Research article

Suitability of coconut bran and biochar as a composite substrate for lettuce cultivation in aquaponic systems

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A B S T R A C T

Growth substrates are essential for aquaponic systems and play an important role in vegetable growth and water quality. In this study, we explored an innovative combination of coconut bran and coconut shell biochar (CSB) as a composite growth substrate for lettuce cultivation in aquaponic systems. The study included the control (100 % coconut bran as the growth substrate) and treatment groups (T1–T5; containing 10 %, 20 %, 30 %, 40 %, and 50 % CSB as the growth substrate, respectively). The substrate properties; lettuce growth performance; and soil enzyme activity, nitrogen content, and abundance of microbial communities in the substrate were analyzed to determine the optimal substrate. Our findings indicated that CSB incorporation significantly altered the properties of the substrate, resulting in increased dry and bulk densities, pH, and water-holding capacity, and decreased electrical conductivity, water-absorption capacity, and porosity. Furthermore, the fresh weight of lettuce was notably increased in the treatment groups. The activities of fluorescein diacetate hydrolase, urease, nitrate reductase, and hydroxylamine reductase initially increased and further decreased, reaching the maximum in the T3 group. Conversely, the activity of nitrite reductase and contents of available nitrogen, nitrate-nitrogen, and ammonium-nitrogen in the substrates initially decreased and further increased, with the minimum values observed in the T3 group. The microbial sequencing results indicated that CSB incorporation significantly increased the microbial diversity and relative abundance of microorganisms associated with nitrogen transformation. Moreover, 30 % CSB incorporation exhibited the greatest effect on lettuce growth, with a 34.5 % and 31.6 % increase in fresh weight compared to the control during the growth and harvest periods, respectively. This study indicated the enormous potential of biochar in the research and development of green technologies for substrate amendment in aquaponic systems.

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1. Introduction

Aquaponics is an innovative agricultural system that combines aquaculture, hydroponics, and beneficial bacteria in a mutually beneficial manner [1]. This technology is being increasingly recognized as a sustainable approach for organic food production with minimal environmental impact [2,3]. With lower daily water consumption than traditional aquaculture and horticulture, aquaponics facilitates the cultivation of abundant food crops in compact spaces [4]. Although aquaponics holds promising potential, its commercialization is hindered by several limitations, particularly by concerns regarding optimal growth performance of plants in this system [5,6].

Selecting an appropriate growth substrate is a pivotal factor for optimal plant growth performance in an aquaponic system because it protects plant roots and ensures stability [7,8]. The application of appropriate substrates for plant cultivation in aquaponic systems is reported to enhance plant productivity and nutrient content [9–11]. Moreover, the substrate is instrumental in retaining and gradually releasing the nutrients to plant roots, as well as in the filtration of fish waste [12]. The growth substrates commonly employed in aquaponics include volcanic lava rocks, gravel, and lightweight expanded clay aggregates (LECAs) [13,14]. Gravels and LECAs are extensively used in commercial aquaponic systems globally, considering their compatibility with popular leafy vegetables [14]. However, the relatively high cost of LECA makes it challenging for local farmers to adopt aquaponic practices [14]. Moreover, volcanic lava rocks and gravel have been the least favorable of their low durability, difficulty in cleaning, bulkiness, and cumbersome operation.

The growth substrate is selected depending on its effect on plant production and as per economic and environmental considerations [15]. Bioresources such as agricultural waste and renewable materials can be used as substrate [16]. Moreover, composite substrates are gaining popularity because of the nutrient richness and stability. Coconut bran, a byproduct of coconut husk fiber processing, is recognized for its renewable nature, cost-effectiveness, stable chemical properties, excellent water absorption, and fertilizer retention [17]. It is widely used in horticultural substrates [17]. The use of coconut bran as a substrate has ecological advantages such as mitigation of resource depletion and minimization of environmental pollution [18]. However, a previous study revealed that exclusive use of coconut bran may result in seedling dehydration and growth retardation; therefore, it should be used as a component of composite substrates [19]. Biochar is produced from agricultural waste under anaerobic conditions through high-temperature pyrolysis, which addresses the aforementioned environmental concerns and helps in enhancing the physical properties of substrate [20, 21]. Because of its rich pore structure and large surface area, biochar can effectively retain water and nutrients; this makes it a good soil conditioner, fertilizer enhancer, and carbon sequestration agent [22]. The unique structure of biochar enables it to adsorb mineral nitrogen from the soil, thereby reducing nitrogen loss through volatilization [23]. Moreover, certain microorganisms may be protected by adhering to the pores of biochar, which enhances the microbial community diversity [24]. Previous studies indicated that the addition of biochar in different growth substrates could significantly enhance the nutritional status and plant growth in aquaponics and hydroponics [14,21,25]. However, the suitability of coconut bran and biochar as substrate materials for the cultivation of leafy vegetables in aquaponic systems is not yet reported.

In this study, we investigated the efficacy of the combination of coconut shell biochar (CSB) and coconut bran as a novel growth substrate for lettuce (*Lactuca sativa*) cultivation in an aquaponic system containing largemouth bass (*Micropterus pallidus*). The physicochemical properties of the composite substrate, soil enzyme activities in the substrate, diversity and structure of the microbial community during lettuce growth, and growth performance of lettuce were studied to evaluate the suitability and performance of the composite growth substrate in aquaponic systems.
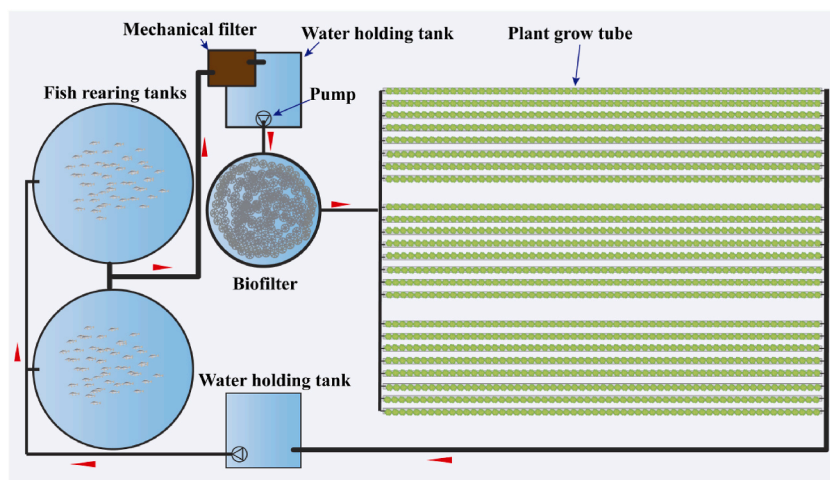

Fig. 1. Schematic of the experimental aquaponic system. The red arrows represent the direction of the water flow.

2. Materials and methods

2.1. Experimental setup and operation

The experiment was conducted at the Laboratory of Aquaponics, Fishery Research Institute, Anhui Academy of Agricultural Sciences, Anhui Province (117°14′ E, 31°53′ N). The aquaponic system comprised two fish rearing tanks (total volume = 6000 L), two water-holding tanks, a mechanical filter, a 2500 L biofiltration tank filled with 90 kg of biofilter media (K3 media, Henan Qianbang Environmental Protection Technology Co., Ltd., Zhengzhou, China), and a plant growing unit consisting of 12-m long planting tubes (n = 24) (Fig. 1). Well water was used to fill the aquaponic system and replenish transpiration and evaporation losses during this study. A water pump was used to deliver water from the fish rearing tank to the plant growing unit. An air pump was used to aerate the fish tanks. Each fish rearing tank was filled with 30 kg/m^3 largemouth bass fish purchased from Nanxun Yufuren Fishery Co., Ltd. (Huzhou, China), which was domesticated for 3 months before the experiment. The fishes received commercial fish feed (Tongwei Group, China) at 1 % of their body weight twice daily at 8:00 and 18:00 during the entire experiment. The feed had particle size of 4.0 mm and composed of 48 % protein. The water environmental conditions were maintained as follows: temperature 25–30 °C, pH 6.5–7, and dissolved oxygen content >5 mg/L. Ammonia-nitrogen and nitrite-nitrogen remaining within safe ranges.

2.2. Experimental materials and design

Seeds of the Batavia variety of lettuce were purchased from Dingfeng Modern Agricultural Development Co., Ltd. (Beijing, China). Desalted coconut bran was purchased from Hezhiyuan Ecological Agricultural Technology Co., Ltd. (Xiamen, China). CSB, with a particle size of 2–3 mm and an iodine adsorption capacity of 805 ± 8.48 mg/g, (determined according to the ASTM D4607-14 standard method [26]), was obtained from Tenghui Water Treatment Materials Co., Ltd. (Zhengzhou, China). CSB was characterized using scanning electron microscopy (SEM; Model SU-8020, Hitachi, Tokyo, Japan) equipped with energy dispersive X-ray spectroscopy (EDS) and Fourier transform infrared spectroscopy (FTIR; Bruker Company, Germany) in the wave number range of 400–4000 cm^{-1} using the KBr pellet technique. An Accelerated Surface Area and Porosimetry system (ASAP 2020; Micromeritics Co., USA) was used to determine the pore properties of CSB, including the Brunauer–Emmett–Teller (BET) specific surface area, pore volume, and pore size distribution. The Barrett-Joyner-Halenda (BJH) method was utilized to analyze the pore size distributions of CSB by calculating the distributions within the mesopores and small macropores range based on experimental N$_2$ isotherms (desorption branch) using the Kelvin model of pore filling [27].

The plants were divided into six groups: the control group (CK; substrate 100 % coconut bran) and treatment groups T1 (10 % biochar and 90 % coconut bran), T2 (20 % biochar and 80 % coconut bran), T3 (30 % biochar and 70 % coconut bran), T4 (40 % biochar and 60 % coconut bran), and T5 (50 % biochar and 50 % coconut bran). The growth substrate for each group was filled in nonwoven seedling bags. Each group contained 40 Batavia lettuce plants arranged as per the randomized complete block design. Lettuce and the growth substrate were sampled on the 15th and 30th days after sowing and were stored at −80 °C for subsequent testing.

2.3. Assessment of the substrate properties and lettuce growth parameters

2.3.1. pH, electrical conductivity, dry density, bulk density, and porosity of the substrate

The air-dried substrate and deionized water were mixed in a ratio of 1:5 (m/v), thoroughly stirred for 2 h, and filtered with filter paper at room temperature. Further, the pH and electrical conductivity (EC) of the solution were measured using a combined EC and pH meter (HQ440d Multi; Hach, Loveland, USA). The dry density is the ratio of the mass of solid particles to the total volume of the material. According to Shi's method [28], the substrate was dried at 105 °C till constant weight was obtained. Further, it was filled into a beaker with a fixed volume (V); the mass per unit volume of dry substrate was calculated as the bulk density. The air-dried substrate was added to a beaker (of weight M0), and the total weight of the dried substrate and beaker of was measured and labelled as M1. To this, water was added until it was saturated; the weight was measured and labelled as M2. Further, the breaker was covered with a wet gauze (the weight measured and labelled as M3), inverted, allowed to stand for approximately 6 h until no water oozed out of the container, and further weighed (labelled as M4). Various parameters were calculated using the following formulae:

$$Total\ porosity = (M2 - M3 - M1)/V \times 100\% \tag{1}$$

$$Ventilation\ porosity = (M2 + M3 - M4)/V \times 100\% \tag{2}$$

$$Water - holding\ porosity = (M4 - M1 - M3)/V \times 100\% \tag{3}$$

2.3.2. Water absorption and water retention

In a nylon mesh bag with suitable pore size, 10 g of naturally air-dried substrate was taken. The bag was sealed and immersed in water. After 12 h, the bag was taken out; the sample was drained until free of water droplets and weighed again. Water absorption was measured as the mass of water absorbed per gram of substrate, with the water absorption of the CK group considered as "1". Accordingly, the water absorption of treatment groups was calculated [29].

The water loss was calculated as follows. A total of 15 g of naturally air-dried substrate was taken and treated in the same way as

described above; 10 g of the substrate saturated with water was accurately weighed for each group, spread in petri dishes, placed in a constant-temperature incubator set at 25 °C, and weighed every 1 h. The water loss of substrate from each group was recorded, and water loss curves were plotted [30].

2.3.3. Assessment of lettuce growth parameters

The growth performance of the lettuce plants was measured on the 15th and 30th days after sowing. Three uniformly growing plants from each group were selected, and their fresh weights were measured using an electronic scale. Shoot height, leaf length, and leaf width were measured using a steel tape. Shoot height was measured from the base to the highest point of the plant. Leaf length and width were measured using the leaf on plant with the largest leaf area. The number of leaves in each sample was determined.

2.4. Measurements of soil enzyme activities and nitrogen contents

The substrate samples were air-dried at 37 °C in a blast-drying oven, ground using a mortar and pestle, and passed through a 40-mesh sieve for subsequent testing. The ammonium-nitrogen, nitrate-nitrogen, and available nitrogen contents and activities of
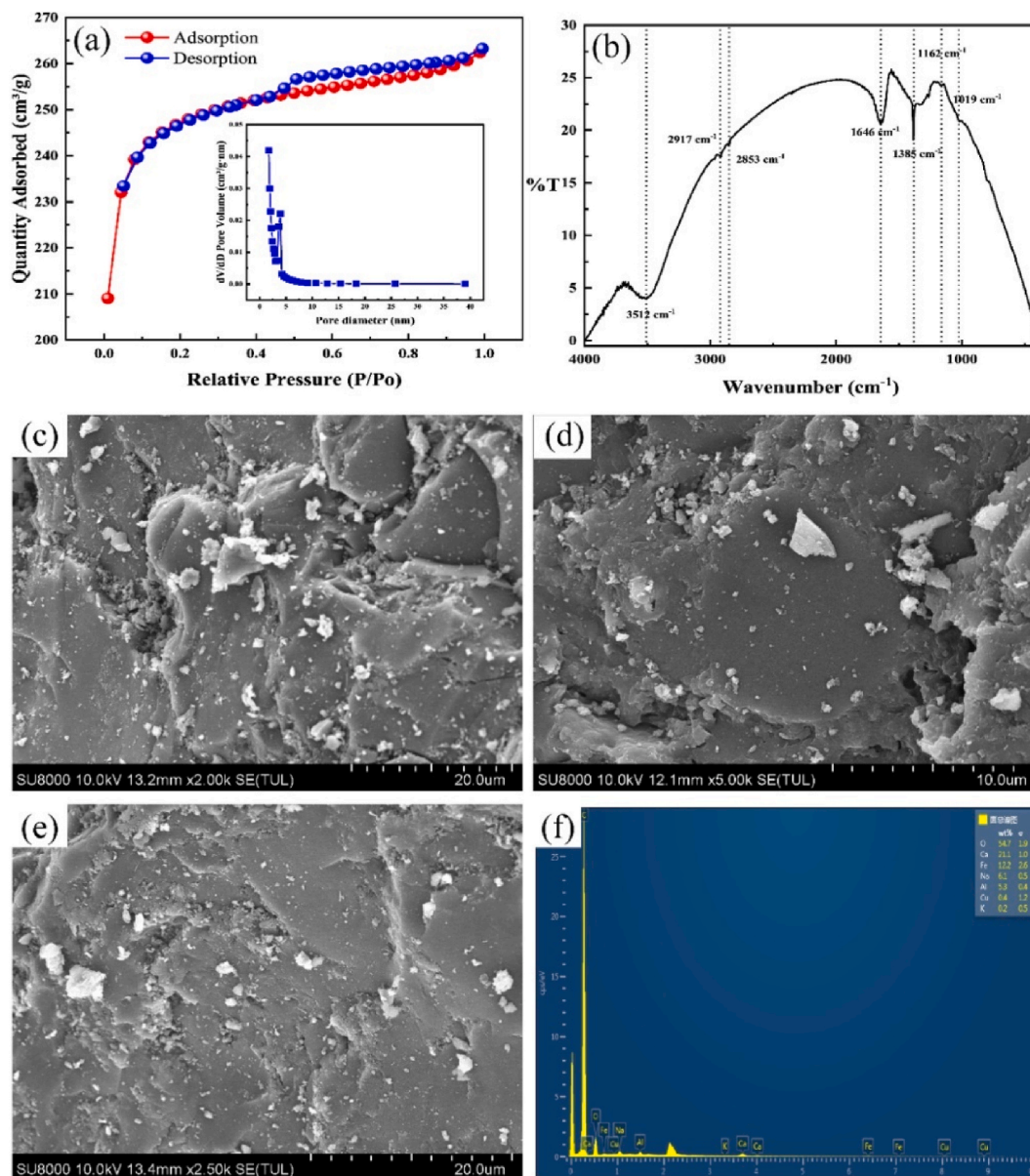

Fig. 2. N_2 adsorption–desorption isotherms, pore size distribution (a), FTIR spectrum (b), SEM images (c, d, and e), and EDS spectrum (f) of coconut shell biochar (CSB).

fluorescein diacetate hydrolase (FDA), urease (UE), nitrate reductase (NR), nitrite reductase (NiR), and hydroxylamine reductase (HyR) in the substrate were measured using respective test kits (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) as per the manufacturer's protocol.

2.5. Assessment of microbial community

Microbial DNA was extracted from the substrate samples using an E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, U.S.) as per the manufacturer's protocol. Subsequently, the V4–V5 region of the bacterial 16S rRNA gene was amplified using PCR (95 °C for 2 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and final extension at 72 °C for 5 min). The primers used were 515F 5'-barcode-GTGCCAGCMGCCGCGG-3' and 907R 5'-CCGTCAATTCMTTTRAGTTT-3', where the barcode is an eight-base sequence unique to each sample. PCR was performed in triplicates. The reaction mixture (20 μL) contained 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The amplicons were extracted from 2 % agarose gels after electrophoresis and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions. Qubit®3.0 (Life Invitrogen) was used to quantify the purified PCR products, followed by sequencing using a MiSeq Illumina platform (Shanghai BIOZERON Co., Ltd., China) as described in the standard methodology and Illumina computer software. The sequences were aligned against the Silva (SSU132) 16S rRNA database for taxonomic classification. Microbial alpha-diversity was determined by calculating the coverage and Shannon and Simpson indices using the online Lingbo Cloud Platform (www.cloud.biomicroclass.com/CloudPlatform). Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) and the FAPROTAX program were used to predict functional alterations in the microbiota across different samples. The sequencing data were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (accession number: PRJNA1095029).

2.6. Data processing and analysis

Statistical analysis of the experimental data and the test of significance of differences (using one-way ANOVA and Duncan's multiple comparisons; P < 0.05) were performed using SPSS21.0. Graphs were plotted using OriginPro2021 and Adobe Illustrator2022.

3. Results and discussion

3.1. The characterization of coconut shell biochar (CSB) and its effects on the substrate properties

The pore properties and surface functional groups of biochar determine its water retention property, sorption of contaminants, and microbial growth in it [27]. Fig. 2a shows the N_2 adsorption–desorption isotherms and pore size distributions of CSB. According to the International Union of Pure and Applied Chemistry (IUPAC), the adsorption isotherms should be Type IV [31]. As the hysteresis loop (irreversible process) was observed during adsorption–desorption, the isotherm shape of CSB was characteristic of mesoporous materials [31]. The pore properties of CSB such as surface area (965.6846 m^2/g), pore volume (0.4072 cm^3/g) and average pore size (3.0121 nm) are shown in Table S1, which again indicated a mesoporous feature of CSB. The SEM images (Fig. 2c, d and 2e) revealed that the surface of CSB exhibited significant irregularities, and no discernible macropores were observed that corresponded to tracheid cells in the conventional biochar [32]. Examination of the chemical composition of CSB using EDS analysis revealed that in addition to carbon and oxygen, CSB included Ca, Fe, K, Na, Cu, and Al (Fig. 2f). FTIR spectra of CSB consisted of O–H stretching vibrations in the hydroxyl groups (approximately 3500 cm^{-1}), C–H stretching vibrations in aliphatic CH_2 and CH groups (2850–2970 cm^{-1}), C=C or C=O stretching vibrations in aromatic groups (1700–1500 cm^{-1}), symmetric deformation band of –CH_3 groups (approximately 1385 cm^{-1}), and hydroxyl group bending vibration in (C–O (H)) compounds (1200-1000 cm^{-1}) (Fig. 2b) [27]. Hence, the mesoporous structure and functional groups present in CSB significantly impact soil pH and its interaction with ionic contaminants, leading to increased cation exchange capacity (CEC) and cation adsorption capabilities particularly for metal ions. These attributes positively contribute to water retention, adsorption capacity, and space for microbial growth [27].

Further, we determined the influence of addition of CSB on the physicochemical characteristics of the substrate such as bulk density, total porosity, water-holding capacity, aeration porosity, pH, and EC, which play a role in its ability to retain moisture, support

Table 1
Physicochemical properties of the substrates with different levels of CSB.

	CK	T1	T2	T3	T4	T5
Dry density (g/cm^3)	0.12 ± 0.01f	0.16 ± 0.01e	0.19 ± 0.01d	0.27 ± 0.02c	0.37 ± 0.02b	0.41 ± 0.01a
Bulk density (g/cm^3)	0.08±0f	0.12 ± 0.01e	0.16 ± 0.01d	0.21±0c	0.27 ± 0.01b	0.29 ± 0.01a
EC value (μS/cm)	585.33 ± 20.74a	458.67 ± 21.55b	323.33 ± 16.17c	218.33 ± 18.45d	178.33 ± 14.57e	150 ± 12.77e
pH	6.77 ± 0.06d	6.87 ± 0.06cd	6.97 ± 0.06BCE	7.03 ± 0.06 ab	7.07 ± 0.06 ab	7.17 ± 0.15a
Water absorption capacity	1 ± 0.02a	0.84 ± 0.04b	0.65 ± 0.03c	0.48 ± 0.01d	0.39 ± 0.03e	0.34 ± 0.01f

Note: Values are presented as the mean ± SD; n = 3. Different lowercase letters indicate significant differences between treatments at P < 0.05 according to Duncan's multiple comparison test.

air circulation, and take up nutrients [33]. These critical characteristics collectively determine the effect of the substrate on water and nutrient uptake by crops, ultimately influencing overall plant growth.

CSB incorporation significantly improved the dry density, bulk weight, and pH (P < 0.05) but significantly reduced the EC values and water absorption capacity (P < 0.05). Compared with the dry density (0.12 g/cm^3) and bulk density (0.08 g/cm^3) of the CK group, those (0.41 and 0.29 g/cm^3, respectively) of the T5 group exhibited more than 3-fold increase (P < 0.05) (Table 1). Generally, bulk density of 0.1–0.8 g/cm^3 is considered optimal for crop growth [34]. Moreover, an increase in bulk density implies greater substrate compactness, resulting in relatively reduced porosity [35]. Notably, the bulk density of the CK group was <0.1 g/cm^3, and CSB incorporation significantly increased the bulk density of substrate, favoring lettuce growth and enhancing resistance to lodging. Biochar, known for its strongly alkaline nature, significantly increased the pH of the composite substrate (P < 0.05), which is consistent with the results of Yang [36]. Soil acidification occurs when plants such as banana take up more cations than anions [37]; this phenomenon is also observed in lettuce cultivation. Therefore, the pH adjustment induced by CSB mitigated substrate acidification during lettuce cultivation. In addition, CSB incorporation significantly reduced EC and water absorption (P < 0.05). Consequently, CSB incorporation significantly altered the physicochemical properties of the substrate. These characteristics likely contributed to improvements in the particle composition, nutrient conditions, and microbial community structure within the substrate, ultimately enhancing plant growth [38].

Fig. 3a shows the total porosity, aeration porosity, and water-holding porosity of various composite substrates. CSB incorporation significantly reduced both total porosity and aeration porosity within the substrate (P < 0.05), which is consistent with previous studies [36,39], and exerted a comparatively weaker influence on water-holding porosity (P < 0.05). The T5 group exhibited 46.11 %, 26.81 %, and 17.83 % reductions in the total porosity, aeration porosity, and water-holding porosity, respectively, compared with those in the CK group. Our results indicated that total and aeration porosities of substrates decreased with CSB incorporation because of the lack of macropores in CSB [32], as evidenced by SEM results. However, substrates with 30–50 % CSB still maintained 60–90 % total porosity and 10–30 % aeration porosity, similar to most soilless growth media [40]. Fig. 3b shows the water loss curves for the composite substrates in each group, demonstrating that CSB incorporation enhanced the water-holding capacity. Overall, CSB incorporation reduced the water absorption capacity and water-holding porosity of the substrate while concurrently enhancing the water-holding capacity. Kaudal et al. [41] reported that the water absorption capacity of substrate decreased with the incorporation of biochar, which is thought to be the cause of the short water absorption treatment time; however, longer exposure to water caused biochar to absorb more water than peat [42]. In the context of this aquaponic system, where the substrate draws nutrients from a continuous liquid source via the pipeline, the water absorption rate of the substrate surpasses the rate of water loss. This systematic advantage compensates for the decrease in water-absorbing capacity induced by biochar. Additionally, the increased water-holding capacity ensures a sustained supply of adequate nutrients to support lettuce growth.

3.2. Effect of CSB application on the growth parameters of lettuce

To determine whether CSB application altered the growth performance of lettuce, the leaf length and width, number of leaves, shoot height, and fresh weight were evaluated on the 15th and 30th days. Throughout the entire growth stage, the T3 group exhibited significantly greater leaf length than the CK group (P < 0.05), whereas no significant differences were observed among other groups (P > 0.05) (Fig. 4a, b, and 4c). This may be attributed to the T3 group having higher soil enzyme activity and nutrient availability. Jabborova et al. reported similar findings confirming significantly enhanced leaf length in spinach (*Spinacia oleracea* L.) and basil (*Ocimum basilicum* L.) after biochar addition via alteration in soil enzymatic activities and nutrient availability [43,44]. Additionally, the leaf width and number of leaves were slightly increases in the T1–T5 groups compared with those in the CK group, although the differences were not significant (P > 0.05). Equal volumes of biochar and perlite enhanced the number of leaves and leaf area in
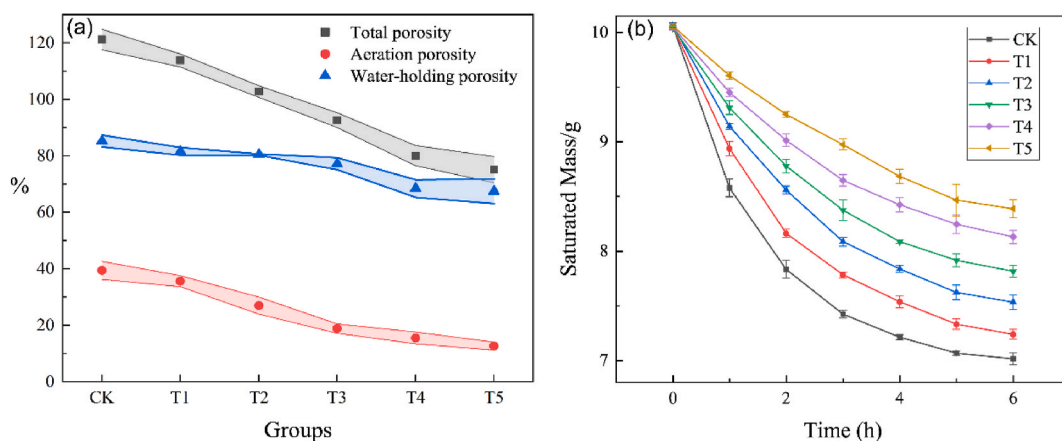

Fig. 3. The total porosity, aeration porosity, and water-holding porosity (a) and the water loss (b) curves of the substrates in substrates with various CSB levels.
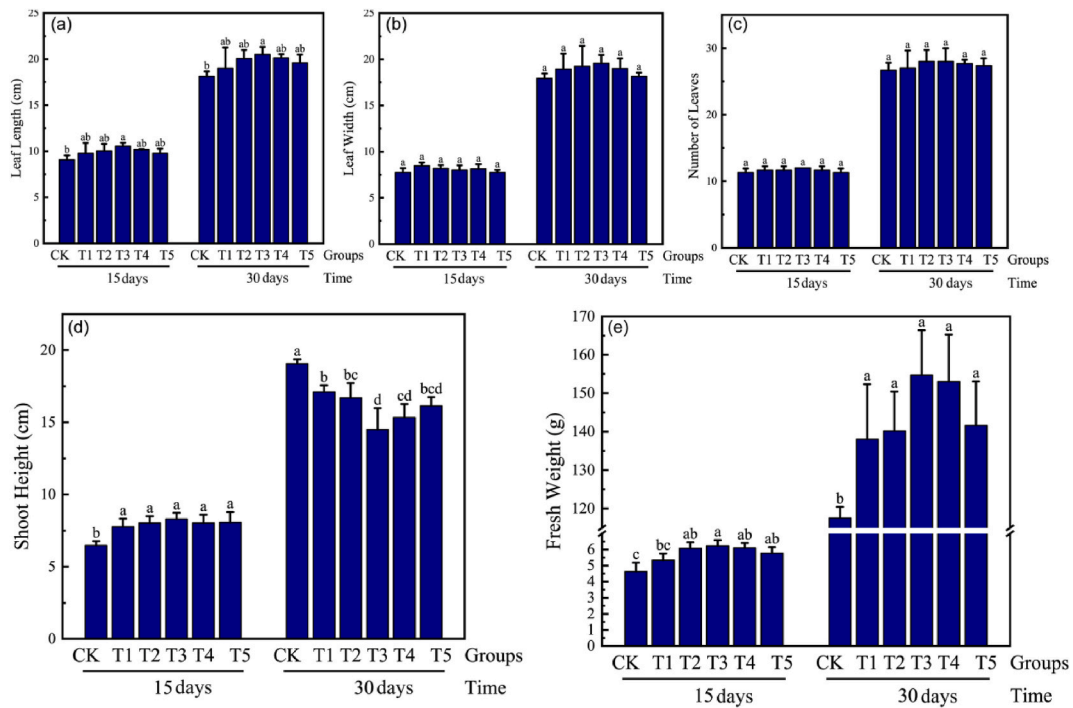
Fig. 4. Lettuce growth parameters in the substrates with various CSB levels on the 15th and 30th days. Leaf length (a), leaf width (b), number of leaves (c), shoot height (d), and fresh weight (e).
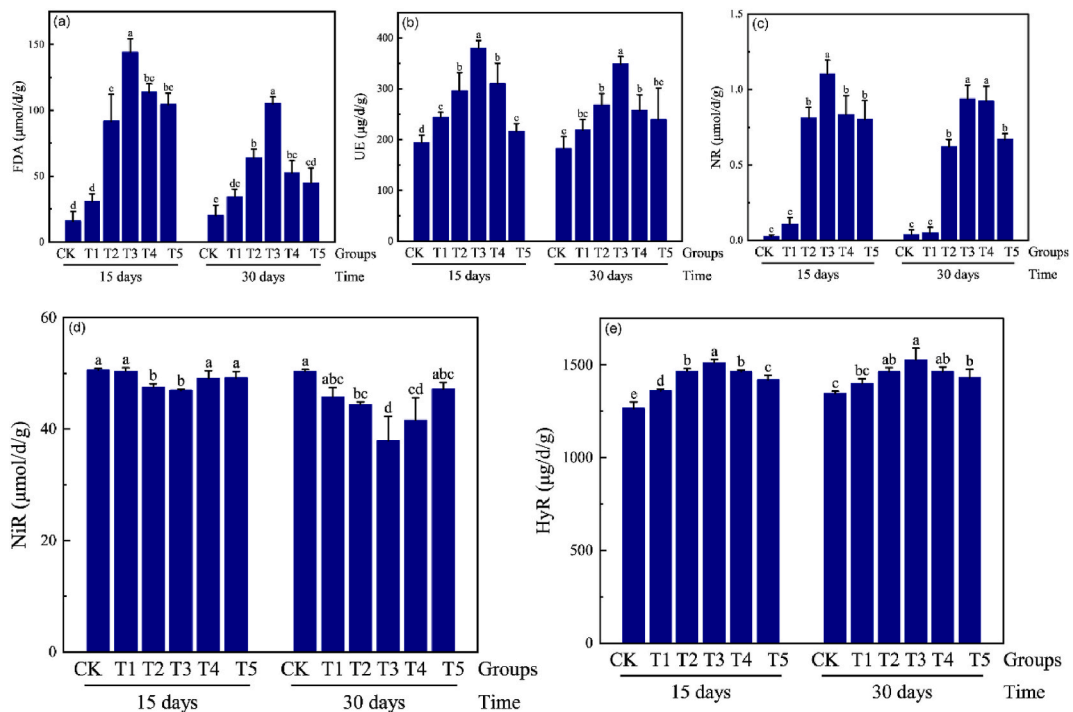

Fig. 5. Effect of CSB application on the soil enzyme activities of the substrate on the 15th and 30th days. The activities of fluorescein diacetate hydrolase (FDA) (a), urease (UE) (b), nitrate reductase (NR) (c), nitrite reductase (NiR) (d), and hydroxylamine reductase (HyR) (e).

various leafy vegetables cultivated in hydroponic systems; however, the results were not significant [21], which is consistent with our results.

Fig. 4d shows the temporal variations in lettuce shoot height. On the 15th days, CSB incorporation significantly increased lettuce shoot height compared with the CK group (P < 0.05); however, no significant differences were observed among the T1–T5 groups (P > 0.05). In contrast, on the 30th day, the shoot heights were significantly lower in the T1–T5 groups than in the CK group (P < 0.05), with the most significant differences observed in the T3 group. A previous study demonstrated that incorporating biochar into a sand substrate had a favorable impact on the development of lettuce during the first 2 weeks, particularly on shoot height, followed by a decreasing trend [45]. This is consistent with our study. Moreover, the application of biochar-based compost decreased the shoot height of crown daisy [46], consistent with the results observed in the subsequent stage of this study. Notably, in common hydroponic cultivation of lettuce, the emergence of bolting represents one of the factors contributing to diminished lettuce yields. This phenomenon is characterized by accelerated longitudinal growth resembling a pagoda shape, culminating in collapse and subsequent growth restriction.

Fig. 4e shows the fresh weight of lettuce across distinct growth stages, revealing a significant improvement (P < 0.05) in all treatment groups compared with the CK group. Remarkably, the T3 group exhibited the highest fresh weight at both time points, with values of 6.23 and 154.7 g, respectively. Carter et al. [47] and Jabborova et al. [43] reported similar results and demonstrated that biochar significantly enhanced the fresh weight and shoot length of lettuce (*Lactuca sativa*), cabbage (*Brassica chinensis*), and spinach (*S. oleracea* L.) compared with the control group. The shoot height and fresh weight of the T3 group were greater than those of other groups on the 15th day; moreover, on the 30th day, the T3 group exhibited the highest fresh weight despite the shortest shoot height. This suggested that optimal growth during the initial stage of lettuce development is associated with increased shoot height. However, excessive height in subsequent stages may hinder growth, which is potentially linked to the bolting phenomenon [48]. Consequently, the promotion of lettuce growth using biochar-containing substrates in aquaponic systems may be attributed to biochar improving the substrate's physicochemical properties to resemble conventional soilless substrates and enhancing nutrient availability [40,49].

3.3. Effect of CSB application on soil enzyme activity and nitrogen content of the substrate

To determine whether the differences in lettuce growth were related to the nutrient status of the substrate in various groups, the soil enzyme activity and nitrogen content were measured. Soil enzyme activity reflects changes in nutrients and overall biological activity and serves as a sensitive indicator for assessing soil health [50]. The enzymatic activities of FDA and UE are thought to represent the major steps in the soil geochemical nutrient cycle for carbon and nitrogen [51]. FDA is recognized as a reliable indicator of microbial activity [52]. FDA activity significantly increased (P < 0.05) across all groups receiving 20%–50 % CSB compared with that in the CK group (Fig. 5a). Notably, the T3 group exhibited the highest enzyme activity (144.15 and 105.09 μmol/day/g on the 15th and 30th days, respectively). FDA activity gradually decreased as the lettuce grew. UE hydrolyzes urea into ammonia and CO_2 [53] and inhibits diseases caused by fungal pathogens under continuous cropping conditions [54]. Similar to FDA activity, UE activity significantly increased after CSB incorporation (Fig. 5b) (P < 0.05) and was the maximum in the T3 group. It was 380.01 and 348.36 μg/day/g in the T3 group on the 15th and 30th days, respectively, exhibiting decreasing tendency with the growth of lettuce. The increase in FDA and UE activities suggested that CSB can affect microbial activity and enhance nutrient transformation in the substrate, thus exerting a positive effect on the growth of lettuce.

NR, NiR, and HyR are essential enzymes that affect N_2O emissions [55]. During denitrification, NR catalyzes the conversion of NO_3^- to NO_2^-, and NO_2^- is further converted to N_2O by NiR [56]. NH_2OH functions as an important modulator of chemical oxidation pathway activity, thereby participating in the formation of soil N_2O, HyR catalyzes the reduction of NH_2OH to NH_4^+ during the dissimilatory nitrate reduction to ammonium (DNRA) process [57]. CSB incorporation significantly increased the NR activity (P < 0.05) of the substrate on the 15th and 30th days (Fig. 5c). Particularly, the NR activity was more by 10-folds in the T3 group than in the CK group. Consistent with the results of FDA and UE activities, the NR activity decreased as lettuce growth progressed. HyR activity first increased and further decreased with increasing CSB content on the 15th and 30th days (Fig. 5e). Notably, unlike the above three enzymes, HyR activity exhibited an increase as lettuce growth progressed. Conversely, the NiR activity initially decreased and further increased with increasing CSB content on the 15th and 30th days (Fig. 5d). Furthermore, the decrease in the NiR activity was particularly notable on the 30th day. However, the activities of soil enzymes such as NR, NiR, and HyR are greatly affected by substrate
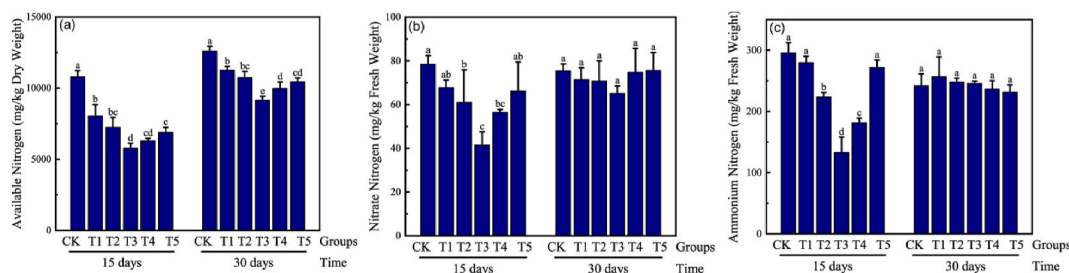

Fig. 6. Available nitrogen (a), nitrate-nitrogen (b), and ammonium-nitrogen (c) contents of the substrate with various CSB levels on the 15th and 30th days.

concentrations [57]. In aquaponic systems, after nitrification, tailwater contains relatively high nitrate and low nitrite-nitrogen levels before entering the vegetable growing unit. Our results are inconsistent with those of previous studies reporting that CSB incorporation decreased soil N_2O release by inhibiting the activities of NR, NiR, and HyR during denitrification [57]. This was mostly because of an increase in N availability for denitrifying microorganisms. Consequently, our results suggested that CSB promotes nitrogen conversion in the substrate and reduces the loss of nitrogen as N_2O, which may enhance the efficiency of nitrogen use in the substrate by lettuce.

To further explore the effect of CSB incorporation on nitrogen use efficiency, the contents of three types of nitrogen (available nitrogen, nitrate-nitrogen, and ammonium-nitrogen) were measured at different times. Fig. 6b and c shows the nitrate-nitrogen and ammonium-nitrogen contents, respectively, in the substrate. The treatment groups exhibited significantly lower nitrate-nitrogen and ammonium-nitrogen contents than the CK group on the 15th day (P < 0.05). However, no significant differences were observed among the groups on the 30th day (P > 0.05). The available nitrogen content was significantly lower across all treatment groups than in the CK group (P < 0.05), whereas the available nitrogen content was greater on the 30th day than on the 15th day (Fig. 6a).

Previous studies have reported that biochar can enhance the nitrogen content of different soils [58], which is inconsistent with our results. This might be because biochar has been generally applied to degraded arable soils in previous studies, which is quite different from the experimental environment and nitrogen input methods used in aquaponic systems. The nitrogen source was uniform across all groups because of the same origin, and nitrate-nitrogen and ammonium-nitrogen were predominant after nitrification. Additionally, lettuce remained in the seedling stage on the 15th day, requiring substantial nutrients for rapid growth [59]. Consequently, the group exhibiting better growth, such as the T3 group, indicated a correspondingly greater reduction in the nitrogen content of the substrate. On the 30th day, the growth rate of lettuce decreased as it reached a harvestable size, resulting in a reduced demand for nutrients. Thus, no significant differences were observed in the contents of nitrate-nitrogen or ammonium-nitrogen among the groups. Our results indicated that the incorporation of appropriate amount of CSB into a substrate can improve the growth of lettuce in aquaponic systems by increasing nutrient absorption, thereby increasing the removal of nitrogen and helping to create a better aquatic environment in aquaponic systems.

3.4. Effect of CSB application on the microbial community of the composite substrate

3.4.1. The taxon number of the microbial community

Microorganisms significantly contribute to nutrient cycling, soil structure maintenance, and crop production through several methods, such as the secretion of soil enzymes [60]. In this study, 16S rRNA gene sequencing was used to investigate the effect of biochar on microbial communities in the substrate. The rarefaction curve reflects whether the sequencing data is reasonable and demonstrates the diversity of species in various samples with different amounts of sequencing data [61]. The Shannon–Wiener curve was constructed using the microbial diversity index of each sample at different sequencing depths to evaluate the volume of sequencing data [62]. In the present study, the rarefaction curve gradually stabilized, whereas the Shannon–Wiener curve exhibited smoothness across different sequencing depths. Our findings indicated that the sequencing results are reliable and accurately represent the majority of microbial information present in the samples (Fig. S1).

Taxonomic statistics for each group were obtained to elucidate the differences in classification among the groups (Fig. 7). These statistics included taxonomic numbers ranging from phylum to species and were normalized to the same sequencing depth. Notably, the taxonomy numbers in each group represented a minor proportion at the phylum level, whereas they constituted a substantial proportion at the genus level. Specifically, the number of taxa across all the samples exceeded 39 % at the genus level and 10 % at the species level, indicating reliable classification annotation quality. The results revealed that the CK group exhibited a lower species count on the 15th day. However, after CSB incorporation (particularly at the 20%–50 %), the species count notably increased (P <
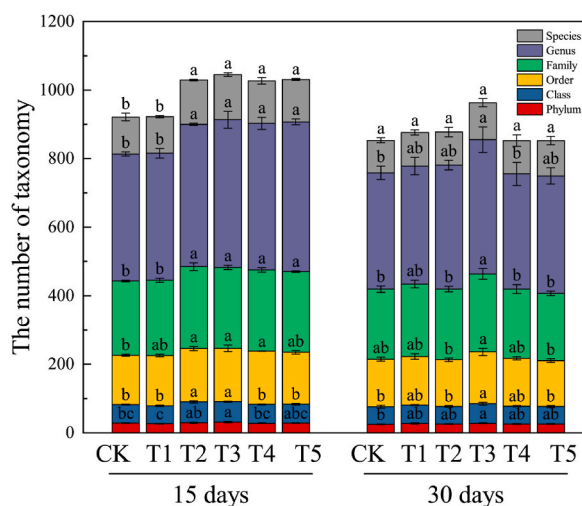

Fig. 7. Statistics of the taxonomic numbers of the microbial communities in the substrate with various CSB levels on the 15th and 30th days.

0.05). Only the T3 group maintained a significantly greater taxonomic number than other groups ($P < 0.05$) on the 30th day.

Microbial community diversity determines microbial adaptability to various environments and the establishment of dominant microbial communities [63]. In this study, diversity parameters were used to thoroughly assess the alpha-diversity of microbial communities. The observed species, Chao1, and ACE indices were used to describe the richness, whereas the Shannon and Simpson indices were used to describe diversity. The evenness and evolutionary diversity, respectively, were assessed by Pielou_J and Pd_Faith. Notably, all diversity parameters displayed a gradually increasing trend on the 15th day, whereas these parameters exhibited an increase followed by a decrease till the 30th day, with the maximum value observed in the T3 group (Table 2). These results indicated that CSB incorporation enhanced microbial community diversity, which is consistent with the findings of Xu [64]. However, almost all diversity parameters were lower on the 30th day than on the 15th day in all groups, implying stabilization of the bacterial environment in the substrate and promotion of the formation of dominant species [65].

3.4.2. Bacterial community structures

To further differentiate the taxonomic characteristics among the groups, the bacterial community diversity was determined via annotation analysis at the phylum level (Fig. 8a). The findings indicated significant differences in the bacterial community between the CK group and the other groups. Notably, 10 phyla (Proteobacteria, Bacteroidota, Verrucomicrobiota, Cyanobacteria, Actinobacteria, Acidobacteria, Bdellovibrionota, Patescibacteria, Myxococcota, and Firmicutes) were particularly active, and their minimum abundance reached >95 % in all groups. In elucidated microbial life history, a framework comprising three strategies, namely, high yield–resource acquisition–stress tolerance (Y-A-S), has been proposed [66]. According to this framework, Y-strategy taxa are speculated to divert organic matter toward anabolic pathways and biomass synthesis in nutrient-rich environments, whereas A-strategists are hypothesized to invest resources in producing extracellular enzymes for depolymerizing complex carbon in environments with poor nutrients [67]. Among the top 10 phyla identified in our study, Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes were classified as Y-strategy taxa, whereas Verrucomicrobiota, Cyanobacteria, and Acidobacteria were classified as A-strategy taxa.

In the CK group, the total relative abundance of Proteobacteria, Bacteroidota, and Verrucomicrobiota exceeded 81 %. This indicated that these three groups were the predominant microorganisms, of which the top two were the Y-strategy taxa. The relative abundances of phyla such as Bacteroidota, Actinobacteriota, and Firmicutes, which follow the Y-strategy, tended to increase. In contrast, phyla such as Cyanobacteria and Acidobacteria, which follow to the A-strategy, exhibited a decrease in relative abundance compared with the CK group. Previous studies have reported that biochar can increase the relative abundance of Proteobacteria in soil by increasing the soil pH, total nitrogen, available phosphorus, and total carbon, which is beneficial for plant growth, yield, and fruit quality [20,68]. Notably, the relative abundance of Proteobacteria appeared to increase on the 30th day, particularly in the treatment groups, compared to that on the 15th day. Bacteroidota are observed to antagonize various plant pathogens in different crops in plant rhizosphere soils [69]. Several bacteria belonging to Bacteroidota have been identified as plant-growth-promoting rhizosphere bacteria in various crops [70–72]. However, the relative abundance of Bacteroidota in the treatment groups exhibited an increasing trend till the 15th day, follow by decreasing trend till on the 30th day. Firmicutes are reported to promote the growth of plants such as tomatoes, because of their genotype [73]. Therefore, our results demonstrated that CSB may promote lettuce growth by enhancing the abundance of bacterial phyla with Y-strategy at different stages.

Fig. 8b illustrates variations in the relative abundances of the top 30 microorganisms at the phylum level. Apart from the top 10 phyla in terms of relative abundance, notable phyla such as Chloroflexi, SAR324, FCPU426, Planctomycetote, Nitrospirota, LCP-89, and Armatimonadota exhibited increased relative abundance but Margulishbacteria exhibited decreased relative abundance after CSB incorporation. Previous studies have reported the involvement of some of these microorganisms in inorganic carbon and nitrogen fixation processes. For instance, Chloroflexi utilizes the 3-hydroxypropionic acid pathway for CO_2 fixation [74]; SAR324 members fix inorganic carbon via the Rubisco pathway in oxygenated waters [75]; FCPU426 demonstrates high nitrogen fixation efficiency [76], and Margulisbacteria uses a fermentation-based metabolism featuring various hydrogenases and streamlined nitrogen-fixing enzymes [77]. Additionally, certain phyla participate in other aspects of the nitrogen cycle. for instance, Planctomycetota includes anaerobic ammonia-oxidizing bacteria [78]; Nitrospirota oxidizes nitrite to nitrate [79], and genetic analysis of LCP-89 indicates its presence in the nitrate reduction pathway [80]. Armatimonadota plays a vital role in organic matter decomposition and humus formation [81],

Table 2
Bacterial alpha-diversity in response to various CSB incorporation levels on the 15th and 30th days.

|	Observed species	Chao1	ACE	Shannon	Simpson	Pielou_J	Pd_faith
CK (15days)	2410.00	3125.56	3226.97	5.52	0.94	0.71	103.46
T1 (15days)	2424.67	3231.73	3273.07	5.46	0.94	0.70	103.99
T2 (15days)	2579.67	3286.21	3360.34	6.14	0.98	0.78	110.91
T3 (15days)	2776.00	3535.52	3608.72	6.54	1.00	0.82	115.11
T4 (15days)	2999.67	3665.69	3851.92	6.44	0.99	0.80	130.16
T5 (15days)	3052.67	3749.70	3903.19	6.51	0.99	0.81	130.90
CK (30days)	2393.33	3058.55	3199.36	5.07	0.90	0.65	98.49
T1 (30days)	2509.67	3325.90	3460.93	5.21	0.91	0.67	102.69
T2 (30days)	2490.67	3320.40	3512.70	4.55	0.86	0.58	108.06
T3 (30days)	2778.67	3564.68	3746.44	5.37	0.91	0.68	120.25
T4 (30days)	2415.33	3156.72	3297.54	4.75	0.86	0.61	100.01
T5 (30days)	2489.00	3264.41	3359.78	5.05	0.90	0.65	98.98
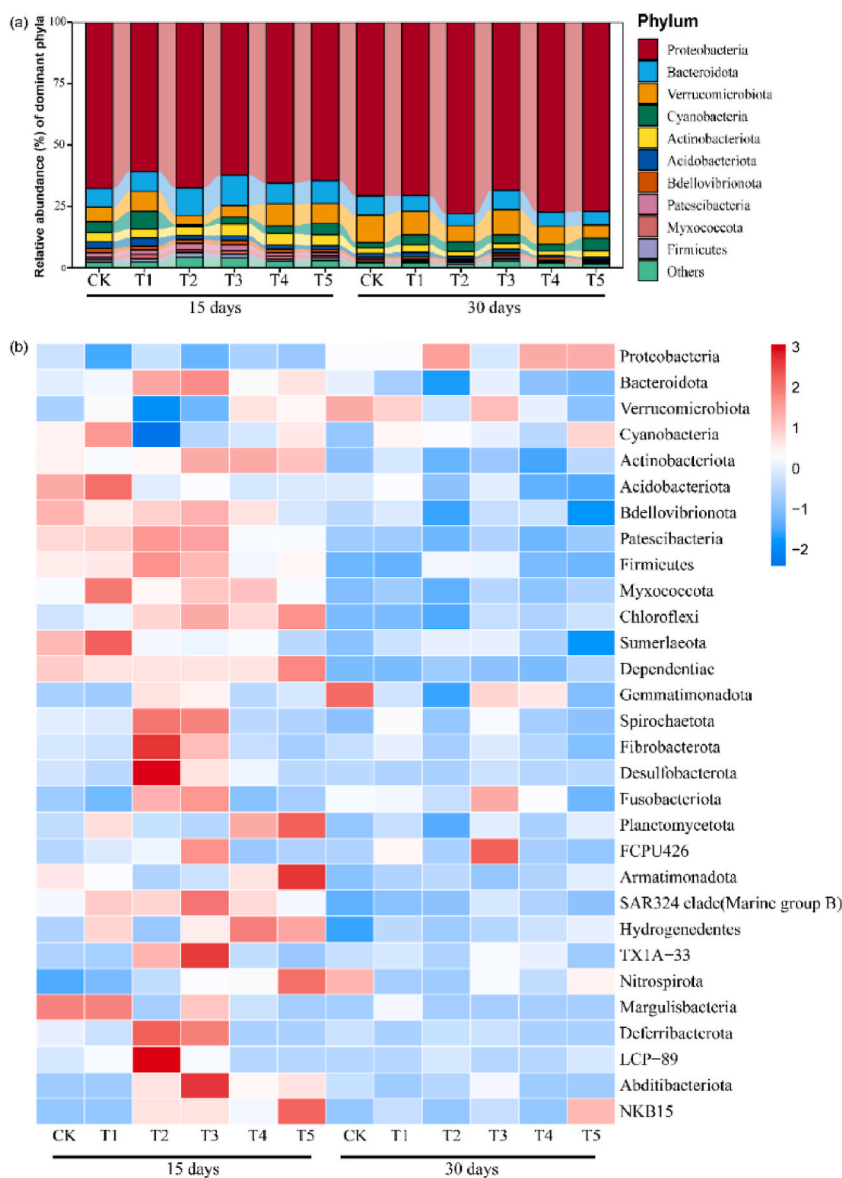
Fig. 8. Relative abundances of taxa at the phylum level in substrates with various CSB levels on the 15th and 30th days. Relative abundance of the top 10 dominant phyla (a). Heatmap of the top 30 dominant phyla (b).

thereby serving as a potent promoter of plant growth because of its physicochemical properties and nutrient richness. Our findings demonstrated that CSB application may enhance the carbon and nitrogen fixation capacity of the composite substrate, improve nitrogen cycling efficiency in the substrate, and boost organic matter decomposition ability, thereby promoting the growth of lettuce.

Additionally, our results indicated that CSB application increased the relative abundances of some nitrogen-cycle-related microorganisms at the family level, including those from Rhizobiaceae, Hyphomicrobiaceae, Magnetosprillaceae, Mycobacteriaceae, and Nocardioidaceae families (Table S2). Rhizobiaceae includes common rhizosphere N_2-fixing and efficient phosphorus-solubilizing bacteria [82]. Biochar can accelerate the dissimilatory reduction of nitrate to ammonia by promoting the growth of members of Hyphomicrobiaceae and Mycobacteriaceae [82]. Magnetosprillaceae and Nocardioidaceae include nitrate-nitrogen reducing bacteria [83]. These results indicated that CSB may enhance nitrogen use efficiency and promote lettuce growth by regulating microbial activity. Thus, It affects nitrogen cycling in the substrate via increasing N_2 fixation in the substrate, promoting the conversion of nitrate-nitrogen to ammonium-nitrogen, and reducing N leaching and N_2O escape [84].

3.4.3. Functional prediction of the microbial community in the composite substrate

Fig. 9a shows the function of the microbial community in the composite substrate predicted using the PICRUSt2 tool. Our results indicated that the microbial community displayed remarkable advantages in terms of metabolic function, particularly in the
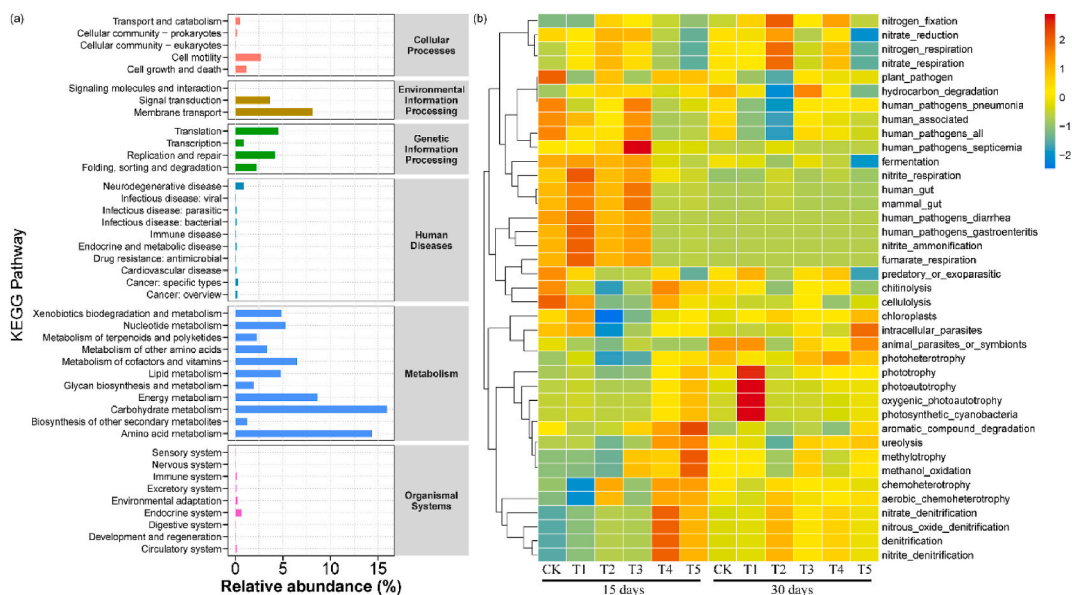
Fig. 9. Functional prediction of the microbial community in substrates with various CSB levels on the 15th and 30th days. Relative abundance of the function of the microbial community (a) and cluster heatmap of the top 40 species (b).

metabolism of carbohydrates, amino acids, and energy, which are closely associated with lettuce growth in aquaponic systems. However, CSB incorporation did not cause any significant differences in the function of the microbial community in our study. Previous studies have demonstrated that CSB incorporation enhances the capacity for xenobiotic biodegradation [85] and various metabolic activities in soil [86], which is inconsistent with our results. This difference may be attributed to variations in the environmental and substrate factors associated with biochar application.

To further determine the impact of CSB incorporation on microbial function in the substrate, the FAPROTAX tool was used to generate functional predictions for each group. CSB incorporation significantly improved the nitrogen cycling efficiency of the composite substrate (Fig. 9b). Specifically, it enhanced nitrogen fixation, nitrogen respiration, nitrate reduction and respiration, nitrite ammonification, and denitrification. This finding is consistent with previous observations regarding the application of biochar to other soil types [84]. Furthermore, biochar application enhanced microbial activity in certain processes, such as phototrophy, photoheterotrophy, and photoautotrophy, which is in consistent with the results of enzyme activity and microbial structure in our study.

4. Conclusion

In the planting unit of the aquaponic system, incorporating CSB significantly improved the physicochemical properties of substrate, such as bulk density, pH, porosity, and water-holding capacity, further enhancing the ability of lettuce roots to obtain nutrients from the substrate. Additionally, CSB influenced soil enzyme activity, increased organic matter decomposition, and altered nitrogen cycling rates. Additionally, CSB significantly increased the diversity of the microbial community in the composite substrate, improved nitrogen conversion efficiency, increased N_2 fixation, reduced nitrogen loss, and dynamically adjusted the microbial community structure to meet the nutrient requirements of lettuce during different growth periods. In conclusion, CSB promotes lettuce growth in aquaponic systems by altering physicochemical properties of the substrate, enhancing soil enzyme activity, and improving the microbial community. Coconut bran supplemented with 30 % CSB was observed to be the optimal growth substrate in aquaponic systems for lettuce. This study provided an important reference for selecting suitable growth substrates for aquaponic systems. Future studies should explore the effects of biochar on other crops in aquaponic system, evaluate its long-term impact on substrate and crop performance, assess the economic feasibility of commercial use, analyze ecological impacts, and optimize the mixing process for efficiency and consistency. Further application of biochar-incorporated substrates can enhance the outcomes of aquaponic systems and promote sustainable agriculture.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Chen Zhu: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Zuo Lin:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wang Fen:** Validation, Resources, Methodology, Investigation, Conceptualization. **Wang Jiajia:** Methodology, Investigation, Data curation, Conceptualization. **Zhou Xiang:** Methodology, Investigation, Data curation, Conceptualization. **Cui Kai:** Validation, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Zhang Yu:** Investigation, Data curation, Conceptualization. **Zhang Kelai:** Methodology, Investigation, Data curation, Conceptualization. **Jiang Yelin:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Krishna R. Salin:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35515.

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