frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 27 January 2022
doi: 10.3389/fevo.2021.798520

Positive Effects on Alfalfa Productivity and Soil Nutrient Status in Coastal Wetlands Driven by Biochar and Microorganisms Mixtures

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Specialty section:
*This article was submitted to Conservation and Restoration Ecology,
a section of the journal Frontiers in Ecology and Evolution*

Received: *20 October 2021*
Accepted: *22 December 2021*
Published: *27 January 2022*

Citation:
Cui Q, Xia J, Peng L, Zhao X and Qu F (2022) Positive Effects on Alfalfa Productivity and Soil Nutrient Status in Coastal Wetlands Driven by Biochar and Microorganisms Mixtures. Front. Ecol. Evol. 9:798520. doi: 10.3389/fevo.2021.798520

Biochar application in reclaiming degraded soils and improving plant productivity has been recognized as a promising technology. Yet, the impacts of biochar and mixtures with compound effective microorganisms (CEM) on alfalfa growth and soil quality in coastal wetlands are poorly understood. A greenhouse experiment was set to systematically reveal the impacts of biochar and biochar combined with CEM on alfalfa growth traits, nutrient uptake, biomass, soil quality, and enzyme activities. Eight treatments were included: (1) control (CK−CEM), (2) 10-g/kg biochar (B_{10}−CEM); (3) 20-g/kg biochar (B_{20}−CEM); (4) 30-g/kg biochar (B_{30}−CEM), (5) CEM without biochar (CK + CEM); (6) 10-g/kg biochar with CEM (B_{10} + CEM), (7) 20-g/kg biochar with CEM (B_{20} + CEM), (8) 30-g/kg biochar with CEM (B_{30} + CEM). The utilization of biochar promoted seed germination, height, and tissue nutrient contents of alfalfa, and the combined biochar with CEM showed greater effects. Alfalfa biomass showed the maximum value in the B_{20} + CEM treatment, and the biomass of root, shoot, leaf in the B_{20} + CEM treatment increased by 200, 117.3, 144.6%, respectively, relative to the CK−CEM treatment. Alfalfa yield in the CK + CEM, B_{10} + CEM, B_{20} + CEM, B_{30} + CEM treatments was 71.91, 84.11, 138.5, and 120.5% higher than those in the CK−CEM treatment. The use of biochar and CEM decreased soil salinity and elevated soil nutrient content effectively. Biochar elevated soil organic carbon (SOC) and microbial biomass carbon (MBC), NH_4^+, NO_3^-, and enzymatic activities, and the positive impacts of biochar combined with CEM were additive. The combined addition of 20-g/kg biochar with CEM showed the pronounced improvement effects on improving soil fertility and nutrient availability as well as soil enzyme activities. Path analysis indicated that the application of biochar mixture with CEM promoted alfalfa biomass by regulating plant nutrient uptake, soil quality (soil nitrogen, SOC, MBC, NH_4^+, NO_3^-), and soil enzymatic activities (sucrase, urease, and alkaline phosphatases). Thus, incorporation of suitable biochar and CEM can serve as an effective measure to promote alfalfa productivity and restore coastal wetlands soils.

Keywords: biochar, alfalfa growth, alfalfa nutrient uptake, soil quality, soil enzyme activities

HIGHLIGHTS

- Biochar addition decreased soil salinity and elevated soil organic carbon, NH_4^+ and sucrase activity effectively.
- Combined use of 20 g/kg biochar with microorganisms mixtures performed well in improving alfalfa growth.
- Alfalfa total biomass increased depend on the rise of plant potassium uptake, soil organic carbon, NH_4^+, and NO_3^-.
- An appropriate dosage of biochar combined with microorganisms mixtures is effective to ameliorate coastal wetlands.

INTRODUCTION

Soil salinization with potentially nutrient deficiency has become a global issue concerning the sustainable development of food security and human livelihoods (Saifullah et al., 2018; Hassani et al., 2020). As a typical part of saline-alkali soil, coastal wetlands are considered as one of the most productive ecosystems on Earth and contributors to valuable service (Kirwan and Megonigal, 2013; Zhao et al., 2018). Recently, anthropogenic activities and seawater encroachment have resulted in high salinization, shallow groundwater, and soil function deterioration, which would cause soil degradation and threaten crop productivity in coastal wetlands (Stagg et al., 2017; Liu et al., 2018). Severe soil depletion, low nutrient deficiency, and poor vegetation coverage occur on coastal wetlands, aggravating plant productivity and unsustainable land use (Huang et al., 2012; Li et al., 2021). Consequently, the restoration of coastal wetlands is particularly critical, and soil amendments with the advantages of being effective, sustainable, and environmentally friendly are urgently needed for improving soil health and rehabilitating vegetation (Luo et al., 2017).

Biochar is a carbonous coproduct generated by pyrolysis organic biomass that has received growing attentions in pieces of research of ecological issues, soil restoration, and soil carbon sequestration (Lehmann, 2007; Chávez-García and Siebe, 2019; Cooper et al., 2020). Biochar has been advocated as a suitable conditioner that can remediate salt-affected soils by reducing salinity stress and promoting soil nutrient status (Kim et al., 2016; Ali et al., 2017; Gunarathne et al., 2020). Biochar has been proved to increase soil aggregate stability and water retention (Palansooriya et al., 2019; Caban et al., 2020), stimulate nutrient utilization efficiency (Biederman and Harpole, 2013; Chen et al., 2018), boost soil microbial proliferation and activities (Pokharel et al., 2020), and improve plant nutrient uptake and productivity (Lashari et al., 2015). Biochar can diminish sodium (Na^+) absorption by plants and mitigate salt stress to plant growth and physiology in salt-affected soils (Akhtar et al., 2015; Saifullah et al., 2018). Furthermore, biochar application can stimulate the plant nutrients absorption ability *via* promoting biochar-root interactions and increasing the efficiency of nutrient absorption by the roots (Olmo et al., 2016; Jeffery et al., 2017). However, inappropriate use of biochar to salt-affected soils may increase salinity and alkalinity and inhibit soil nutrient supply, which have an adverse impact on plant performance (Sigua et al., 2016; Luo et al., 2017). Thus, the effect of biochar on soil and plant quality under salinity conditions depends on soil type, plant feature, biochar feedstock type, biochar pyrolysis terms, and biochar addition rate.

Recently, compound effective microorganisms (CEM), a wide range of beneficial and non-pathogenic microorganisms, has been adopted as microbial fertilizers to improve soil quality (Talaat et al., 2015). The benefits of CEM have been highlighted in salt-affects soils for diminishing the detrimental impact of saline stress, enhancing beneficial microbial activities and soil nutrient cycling, accelerating decay of soil organic matter, which are favorable for plant productivity (Hu and Qi, 2013; Olle and Williams, 2013; Talaat, 2019). The amendment of biochar within microorganism mixtures showed encouraging results in promoting plant yield under saline conditions than single addition of biochar or other soil amendments (Akhtar et al., 2015; Talaat et al., 2015; Wang et al., 2021). For example, El-Mageed et al. (2020) found that the combined use of biochar and CEM maximized pepper growth and production more by improving favorable soil characteristics, photosynthetic availability, and nutritional status than biochar addition alone. Wang et al. (2021) reported that biochar mixture with plant growth-promoting rhizobacteria improved microbial diversity, enzyme activity, and nitrogen availability from tomato soil. In general, biochar mixture with microorganisms provides a favorable environment for microorganisms and produces remarkable effects on soil quality and plant growth. Thus, the combination of biochar with microorganisms is considered as a promising and effective measure for improving plant yield and remediating nutrient-poor soil.

Adding soil conditioner is an effective and eco-friendly approach to remediate soil degradation and improve salinity soil function of coastal wetlands (Sun et al., 2016). The additive positive effects on soil nutrient status and plant growth have been proved in salt-affected soils amended with the mixture of biochar and other fertilizer or microorganisms (Luo et al., 2017; Saifullah et al., 2018). Furthermore, planting halophytes in coastal wetlands is a bioremediation strategy to restore the degraded soil and improve the efficiency of land usage (Cai et al., 2021). Alfalfa is a preferred halophyte to adapt to saline environment and develop grass and livestock breeding in salt-affected regions. Alfalfa is a considerable important forage crop and has many prominent advantages, including high yield, nutritional quality, drought tolerance, and adaptation to infertile habitat (Cao et al., 2012; Zhang et al., 2019). In addition, combinations of biochar with microorganism mixtures or other amendments applied to soil showed effective effects on alfalfa yield (Liu et al., 2020; Raklami et al., 2021). However, previous reviews on restoring coastal wetlands soils have focused on sole biochar addition or combined with other organic materials (Kim et al., 2016; Luo et al., 2017). It is still unclear if the use of biochar with microorganism mixtures affects soil nutrient status and plant yield in coastal wetlands. Here, we hypothesize that biochar combined with CEM would improve soil nutrient availability, enhance soil enzyme activities, and promote plant nutrient uptake and alfalfa growth. The main objectives of this study were: (1) to clarify the changes in plant growth traits, plant nutrient content, and soil nutrient content, following biochar and CEM inputs, and (2) to evaluate the potential mechanisms affecting alfalfa growth in coastal wetlands.

MATERIALS AND METHODS

Soil Sample and Experiment Preparation

The soil was randomly sampled from topsoil samples (0–20 cm) of the coastal wetlands in the Nature Reserve of the Yellow River Delta (37°45′N, 118°59′E) in the Shandong Province, China. Soil samples were transferred to a laboratory, and stones and roots were removed. The soil samples were air-dried and passed through a 2-mm sieve; then, all the samples were homogenized thoroughly. The soil had a salt content of 0.6%, pH of 7.49, soil organic matter of 9.23 g/kg, total nitrogen (TN) of 0.42 g/kg, available phosphate (AP) of 3.9 mg/kg.

Phragmites australis was chosen as a raw material for biochar mainly due to well-developed aerenchym and high carbon fixation capacity, which is suitable for the requirement of biochar (Liang et al., 2019). Biochar was produced from *Phragmites australis* shoot by combustion anaerobically for 2 h at 400°C in a muffle furnace (Leibo Terry Equipment Co., Ltd., Tianjin, China). The pyrolysis temperature of 400°C was selected due to its highest yield and relatively stable properties. The biochar sample was passed through a 2-mm sieve and homogenized completely before addition. Biochar contains 40.5% yield, 58.34% total carbon (TC), 1.18% TN, 4.11% hydrogen (H), 36.75% oxygen (O), 14.19 C/H, 0.70 (O + H)/C, an 8.65 m^2/g specific surface area, 1.83-nm average pore width, with a pH of 7.65. CEM contains a series of composite strains, and the main strains are photosynthetic bacteria, lactic acid bacteria, fermenting fungi, yeast, and actinomycetes. The quantity of colony forming units (CFU) units in each strain exceeded 10^4/ml.

Experiment Design and Measurements

This pot experiment comprised eight treatments in five replicates. These treatments were: (1) soil alone (CK−CEM); (2) 10-g/kg biochar (B_{10}−CEM, w/w); (3) 20-g/kg biochar (B_{20}−CEM); (4) 30-g/kg biochar (B_{30}−CEM); (5) CEM without biochar (CK + CEM); (6) 10-g/kg biochar with CEM (B_{10} + CEM); (7) 20-g/kg biochar with CEM (B_{20} + CEM); (8) 30-g/kg biochar with CEM (B_{30} + CEM). Soils samples with or without biochar addition were weighed (450-g dry mass) into a series of plastic pots (15 cm × 13 cm × 10 cm) with a hole and a tray in the bottom. Each pot was filled with deionized water to adjust the soil moisture to 60–70% of its water-holding capacity during the experiment. The CEM solution was diluted with deionized water to a concentration of 0.5 and then added to each pot. This diluted concentration was chosen because the alfalfa seed germination rate showed the highest in the pre-experiment. The dosage of the CEM was 4 ml/pot from the CEM solution in the treatments with CEM at the age of irrigation, while the treatments without CEM were added with double distilled water.

This experiment was conducted in a greenhouse. Alfalfa seeds were surface sterilized with 10% H_2O_2 for 0.5 h and rinsed. Fifteen alfalfa seeds were sown and then thinned to five plants per pot after short-term growth (3- to 5-cm height aboveground). The alfalfa grew in May 2019 and harvested in September 2019. The alfalfa was watered daily, and weeds in each pot were eradicated to ensure the growth of alfalfa. The number of seed germination was counted after the seeds were germinated. The height of alfalfa was measured in each plastic pot before harvesting. After harvesting, alfalfas aboveground parts and roots were rinsed, wiped with absorbent paper, and oven-dried at 70°C for 48 h. The soil sample in each pot was collected, mixed, and stored at 4°C for the measurement of soil property.

Soil pH was measured by deionized water at a 1:5 ratio (soil/water) with a pH meter, and soil salt content was measured using the gravimetric method. An elemental analyzer was used to analyze soil TC and TN. The Olsen method was conducted to measure AP, and the NH_4Ac extraction plus flame photometry method was used to analyze available potassium (AK). Soil organic carbon (SOC) was analyzed by the elemental analyzer after being treated with hydrochloric acid (HCl, 1 mol/L) for removal of inorganic carbon. The chloroform fumigation-extraction method was adopted to determine microbial biomass carbon (MBC; Vance et al., 1987). After the soil samples were extracted with potassium chloride (KCl) solution, an AA3 automated flow injection analysis (Auto Analyzer III, Seal, Germany) was used to determine soil NH_4^+ and NO_3^-. Soil sucrase, urease, and alkaline phosphatase activities were measured based on these methods and procedures (Tan et al., 2014). An elemental analyzer was used to analyze plant TN, the molybdenum-antimony colorimetry method was used to test plant total phosphorus (TP), and the flame photometer was used to determine plant total potassium (TK).

Statistical Analysis

All statistical analyses were conducted *via* SPSS 19.0. The influences of biochar, CEM, and their interactions on alfalfa seed germination, height, biomass, nutrient contents, and soil fertility change were tested by two-way ANOVA. The significance of differences in alfalfa growth parameters, soil properties, and soil enzymatic activities was analyzed *via* one-way ANOVA. Pearson correlation analysis was used to explore the relationship of plant parameters with soil quality. Path analysis was performed to ascertain hypothetical pathways by which soil quality and plant nutrient might explain plant biomass. Path analysis was conducted using the AMOS 21.0 version (IBM SPSS Amos 21.0 software). The data were fit to the model using maximum likelihood estimation. We selected the χ^2 test and the root mean square error of approximation to assess the fitness of the model.

RESULTS

Plant Growth, Biomass, and Nutrient Status

Compared with the CK−CEM treatment, seed germination significantly increased by 50–81.25% and 56.25–118.7% under −CEM treatments and +CEM treatments, respectively ($p < 0.05$, **Figure 1A**). The application of biochar and CEM increased alfalfa height among different treatments, and the highest plant height in

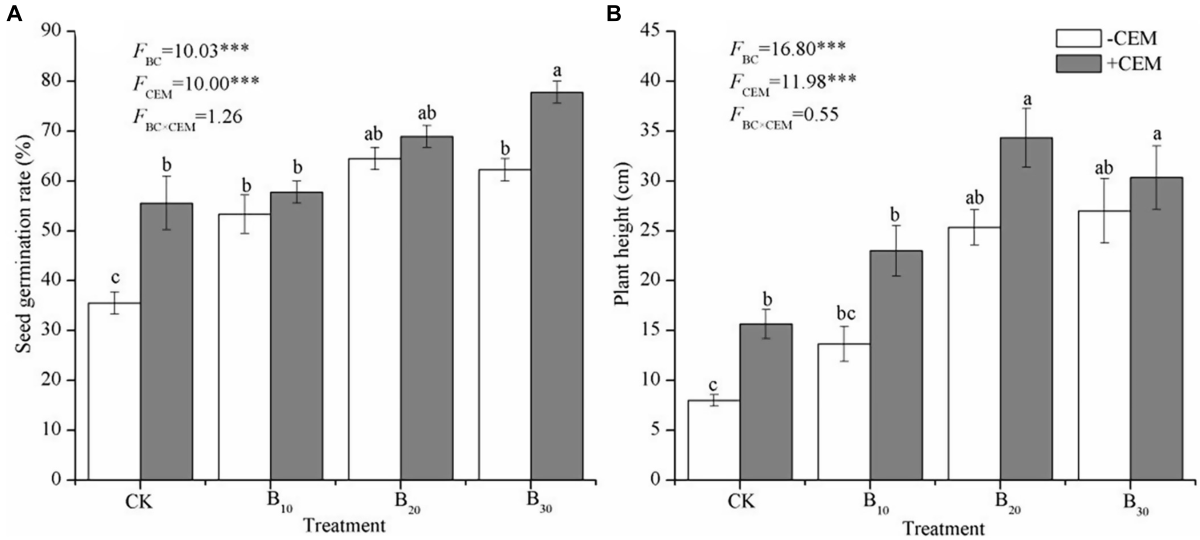

FIGURE 1 | Alfalfa seed germination rate **(A)** and alfalfa plant height **(B)** under different treatments. Bars and error bars show means ± SE (*n* = 5). Different lowercase letters referred to significant difference between treatments ($p < 0.05$). The F_{BC}, F_{CEM}, and $F_{BC \times CEM}$ are *F* and the degree of freedom of two-way ANOVAs. ***$p < 0.001$.

the B_{20} + CEM treatment reached to 34.33 ± 2.96 cm ($p < 0.05$, **Figure 1B**). The influences of biochar and CEM on plant biomass (root, shoot, leaf, aboveground, and total) varied with a biochar level (**Figure 2**). Alfalfa biomass enhanced with the increase of the biochar level in −CEM treatments, and the alfalfa biomass elevated and then declined with the increase of the biochar level in +CEM treatments. The B_{20} + CEM treatment showed the highest biomass among all the treatments, and the biomass of root, shoot, leaf, aboveground, and total was significantly higher than the CK−CEM treatment by 200, 117.3, 144.6, 123.2, and 138.5%, respectively ($p < 0.05$).

The contents of root TN, shoot TN, and leaf TN in other treatments were 60.24–226.6%, 46.46–143.5%, and 33.98–93.73% higher, respectively, than those in the CK−CEM treatment ($p < 0.05$, **Figures 3A–C**). Single biochar amendment or mixture with CEM significantly increased root TP concentrations by 57.58–155.3%, shoot TP concentrations by 36.89–117.6%, leaf TP concentrations by 31.81–108.8%, root TK concentrations by 41.28–138.9%, shoot TK concentrations by 64.55–209.8%, and leaf TK concentrations by 20.85–98.32%, respectively ($p < 0.05$, **Figures 3D–I**), compared with the CK−CEM treatment. Biochar elevated the contents of plant TN, TP, and TK in the −CEM treatments, while biochar increased the concentrations of plant TN, TP, and TK first and then decreased in +CEM treatments. The maximum TN, TP, and TK contents in plant tissues were detected in the B_{20} + CEM treatment. The interactive application of biochar and CEM led to more improved performance on alfalfa growth and nutrient absorption than the individual addition of either biochar or CEM.

Soil Properties and Fertility

Biochar addition with or without CEM had no significant influence on soil pH (**Table 1**). The soil salinity in the B_{10} + CEM, B_{20} + CEM, and B_{30} + CEM treatments was significantly reduced by 38.76, 44.14, and 45.48% than the CK−CEM treatment ($p < 0.05$, **Table 1**). The use of biochar and CEM improved soil TC, and the highest value in the B_{20} + CEM treatment was 50.13% higher than the CK−CEM treatment. Compared with that of CK−CEM treatment, soil TN treated with biochar application alone increased by 17.89–70.36%, while soil TN treated with biochar combined with CEM enhanced by 60.12–114.7% ($p < 0.05$, **Table 1**). Soil AP and AK in the B_{20} + CEM treatment significantly elevated by 44.06 and 18.35% ($p < 0.05$), respectively, compared with the CK−CEM treatment. Biochar amendment alone enhanced soil fertility, whereas the use of 30-g/kg biochar combined with CEM exerted a negative influence on soil fertility.

Soil Nutrient Availability and Enzyme Activities

Compared with the CK−CEM treatment, the B_{10}−CEM, B_{20}−CEM, B_{30}−CEM, B_{10} + CEM, B_{20} + CEM, B_{30} + CEM treatments enhanced SOC by 15.58, 65.07, 135.9, 12.65, 34.95, 157.94, and 140.3%, respectively (**Figure 4A**). Likewise, biochar addition alone significantly enhanced soil MBC by 41.44–86.50%, while biochar mixture with CEM enhanced soil MBC by 43.31–97.33% ($p < 0.05$, **Figure 4B**). Under the −CEM and +CEM treatments, soil NH_4^+ and NO_3^- were significantly increased by 44.95–198.1% and 36.42–104.7% relative to the CK−CEM treatment, and the enhanced effects on soil NH_4^+ were greater than soil NO_3^- ($p < 0.05$, **Figures 4C,D**). Biochar, CEM, and their interaction enhanced soil sucrase activity by 23.64–125.7%, urease activity by 19.35–74.45%, and alkaline phosphatase activity by 13.86–113.8%, as compared to the CK−CEM treatment, respectively (**Figure 5**). Notably, the increase amplitude of soil sucrase activity was more pronounced

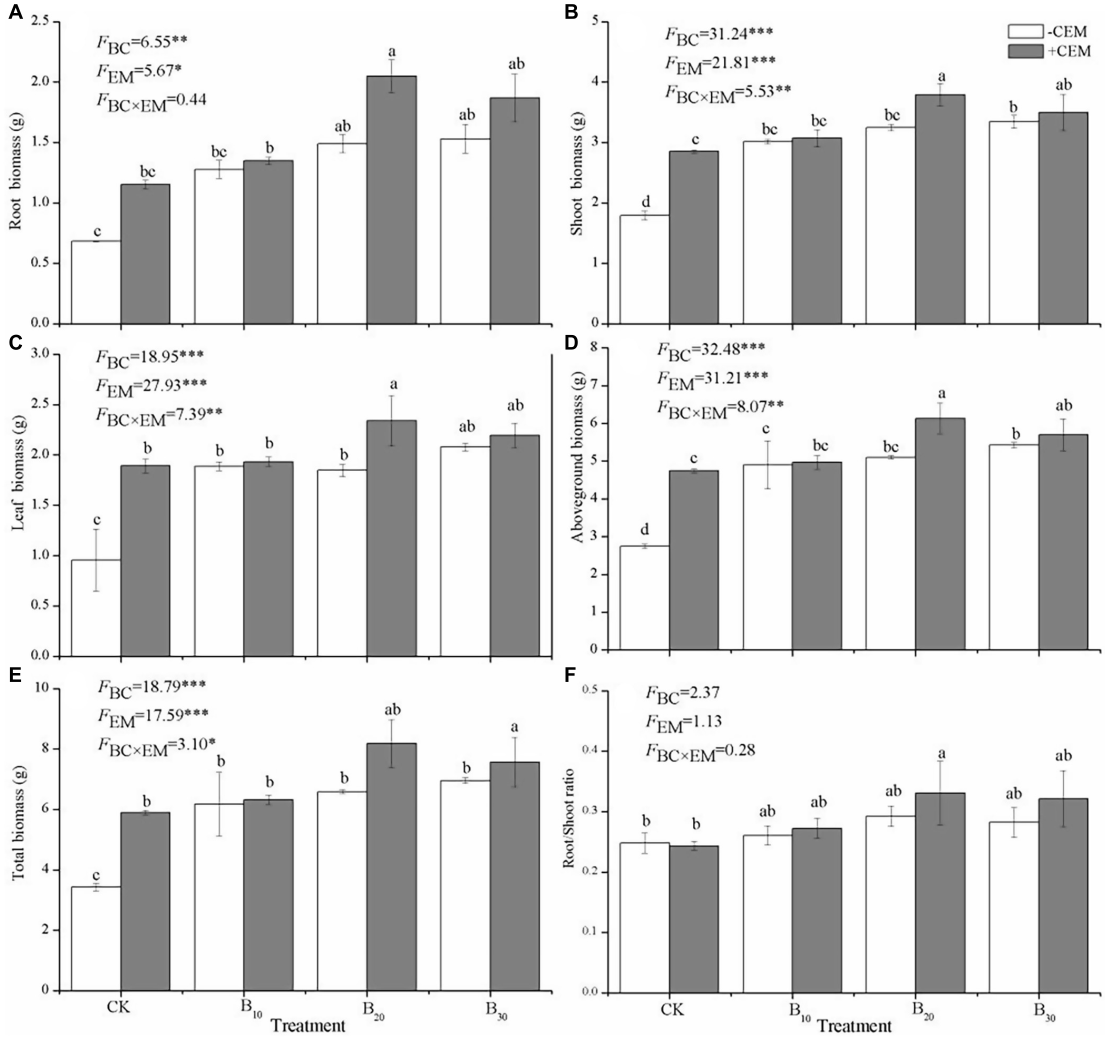

FIGURE 2 | Root biomass **(A)**, shoot biomass **(B)**, leaf biomass **(C)**, aboveground biomass **(D)**, total biomass **(E)**, and root/shoot ratio **(F)** under different treatments. Different lowercase letters referred to significant difference between treatments ($p < 0.05$). The F_{BC}, F_{CEM}, and $F_{BC \times CEM}$ are *F* and the degree of freedom of two-way ANOVAs. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

than alkaline phosphatase and urease activity, and the B_{20} + CEM treatment emerged the highest value among all the treatments.

Linkages Between Soil Nutrient Status and Plant Biomass

Significant positive correlations between plant biomass with nutrient contents of plant tissue, soil TN, SOC, MBC, NH_4^+, and NO_3^- were detected in this experiment (**Table 2**). The various pathways of biochar and CEM addition on soil nutrient and further on alfalfa biomass were quantified by SEM. The root mass, shoot mass, leaf mass, and total mass were explained by 54, 62, 70, and 72% using the path analysis (**Figure 6**). The amendment of biochar and CEM enhanced the contents of soil enzyme activities, TN, SOC, MBC, NH_4^+, and NO_3^-, and then positively promoted plant tissue nutrient absorption, and hence improved plant biomass. Soil TN, SOC, MBC, NH_4^+, and NO_3^- positively affected root TN and TK, and subsequently, root TN and TK strongly impacted root mass (**Figure 6A**). Likewise, soil NH_4^+, NO_3^-, and SOC were positively correlated with shoot TK, and hence, shoot TK mainly affected shoot mass (**Figure 6B**). Soil TN, MBC, NH_4^+, and NO_3^- positively influenced leaf TN, and SOC, NH_4^+, and NO_3^- positively affected leaf TK, and thus, leaf mass was positively driven by leaf TN and TK (**Figure 6C**). With regard to total biomass, soil NH_4^+, NO_3^-, and SOC positively affected total TK, and hence, total mass was explained by total TK (**Figure 6D**).

DISCUSSION

Promotion of Alfalfa Growth and Nutrient Absorption

In accordance with our hypothesis, appropriate application of biochar and CEM in coastal wetland soils resulted in an increase

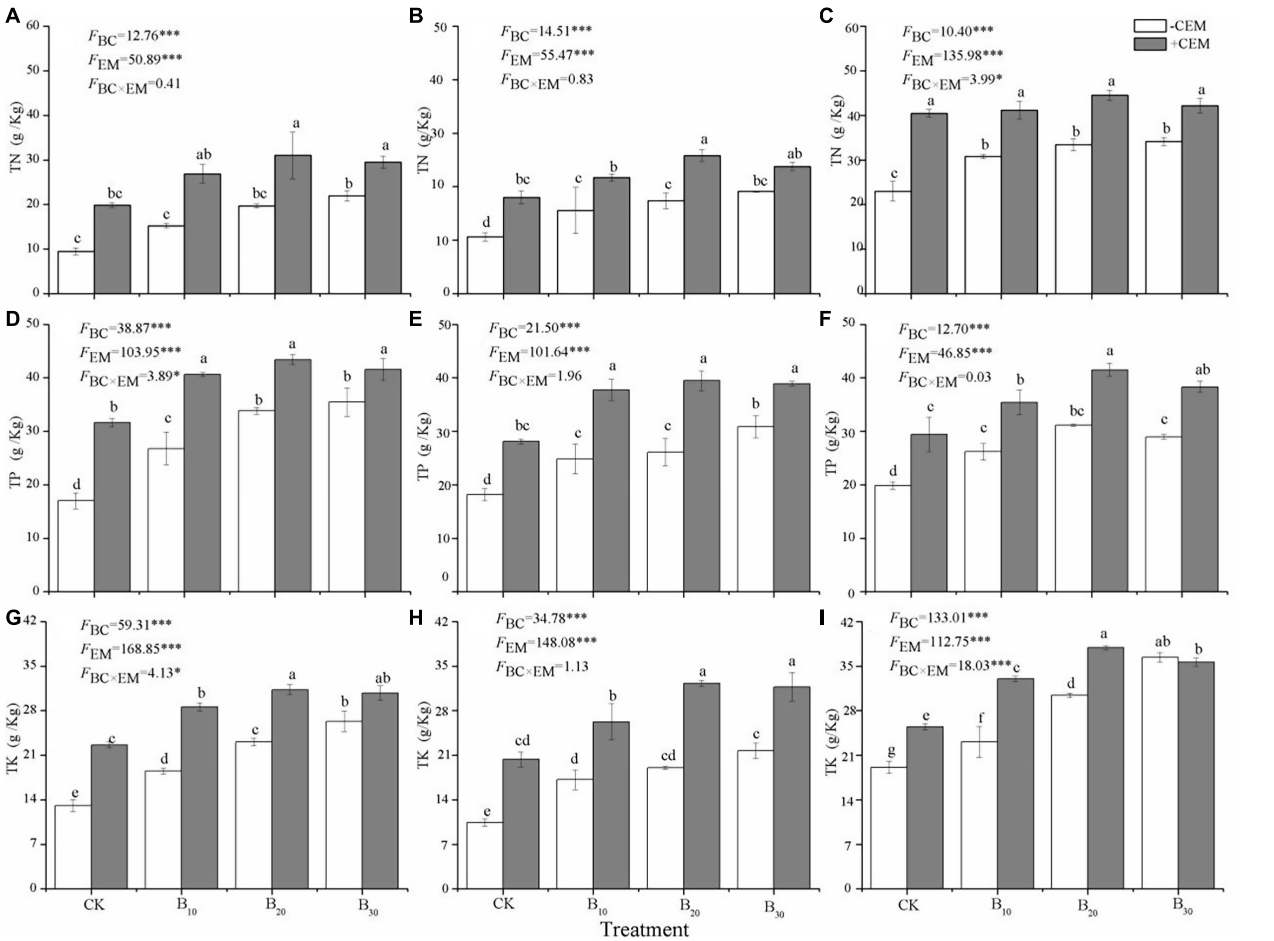

FIGURE 3 | Root TN **(A)**, shoot TN **(B)**, leaf TN **(C)**, root TP **(D)**, shoot TP **(E)**, leaf TP **(F)**, root TK **(G)**, shoot TK **(H)**, and leaf TK **(I)** under different treatments. TN, total nitrogen; TP, total phosphorus; TK, total potassium. Different lowercase letters referred to significant difference between treatments ($p < 0.05$). The F_{BC}, F_{CEM}, and $F_{BC \times CEM}$ are *F* and the degree of freedom of two-way ANOVAs. ***$p < 0.001$ and *$p < 0.05$.

in alfalfa seed germination, height and biomass, indicating that improved alfalfa growth was regulated by many benefits from the presence of biochar (Zhang et al., 2019; Liu et al., 2020). These results were similar to previous studies, which documented that biochar promoted plant growth, yield, and soil quality in salinity soils (Kim et al., 2016; Saifullah et al., 2018). The improved soil physicochemical and biological properties and elevated soil nutrient induced by biochar may be responsible for these results, which can provide nutrient supply with ongoing alfalfa growth (Agegnehu et al., 2017; Ali et al., 2017; Mehmood et al., 2020). As revealed by the path analysis, the improvement of plant biomass may be due to the stimulated nutrient cycling of alfalfa caused by the enhanced soil quality and enzyme activities. These following factors dominated the promotion of biochar on alfalfa growth: (i) improved soil TN, NH_4^+, NO_3^-, SOC, MBC, soil sucrase, urease, and alkaline phosphatase activities (El-Naggar et al., 2019); (ii) stimulated plant TN, TP, and TK concentration (Agegnehu et al., 2017); (iii) decreased soil salinity stress to alfalfa (Lashari et al., 2015).

The promotion impacts of biochar mixture with CEM on alfalfa growth were stronger than sole biochar addition, implying that biochar combined with CEM can have a stronger cooperative effect. These profound lifting effects could be ascribed to the increased beneficial microorganism activity induced by CEM, thus facilitating alfalfa growth through accelerating decomposition of soil organic matter (Talaat, 2019). Moreover, the interior of biochar could offer suitable habitats for the survival of microorganisms, leading to boosted microbial activities and nutrient release. The combined use of biochar and CEM can further promote alfalfa growth through alleviating salt stress and increasing photosynthetic capacity in salted-affected soils (Akhtar et al., 2015; El-Mageed et al., 2020). However, the CEM + B_{20} treatment accelerated alfalfa growth, while the CEM + B_{30} treatment inhibited alfalfa growth. Therefore, the combined use of biochar with CEM should be kept at an appropriate level, which may generate greater noticeable effects.

The amendment of biochar and CEM caused a significant increase in concentrations of alfalfa tissue nutrient (TN, TP, and TK), identifying the positive response of biochar in contributing to plant nutrient uptake as proved by several findings (Biederman and Harpole, 2013; Sigua et al., 2016). Improved soil properties, soil nutrient availability, and plant

TABLE 1 | Soil properties and fertility under different treatments.

Treatment	pH	Soil salt content (%)	TC (g/kg)	TN (g/kg)	AP (mg/kg)	AK (mg/kg)
CK−CEM	7.25 ± 0.04[a]	0.58 ± 0.01[a]	18.70 ± 0.62[d]	0.54 ± 0.08[b]	17.70 ± 0.75[d]	199.23 ± 3.46[c]
B_{10}−CEM	7.16 ± 0.01[a]	0.50 ± 0.03[b]	19.39 ± 0.10[d]	0.64 ± 0.06[b]	21.1 ± 0.65[bc]	210.16 ± 5.31[bc]
B_{20}−CEM	7.16 ± 0.03[a]	0.42 ± 0.03[c]	21.70 ± 0.70[c]	0.65 ± 0.08[b]	22.07 ± 0.52[b]	215.53 ± 6.79[b]
B_{30}−CEM	7.21 ± 0.04[a]	0.39 ± 0.02[cd]	26.05 ± 0.77[ab]	0.92 ± 0.09[ab]	23.63 ± 0.84[ab]	225.50 ± 3.55[b]
CK + CEM	7.17 ± 0.02[a]	0.36 ± 0.01[cd]	21.95 ± 0.90[bc]	0.76 ± 0.21[b]	18.27 ± 0.52[cd]	216.46 ± 3.98[b]
B_{10} + CEM	7.15 ± 0.03[a]	0.35 ± 0.01[d]	24.09 ± 0.69[b]	0.86 ± 0.03[ab]	20.00 ± 0.75[c]	221.80 ± 2.90[b]
B_{20} + CEM	7.12 ± 0.03[a]	0.32 ± 0.01[d]	28.08 ± 0.90[a]	1.16 ± 0.13[a]	25.50 ± 0.31[a]	235.80 ± 1.90[a]
B_{30} + CEM	7.13 ± 0.03[a]	0.31 ± 0.01[d]	27.91 ± 0.86[ab]	1.00 ± 0.15[ab]	24.03 ± 0.67[a]	228.96 ± 4.56[ab]
BC	1.38	12.86***	32.40***	2.98	37.99***	8.78**
CEM	4.22	84.89***	58.61***	9.84**	3.24	18.56**
BC × CEM	0.55	4.43*	3.35*	1.11	4.27*	1.46

*Different lowercase letters behind values referred to significant differences between the same columns ($p < 0.05$). The given are F values of two-way ANOVAs. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. Data were mean values with standard deviation in parentheses (n = 5).*

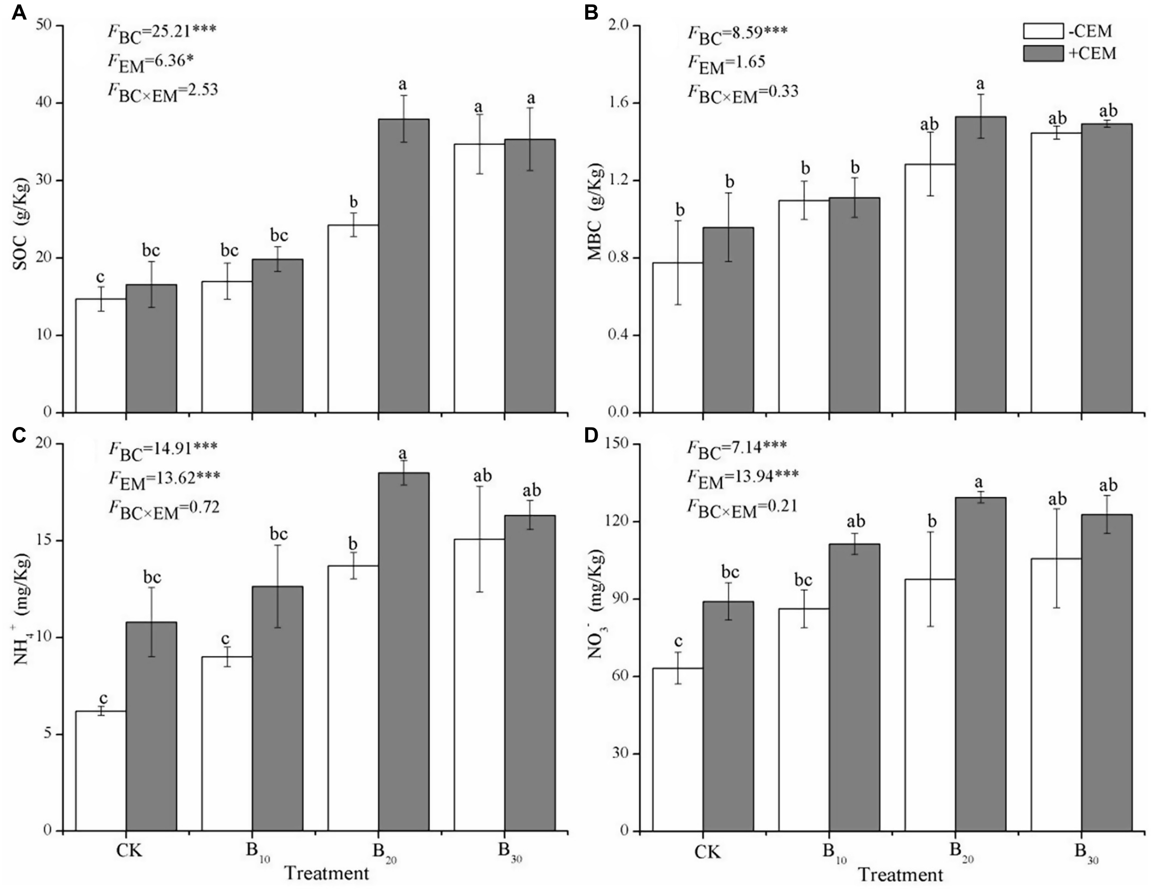

FIGURE 4 | SOC **(A)**, MBC **(B)**, NH_4^+ **(C)**, and NO_3^- **(D)** under different treatments. SOC, soil organic carbon; MBC, microbial biomass carbon; NH_4^+, ammonium nitrogen; NO_3^-, nitrate nitrogen. Different lowercase letters referred to significant difference between treatments ($p < 0.05$). The F_{BC}, F_{CEM}, and $F_{BC \times CEM}$ are F and the degree of freedom of two-way ANOVAs. ***$p < 0.001$ and *$p < 0.05$.

photosynthetic parameter after biochar is applied may contribute to the boosted plant nutrient uptake (Palansooriya et al., 2019). Notably, biochar combined with CEM was more effective in stimulating alfalfa nutrient uptake compared to single biochar application. The CEM could provide photosynthetic bacteria for soil to promote photosynthesis and endogenous phytohormones synthesis (Talaat, 2019). Thus, the EM combined with biochar could alleviate salinity stress and elevate the contents of plant TN, TP, and TK. Hence, biochar combined with CEM could provide a survival advantage for alfalfa growth by acquiring

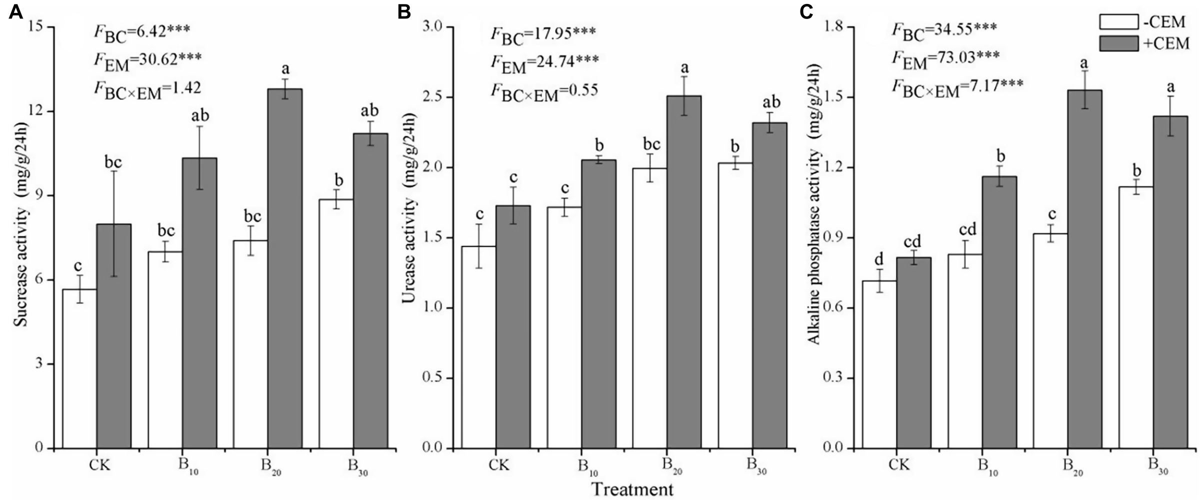

FIGURE 5 | Soil sucrase activity **(A)**, soil urease activity **(B)**, and soil alkaline phosphatase activity **(C)** under different treatments. Different lowercase letters referred to significant difference between treatments ($p < 0.05$). The F_{BC}, F_{CEM}, and $F_{BC \times CEM}$ are *F* and the degree of freedom of two-way ANOVAs. $^{***}p < 0.001$.

more nutrients in soil and improving plant absorption (Agegnehu et al., 2017). In general, nutrient contents of alfalfa tissues could generate dramatically active effect on improving alfalfa growth by modulating nutrient supply (Zhang et al., 2019).

Amelioration of Soil Salinity and Fertility

As an excellent soil conditioner, biochar plays a crucial role in reducing soil salinity *via* improving leaching of soluble salts and declining salt accumulation of surface soils (Saifullah et al., 2018). Biochar addition or combined with CEM reduced soil salinity by 12.97–45.48%, suggesting that biochar addition can alleviate salt stress and offer a suitable environment for alfalfa growth. Moreover, the adsorption of salts on the biochar surfaces or fine pores might be a reason for reducing soil salinity. Another possible explanation for decreased soil salinity could be the cover of biochar that helped downward saline water movement, contributing to the falling soil salinity in topsoil (Hammer et al., 2015; Gunarathne et al., 2020). Biochar addition could facilitate salts leaching by meliorating soil hydraulic conductivity and porosity (Chaganti et al., 2015). Moreover, extensive results carried out confirmed that planting alfalfa was an effective plant-assisted strategy to prevent salinization and promote soil quality in inland regions (Qadir et al., 2001; Cao et al., 2012). Thus, alfalfa planting combined with the amendment of biochar and CEM could also help inhibit salt accumulation in coastal wetlands.

Biochar makes a critical difference in improving soil fertility and rehabilitating salt-affected soils *via* the improvement in soil functions, such as aggregation formation, a nutrient cycle, and microorganism development (El-Naggar et al., 2019; Gunarathne et al., 2020). Our analysis confirmed that biochar application elevated soil content of TC, TN, AP, and AK, and the enhanced effects become even more significant when biochar is combined with CEM. The high content of nutrients in biochar may elevate soil nutrient status and promote nutrient transformation considerably (Kim et al., 2016; Gunarathne et al., 2020). Besides, the porous structure and super absorption of biochar may enhance soil fertility through reserving nutrients and releasing nutrient slowly (Ding et al., 2016). The increased soil aggregation and water holding ability induced by biochar addition were responsible for stabilizing soil structure, which was conducive to decreasing nutrient leaching and increasing nutrient contents (Agegnehu et al., 2017; El-Naggar et al., 2019; Zhao et al., 2020). In addition, the more pronounced improvement of soil fertility caused by the addition of biochar with CEM may also ascribe the beneficial microorganisms that boost soil nutrient cycling and organic matter decomposition, thereby improving soil nutrient supply (Talaat, 2019; El-Mageed et al., 2020).

TABLE 2 | Pearson correlations analyses between plant parameters and soil quality.

Parameters	Height	RB	SB	LB	TB
Root TN	0.67**	0.56**	0.79**	0.79**	0.72**
Shoot TN	0.72**	0.64**	0.72**	0.75**	0.75**
Leaf TN	0.66**	0.61**	0.91**	0.76**	0.73**
Root TP	0.75**	0.64**	0.68**	0.64**	0.76**
Shoot TP	0.67**	0.65**	0.90**	0.78**	0.76**
Leaf TP	0.69**	0.61**	0.69**	0.69**	0.70**
Root TK	0.81**	0.68**	0.94**	0.81**	0.81**
Shoot TK	0.78**	0.73**	0.91**	0.78**	0.80**
Leaf TK	0.85**	0.70**	0.68**	0.78**	0.80**
Soil TN	0.55**	0.63**	0.88**	0.73**	0.68**
SOC	0.77**	0.56**	0.82**	0.61**	0.64**
MBC	0.63**	0.58**	0.90**	0.59**	0.65**
NH_4^+	0.85**	0.62**	0.87**	0.71**	0.75**
NO_3^-	0.67**	0.65**	0.81**	0.71**	0.72**

RB, Root biomass; SB, Shoot biomass; LB, Leaf biomass; TB, Total biomass. $^{**}p < 0.01$.

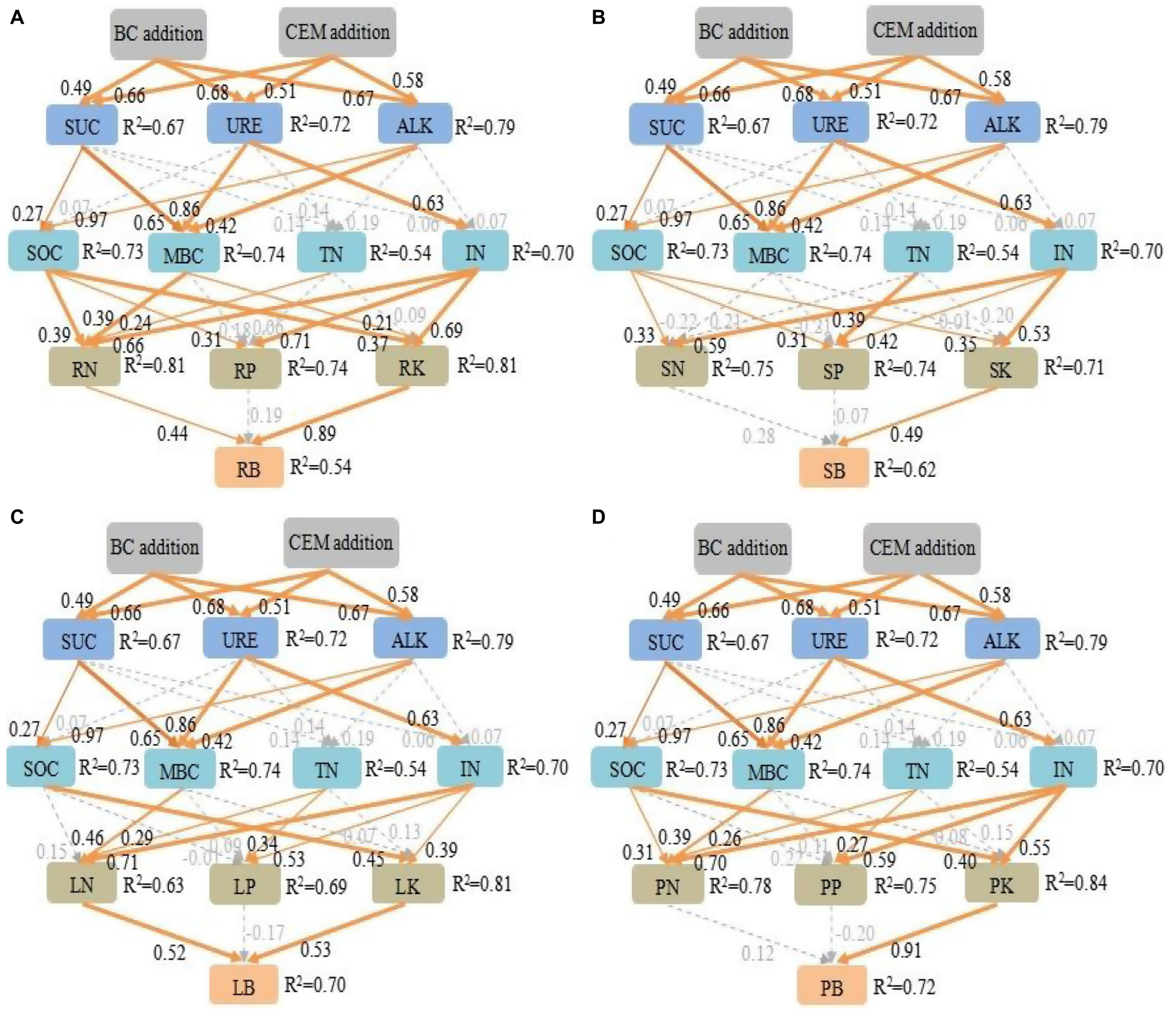

FIGURE 6 | The structure equation model investigates the plausible pathways by which biochar addition and CEM addition affected root biomass **(A)**, shoot biomass **(B)**, leaf biomass **(C)**, and plant total biomass **(D)**. Goodness-of-fit statistics for the model: root biomass: $\chi^2 = 118$, df = 45, $p < 0.05$, root mean square error of approximation (RMSEA) = 0.26. Shoot biomass: $\chi^2 = 127$, df = 45, $p < 0.05$, shoot mean square error of approximation (RMSEA) = 0.28. Leaf biomass: $\chi^2 = 113.6$, df = 45, $p < 0.05$, leaf mean square error of approximation (RMSEA) = 0.25. Plant total biomass: $\chi^2 = 153.9$, df = 45, $p < 0.05$, total mean square error of approximation (RMSEA) = 0.32. Orange solid arrows from thick to thin indicate positive pathways ($p < 0.001$, $p < 0.01$, and $p < 0.05$) and light dashed gray arrows indicate non-significant relationships ($p > 0.05$). The numbers next to arrows are standardized path coefficients, and the arrow width represents the strength of the relationship. The proportion of the variation (R^2) values occurs aside each response variable in the model. SUC, sucrase activity; URE, urease activity; ALK, alkaline phosphatase activity; SOC, soil organic carbon; MBC, microbial biomass carbon; TN, total nitrogen; IN, NH_4^+ and NO_3^-; RN, root total nitrogen; RP, root total phosphorus; RK, root total potassium; SN, shoot total nitrogen; SP, shoot total phosphorus; SK, shoot total potassium; LN, leaf total nitrogen; LP, leaf total phosphorus; LK, leaf total potassium; PN, plant total nitrogen; PP, plant total phosphorus; PK, plant total potassium; RB, root biomass; SB, shoot biomass; LB, leaf biomass; PB, plant total biomass.

Enhancement of Soil Organic Carbon, Microbial Biomass Carbon, NH_4^+, and NO_3^- Availability

Considerable reviews from field or laboratory experiments confirmed that biochar could be used to reduce carbon mineralization and sequestrate soil carbon (Lehmann, 2007; Agegnehu et al., 2016; Liu et al., 2021). We showed that SOC and MBC were facilitated under the application of biochar and CEM, as supported by a meta-analysis study by Liu et al. (2016), who revealed biochar addition elevated SOC and MBC by 40 and 18%, respectively. The increased soil SOC and MBC following biochar and CEM addition may be explained by several reasons: (i) the inertness and rich polychromatic acids of biochar may store carbon in soil and increase the mineralization of SOC *via* activating soil microbial activity and preserving labile soil organic matter (Cooper et al., 2020); (ii) the amendment of biochar and CEM would be more beneficial for accelerating soil carbon decomposition through improving soil aeration and modulating microbial diversity (Talaat, 2019; Liu et al., 2021); (iii) the promoted plant biomass and endogenous carbon formation or exogenous carbon input induced by biochar and

CEM would increase soil aggregation formation and strengthen fertility conservation (Liu et al., 2016; Caban et al., 2020).

Amendment of biochar and CEM led to improvements in soil NH_4^+ and NO_3^- availabilities, which may be closely connected with stimulated soil N recycling in coastal wetland soil. Increases in soil NH_4^+ and NO_3^- may have been linked to the alteration of soil N mineralization to immobilization and the negatively charged biochar surface, in turn influencing plants N absorption (Jones et al., 2012; Lentz et al., 2014; Agegnehu et al., 2017). The improvement of N immobilization and the reduction of N leaching following biochar addition may also be responsible for altering the nitrification rate and enhancing NH_4^+ content (Sun et al., 2017). The CEM supplement modified the diversity and abundance of beneficial microorganisms in salt-affected soils, which, in turn, stimulated soil organic matter turnover and N availability (Olle and Williams, 2013; Talaat, 2019). The path analysis suggested that soil NH_4^+ and NO_3^- were closely correlated with enzyme activities, indicating that biochar and CEM addition may accelerate N transformations by enhancing soil enzyme and microbial activities (Lehmann et al., 2011; Ameloot et al., 2015). Furthermore, the stimulated soil inorganic N availability after biochar and CEM addition was beneficial for alfalfa growth and productivity.

Stimulation of Soil Enzyme Activities

In this study, the mixture of biochar and CEM exerted a beneficial impact on soil sucrase, urease, and alkaline phosphatase activities. Biochar application directly drives changes in soil enzyme activities by targeting diverse soil substrates for the decomposition of SOC (Sinsabaugh, 2010). The priming effect on soil microbial and enzyme activities induced by biochar may stimulate microbial growth by increasing the SOC bioavailability (Pokharel et al., 2020). Significantly, the most likely mechanisms promoting enzyme activities included the following parts: (i) improved soil aggregate formation and water retention (Palansooriya et al., 2019); (ii) enhanced nutrients supply for microbes through improving flows of air, water, and nutrients within soil substrates (Liu et al., 2016); (iii) decreased salinity and provided sufficient carbon sources for microorganisms (Mehmood et al., 2020; Liu et al., 2021). In addition, CEM as a microbial inoculant contained many species of microorganisms and produced biologically active agents that can stimulate the decomposition of organic materials and soil enzyme activities (Talaat, 2019). Soil enzymes are good indicators of soil quality due to the crucial role in influencing microbial activity and nutrient cycling (Pokharel et al., 2020). In our case, soil sucrase, urease, and alkaline phosphatase activities were directly associated with SOC, MBC, and inorganic N, implying that the elevated soil enzyme activities and nutrient availability would be helpful to ameliorate soil quality and promote alfalfa growth (Chávez-García and Siebe, 2019; El-Naggar et al., 2019). Thus, the use of biochar combined with CEM in coastal wetlands soils showed encouraging results, resulting in increased soil enzyme activities, ameliorated soil quality, and improved alfalfa nutrient absorption and growth.

CONCLUSION

Biochar addition alone and combined with CEM decreased soil salt and improved alfalfa growth, nutrient uptake, soil fertility, SOC, MBC, NH_4^+, NO_3^-, and enzyme activities in coastal wetlands. As compared with the CK−CEM treatment, alfalfa mass in the B_{10}−CEM, B_{20}−CEM, B_{30}−CEM, CK + CEM, B_{10} + CEM, B_{20} + CEM, B_{30} + CEM treatment enhanced by 20.01, 91.93, 102.8, 71.91, 84.11, 138.5, and 120.5%, respectively. The B_{20} + CEM treatment could achieve the best performance in terms of elevating soil quality and enzyme activities, contributing to the highest nutrient concentration and growth of alfalfa. Alfalfa biomass was regulated by elevated plant nutrient uptake through promoting soil quality, such as SOC, MBC, TN, NH_4^+, NO_3^-, soil sucrase, urease, and alkaline phosphatase activities. Together, applying biochar compositing with CEM into coastal saline-alkali soils could take as an efficient management practice to accelerate soil nutrient cycling and improve alfalfa productivity. This study could provide effective coastal wetlands restoration approach, and further research should be confirmed in the field.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

QC contributed to data curation, formal analysis, methodology, and writing of the original draft. JX contributed to data curation and supervision. LP contributed to visualization, methodology, and investigation. XZ contributed to conceptualization and formal analysis. FQ contributed to review and editing. All authors reviewed, edited, and approved the manuscript.

FUNDING

This study was funded by the Joint Funds of the National Natural Science Foundation of China (U2006215), the National Natural Science Foundation of China (41901118), Natural Science Foundation of Shandong Province (ZR2019PD012), Shandong Key Laboratory of Coastal Environmental Processes, YICCAS (2019SDHADKFJJ10), and Taishan Scholars Program of Shandong Province, China (No. TSQN201909152).

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