasional snowfall at places.

**Study area**

Extensive surveys and all measurements were performed in 32 wetlands located in five districts with diverse agro-climatic regions of West Bengal (Figure 1). Ten ponds located in Gangetic alluvial zones in both Hooghly and Nadia, five ponds located in coastal regions of the Bay of Bengal in Midnapore and four cold-water lakes in Hilly Darjeeling were selected for investigation during the period of August 2011 to March 2014. The water area of these wetlands ranged from 0.5 to 16.9 ha with a mean depth from 1.5 to 8 m and other physiographic features (Table 3). All the perennial wetlands have different levels of productivity and are used mostly for fish farming except the four sites of coldwater Mirik Lake in Darjeeling which are popular sites of tourism, recreation, sports, boating, jogging, etc. Mirik Lake in the undulating highland is one of the famous hill resorts in the Darjeeling Himalaya at an altitude of 1,767 m. It is an artificial reservoir of Mirik town and was constructed in 1979. The lake margin is dominated by sand, grain and pebble. A dense forest of alpine trees (*Cryptomeria japonica*) covers the south east part of the lake. Considering the vast area (>16 ha) and different physiographic features, four distinct sites were selected and designated as lake-1, lake-2, lake-3 and lake-4.

**In-situ limnocorral study**

**Experimental**

Two wetlands with contrasting ecological characteristics were selected for the study, both were located within a 5 km radius of the University of Kalyani. Of the two wetlands, one (K R Steel Beel – KRS Beel) was large, moderately mesotrophic and fed by rain and sometimes by treated effluents from some industries including a brewery, and the other one was fed by partially treated sewage effluents and was highly eutrophic with a dense population of blue green algae.

Six limnocorals (1.5 m dia) were placed in the two wetland system in such a way that at least 50 cm top end of the limnocorals was exposed to atmospheric air over the water surface. The top of 3 limnocorals were covered with transparent polythene that mimic a solar heated greenhouse structure, whereas the remaining 3 limnocorals were open on the top and served as control. The open bottom of the limnocorals was deeply entrenched in the pond bottom so that pond mud inside the limnocoral was in direct contact with its overlying water.

**Sample collection and analysis**

Samples of surface water were collected from each of the limnocorals placed in the two local wetlands (KRS Beel, and sewage-fed pond) and 32 regional wetlands (ten each of Hooghly – HG and Nadia – ND, five in 24 Parganas – 24 P, three in Midnapore – MP and four in Darjeeling – DJ) at different time intervals during the course of the investigation (August 2011–March 2014) and analyzed for different water quality parameters (temperature, pH, free CO₂, CO₃, total alkalinity, hardness, dissolved oxygen, phosphate-P ammonia-N, nitrite-N, nitrate-N and conductivity) following the standard methods described in American Public Health Association (APHA 2005). Chlorophyll content of microalgae was estimated according to the method of the Environmental Protection Agency (EPA 1991). For estimation of chlorophyll content of microalgae, collected water samples were vacuum filtered through a finer grade of glass fibre filters and transferred into an airtight glass stoppered corning tube.
Into this was added 5 ml of 90% acetone (aqueous mixture) and the filter paper and this was completely ground to obtain the chlorophyll extract. The tube was refrigerated for 24 hours after which the suspension was centrifuged for 10 minutes and the clear supernatant solution was transferred into a 1 cm cuvette. The optical density of the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hooghly (HG)</th>
<th>24 Parganas (24 P)</th>
<th>Nadia (ND)</th>
<th>Midnapore (MD)</th>
<th>Darjeeling lakes (DJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of ponds</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Water area (ha)</td>
<td>0.25–0.75</td>
<td>0.30–1.20</td>
<td>1.0–2.5</td>
<td>0.5–1.0</td>
<td>3.5–8.5</td>
</tr>
<tr>
<td>Water depth (m)</td>
<td>1.5–3.0</td>
<td>1.0–3.0</td>
<td>1.5–2.5</td>
<td>1.75–3.0</td>
<td>1.0–8.0</td>
</tr>
<tr>
<td>Atmospheric</td>
<td>29.80–34.60</td>
<td>32.60–34.50</td>
<td>29.10–34.80</td>
<td>30.10–34.80</td>
<td>20.3–26.3</td>
</tr>
<tr>
<td>temperature (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Altitude (m)</td>
<td>18</td>
<td>13</td>
<td>13</td>
<td>6</td>
<td>1,767</td>
</tr>
<tr>
<td>Annual rainfall (mm)</td>
<td>1,311</td>
<td>1,354</td>
<td>1,250</td>
<td>1,500</td>
<td>3,092</td>
</tr>
<tr>
<td>Nature of soil</td>
<td>Gangetic alluvial</td>
<td>Vindhya alluvial</td>
<td>Gangetic alluviumlateritic, red and gravelly</td>
<td>Alluvial loamy</td>
<td>Alluvial</td>
</tr>
</tbody>
</table>

Figure 1 | Map of land use land cover (50 K): 2005–06 showing the locations of wetlands in different districts of West Bengal, India: (a) – Darjeeling (DJ), (b) – Nadia (ND), (c) – Midnapore (MD), (d) – 24 Parganas-N (24-P) and (e) – Hooghly (HG) (source: http://bhuvan.nrsc.gov). (key: red/orange – built up; yellow – agriculture; green – forest, grass or grazing; purple – barren, unculturable, wasteland; blue – wetlands, waterbodies. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wcc.2019.102.

Table 3 | Physiographic features of wetlands surveyed from 5 districts of West Bengal

for 24 hours after which the suspension was centrifuged for 10 minutes and the clear supernatant solution was transferred into a 1 cm cuvette. The optical density of the
chlorophyll extract of phytoplankton was measured at 665 nm in a Shimadzu UV Spectrophotometer (model UV 1601) using 90% acetone as blank. Then 2 drops of 1 N HCl acid was added to the cuvette containing the extract and the entire solution was shaken, after which the optical density of this solution was again determined at the same wavelength in the Spectrophotometer. The results were expressed in mg/m³ using the formula of Strickland & Parsons (1968).

**Water quality signature**

Assessment of water quality signature (WQS) was based on the standard values of pH, total inorganic carbon (free carbon dioxide, carbonate and bicarbonate), total dissolved solids as well as nutrient loads (phosphate, nitrate and specific conductivity) recommended for fish culture (BIS 1983, 1993; WHO 1992). The weighted arithmetic index methods (Brown et al. 1972) were followed for the calculation of WQS for inorganic carbon and nutrient load separately. Quality rating or sub index \( q_n \) was calculated using the following expression:

\[
q_n = 100 \left( \frac{V_n - V_{io}}{S_n - V_{io}} \right)
\]

where \( q_n \) = quality rating for the \( n \)th water quality parameter; \( V_n \) = estimated value of the \( n \)th parameter at a given sampling site; \( S_n \) = standard permissible value of the \( n \)th parameter; \( V_{io} \) = ideal value of the \( n \)th parameter in pure water.

Unit weight was calculated by a value inversely proportional to the recommended standard value \( S_n \) of the corresponding parameter,

\[
W_n = K/S_n
\]

where \( W_n \) = unit weight for the \( n \)th parameter; \( S_n \) = standard value for \( n \)th parameters; and \( K \) = constant for proportionality.

The overall water quality signature (WQS) was calculated by aggregating the quality rating with the unit weight linearly,

\[
WQS = \sum q_n W_n / \sum W_n
\]

Water quality signature (WQS) was calculated separately using: (i) pH; (ii) total alkalinity, total hardness and total dissolved solids; and (iii) nitrate and conductivity of water in different wetlands.

**Statistical analysis**

The data were statistically evaluated; one way analysis of variance was applied to find the differences of the mean of different parameters among the wetlands located in different geographical locations of West Bengal as well as between the closed and open system of limnocorral, if any, using SPSS Package. Multivariate analysis of the Akaike information criterion (AIC) model was performed between the phytocarbon of microalgae as a dependent variable and physico-chemical factors (water temperature, pH, free CO\(_2\), CO\(_3\), total alkalinity, hardness, dissolved oxygen, phosphate-P, ammonia-N, nitrite-N, nitrate-N and conductivity) as independent variables. The stepwise regression model was used to find the subset of variables in the data set resulting in the best suited model. The best model was chosen on the basis of minimum value of Akaike information criterion (AIC). The variable selection was performed using the direction ‘forward’ and only considered in the final model if the variable had a significance level of <5%. Statistical analysis was performed using a function step (AIC) of MASS package which was used for computing stepwise regression in statistical language ‘R’. All the selected variables of the final model were significant according to \( P \)-value of the corresponding ‘t’ test.

**RESULTS**

**Limnocorral experiment**

**Light and temperature**

The transudation of light, as expected, was elevated in the open limnocorral ranging from 800–1,200 × 100 and 740–829 × 100 lux in the mesotrophic and eutrophic wetlands, respectively, compared with their top-covered solar-heated structures (mesotrophic: 790–820 × 100 lux; eutrophic: 735–790 × 100 lux). As a result, top-covered
limnocorals gained at least 1 °C rise in water temperature in both mesotrophic (28.2 °C) and and eutrophic (28.72 °C) wetlands. The water temperatures of both wetlands were slightly less than the open limnocorral.

**Free CO₂–pH–carbonate–bicarbonate system**

The responses of the pH–carbonate–bicarbonate system to open and closed conditions were different in two ecologically contrasting wetlands. While the pH of water was little reduced (8.32) accompanied with elevated CO₂ (1.4 mg/l), bicarbonate (179.0 mg/l) and hardness (179.4 mg/l) in the top-covered limnocorral of eutrophic wetland, the water in the parallel set placed in the mesotrophic wetland did not display a marked difference from the open ones (Table 4). The mean concentration of inorganic carbon of water mesotrophic wetland (299.0 mg/l) did not differ from those of open (298.0 mg/l) or closed (297.0 mg/l) ones. The mean concentration of total inorganic carbon in the open limnocorral was reduced by mere 8% (169.5 mg/l) compared with closed (184.4 mg/l) or eutrophic wetland (185.6 mg/l) showing hardly any difference from each other.

**Nutrient parameters**

Top covering the limnocorral placed in mesotrophic wetland resulted in an increase in the concentrations of ammonium-N (0.72 mg/l), nitrite-N (0.11 mg/l) and nitrate-N (0.23 mg/l) mg/l compared with corresponding values in the open (ammonia-N 0.60 mg/l; nitrite-N 0.04 mg/l and nitrate-N 0.20 mg/l). In the eutrophic wetland, though the concentration of ammonia-N in the closed system remained significantly higher (P < 0.05) than those in the open, no marked differences (P > 0.05) between them were registered for the values of nitrite-N and nitrate-N. This was attributed to the lack of difference in the rate of transformation of ammonium into nitrite-N or nitrate-N. Top cover of the limnocorral resulted in significant reduction (P < 0.05) of phosphate in mesotrophic (1.12 mg/l), but increase in eutrophic (0.438 mg/l) compared with their open structures (mesotrophic 1.47 mg/l; eutrophic 0.34 mg/l).

**Primary productivity and chlorophyll**

Top cover of the solar-heated structure (limnocorral) resulted in a 30% rise in the amount of chlorophyll content of microalgae (2.125 mg/M³) in the mesotrophic pond, whereas it was reduced by 10% (56.75 mg/M³) in the highly eutrophic pond. Reduction to the tune of 64% (P < 0.05) in the overall mean values of chlorophyll-a content of microalgae was registered as a result of top cover of the limnocorral in the mesotrophic wetland, but the reverse response was recorded in the highly eutrophic wetland (Figure 2). Interchange of exposure conditions performed on day 42 (i.e., top covered limnocorral was made open on day 42 and vice versa) exhibited the algal and chlorophyll responses of the preceding condition.

**Wetland study**

**Water temperature**

As anticipated, water temperature of four coldwater lakes in Darjeeling (DJ) remained distinctly lower (20.3–23.4 °C) than the rest of the ponds located in the other agro-climatic regions (HG, MP, ND, 24P) investigated. The next higher temperature range (23.5–26.6 °C) occurred in one of the four lakes in DJ (9.93%), but was hardly observed in other locations. The third categories of temperature range (26.7–29.8 °C) were more frequent in HG ponds where it occurred in seven out of ten ponds investigated. The highest temperature range of 29.9–33.0 °C was more common in two ponds in MD (16.31%), followed by seven ponds in ND (13.48%), three ponds in HG (9.93%) and five ponds in 24 P (7.09%). The overall mean temperature of water remained significantly lower in DJ lakes (Figure 3) than ponds in other regions (F₄,136 = 100.139; P < 0.001).

**Hydrogen ion concentration**

Water pH ranged from 6.3 to 9.5 in all the 32 ponds investigated across the five districts of West Bengal. Four coldwater lakes in DJ showed the highest frequency (20%) of pH range of 6.3–7.3, but lower frequency was observed in other districts (Figure 3). The next highest range of pH (7.4–8.4) was skewed towards maximal occurrence in about 25%
<table>
<thead>
<tr>
<th>Parameters</th>
<th>KRS Pond – moderately mesotrophic</th>
<th>Sewage-fed pond – highly eutrophic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Light intensity (lux × 100)</td>
<td>800–1,200</td>
<td>1,025.00</td>
</tr>
<tr>
<td>Air Temp (°C)</td>
<td>29–29.5</td>
<td>29.23</td>
</tr>
<tr>
<td>Water Temp (°C)</td>
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<td>27.95</td>
</tr>
<tr>
<td>pH</td>
<td>7.6–8.2</td>
<td>7.95</td>
</tr>
<tr>
<td>Free CO₂ (mg/l)</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<tr>
<td>Nitrite-N (mg/l)</td>
<td>0.003–0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Nitrate-N (mg/l)</td>
<td>0.2–0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>396–461</td>
<td>429.25</td>
</tr>
</tbody>
</table>

The results of natural wetlands are also shown. Each mean represents the data for 16 observations during the course of investigation (October 2011–March 2014).
samples collected from two ponds in MP, followed by seven ponds in HG (16.31%), six ponds in ND (12.77%), three ponds in 24 P (4.26%) and least 2.84% occurred in three DJ lakes suggesting a dominance of the acidic range of pH in coldwater lakes. Though the higher range of pH (8.5–9.5) was conspicuous by its absence in DJ lakes and 24 P ponds, it occurred, albeit less frequently, in two ponds in HG (4.26%), one pond in MP (4.26%) and two ponds in ND (5.5%). The overall mean pH of water remained significantly lower in DJ lakes (6.91) than ponds in other regions ($F_{4,136} = 30.596; P < 0.001$).

District wise, all the ponds located in HG and ND represented the pH values covering all the class groups, whereas the coldwater lakes in Darjeeling had pH between 7.4 and 8.4. The ponds of the 24 P showed wide fluctuations in pH between 6.3–8.4 and 7.4–9.5 in MP ponds.

**Free CO₂–HCO₃–CO₃ system**

Free CO₂ – The amount of free CO₂ of water ranging from 0–6 mg/l was less common in three ponds of HG (7.81%), five ponds of ND (9.22%), but more frequent in two ponds of MP (24.82%). The next highest concentration range (6.1–12.1 mg/l) occurred more frequently in three coldwater lakes in DJ (17.02%), followed by five ponds in HG (10.64%), three ponds in ND (7.80%), two ponds in 24 P (2.84%) and one in MP (1.42%). The highest concentration range of 12.2–24.3 mg/l was almost uniformly distributed in the ponds of almost all the districts except in Darjeeling and MP. The overall mean free carbon dioxide of water (Figure 3) was significantly different from each of the others ($F_{4,136} = 7.327; P < 0.001$).

CO₃ alkalinity – Since the water of the ponds in general is in the acidic range, the concentration of carbonate alkalinity of water remained towards the lower range, varying from 4 to 12.2 mg/l in all the ponds in ND (21.28%). Distribution of alkalinity was also skewed towards the lower range (4.1–8.1 mg/l) in almost all the ponds investigated. The overall mean CO₃ alkalinity of water remained significantly different from each other ($F_{4,136} = 2.534; P < 0.05$).

HCO₃ alkalinity – The values of bicarbonate ranged from 12 to 297.2 mg/l in 32 ponds investigated across five districts of West Bengal. The class range of 12.0–107 mg/l was highest in four lakes in DJ (22.69%) and about 27.66% of the total samples in nine ponds of the remaining districts. The next highest concentration range (107–202 mg/l) was absent in DJ lakes, but occurred in two ponds in MP, (17.73%), six ponds in ND (11.35%) and five ponds in HG (10.64%). Though the highest concentration range occurred less, it occurred in the ponds representing three districts (HG, ND, and 24 P) of the state. The overall mean CO₃ alkalinity of water (Figure 3) remained significantly different from each other ($F_{4,136} = 26.210; P < 0.001$).

**Total inorganic carbon**

The amount of total inorganic carbon (TIC) was in the range of 25.3–503. mg/l among all the 32 ponds investigated. All
three class ranges (25.3–117.8 mg/l; 117.9–210.4 mg/l and 210.5–303 mg/l) of inorganic carbon occurred in ten ponds in HG, (21.28%), five ponds in 24 P (7.09%) and ten ponds in ND (21.3%). However, three ponds in MP and four lakes in DJ contain a lower range of inorganic carbon (25.3–210.4 mg/l). The overall mean inorganic carbon in water (Figure 3) remained significantly higher in HG ponds and lower in DJ lakes ($F_{4,136} = 27.013; P < 0.001$).
Organic carbon

The amount of organic carbon (OC) varied in the range of 0.6–9.3 mg/l in the 32 ponds investigated. The concentration range of 0.6–2.7 mg/l was most frequent (77.29%) followed by next class (2.8–4.9 mg/l) in all the districts (1.71%) except in 24 P where this class range was absent. The third (5–7.1 mg/l) and fourth (7.2–9.3 mg/l) class ranges occurred only twice in one pond in HG and one or two ponds in ND. The overall mean concentration of organic carbon in water (Figure 3) was distinctly higher in HG ponds and lower in 24 P ponds or DJ lakes ($F_{4,136} = 2.999; P < 0.05$).

Total carbon

The concentrations of total carbon (TC) of water showed more than one order of magnitude variations ranging from 27 to 308 mg/l among the 32 water bodies in all 142 observations. One of the characteristic features of four cold water lakes in DJ was the occurrence of maximal frequency (22.70%) of low concentration range (27.7–83.7 mg/l) followed by a pond in MP (7.09%), three ponds in ND (5.67%) and one pond in HG (1.42%). Such range did not occur in 24P ponds. The next highest range (83.8–139.8 mg/l) occurred less frequently, ranging from 4.26 to 12.77% in all the district ponds except in DJ lakes which were conspicuous by their absence. The third (139.9–195.9 mg/l) and fourth class range (196–252.0 mg/l) were further reduced in their occurrences especially in MP ponds where the fourth class range was absent. The fifth and highest class range (252.1–308.1 mg/l) did occur (3.13%) in a single pond in HG. The overall mean concentration of total carbon in water (Figure 3) was distinctly higher in HG ponds and lower in 24 P ponds or DJ lakes ($F_{4,136} = 27.657; P < 0.001$).

Total dissolved solids

Total dissolved solids (TDS), which is almost equivalent to salinity, showed two orders of magnitude variations ranging from 33 to 678.2 mg/l among all the 32 ponds investigated across five districts of West Bengal. The lowest concentration range of 33–248 mg/l occurred mostly in four cold water lakes in DJ (22.69%) and two ponds in MP (23.4%). The next highest values (248.1–463.1 mg/l) were skewed toward lower frequencies in MP (4.25%), 24P (6.38%) and ND (7.80%) ponds; six ponds in HG had 10.64% of total samples examined. The next highest concentration range (463.2–678.2 mg/l) occurred only in three ponds in HG (8.51%) and four ponds in ND (8.51%). The overall mean values of TDS in water (Figure 3) remained significantly different from each other ($F_{4,136} = 65.470; P < 0.001$).

Hardness

The water of the ponds has been categorized with hardness values ranging from 34 to 286.3 mg/l. All ten ponds in HG had values of hardness across all four categories (21.28%), whereas the ponds of 24 P were limited to the first three categories (7.1%). The MP ponds displayed hardness in the lower two categories (34.0–160.1 mg/l) amounting to 27.66%. All ten ponds of ND showed the highest three categories (97.1–286.3 mg/l) of hardness (22.69%). Four coldwater lakes in DJ had the occurrence in the lower two categories (34.0–160.1 mg/l) of hardness (22.69%). The overall mean values of total hardness of water (Figure 3) remained significantly different from each other ($F_{4,136} = 24.341; P < 0.001$).

Dissolved oxygen

The concentrations of dissolved oxygen (DO), ranging from 3.08 to 5.68 mg/l, occurred more frequently in two ponds in MP (25.53%), nine ponds in ND (20.57%), seven ponds in HG (14.18%), five ponds in 24 P (7.09%) and one lake in DJ (2.84%). Higher concentrations of DO (5.69–8.29 mg/l) were recorded occasionally in DJ (9.93%), three ponds in HG (7.90%), one in each of MP (2.13%) and ND (0.7%). The highest range of 8.3–10.9 mg/l occurred in two lakes in Darjeeling (9.93%). The overall mean values of DO in water (Figure 3) remained significantly different from each other ($F_{4,136} = 64.072; P < 0.001$).

Phosphate-P

The concentrations of phosphate showed more than three orders of magnitude variations among the 32 ponds
investigated across five districts of West Bengal. About 90% (126 samples) of the total of 141 had the lowest concentration range of 0.03–0.34 mg/l and that occurred mostly in two MP ponds (24%) and in three cold water lakes in DJ (21%) while the rest were represented in eight ponds in ND (19%), nine ponds in HG (18%) and four ponds in 24 P (6.38%). The next highest concentration range of 0.35–0.66 mg/l was entirely absent in four DJ oligotrophic lakes, but occurred in other districts (8.52%). The highest range of 0.67–0.98 mg/l occurred only once in a single pond in ND (0.77%). The overall mean concentration of phosphate in water (Figure 2) remained significantly lower in DJ lakes and higher in HG ponds ($F_{4,136} = 5.302; P < 0.001$).

**Ammonical-N**

The concentrations of NH$_4$-N were highly variable ranging from 0.03 mg/l to 4.85 mg/l in 32 ponds investigated during the period of study. The lower concentration range (0.03–1.63 mg/l) was most common in the majority of ponds (26) including four coldwater lakes representing DJ. The second (1.64–3.24 mg/l) and third (3.25–4.85 mg/l) highest values occurred in one pond of HG (3.13%) and one pond of ND, respectively. The overall mean concentration of ammonical-N in water (Figure 3) remained significantly different from each other ($F_{4,136} = 4.078; P < 0.05$).

**Nitrate-N**

The concentration range of nitrate-N was between 0–1.9 mg/l registered in more than 90% of ponds (29) investigated with maximum occurrence in three ponds in MP (27.66%), four lakes in DJ (22.69%) and eight or nine ponds in each of HG (19.15%) and ND (19.15%), respectively. The second (1.91–3.81 mg/l) class range were registered in one pond in each of HG (2.12%) and ND (0.71%), whereas the fourth class range (5.73–7.63 mg/l) was found only in one pond in ND (1.41%). The overall mean concentration of nitrate-N in water (Figure 3) remained significantly higher in ND ponds ($F_{4,136} = 2.813; P < 0.05$).

**Total inorganic-N**

Total inorganic N (TIN) samples collected from ten ponds in ND (21.28%) showed all the class ranges of nitrate-N. Ten ponds in HG (21.27%) displayed the lowest three concentration ranges (0.05–4.57 mg/l), whereas five ponds in 24 P (0.05–3.06 mg/l) and four lakes in DJ belonged to lowest two class range (29.79%). Three ponds in MP (27.66%) had the lowest class range of 0.05–1.55 mg/l. The overall mean concentration of total inorganic-N in water (Figure 3) remained significantly different from each other ($F_{4,136} = 7.160; P < 0.001$).

**Conductivity**

The lower range of conductivity (3–358 μS/cm) occurred more frequently in four coldwater lakes in DJ (22.70%) compared to one pond in MP (12.06%) and one pond each in ND (2.84%) and in HG (1.42%). Next highest values (358.1–679.1 μm/cm) were more common in almost all but Darjeeling lakes. Still higher values (679.2–1,000.2 μm/cm) were encountered in three ponds in HG (8.51%) and two ponds in ND (6.38%). The overall mean concentration of specific conductance of water (Figure 3) remained significantly lower in DJ lakes than the ponds in other regions ($F_{4,136} = 78.343; P < 0.001$).

**Chlorophyll**

The variability of chlorophyll was extremely high, ranging from 0.01 to 0.16 in all the ponds investigated. The ten
ponds in both HG and ND showed chlorophyll values in all four class ranges (0.01–0.04, 0.05–0.08, 0.09–0.12 and 0.13–0.16 mg/m³). The ponds of 24 P and Darjeeling had measured values in the three lower class ranges of chlorophyll, but those of MP ponds exhibited the next three higher levels (0.05–0.16 mg/m³). The overall mean concentration of chlorophyll content of microalgae (Figure 3) was significantly higher in MP ponds in the coastal region ($F_{4,136} = 10.921; P < 0.001$).

**Phytocarbon of microalgae**

The amount of phytocarbon could be grouped into three class ranges from 0.5–2.85, 2.86–5.21 and 5.22–7.57 mg/m³) and they occurred in all 32 ponds distributed across the five districts of West Bengal. The range of frequency was greatest in three ponds in MP (27.665 mg/m³), followed by four lakes in DJ (22.69%), ten ponds in ND (21.28%), ten ponds in HG (20.28%) and five ponds in 24-P (7.1%). The overall mean concentrations of phytocarbon in water remained significantly different from each other ($F_{4,136} = 10.921; P < 0.001$).

**Water quality signature**

Determination of water quality signature (WQS) using pH values of 141 samples of water across the five districts of West Bengal (see Table 2) showed a preponderance of acidic state with lowest value of WQS (17.40) in a high altitude DJ lake and highest WQS (64.87) in the Gangetic alluvial ponds in Hooghly with an alkaline state. The second WQS expression using total alkalinity, total hardness and total dissolved solids also points to the lowest (11.09) for DJ lakes and highest (95.2) for Gangetic alluvial ponds of Nadia (Table 5). With the third expression using nutrient, nitrate and total ionic load, conductivity registered the lowest for DJ lakes (23.51) and highest for ND ponds (199.79).

**DISCUSSION**

Heterogeneity of measured water quality signature among the investigated ponds across different agro-climatic regions of the state of West Bengal was increasingly apparent. This was manifested by the significant differences in the frequency distribution of different class ranges of physicochemical criteria used as signature molecules for carbon sequestration by microalgae in the 32 wetlands investigated. The results of multivariate analysis using the Akaike information criterion (ACI) model also lent credence to the theory that carbon sequestration by microalgae could be predicted from location-specific water quality variables.

Carbon flux in natural water bodies, eminently contingent on altitude, temperature and biological interactions, plays a key role in the cycling of carbon. Pond soil acts as a buffer, either as a functional response-mediated sink or as a source of carbon resulting from the net balance between opposing kinetics of CO$_2$ fluxes attributable to community assimilation and respiration of the system as a whole.

As is known, the proportion of three forms of inorganic carbon (CO$_2$, H$_2$CO$_3$, HCO$_3$ and CO$_3$) required for photosynthesis of microalgae is highly dependent upon the pH of water that, in turn, was regulated by the agro-geo-biological interactions between nutrient-driven microalgal biomass and heterotrophic consumption of the ecosystem. The atmospheric carbon that gets dissolved in water undergoes chemical transformations involving the formation of the

<table>
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<th>Table 5</th>
<th>Summary of the calculated values of Water Quality Signatures (WQS) for five different districts of wetlands located under divergent agro-climatic conditions of West Bengal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Quality Signature</td>
<td>Hooghly (HG)</td>
</tr>
<tr>
<td>(i) pH</td>
<td>64.87</td>
</tr>
<tr>
<td>(ii) Total alkalinity, total hardness and total dissolved solids</td>
<td>80.1</td>
</tr>
<tr>
<td>(iii) Nitrate and conductivity</td>
<td>188.18</td>
</tr>
</tbody>
</table>

WQS was calculated using: (i) pH, (ii) total alkalinity, hardness and total dissolved solids, and (iii) nitrate and conductivity separately.
CO$_2$–H$_2$CO$_3$–HCO$_3$–CO$_3$ system leading to lowering of the pH, a situation that is vital for capturing atmospheric carbon and impacting positive feedback to climate change.

The thylakoid discs of chloroplasts of microalgae are the site of two steps of photosynthesis reactions converting photon energy into chemical energy in the form of glucose. It is stated that microalgae are able to capture as much as 90% of carbon dioxide or bicarbonate in open ponds though this ability may vary among species (Hanagata et al. 1992; Bhakta et al. 2015; Chang et al. 2016). The captured carbon is pumped into the cell by bicarbonate transporters present in both the plasma membrane and in the chloroplast envelope of eukaryotic algae (Spalding et al. 2008). Inside the chloroplast, bicarbonate is concentrated, dehydrated spontaneously or by carbonic anhydrase through Calvin-cycle activity, finally yielding algal biomass. The RuBisco enzyme plays a great role in carbon fixation; it is capable of utilizing both CO$_2$ and O$_2$, leading to formation of carboxylation in the presence of CO$_2$ and oxygenation of RuBP in the presence of oxygen, respectively. When concentration of O$_2$ is high and CO$_2$ is low, RuBisCO acts as an oxygenase and further catalyzes the photorespiration reaction resulting in reduced carbon fixation due to production of the two-carbon molecule phosphoglycolate (Peterhansel et al. 2010). In essence, RuBisCo enzyme loses a carbon that reduces the Calvin cycle ability to regenerate the five-carbon sugar substrate ribulose bisphosphate which is essential for CO$_2$ fixation by RuBisCo (Peterhansel et al. 2010). In another adaptation for reducing the competitive inhibition of oxygen on carbon fixation by RuBisCo, microalgae actively pump sufficient amounts of bicarbonate from the water phase into cells increasing the internal CO$_2$ concentrations to levels above the possible equilibrium with air, and thus competitively inhibit photorespiration (Badger & Price 1994). That heterogeneity of inorganic carbon interacting with nutrient level in different geographical locations would eventually select the qualitative and quantitative abundance of microalgae in that specific wetland. When water becomes saturated with CO$_2$, it not only reduces the pH, but depletes the calcium carbonate sources as well which is not conducive to the growth of shell-forming animals, especially shrimps (www.foindriest.com/environmental measurements/parameters/water quality).

Multivariate analysis using the ACI model between phytocarbon of microalgae as a dependent variable and different water quality parameters as independent variables revealed that all the three forms of dissolved inorganic carbon (free carbon dioxide, bicarbonate, carbonate) and inorganic nitrogen (NH$_4$-N or NO$_3$-N) appeared to be major factors regulating the phytocarbon content of microalgae in wetlands located in different geographical regions of the state West Bengal (Table 6). The wetlands located in the Gangetic alluvial tropical or semi-coastal areas (Hooghly, 24-Parganas, Nadia, Midnapore) were alkaline (7.52–7.97) where half-bound carbon dioxide comprised the major component (18–26%) of total inorganic carbon with moderate to eutrophic (PO$_4$: 0.16–0.23 mg/l) states having negative feedback to global warming. Interaction of trophic state and temperature was evident from the in situ limnocorals study exhibiting contrasting responses of microalgae to solar-heated greenhouse temperature in two ecologically contrasting wetlands. The heterogeneity of measured water quality signature consolidated the sanative nature of wetlands through their complex functional attributes with agro-climatic, biogeochemical and soil-water-biological interactions.

The coldwater lakes in Darjeeling were conspicuous by their distinctly lower ratios for total inorganic carbon to organic carbon, phosphate to chlorophyll, total inorganic carbon to hardness, or the absolute concentrations of total

<table>
<thead>
<tr>
<th>Sites</th>
<th>Equations</th>
<th>AIC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darjeeling</td>
<td>Phyto.C = 0.029140(Hardness) – 2.858930(pH) – 0.478525(F.C02) – 0.557907(DO) + 28.762115</td>
<td>4.18</td>
</tr>
<tr>
<td>Nadia</td>
<td>Phyto.C = 1.102543(pH) + 0.003362(TDS) – 6.77597</td>
<td>18.56</td>
</tr>
<tr>
<td>Hooghly</td>
<td>Phyto.C = 0.519520(CO2) – 0.014517(HCO3) + 0.005937(TDS) + 1.139549(NH4) – 4.705385</td>
<td>12.14</td>
</tr>
<tr>
<td>Midnapore</td>
<td>Phyto.C = 0.127285(F.C02) – 4.329651(NO3) + 6.228474</td>
<td>2.52</td>
</tr>
</tbody>
</table>

*Water quality parameters: water temperature, pH, free CO2 (f.C02), CO2, HCO3, carbonate, pH, DO, total dissolved solids (TDS), NH4-N, NO2-N, NO3-N, PO4-P, conductivity.
dissolved solids, or conductivity and oligotrophic state as evidenced by at least a seven-fold reduction in the values of conductivity per degree Celcius water temperature compared with wetlands located in other agro-climatic regions. Furthermore, DJ lakes had distinctly low values of all three expressions of WQS (see Table 5) that led to credence that acidic conditions were prevalent in the sub-temperate high altitude lakes of Darjeeling ascribable to their oligotrophic, high altitude and low temperature (23.15°C) effects. The HCO₃ that comprised the major component of dissolved inorganic carbon delineated a mere 6% of TIC in DJ lakes and also strengthened their more frequent acidic conditions with a concomitant rise in H₂CO₃ – a situation favourable for the dissolution of atmospheric carbon by selecting the green algae and their abundance, though the production was somewhat limited by the oligotrophic state of the high altitude lakes. Mondal et al. (2012) also reported mostly the acidic range (6.3–8.1) in different sites of Mirik Lake water during October 2005 to 2007.

All the three forms of dissolved inorganic carbon (free CO₂, HCO₃ and CO₃) also appeared to be significant contributors in the wetlands located in the Gangetic alluvial tropical zones of ND and HG and semi-coastal areas of MD, registering higher ratios of total inorganic carbon to organic carbon, phosphate to chlorophyll, total inorganic carbon to hardness or the absolute concentrations of total dissolved solids, or conductivity. This shows that the Gangetic alluvial ponds and coastal ponds in the tropical regions of all these districts were more alkaline and were moderately to highly eutrophic which favours massive growth of microalgae where the bicarbonate or half-bound carbon dioxide comprising 18–26% of the TIC was the main source of carbon for photosynthesis. Freshwater lakes, ponds and streams exhibit the pH range of 6–8.5 and most recommended pH levels for fish farming are between 6.5 and 8.5, though some fish especially the American char (Svobodová et al. 1993) or Osorezan dace or Japanese dace, are resistant to acidic water and thrive well in acidic waters at pH = 4.5–5.0 (Svobodová et al. 1993) or between pH = 3.5 and 5.0 as a result of their reduced metabolic rates under acidic conditions.

It is evident from the results of in situ limnocorral studies that chlorophyll or phytocarbon content of microalgae was triggered when exposed to simulated greenhouse conditions and vice versa in mesotrophic wetland. Such responses were reversed in the highly eutrophic wetland due to competitive inhibition for light. This suggests clear cut interactions of temperature and the nutrient state of water that have a pivotal role, not only in regulating the density of microalgae, but also in the temperature-driven dissolution of atmospheric carbon into the water phase. Thus, the heterogeneity of measured water quality signature among the investigated ponds across the diverse agro-climatic regions of the state of West Bengal consolidated the sanative nature of wetlands for their complex functional attributes with agro-climatic, biogeochemical and soil–water–biological interactions.

CONCLUSIONS

It is reasonable to conclude that water quality signature can be used to monitor the carbon status of the wetlands located in different agro-climatic regions. This will reflect the carbon capture potential of the wetland under a given set of conditions. This present study reveals the heterogeneity of measured water quality signature among the investigated ponds across the diverse agro-climatic regions of the state of West Bengal. This lends credence to the sanative nature of wetlands for their complex functional attributes with agro-climatic, biogeochemical and soil–water–biological interactions, but further research is need in this direction.

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