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Microalgae-derived hydrochar application on rice paddy soil: Higher rice yield but increased gaseous nitrogen loss

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Highlights

- *Chlorella vulgaris* hydrochars (CVH) were fabricated by hydrothermal carbonization
- CVH addition improved N use efficiency, sugar content, and grain yield of rice
- CVH addition stimulated NH_3 volatilization and N_2O emission from paddy soil
- Compared to direct addition of CV, CVH addition inhibited NH_3 volatilization
- Increasing gaseous N loss results from physiochemical and microbiological factors

Abstract

Hydrothermal carbonization represents a promising technique for transforming microalgae into the hydrochar with abundant phytoavailable nutrients. However, the effects of microalgae-derived hydrochars on the gaseous nitrogen (N) loss from agricultural field are still unclear. *Chlorella vulgaris* powder (CVP) and two *Chlorella vulgaris*-derived hydrochars that employ water (CVHW) or citrate acid solution (CVHCA) as the reaction medium were applied to a soil column system grown with rice. The temporal variations of nitrous oxide (N_2O) emissions and ammonia (NH_3) volatilization were monitored during the whole rice-growing season. Results showed that CVHW and CVHCA addition significantly increased the grain yield (by 13.5-26.8% and 10.5-23.4%) compared with control and CVP group, while concomitantly increasing the ammonia volatilization (by 53.8% and 72.9%) as well as N_2O emissions (by 2.17- and 2.82-fold) from paddy soil compared to control. The microbial functional genes (*AOA*, *AOB*, *nirk*, *nirS*, *nosZ*) in soil indicated that CVHW and CVHCA treatment stimulated the nitrification and denitrification, and inhibited the N_2O oxidation in soil. Notably, CVHW was recommended in the view of improving yield and controlling NH_3 volatilization because no significant difference of the yield-scale NH_3 volatilization was detected between control and CVHW treatment. This study for the first time uncovered that *Chlorealla vulgaris*-derived hydrochars have positive effects on rice N utilization and growth but negative effects on the atmospheric environment.

Keywords: Ammonia volatilization; *Chlorella vulgaris*; Hydrothermal carbonization**;** Nitrogen use efficiency; Nitrous oxide emission; Non-point pollution

1. Introduction

Blue and green microalgae are predominant biological pollutants of harmful algal blooms in eutrophicated waterbodies that have severe impacts on aquatic ecosystems and human health (O'Neil et al., 2012; Zhang et al., 2016). These microalgae are nutrients-rich, can store the inorganic nitrogen (N) and phosphorus (P) in excess within the cells in the form of protein and polyphosphate (Solovchenko et al., 2016), and thus own potential to be transformed from biowaste to biofertilizer (Ray et al., 2013; Mukherjee et al., 2015; Santos and Pires, 2018). *Chlorella*, a green microalga, contains significant quantities of N and P (up to 7%–12% and 1%–3% of their cell dry weight, respectively) (Powell et al., 2009; Cabanelas et al., 2013; Zhu et al., 2015). However, direct application of microalgae did not deliver significant difference on the growth of wheat (Schreiber et al., 2018) or rice (Ray et al., 2013; Mukherjee et al., 2015), because the dominant forms of the stored N and P in microalgae are proteins and polyphosphates that are difficult to decompose in soil and unable to be directly utilized by plants. Hydrothermal carbonization (HTC), which can transform the microalgae biomass into hydrochars, is recommended because has been demonstrated to transform most polyphosphates and proteins from lignocellulosic feedstock (Funke et al., 2013; Kruse et al., 2016) and biosolids (Huang and Tang, 2015; Huang et al., 2017; Yu et al., 2019) into orthophosphate and ammonium or nitrate. Also, HTC is cost-effective to avoid dehydrating the microalgae collected from the wastewater.

Rice (*Oryza sativa* L.) is a primary food source for more than half of the world's population (Khush, 2005). When applying microalgae-derived hydrochar to rice paddy fields as a fertilizer, the effects on atmospheric environment should be considered, because rice fields have been confirmed as a major emission source of ammonia (NH_3) volatilization and nitrous oxide (N_2O) emissions (Bhattacharyya et al., 2013; Kim et al., 2015; Wang et al., 2018). Ammonia (NH_3) volatilization from agricultural fields is a major non-point pollution (Norse, 2005) because it is widely dispersed to the atmospheric environment, leading to atmospheric pollution, such as forming particulate matters (e.g., $PM_{2.5}$, with diameters ≤ 2.5 μm) (Zhao et al., 2017; Dubache et al., 2019). In paddy fields, N is often applied in excess of plant demand. As a result, large proportions of overused N fertilizers are lost via NH_3 volatilization (Sun et al., 2017; Sun et al., 2019; Hayashi et al., 2008). NH_3 volatilization from agricultural fields accounts for 10-60% of the total nitrogen input (Tilman et al., 2011). In addition, the midseason and final drainage periods stimulate nitrification, thus caused substantial nitrous oxide (N_2O) emissions from paddy fields (Bhattacharyya et al., 2013; Ali et al., 2015; Zhou et al., 2018).

Generally, biochar application can reduce soil pH, and increase porosity, aeration, and redox potential, thus reducing NH_3 volatilization or N_2O emission by altering soil properties such as NH_4^+-N adsorption and activity of nitrobacterium and denitrobacterium (Nelissen et al., 2014; Feng et al., 2017; Mandal et al., 2019; Sha et al., 2019). However, these controversial results also indicate that biochar application can potentially promote NH_3 volatilization (Sun et al., 2017; Feng et al., 2018b; H. Sun et al., 2019b) or N_2O emissions (Duan et al., 2018; Senbayram et al., 2019; Zhang et al., 2019), depending on different properties of soil or feedstock of biochars. Microalgae-derived biochars own a number of distinctive attributes compared with biochars from other feedstocks. For instance, the surface area of *Chlorella vulgaris*-derived biochar was markedly lower than biochar produced from lignocellulosic biomass (Wang et al., 2013), which may reduce the adsorption capacity to ammonium (NH_4^+). The hydrolysis via HTC promotes the chain breakage in macromolecules, which may provide more labile carbon (C) and N pools to nitrobacteria and denitrobacteria. Therefore, it is necessary to investigate the effects of microalgae-derived hydrochar application on gaseous N loss from rice paddy soils.

In this study, the primary objective was to investigate the response of NH_3 volatilization and N_2O emissions when applying microalgal hydrochar as fertilizers. We also aimed to identify the key biogeochemical factors influencing gaseous N loss in rice paddy soil. We hypothesize that the addition of microalgae-derived hydrochars would increase N gasification loss from paddy soil because of introduced labile C and N sources to soil.

2. Materials and Methods

2.1． Hydrochar production

Chlorella vulgaris, a common microalgal strain existing in eutrophication, was employed in this study and acquired from Guangyu BioScience Limited, Shanghai. The inoculum was maintained in 2 L borosilicate bioreactors using sterilized medium, 3N-BBMV. The operational conditions were as follows: constant aeration using 2.5% CO_2 at 0.2 vvm, a photoperiod of 14:10 light : dark cycles, 150 μmol/m^2/s of luminance and temperature of 25 ± 1 °C. Microalgal cells were collected from a cultivation broth using centrifugation at 8,000 *g* for 5 min at 4 °C, washed using distilled water and lyophilized.

HTC was conducted in a high-pressure (approximate 8 Mpa, auto-generated during HTC) hydrothermal reactor, using a solid:liquid ratio of 1:10 (w/w). The structure of high-pressure hydrothermal reactor is shown in **Fig S1**. The reactor was sealed and heated at 260 °C for 1 h and then allowed to naturally cool down to room temperature overnight. The solid hydrochars produced by HTC were collected by centrifugation, and dried at 70 °C until no further weight loss. Two hydrochars were produced using different reaction media: CVHW (employing deionized water) and CVHCA (employing 1 wt.% citric acid). Citrate acid was added to increase the hydrochar yield (Heilmann et al., 2010), reduce hydrochar pH and promote the degradation of proteins and polyphosphates by acidic hydrolysis (Huang et al., 2017).

2.2. Soil column experiment design

Polyvinyl chloride sleeves (diameter: 30 cm diameter; height: 50 cm) were filled with dry paddy soil each. The paddy soil, which was classified as hydroagric Stahnic Anthrosol, was collected from a paddy field in Yixing, Jiangsu Province in China. During the rice season of the paddy field in 2017, the local average temperature was 25.7 °C and total precipitation was 1082.5 mm. Soil-column experiments were performed in the glasshouse of Jiangsu Academy of Agriculture Science, with natural light and temperature. Each soil column was filled with 35 kg of paddy soil. The soil was air-dried and ground to pass through a 2 mm sieve. The soil had the following properties: pH 6.42 (soil : water, 1 : 2.5), organic matter content, 2.28%, total N, 1.56 g kg^{-1}, total P 0.96 g kg^{-1}, and total potassium 4.12 g kg^{-1}. Rice (*Oryza sativa* L., Nangeng 46) was grown in the soil-column with three plants. All experiments were performed in triplicate.

Prior to sowing, the *Chlorella vulgaris* powder (CVP), CVHW or CVHCA were mixed with paddy soil at an application rate of 1%, respectively. Rice plants were transplanted on June 29, 2018 and harvested on November 9, 2018. All treatments were applied with 240 kg urea–N ha^{-1} throughout the rice-growing season. This amount of N fertilizer was applied thrice: as a basal fertilizer (BF) prior to transplanting, as a first supplementary fertilizer (SF1) after tillering and as a second supplementary N fertilizer (SF2) after panicle formation at a proportion of 4:4:2. The BF was applied at a rate of 96 kg N ha^{-1}, 96 kg P_2O_5 ha^{-1} and 192 kg K_2O ha^{-1} in the form of urea, calcium superphosphate, and potassium chloride. No hydrochar was applied to the control, but chemical fertilizers were applied in the same rate as other treatments. All plants were flooded to a water level of 3–5 cm and a mid-season drainage was done during the period from 7-18 August, 2018.

2.3. Characterization of hydrochar

The pH value of hydrochar samples was determined using a solid/Milli-Q water ratio of 1:2.5 (w/v). Total C, N, hydrogen (H), and sulfur (S) contents of hydrochar were determined by an Elemental Analyzer (EL III; Elementar Analysensysteme GmbH, Germany). The surface morphologies of the hydrochars were visualized by scanning electron microscopy (SEM, Quanta200, FEI, Netherlands) at 5000×. The surface functional groups were characterized by Fourier transform infrared spectroscopy (FTIR) on an Agilent Cary 660 FTIR Analyzer (California), as described in a previous study (Feng et al., 2018a). The surface elements contents (C, O, N, P, Ca, Si) on the surface of CVP, CVHW, and CVHCA were characterized using X-ray photoelectron spectroscopy (XPS) technology (the details of analysis are shown in Supplementary Materials). The specific surface area (SSA), porous diameter, and porous volume for adsorption were measured using a NOVA 1200 analyzer, and the parameters were calculated using the Brunauer–Emmett–Teller (BET) method, using a surface area analyzer (Quadrasorb SI, America) (Yu et al., 2019; Chu et al., 2020).

2.4. Measurement of rice growth index, grain N content and grain yield

Rice plants were manually harvested from each pot at physiological maturity to determine the grain N content and grain yield. The grain N content was determined using the Kjeldahl method, as described in previous study (Chu et al., 2016b). Furthermore, The N use efficiency was calculated as the percentage of applied fertilizer N recovered in aboveground biomass minus that of the control treatment without N fertilizer application (H. Sun et al., 2019a). The N content of aboveground biomass in the control treatment without N fertilizer application was 1.18 g pot^{-1}.

2.5. Monitoring NH_3 volatilization and N_2O emissions from paddy soil

NH_3 volatilization was measured in parallel with floodwater sampling. The continuous air-flow enclosure method was used to estimate daily NH_3 volatilization fluxes, as described by Feng et al. (2017). In brief, volatilized NH_3 was captured in a Plexiglas chamber with an inner diameter of 15 cm and 20 cm height using a mixture of 80 mL 2% boric acid, an indicator of methyl red, bromocresol, and ethanol as NH_3 absorbent. The NH_3-containing solution was titrated against with 0.01 M H_2SO_4.

The cumulative volatilized NH_3 was calculated as the sum of daily NH_3 volatilization amounts during the monitoring period (The NH_3 volatilization mainly derives from the urea addition and thus the first 7 days after urea application at three fertilization dates was applied) after BF, SF1, and SF2, respectively.

The monitoring of N_2O, as well as gas flux measurement, were conducted as described in previous study (Zhou et al., 2018). N_2O concentrations was determined by gas chromatography (Agilent 7890, USA). The yield-scale NH_3 volatilization or N_2O emissions were calculated using the aforementioned cumulative NH_3 volatilization loss or N_2O emission loss by dividing it, respectively, with the rice grain yield of the corresponding treatment.

2.6. Analysis of soil N and microbial biomass C and N

Fresh soil samples were collected from the top layer (0-20 cm) of soil at BF, SF1, and SF2, respectively. The soil samples from each plot were mixed and homogenized, grounded to < 2 mm, and then divided into two aliquots. The first section was frozen immediately in liquid N_2 and stored at −80°C for molecular analysis; the remaining section was stored at −20°C for nitrate (NO_3^--N) and ammonium (NH_4^+-N) analysis, as well as microbial biomass C and N.

Soil pH was determined by the same method as described above. The NH_4^+-N and NO_3^--N extracted from the soil by 2.0 M KCl were measured using a San++ Continuous Flow Analyzer, as previously described (Chu et al., 2016a; Feng et al., 2017). Soil microbial biomass C and N were measured by the chloroform-fumigation-extraction method described by (Moore et al., 2000). All results were reported as the averages of the duplicated analyses and were expressed on a moisture-free basis. Moisture was determined after drying at 105°C for 48h.

2.7. DNA extraction and quantitative polymerase chain reaction (qPCR)

Total DNA was isolated from soil samples (*ca*. 0.5 g) using the mericon DNA Bacteria Kit and mericon Bacteria Plus Kit (Qiagen, Germany) according to the manufacturer's instructions. PCR amplifications of genes of archael NH_3-oxidizers (*AOA*), bacterial NH_3-oxidiziers (*AOB*), nitrite reductase (*nirK* and *nirS*) and N_2O reductase (*nosZ*) were performed for primers as shown in **Table S1**. The abundance of these genes was quantified by qPCR using an AB17500 thermocycler (Applied Biosytems Inc., USA) and was described as the gene copy number per gram of dry soil. The details of this process are shown in the Supplementary Information.

2.8. Statistical analyses

Statistical analyses were performed using the SPSS version 18.0 (SPSS Inc. Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to evaluate the results at $P < 0.05$ probability. Duncan's multiple range test was employed only when the ANOVA F-test indicated significant treatment effects at the significance level ($P < 0.05$).

3. Results

3.1. Characteristics of hydrochars

Physiochemical characteristics of CVP, CVHW, and CVHCA are listed in **Table 1**, where the major elements, including C, H, N, and S of the hydrochars are presented. CVHCA exhibited an obviously lower pH than CVP or CVHW. Compared with CVP, CVHW and CVHCA hydrochars had higher C and lower N and S content. The C/N and H/C ratio established whether hydrochars were susceptible to mineralization and degradation by soil microorganisms (Zimmermann et al., 2012; Mukome et al., 2013; Senbayram et al., 2019). By employing HTC, CVHW and CVHCA improved C/N ratios by 2.15- and 1.99-fold, respectively, and the H/N ratio indicated a 2.09- and 1.69-fold improvement compared with CVP, respectively. In addition, the surface atomic concentration was analyzed by XPS technology (**Table S1**). CVHW and CVHCA both increased surface O concentration and decreased surface N and P concentration compared to CVP, suggesting the possible variation on the according surface O-, N-, and P-functional groups. Surface Ca concentration was only detected in CVHW.

High SSA and porous volume levels are extremely desirable for enhancing nutrients retention in soil because these features facilitate high mass transfer fluxes and adsorption loading. With respect to adsorption capacity, CVHW and CVHCA had significantly larger SSA (6.71- and 7.76-fold, respectively), and porous diameter (4.33- and 4.54-fold, respectively) than CVP. The porous volume of CVHW was 1.93-fold larger than CVHCA, and that of CVHCA was 3.26-fold larger than CVP. Furthermore, SEM structural image showed that more obvious crystal structures formed in the CVHW and CVHCA hydrochars than in the CVP (**Fig. S2**). A larger porous diameter and more abundant pores were observed in the SEM images of CVHW and CVHCA, which was supported by analysis using the BET method.

The FTIR spectra of the hydrochars revealed the functional groups present on their surfaces (**Fig. S3**). The differences between treatments were detected in the peak intensity of the aliphatic C–H at 2923.5 cm^{-1} and 2852.7 cm^{-1} wave numbers, in the –COOH peak at a wavenumber of 1700 cm^{-1}, in aliphatic C–O/C–O–C at 1028 cm^{-1}, in aromatic C-H at 825 cm^{-1}, and phenolic hydroxyl group (phenolic –OH) at 3420 cm^{-1}. The peak intensity for all these functional groups for the CVHW and CVHCA hydrochars was markedly lower than those for CVP.

3.2. Effects of CVP and hydrochars on the rice growth, quality and yield

The effects of hydrochars application on grain yield (dry weight, [DW]) and quality are listed in **Table 2.** Compared with the control, CVHW and CVHCA significantly increased grain yield by 26.7% and 21.2%, respectively. A similar trend was also detected for harvest index and soluble sugars. The application of CVHW and CVHCA significantly improved grain N assimilation by 48.7% and 49.6% compared with control, and by 23.9% compared with CVP addition, respectively. Conversely, the application of CVHW and CVHCA significantly reduced straw N assimilation by 42.9% and 59.1% compared to control. These results suggested that the addition of hydrochars promoted the N partition to edible part of rice. Moreover, the application of CVHW and CVHCA significantly improved N use efficiency by 51.5% and 38.6% compared with control, and by 38.5% and 32.7% compared with CVP addition. Similar trend was detected in grain soluble sugars content among treatments. No significant difference was detected in crude fiber or starch levels.

3.3. Effects of CVP and hydrochars on NH_3 volatilization

The effects of CVP and hydrochars on NH_3 volatilization from rice paddy soil are shown in **Fig. 1**. The trends of NH_3 volatilization flux from paddy soil were quite similar among all treatments (**Fig. 1A**). After BF the peak flux appeared at the second, third, or fourth day and declined shortly afterwards. After SF1 the peak flux only appeared at the second day and declined shortly afterwards. After SF2 no peak flux was detected. CVHCA treatment frequently resulted in the maximum peak value of NH_3 volatilization. Also, CVP and CVHW treatment increased NH_3 volatilization compared to control. Following BF, CVHCA addition significantly increased cumulative NH_3 volatilization by 2.29-, 1.51-, and 1.30-fold compared with the control, CVP, and CVHW, respectively (**Fig. 1B**). Following SF1, CVHW and CVHCA application significantly increased cumulative NH_3 volatilization by 61.7% and 62.6% compared with the control but reduced it by 35.7% and 34.9% compared with CVP application. Following SF2, no significant difference was detected for cumulative NH_3 volatilization. Yield-scale NH_3 volatilization is the quantification of cumulative NH_3 volatilization over the entire growth stage of rice based on yield production. Yield-scale NH_3 volatilization was comparable between the control and CVHW groups (**Fig. 1C**).

Compared with the control, CVP and CVHCA treatment significantly increased yield-scale NH_3 volatilization by 51.5% and 42.4%, respectively.

3.4. Effects of CVP and hydrochars on N_2O emissions

The dynamics of N_2O emissions during rice-growing seasons under different treatments are shown in **Fig. 2A**. Different treatments showed the similar temporal trends in N_2O fluxes during the rice-growing season. Obvious emission peaks were observed approximately 30, 45, 80, and 95 days following transplantation. The highest emission peaks of CVHW and CVHCA were observed on the 45 days after transplantation, and the emissions under CVHW and CVHCA were 3.08- and 2.78-fold, and 4.61- and 4.17-fold higher than under control and CVP, respectively. The cumulative N_2O emissions after three fertilization dates affected by different treatments are shown in **Fig. 2B**. Compared to control, CVP, CVHW and CVHCA all significantly increased cumulative N_2O emissions and the significantly highest N2O emissions were observed in CVHCA treatment; CVHCA application significantly increased cumulative N_2O emissions by 3.20-, 1.46-, and 1.47-fold compared with control, CVP, and CVHW, respectively. Moreover, CVP, CVHW and CVHCA all significantly increased yield-scale N2O emissions as well, however, no significant difference was detected between CVP and CVHW, or between CVP and CVHCA (**Fig. 2C**).

3.5. Effects of CVP and hydrochars on soil pH, NH_4^+–N and NO_3^-–N

The effects of CVP and hydrochars application on soil NH_4^+–N and NO_3^-–N concentrations after BF, SF1 and SF2 are shown in **Fig 3.** Following BF and SF1, the CVP, CVHW, and CVHCA all significantly improved the soil NH_4^+ concentration compared with control (**Fig 3A**). Compared with CVP, CVHCA significantly reduced soil NH_4^+ concentration by 25.1% and 22.8% after BF and SF1, respectively. However, after SF2, soil NH_4^+ concentration was comparable among the control, CVHW, and CVHCA groups; only CVP significantly improved the soil NH_4^+ concentration. Moreover, CVHCA significantly increased soil NO_3^-–N concentrations compared with the control, CVP and CVHW, irrespective of fertilization stages. Furthermore, following BF CVHW significantly increased soil NO_3^-–N concentrations by 46.5% compared with the control; however, after SF1, CVHW significantly reduced soil NO_3^-–N concentrations by 2.06-fold. In addition, the effects of CVP, CVHW, and CVHCA application on soil pH were consistent with the pH of materials; CVP, CVHW, and CVHCA all significantly increased soil pH after different fertilization stage and the strongest effect was detected for CVP treatment (**Table. S3**).

3.6. Effects of CVP and hydrochars on soil microbial C and N, and the abundance of microbial functional genes controlling N_2O emission

The effects of CVP and *Chlorella vulgaris* hydrochar application on soil microbial C and N are shown in **Fig. 4**. CVHW and CVHCA application significantly reduced microbial C content compared with CVP. Compared with the control, CVP, CVHW, and CVHCA all significantly reduced microbial N content. The effects of CVP and *Chlorella vulgaris* hydrochars application on the abundance of microbial functional genes controlling nitrification (*AOA* and *AOB*), denitrification (*nirK* and *nirS*) and N_2O oxidation (*nosZ*) are shown in **Fig. 5**. Compared with the control, only CVHCA treatment significantly improved the abundance of *AOA*. Also, CVHCA treatment significantly improved the abundance of *AOB* by 2.56-, 1.52-, and 4.26-fold compared with the control, CVP, CVHW treatment. The *Chlorella vulgaris* hydrochars, CVHW and CVHCA, significantly improved the abundance of *nirK* compared with CVP and control. By contrast, the *Chlorella vulgaris*-derived hydrochars, CVHW and CVHCA, significantly improved the abundance of *nirK* compared with CVP and control. CVHCA treatment significantly ameliorated the abundance of *nirS* by 3.48-, 1.68, and 1.27-fold compared with the control, CVP, and CVHW treatment, respectively. Compared with the control, CVP application significantly improved the abundance of *nosZ* by 68.3%; however, CVHW and CVHCA application significantly reduced *nosZ* levels by 44.8% and 56.1%, respectively. On the basis of these results, the application of CVP and hydrochars had marked impacts on the activities of soil microorganisms that are responsible for nitrification and denitrification.

4. Discussions

CVHW and CVHCA addition both significantly improved the grain yield of rice (**Table. 2**), suggesting that HTC is an effective way to transform the *Chlorella vulgaris* into a fertilizer. The important drivers of increased yield production resulting from microalgae-based hydrochar addition is increased soil NH_4^+-N concentration (**Fig. 3**) and N use efficiency by rice plant (**Table. 2**). N is one of the most important mineral nutrients for plant growth comprising 40-50% of dry matter of protoplasm, and is a constituent of amino acids and chlorophyll, and the building blocks of protein (Marschner, 2011; Chu et al., 2019). HTC was also shown to transform the bulk of organic N compounds to inorganic N (Funke et al., 2013; Kruse et al., 2016; Yu et al., 2019). The increased soil NH_4^+ concentration may stimulate rice growth because NH_4^+–N is the dominant N source for rice. Similar results of increased grain yield derived from increased soil N retention and plant N use efficiency after hydrochar addition have been reported previously (Sun et al., 2017; Yu et al., 2019; Chu et al., 2020). Hydrochars can release an abundant amount of macro and micronutrients, most of which are necessary and beneficial minerals for photosynthesis and plant growth (Feng et al., 2018a; Joseph et al., 2018; Li et al., 2019). The lager SSA of CVHW and CVHCA (8.31- and 9.36-fold higher than CVP, respectively) potentially plays an important role in improving N and nutrients adsorption (Feng et al., 2017; Mandal et al., 2018). The increased grain soluble sugar content (**Table. 2**) might be associated with the stimulated photosynthesis resulting from mineral nutrients introduced by hydrochars, and this may partly explain the increased yield (Chu et al., 2016a).

Chlorella vulgaris-derived hydrochars increased soil NH_4^+ concentration (**Fig. 3A**), NH_3 volatilization (**Fig. 1**), N_2O emission (**Fig. 2**), and plant N use efficiency (**Table.

2). Although the reduced soil microbial N can partly explain the N balance, the increased N gasification and plant N uptake was more likely attributed to the increased soil N mineralization. The N content in CVP and hydrochars was quite high (7.25-12.29%, **Table 1**) and thus increased the N source for soil microorganisms and plants. However, the low C/N ratio of CVP and hydrochars (3.92-8.44) were able to stimulate soil microbial activity and further accelerated the decomposition of soil organic N (Zimmermann et al., 2012; Mukome et al., 2013; Senbayram et al., 2019). Therefore, the abundant N compounds in hydrochars may serve as a paradoxical aspect for improving both plant N utilization and gaseous N loss to the atmospheric environment. The increased NH_3 volatilization exacerbated the non-point pollution.

The introduction of more N and H sources into soil, together with an increase in pore conductivity that allows for gas exchange, may be important factors for promoting NH_3 volatilization, as shown in previous studies on biochars (Sun et al., 2017; Feng et al., 2018b; H. Sun et al., 2019b). Soil pH is a primary factor that affects NH_3 volatilization because an acidic state may promote the negatively charged organic functional groups to become the main adsorption sites for ammonium, thereby preventing NH_3 volatilization (Sha et al., 2019). CVP, which had the highest pH, caused the highest volume of NH_3 volatilization loss after BF (**Fig. 1**). HTC reduced pH of hydrochars, particularly in the citric acid medium (CVHCA), thus helping reduce NH_3 volatilization loss at BF compared with CVP. Similar studies have also indicated an increase in NH_3 loss following biochar amendment to rice paddy and coastal saline soils due to the increased soil pH (Schomberg et al., 2012; Feng et al., 2018b). Following SF1, the short-term acidifying disturbance became weak and NH_3 volatilization loss became stronger under CVHW and CVHCA treatments. Furthermore, CVHW and CVHCA treatments both increased SSA compared with CVP, and thus were also expected to increase the NH_3 retention rather than NH_3 volatilization. The larger porous volume and porous diameter likely led to higher moisture retention in a bid to compete with NH_4^+–N adsorption (Sarkar et al., 2011; Mandal et al., 2019). Furthermore, the reduced abundance of a carboxyl group in CVHW and CVHCA compared with CVP, as shown in FTIR spectra (**Fig. S3**), may have resulted in the increased NH_3 volatilization because NH_3 is an alkaline gas and the acidic surface functional groups on the hydrochar, such as the carboxyl and carbonyl group, can protonate NH_3 gas to NH_4^+ ions (Spokas et al., 2012; Mandal et al., 2018). Therefore, the increased pH and reduced abundance of acidic surface groups impart *Chlorella vulgaris* hydorchars to increased NH_3 volatilization loss. Additionally, due to the increased yield by CVHW addition, no significant difference of the yield-scale NH_3 volatilization was detected between control and CVHW treatment. Therefore, in view of improving yield and avoiding NH_3 volatilization, CVHW is a better recommendation.

Furthermore, CVP and *Chlorella vulgaris* hydrochars all significantly increased N_2O emissions compared with the control group (**Fig. 2**). Obvious emission peaks were observed at the mid-season drainage (30 and 45 days after transplantation) and close to harvest (80 and 95 days after transplantation). The mid-season drainage changed the soil aeration and thus stimulate nitrification, thus caused substantial N_2O emissions from paddy soil (Bhattacharyya et al., 2013; Ali et al., 2015; Zhou et al., 2018). The peaks of N_2O emissions close to harvest was possibly caused by the accumulated N source from the slow decomposition of CVP or hydrochars. In addition, contradictory results that are related to the mitigation effects of biochar application on N_2O emission have been reported (Zhou et al., 2018; Borchard et al., 2019; Wu et al., 2020). The liming effect of the CVP and hydrochars may also be responsible for increased N_2O emission, because increased soil pH above a neutral state enables the NH_4^+ from the adsorption sites of soil minerals to further promote the nitrification (Mandal et al., 2018; Senbayram et al., 2019). However, the inconsistent results between a lower pH in CVHCA and increased N_2O emission suggest that other factors are involved. Compared with CVP, CVHCA significantly reduced the soil NH_4^+ concentration and increased soil NO_3^- concentration, which is consistent with increased abundances of *AOA* and *AOB* (**Fig. 5A and B**). Therefore, CVHCA application improved the nitrification and potentially promoted the generation of more N_2O. Additionally, the increased abundances of *nirK* and *nirS* by CVHCA and CVHW addition suggested the promotion of nitrite reduction, as well as the production of N_2O (**Fig. 5C and D**). *Chlorella vulgaris* hydrochars readily released the labile C and N pool to the soil, which possibly stimulates the activity of nitrobacteria and denitrobacteria. Similar results were reported in previous studies using other biochars (Duan et al., 2018; Senbayram et al., 2019; Zhang et al., 2019). Furthermore, the expression of *nosZ* was increased by CVP but was inhibited by CVHW and CVHCA (**Fig. 5E**) which reflects the inhibition of CVP on the oxidation of N_2O (Krause et al., 2018). This difference may serve as an important reason for the increased N_2O emission by CVHW and CVHCA compared with CVP.

5. Conclusions

The results of this study demonstrated that *Chlorella vulgaris*-derived hydrochars that employ a water or citrate acid solution as a reaction medium in HTC improved the yield and soluble sugars of rice grains while concomitantly increasing the NH_3 volatilization and N_2O emissions from rice paddy soil. The positive effects on rice growth and yield indicate that *Chlorella vulgaris*-derived hydrochars have the potential to serve as a fertilizer that can recycle nutrients from wastewater (enriched by microalgae) into the crop-soil system; this approach is superior to the direct application of CVP. However, the relatively lower C/N and H/C ratio of *Chlorella vulgaris*-derived hydrochars may readily provide a labile C pool for soil microorganisms to stimulate gaseous N loss. In future studies the additional modifications on *Chlorella vulgaris* hydrochars must be attempted to find out a way to improve the plant nutrients utilization without polluting the atmospheric environment.

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Conflicts of Interest

The authors declare no conflict of interest.

Electronic supplementary material

The online version of this article contains supplementary material, which is available to authorized users.

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