Coconut shell derived biochar to enhance water spinach (*Ipomoea aquatica* Forsk) growth and decrease nitrogen loss under tropical conditions

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Farms usually apply excessive nitrogen (N) fertilizers, especially in a vegetable production system, resulting in severe N leaching loss. Although there have been some reports on the impact of biochar on the N leaching in farmlands, most of them focused on field crops in temperate or subtropical regions. Limited information about N leaching in the tropical vegetable system is available regarding the quantitative data and effective countermeasures. A field experiment was conducted to quantify N leaching in a tropical leafy production system (*Ipomoea aquatica* Forsk) and to evaluate the effects of coconut shell biochar on N loss and crop growth. The results showed that compared to conventional fertilization with the 240 kg N ha⁻¹ application rate (NPK), biomass yield of water spinach increased by 40.1% under the high biochar application rate of 48 t ha⁻¹ (HBC), which was significantly higher than that of NPK treatment. Moreover, The HBC treatment decreased N leaching by 34.0%, which can be attributed to enhanced crop uptake which increased by 40.3% as compared to NPK treatment. The $\text{NH}_4^+ / \text{NO}_3^-$ ratio in leachates was between 0.01 and 0.05. It was concluded that coconut shell derived biochar improved the biomass yields of water spinach and reduced the leaching N loss, which provides a promising amendment in tropical regions.

Excessive chemical fertilizers are often applied to maintain or enhance agricultural productivity under the pressures of shrinking land area per capita. The average chemical fertilizer usage per hectare in China has increased from 86.7 kg ha⁻¹ in 1980 to 359 kg ha⁻¹ in 2016, about 3.3 times that of the United States and 3.6 times that of the world average⁴. The average N input for vegetable crops was raised to 588 kg ha⁻¹ according to the 2013–2015 survey, which was 2.7 times the recommended for open-field vegetables in China, respectively⁵. Excessive chemical fertilizers have caused many environmental problems, including soil acidification, water and air pollution⁶. One of the most common environmental concerns is NO₃⁻ leaching into underground water and caused great risk for human health⁷.

Biochar is a carbon-rich product made by pyrolysis of organic matter under partial or complete exclusion of oxygen⁸. Biochar can be produced from various feedstocks. It is composed of condensed aromatic groups that are partially responsible for its highly biochemical recalcitrance than many other forms of organic matter in soil⁹. Biochars are highly porous, usually alkaline, and exhibit large specific surface area¹⁰. Owing to these inherent physicochemical properties, biochar affects many soil properties including soil pH, organic matter, water holding capacity and microbial composition and diversity¹¹,¹². In addition, oxidation of biochar at a slow rate in soil leads to the production of negatively-charged functional groups, such as carboxyl and phenolic groups¹³,¹⁴. The presence of these groups implies a high density of functional groups at the surface of biochar particles that interact

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concentration in the leachate was 42.5 mg L$^{-1}$

**Results**

**Growth of water spinach and N use efficiency.** The biomass of water spinach was harvested three times during the experiment (Fig. 1). The first harvest had obvious biomass due to the short growth period (26 days) than that of the other two times (33 days). Significant differences occurred in every harvest dry biomass between the treatments. Biochar amendment promoted the growth of water spinach. The accumulative dry biomass yields of spinach amended with biochar was from 1357 to 1678 kg ha$^{-1}$, which was 13.3–40.1% more than that of chemical fertilizer treatment (NPK, 1197 kg ha$^{-1}$). The difference in biomass yield between the treatments of NPK plus a high biochar rate (HBC, 48 t ha$^{-1}$) and NPK was significant ($P \leq 0.05$), whereas the difference was not significant when manure was applied, although the increase was 18%, as compared to NPK treatment.

Biochar addition increased N use efficiency (NUE) by water spinach (Fig. 2). The NUE was ~6% higher with biochar at the high application rate than that of NPK treatment ($P \leq 0.05$), and there were significant differences between them. Medium rate of biochar had a similar NUE of NPKM, and slightly higher than that of low biochar rate.

**N leaching.** The amount of leached N from different treatment was determined by its concentration in the leachate multiplied with the amount of leachate. The cumulative amount of leached N reflected the concentration of N in the leachate and the leachate volume. Due to minor differences in leachate volume among all the treatments (Supplementary Fig. 1), the leaching N was more related to mineral N concentration (Supplementary Fig. 2). The amount of leached N was highest in the 7 days after planting before root systems of water spinach; (2) determining whether biochar could effectively reduce N leaching.

**Figure 1.** Harvested biomass yields of water spinach during the experiment. The same letter on the top of vertical bars indicates no significant difference in the accumulative dry biomass yield of water spinach at the 0.05 level. CK-control (without N fertilizer and soil amendments); NPK- conventional chemical fertilization; LBC, MBC and HBC- chemical fertilizer (NPK) plus 12 t ha$^{-1}$, 24 t ha$^{-1}$, 48 t ha$^{-1}$ biochar, respectively; NPKM-NPK plus 12 t ha$^{-1}$ manure.

with nutrient ions by electrostatic, complexation, or capillary forces$^{15,16}$. These properties of biochars can reduce the leaching of nutrients from soil and subsequent accessibility of nutrients to crops$^{17}$.

As a promising soil amendment, there has been an increased interest in biochar research in recent years due to its potential beneficial effects on soil properties and water remediation$^{18,19}$. It has the potential to be a low cost and efficient sorbent for $\text{NH}_4^+$ and $\text{NO}_3^−$ and may reduce N leaching and increase crop yields$^{20,21,22}$. $\text{NH}_4^+$ could be adsorbed on the surface of biochar by cation exchange and entrapped in its pore structures. Furthermore, $\text{NO}_3^−$ could be also adsorbed as a result of exchange reactions between base functional groups$^{20,23,24}$. However, biochar addition to soil showed inconsistent results on crop growth, soil properties and nutrient N leaching$^{10,23–26}$ because biochar properties vary widely, depending on the biomass source, pyrolysis temperature (350–900 °C) and application rate (4–90 t ha$^{-1}$)$^{20,27,28}$.

In many tropical regions, organic wastes from the processing of coconut are widely generated and mostly they are destined for landfills$^{29}$. An alternative way to reuse the coconut shells may be the production of biochar to keep a sustainable organic matter cycle. Water spinach (*Ipomoea aquatica* Forsk) is a kind of common leafy vegetables in the South of China. There is limited information about the impacts of biochar on water spinach and tropical vegetable production system. The present research was aimed at (1) comparing the efficiency of soil amendments in the South of China. There is limited information about the impacts of biochar on water spinach and tropical vegetable production system. The present research was aimed at (1) comparing the efficiency of soil amendments in the South of China.
Soil mineral N content at the 0–30 cm soil depth before the experiment was 8.82 mg kg\(^{-1}\) and decreased to 6.81 mg kg\(^{-1}\) for CK at the end of the experiment. Soil NO\(_3^-\)-N content for HBC treatment was 5.72 mg kg\(^{-1}\) and was significantly higher than CK and NPKM treatments (Fig. 6). Soil NH\(_4^+\)-N content was from 5.64 mg kg\(^{-1}\) to 12.4 mg kg\(^{-1}\); there was no significant difference between the fertilization treatments. The NH\(_4^+\)/NO\(_3^-\) ratios of biochar treatments (2.1–2.5) were lower than those of NPK and NPKM treatments (2.8–3.0).

**N balance.** The N balance in the open-field water spinach production system was greatly affected by the treatments (Table 1). N input (which was the sum of N from fertilizer, soil mineralization, and mineral N before planting) ranged from 64.3 kg ha\(^{-1}\) for CK to 304 kg ha\(^{-1}\) for the other treatments. Fertilizer was the dominant N input. Compared with NPK, N output of biochar treatments increased by 5.27%-18.4%.

N losses caused by leaching ranged from 19.2 to 26.4%. In both years, N leaching loss was highest in the system managed according to the conventional farming practice (NPK) and was lowest in the system of HBC. Crop uptake N was highest for MBC, although there were no significant differences between fertilization treatments. Compared with NPK, biochar addition increased crop N uptake by 12.6–40.1%, reduced N leaching by 17.3–34.0%, and total N loss by 2.4–7.8%.

**Discussion**

**Biochar increased vegetable yield in tropical religion.** Biochar has been proven a renewable resource and eco-friendly material for improving soil fertility and increasing crop yields\(^{30}\). Although there are controversies regarding the extent and cause of positive benefits, biochar is widely used to boost crop yield\(^{31}\). In the tropics, water spinach commonly grows in acidic Oxisols with a low content of soil organic C and nutrients. Biochar...
**Figure 4.** The leaching NH\(_4^+\)-N as affected by different treatments during the water spinach growing season. Different lowercase letters on the top of vertical bars indicate significant differences between treatments (P < 0.05). CK-control (without N fertilizer and soil amendments); NPK- conventional chemical fertilization; LBC, MBC and HBC- chemical fertilizer (NPK) plus 12 t ha\(^{-1}\), 24 t ha\(^{-1}\), 48 t ha\(^{-1}\) biochar, respectively; NPKM-NPK plus 12 t ha\(^{-1}\) manure.

**Figure 5.** The leaching NO\(_3^-\)-N as affected by different treatments during the water spinach growing season. Different lowercase letters on the top of vertical bars indicate significant differences between treatments (P < 0.05). CK-control (without N fertilizer and soil amendments); NPK- conventional chemical fertilization; LBC, MBC and HBC- chemical fertilizer (NPK) plus 12 t ha\(^{-1}\), 24 t ha\(^{-1}\), 48 t ha\(^{-1}\) biochar, respectively; NPKM-NPK plus 12 t ha\(^{-1}\) manure.

**Figure 6.** Soil mineral N and NH\(_4^+\)/NO\(_3^-\) ratio at the end of the experiment. Different lowercase letters on the top of vertical bars indicate significant differences between treatments (P < 0.05). CK-control (without N fertilizer and soil amendments); NPK- conventional chemical fertilization; LBC, MBC and HBC- chemical fertilizer (NPK) plus 12 t ha\(^{-1}\), 24 t ha\(^{-1}\), 48 t ha\(^{-1}\) biochar, respectively; NPKM-NPK plus 12 t ha\(^{-1}\) manure.
amendment increased water spinach biomass by 13–40%, as compared to control (Fig. 1). The yield increase by biochar can be attributed to (i) increased nutrient supply for crop growth; (ii) enhanced water availability; (iii) improved soil quality; (iv) suppressing of soil-borne plant diseases. Moreover, yield response to biochar has shown to be more evident on acid soils in the tropical regions than on neutral/alkaline soils in the temperate. The global-scale meta-analysis by Jeffery et al. (2017) indicated that biochar had on average no effect on crop yield in the temperate regions, yet elicited a 25% average increase in crop yield in the tropics. Moreover, the physicochemical properties of biochar also affected the extent of crop yield benefits. For instance, the coconut shell biochar with higher pH and micropore specific surface area was more useful for improving soil fertility, nutrient availability and crop growth.

**N leaching in vegetable systems.** Application rates of fertilizers in vegetable production systems often exceed crop requirements, resulting in a high accumulation of nutrients in the soil. Soil NO$_3^-$ leaching was influenced by crop type, soil properties, irrigation management, and rainfall. In this study, N leaching in the water spinach production system amounted to 36.1–54.7 kg ha$^{-1}$ during the 90 days of the experiment (Fig. 5), which is consistent with the report by Chotangui et al. Biochar addition has been shown to increase nutrient retention in highly weathered soils through different mechanisms including sorption of NO$_3^-$, NH$_4^+$ and organic-N; alteration of cation and anion exchange capacity and changes in microbial processes and activities. Significant NO$_3^-$ adsorption occurred at pyrolysis temperatures $\geq$ 700 °C. In the present study, as compared to control (application of 240 kg N ha$^{-1}$ without biochar), biochar-amended treatment decreased N leaching by 17.3–34.0% (Fig. 5). Related research showed that negatively charged functional groups on biochar surface could adsorb positively charged NH$_4^+$ and NO$_3^-$ more prone to leaching than NH$_4^+$, therefore, NH$_4^+$ concentration in leachates was far lower than NO$_3^-$, and the NH$_4^+$/NO$_3^-$ ratio was only 0.01 to 0.05 (Figs. 5 and 6), which is consistent with the report by Zhang et al.

**Biochar decreased N leaching loss.** Biochar addition has been shown to increase nutrient retention in highly weathered soils through different mechanisms including sorption of NO$_3^-$, NH$_4^+$ and organic-N; alteration of cation and anion exchange capacity and changes in microbial processes and activities. Significant NO$_3^-$ adsorption occurred at pyrolysis temperatures $\geq$ 700 °C. In the present study, as compared to control (application of 240 kg N ha$^{-1}$ without biochar), biochar-amended treatment decreased N leaching by 17.3–34.0% (Fig. 5). Related research showed that negatively charged functional groups on biochar surface could adsorb positively charged NH$_4^+$ and NO$_3^-$. It was reported that NO$_3^-$ retention driven by anion exchange with oxonium and pyridinium groups also occurred. Kameyama et al. reasoned that the adsorption of NO$_3^-$ was a result of base functional groups and not a result of physical adsorption since surface area and micropore volumes followed different trends. Other mechanisms for reducing N leaching loss in the biochar-amended soils included enhanced N assimilation by crops and improved soil structure. Enhanced N assimilation by water spinach was observed in the present study (Table 1). Biochar increased N uptake by 12.6–40.3%, as compared to control.

**NUE and N balance.** The high-yield vegetable production systems in China were dependent on the intensive input of fertilizers over the past decades, especially N fertilizers, which have lowered NUE in croplands. In this present study, NUE of water spinach was 10.3–16.2% (Fig. 2), which is consistent with previous studies, but lower than the average NUE of open-air and greenhouse vegetable production systems in China. Nitrogen balance, defined as the difference between N inputs and outputs, can be used as an indicator to reveal N loss in the vegetable cropping systems. In this present study, N leaching accounted for 19.2–26.4% of total N losses (Table 1), and other loss could include runoff, NH$_3$ volatilization, and gaseous emissions from nitrification and denitrification. Previous studies indicated that NH$_3$ volatilization loss to N input accounted for 18–24% of the total N input in the vegetable production systems, and the proportion increased with the application of urea. The nitrous oxide emissions (N$_2$O) for vegetable fields were only around 0.94% of applied N fertilizer.
Conclusion

Concerns about nitrate contamination to groundwater and agricultural sustainable development have been impelling us to apply effective technologies to decrease N environmental loss and promote N use efficiency. In the present study, compared to conventional chemical fertilization treatment, biomass yield of water spinach increased by 13.3–40.1% with biochar amendment. Biochar addition also decreased N leaching by 17.3–34.0%, which is mainly attributed to enhanced crop uptake of N by 12.6–40.3% as compared to control. It was concluded that coconut shell derived biochar improved the biomass yields of water spinach and reduced the leaching N loss, which provides a promising amendment in tropical regions.

Materials and Methods

Experiment site. The field experiment was conducted on the Danzhou Experiment Station of Chinese Academy of Tropical Agricultural Science (19°30′36″ N, 109°29′40″ E). This region is characterized by a tropical monsoon climate with an average annual rainfall of 1815 mm and a mean annual temperature of 23.5 °C. The soil was derived from granite material, classified as Oxisol with a sandy loam texture. The surface soil (0–30 cm) had pH (H2O) 6.02, organic C 5.61 g/kg, total N, P and K of 0.96, 0.24 and 0.29 g kg−1, available N, P and K of 60.7, 9.28 and 41.6 mg kg−1.

Experiment design and agricultural management practices. Water spinach (Ipomoea aquatica Forsk) is a kind of typical leafy vegetable in tropical region of China, which is most commonly grown in East, South and Southeast Asia. The experiment was conducted from July 1 to September 30, 2017, with three water spinach harvests. The seeds of water spinach (var. Ching Quat) were tested. The experiment was a completely randomized design with six treatments and three replications. Six treatments were: no N fertilizer and no soil amendments (CK), conventional chemical fertilizer (NPK), NPK plus a low biochar rate (LBC, 12 t ha−1), NPK plus a high biochar rate (HBC, 48 t ha−1) and NPK plus manure (NPKM, 12 t ha−1). Agricultural practices except for fertilizer management were identical for all the treatments. According to the local recommendation of leafy vegetable, all the treatments except for CK had the same N, P2O5 and K2O input, which were 240, 96 and 192 kg ha−1, respectively; 40% N, 100% P and 40% K are applied as base fertilizers by mixing with the topsoil (0–30 cm) and 100% amendments (biochar or manure) as urea, calcium superphosphate and potassium sulfate. 30% N and 30% K are applied after the second and third harvest, respectively; 40% N, 100% P and 40% K are applied as base fertilizers by mixing with the topsoil (0–30 cm) and 100% amendments (biochar or manure) as urea, calcium superphosphate and potassium sulfate. 30% N and 30% K are applied after the second and third harvest, respectively; 40% N, 100% P and 40% K are applied as base fertilizers by mixing with the topsoil (0–30 cm) and 100% amendments (biochar or manure) as urea, calcium superphosphate and potassium sulfate.

Collection of soil and plant samples and measurements. All the aboveground tissues of water spinach were harvested three times during the experiment and the harvested biomass from each plot was weighed. The subsamples were oven-dried to a constant weight at 75 °C to calculate the water content of biomass, and the dried plant samples were powdered and analyzed for total N concentration using a CHNS element analyzer (Vario EL III, Elementary, Hanau, Germany)34.

After harvest, the 0–30 cm soil layer was sampled using a stainless steel auger (15 mm interior diameter). Soil pH was determined in water at the 1:2.5 soil to solution ratio. Mineral N in soil (NH4+-N and NO3−-N) was extracted by 1 M KCl and NH4+-N concentration in extracts was determined with the colorimetric-indophenol blue method58 and NO3−-N concentration using a dual-wavelength spectrophotometry (Hitachi U-2100; Hitachi, Tokyo, Japan)56.

Soil leachate sampling and analysis. Eighteen lysimeters (one per plot) were installed in the field before the basal fertilizer was applied57. Each lysimeter consisted of a 350-mm-diameter polyvinyl chloride (PVC) cover and a plastic bucket. The PVC cover was at 600-mm-depth below the ground with holes, filtering mesh and sandy gravels filled up to 50-mm-depth. The plastic bucket was used for collecting leachates. This allowed soil solution to drain from the sandy gravel cover and to be collected as leachate in the buckets by vacuum pump. The leachate was collected on day 7, 14, 35, 56 and 90. Total leachate volume was recorded, and subsamples were analyzed for NH4+-N and NO3−-N concentration with the colorimetric-indophenol blue method and dual-wavelength spectrophotometry, respectively.

Data analysis. N use efficiency (NUE) of water spinach was calculated with the following equation58:

$$\text{NUE} = (N_{\text{treat}} - N_{\text{control}})/N_{\text{fert}}$$

where $N_{\text{treat}}$ and $N_{\text{control}}$ are crop N uptake by the aboveground biomass with and without N fertilization, respectively. $N_{\text{fert}}$ is N application rate.

Apparent N loss from the water spinach production system was calculated according to Widowati et al. and Zhang et al.59:

$$\text{Apparent N loss} = N_{\text{initial}} + N_{\text{fert}} + N_{\text{min}} - N_{\text{end}} - N_{\text{crop}}$$

where $N_{\text{initial}}$ and $N_{\text{end}}$ are soil mineral N (sum of NH4+-N and NO3−-N) at the 0–30 cm depth at the initial and end of the experiment. $N_{\text{crop}}$ is the N uptake by the aboveground of water spinach. $N_{\text{min}}$ is the soil N mineralized within the vegetable growing period, and was calculated as follows41:
$N_{\text{min}} = (N_{\text{end}} + N_{\text{crop}} + N_{\text{leaching}} - N_{\text{initial}} - N_{\text{fert}})$ of no N application treatment.

All data were analyzed by one-way analyses of variance (ANOVA) with SPSS (version 16.0). When an ANOVA was significant, means were compared using the Duncan multi-range test. The significant level was set at $P < 0.05$. Figures and tables were generated with Microsoft Excel 2010 and Origin 8.0. The data were expressed as mean± standard deviation ($n = 3$).

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Author contributions
F.Z. and G.Z. conducted the experiment. G.Z., Y.S., Z.D. and M.D. were involved in the field experiment. F.Z. and Z.H. analyzed the data and prepared the manuscript.

Competing interests
The authors declare no competing interests.

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