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Intact and washed biochar caused different patterns of nitrogen transformation and distribution in a flooded paddy soil

Mengjie Yu [a, b], Wei-qin Su [a, b], Sanjai J. Parikh [c], Yong Li [a, b], Caixian Tang [d], Jianming Xu [a, b, *]

[a] Institute of Soil and Water Resources and Environmental Science, College of Environment and Resource Sciences, Zhejiang University, Hangzhou, 310058, China
[b] Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Hangzhou, 310058, China
[c] Department of Land, Air and Water Resources, University of California, Davis, CA, 95616, USA
[d] Department of Animal, Plant & Soil Sciences, Centre for AgriBioscience, La Trobe University (Melbourne Campus), Bundoora, VIC, 3086, Australia

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ABSTRACT

Biochar soil amendment has been proposed as a novel way of mitigating greenhouse gas emissions and improving crop productivity. However, the effects of biochar on N_2O emissions and rice growth are inconsistent. Here, we investigated how biochar and its residue after washing affected nitrogen (N) transformation and rice growth and how far these effects remain from biochar using novel three-compartment pots. Intact biochar, washed biochar, washed biochar plus extract were placed in the central compartment of the pot, while rice plants were grown in the near-char zone (<5 cm, from the central compartment) or far-char zone (>5 cm). The addition of biochar and its extract generally increased N concentration in the roots and grains for the plants grown in the near-char zone but decreased the N concentrations for those in the far-char zone. The intact biochar increased the above-ground biomass, especially for the plant grown in the near-char zone, whereas the washed biochar had little influence on plant growth at the maturity and on dissolved organic C, total organic N, NH_4^+-N and available P. However, washed biochar, as well as intact biochar, still lowered N_2O emissions from the near- and far-char zones. The treatments, sampling times and rice growth all played important roles in regulating N-related functional genes, while sampling zones only affected the abundances of *amoA* and *nifH* genes. The 16S rRNA, bacterial *amoA* and *narG* gene abundances were highest with the intact biochar. The abundance of *nirS* and *nirK* genes increased with the washed biochar plus its extract but not with washed biochar alone. The N-functional microorganisms responded to the intact biochar, its soluble component and washed biochar differently and were closely correlated to microbial biomass C, NH_4^+-N, available P and electrical conductivity. Future research should be devoted to studying the effects of soluble component from biochar and the residual effects of biochar loading on plant growth, soil nutrients and N_2O emissions.

1. Introduction

Global rice production meets the dietary demands for more than half of the world's population, requiring substantial N fertilizer application (Zhang et al., 2019). The current usage of N fertilizer is approximately 100 Tg N per year worldwide, and a large portion of soil N inputs are lost to the air and water (Liu et al., 2018). Clearly, reducing N losses is an urgent task to relieve environmental stress and promote sustainable agriculture. Biochar production and application has been considered as an effective way of recycling biomass while increasing soil alkalinity (Oliveira et al., 2017), improving utilization of soil nutrients including N (Dai et al., 2017) and mitigating climate change (Zhang et al., 2020). Biochar is an eco-friendly product obtained from the thermal conversation (e.g., pyrolysis, gasification) of biomass or manure at different temperatures (from 250 °C to 700 °C) under oxygen-limited conditions (Lehmann et al., 2015). The unique properties of biochar, such as

* Corresponding author. Institute of Soil and Water Resources and Environmental Science, College of Environment and Resource Sciences, Zhejiang University, Hangzhou, 310058, China.
E-mail address: jmxu@zju.edu.cn (J. Xu).

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high alkalinity, porosity and labile nutrient content, alter "charosphere" environment while favoring the nutrient demands of specific microbial communities and further regulating the microbe-controlled biological pathways (Yu et al., 2019). The nitrogen transformation processes including N_2-fixation, nitrification, and denitrification are largely driven by various soil microbes (Gul and Whalen, 2016). Since relatively low N availability in agriculture systems limits rice production, management techniques, including biochar application, are used to regulate N forms in soils and N transformation in the atmosphere-soil-plant continuum through affecting the functional microorganisms (Xiao et al., 2019). Therefore, it is crucial to understand how biochar influences microbial communities related to primary N transformation processes.

The abundance and diversity of N-related functional microbes are often presented as the abundance of N-related functional genes. The gene *nifH* in soil N_2-fixing encodes the enzyme to convert N_2 to ammonia (NH_3) (Ducey et al., 2013). Nitrification is an oxidation process of ammonia (NH_3) to nitrate (NO_3^-) via nitrite (NO_2^-). Ammonia-oxidizing archaea (AOA) and bacteria (AOB) dominate the first and most rate-limiting step of nitrification. The key enzyme of this step is encoded by the gene archaeal and bacterial *amoA* (Afzal et al., 2019). Denitrification is the stepwise reduction of NO_3^- or NO_2^- to N_2 through a sequence of intermediates, including NO and N_2O (Lin et al., 2017). These steps are performed by many facultative and anaerobic bacteria. The enzymes involved in denitrification are encoded by *narG* (NO_3^- to NO_2^-), *nirS*/*nirK* (NO_2^- to NO) and *nosZ* (N_2O to N_2) (Edwards et al., 2018). Since some denitrifiers lack *nosZ* gene and N_2O reductases is sensitive to high oxygen and pH, denitrification becomes one of the major N_2O-producing pathways (Harter et al., 2016). The magnitude and direction of change in N-related functional genes following biochar addition varied among studies. Harter et al. (2014) found that biochar increased the abundance of *nifH* and the transcript copy number of *nosZ*-encoded bacterial N_2O reductase, which not only elevated the ability of N_2-fixing to increase N source, but also contributed to the complete denitrification. Yu et al. (2019) indicated that biochar favored the growth of AOB rather than AOA. Other studies have also shown that the biochar stimulated N-related functional genes by 1) increasing soil pH (Xiao et al., 2019); 2) providing a habitat for microbial growth (Gul and Whalen, 2016); 3) immobilizing soil N (Ducey et al., 2013); 4) serving as an input of labile C and nutrients (Yu et al., 2019). However, other work has shown the conflicting results. For instance, the adsorption of NH_4^+ or NH_3 by biochar may reduce their availability for nitrification and inhibit the reduction of NO_3^- for follow-up denitrification (Nelissen et al., 2014). Additionally, the release of toxic compounds such as phenolic compounds could inhibit the activity of soil microbes (Bandara et al., 2020).

Despite the mechanistic contrasts and unknowns on the biochar effects on N transformation, the critical factor that has attracted more attention is dissolved organic matter (Dai et al., 2019). Dissolved organic matter is believed to be a crucial C and N pool for soil-plant-microbe interactions (Cookson and Murphy, 2004). The initial release of dissolved organic matter from biochar provides labile C to soil microorganisms that dominate N cycling. However, the majority of studies have not examined the residual effects of biochar and spatial variability. Although biochar is much more stable compared to plant or animal biomass, the geochemical degradation process known as "aging" still happens to the incorporated biochar over time (Ghaffar et al., 2015). The dissolved organic matter released from biochar is one of the biochar aging products, which has aroused wide interests lately (Fan et al., 2020). Due to high content of dissolved organic matter and other exchangeable cations in biochar, microorganisms in soils could receive considerable labile C and essential elements (Yu et al., 2018). For example, denitrifiers could use electrons from dissolved organic matter to complete denitrification, an electron-consuming and heterotrophic process (He et al., 2019). Dai et al. (2019) reported that the easily mineralizable C in manure biochar was one of the dominate factors affecting N_2O flux and N-cycling genes. Meanwhile, our previous study showed a spatial variation in soil properties and gene abundance with respect to distance from biochar at millimeter increments. The shift of microbes in "charosphere", soil region in the proximity of biochar particles, was also closely related with the dissolved organic matter (Yu et al., 2019). Nevertheless, the initial effect of biochar, caused by the intrinsic addition of C and nutrients, is likely to dissipate with time (He et al., 2019). The loss of dissolved organic matter from biochar is significant, especially in paddy soils with diffusion (Nie et al., 2018). The residual effect of biochar on the distribution of N and N-related microbes with the absence of dissolved organic matter remains largely unknown.

Therefore, it is important to elucidate the dominant mechanisms by which biochar and its extract (dissolved organic matter) affect N transformation in soils with rice growth and associated spatial variability. Washed biochar was also used to simulate the aged biochar with minimal amount of dissolved organic matter. The aqueous extract of biochar was then added to soil as a separate treatment to extend the direct effect of biochar. The specific objectives of this study are: 1) to assess the impacts of biochar and its extract on the distribution of N in soil, plant and atmosphere; 2) to investigate the effect of biochar on N-related functional genes with respect to the spatial variability; 3) to evaluate the effects of dissolved organic matter and residual biochar on N transformation.

2. Materials and methods

2.1. Soil and biochar characteristics

Soil samples (sandy loam), classified as Acrisols (World Reference Base for Soil Resources), were collected from the surface layer (depth 0–20 cm) of an upland field in Quzhou, Zhejiang Province, China (E119°20′, N29°17′). Soil samples were air-dried and sieved < 2 mm for subsequent analysis and incubation. The soil had the following basic properties: pH (1:2.5 H_2O), 4.8; organic C, 4.70 g kg^{-1}; total N, 0.48 g kg^{-1}; available P, 3.07 mg kg^{-1}.

Intact biochar used in this experiment was derived from chipped corn straw, pyrolyzed at 300 °C for 2 h under O_2-limited conditions in a muffle furnace (SXL-1208, Hangzhou Zhuochi Co., Ltd). After cooling, biochar was milled and sieved to 0.25–2 mm. Washed biochar was created by soaking biochar in 18.2 MΩ cm water (Milli-Q, Millipore) at a ratio of 1:10. The mixture was shaken at 120 rpm and room temperature for 2 h and then filtered through nylon mesh bags (pore size < 25 μm) (Smith et al., 2016). The extraction process was repeated at least eight times until the concentration of dissolved organic C in the last aqueous extract was <10 mg C L^{-1}. The washed biochar was then oven-dried at 60 °C to constant weight for further use. Aqueous extracts of the first five times were collected and combined as an extract, which was added to soil during incubation period in washed biochar + extract treatment. The physicochemical properties of the biochars and its extract are listed in Table 1. The concentrations of the 16 US Environmental Protection Agency (USEPA) priority polycyclic aromatic hydrocarbons (PAHs) in biochar extract were presented in Table S2.

2.2. Seedling preparation and pot experiment

The pot (18-cm diameter, 20-cm height) used in this experiment was divided into three compartments which were separated by nylon mesh bags (pore size < 25 μm): a biochar compartment (central zone), inner and outer soil compartments (Fig. S1). The

Table 1
Physicochemical properties of intact biochar, washed biochar and its extract (Means ± S.D., n = 3).

Sample	pH	EC (μs cm^{-1})	DOC (g kg^{-1})	AP (mg kg^{-1})	Element contents (%)				Surface area (m^2 g^{-1})	Pore volume (cm^3 g^{-1})
					C	H	N	S		
Intact biochar	8.78 ± 0.02	476 ± 21	5.98 ± 0.14	459 ± 82	50.7 ± 0.4	3.92 ± 0.01	2.84 ± 0.06	0.08 ± 0.01	6.79 ± 0.31	0.018 ± 0.001
Washed biochar	7.75 ± 0.03	103 ± 12	0.71 ± 0.02	200 ± 30	47.7 ± 0.5	3.56 ± 0.12	2.99 ± 0.04	0.12 ± 0.01	8.83 ± 0.49	0.021 ± 0.003
Extract	8.17 ± 0.05	253 ± 16	270 ± 2 (mg L^{-1})	48.2 ± 4.3 (mg L^{-1})						

*EC, electrical conductivity; DOC, dissolved organic C; AP, available P.
*Pore volume: total pore volume of pores less than 200 nm diameter.

inner soil compartment, which encircled the biochar compartment, was referred to as the near-char zone (< 5 cm), while the outer compartment as the far-char zone (> 5 cm). On June 22, 2017, 60 g biochar (intact biochar or washed biochar), or quartz sand (0.6–0.9 mm) as the control, were used to fill the central zone. Meanwhile, 1-cm thick quartz sand was placed on the top of the biochar to prevent floating after flooding. The quartz sand was washed by 18.2 MΩ cm water for 5 times and then oven-dried at 105 °C to constant weight before use. Six kg soil was filled in soil compartments. Soil in each pot was added with standard fertilizers (180 mg kg^{-1} $CO(NH_2)_2$, 190 mg kg^{-1} KH_2PO_4, 160 mg kg^{-1} KNO_3) and saturated with deionized water. KNO_3, instead of KCl or K_2SO_4, was selected to avoid introducing Cl^- and SO_4^{2-} into the soil. A total of 48 pots (2 sampling times, with or without rice cultivation, 3 replicates for each treatment) in 4 treatments were used in this study. The 4 treatments were control, intact biochar (BC), washed biochar (WB) and washed biochar + extract (BE). The soils were under constant flooding regime (around 3 cm above soil surface) to avoid disturbance on soil microorganisms and lateral diffusion of soluble components caused by flood and draining cycles. About 60 mL biochar extract was added to each pot along with irrigated water in the BE treatment (with washed biochar in the central zone) every two days during the whole period of rice cultivation. The total volume of added extract was 3 L, which was equal to total volume of 60 g biochar extract (60g * 10 * 5 times extraction = 3 L).

Rice seeds of *Zhonghan 39* were surface-sterilized by using 5% NaClO for 20 min and rinsed five times with sterile water. They were then spread on moist filter papers to germinate in dark at 30 °C. After 3 days, the seedlings were cultivated in 0.25 Hoagland's solution (Hoagland and Broyer, 1936) in the growth incubator at 30 °C with 16 h of light and 8 h of dark period. A week later, plants were transferred to 0.5 Hoagland's solution. Uniform 14-d-old seedlings were transplanted into the two soil compartments, respectively (4 seedlings per compartment; 8 seedlings per pot, Fig. S1). The interference between near- and far-char zones caused by the rice arrangement was neglected. After transplanting, the plants were grown at a temperature of 28–36 °C in a greenhouse and no fertilizer was added during plant growth. At tillering stage (64 days after transplanting) and maturity stage (116 days after transplanting), the nylon mesh bags were carefully removed to collect roots, stems and grains. The biochars and soils were destructively collected for further analyses.

2.3. N_2O measurement and physicochemical analyses

After the first harvest at the tillering stage, a modified soil cover method (Mosier and Hutchinson, 1981) was employed to estimate N_2O emissions by using 50 mL plastic syringe, which was attached with a septum, a three-way valve and a needle (Di et al., 2014). The plastic syringes were inserted about 2 cm into the soils both in near- and far-char soil zones and used as chambers to collect N_2O. They were vented at all the times, except during sampling periods. The N_2O samples were collected every 5 days until the end of the experiment. At each sampling time, two gas samples were taken 40 min apart (0 min and 40 min) from the syringe and injected into 7-mL serum vials for further analysis. N_2O of samples was determined via gas chromatography (GC-2010 Plus SHIMADZU, Japan).

Soil physicochemical properties included pH, electrical conductivity (EC), total organic C and N, dissolved organic C, NH_4^+-N, NO_3^--N, available P and microbial biomass C. Biochar properties included pH, EC, elemental composition, surface area, pore volume and PAHs concentrations in extract. Plant properties included aboveground biomass and elemental composition. Detailed information on analyses is presented in the Supplementary Information (SI).

2.4. Real-time quantitative polymerase chain reaction (qPCR)

Detailed information on soil DNA extraction is presented in SI. The gene copy numbers of bacterial 16S rRNA, *nifH*, archaeal *amoA*, bacterial *amoA*, *narG*, *nirK*, *nirS* and *nosZ* genes in soil extracts of near- and far-char zones taken at two sampling times, were determined in triplicate by quantitative real-time PCR on a LightCycler®480II (Roche, Germany). The details of primers and reaction conditions used for all assays are described in Table S1. Data was analyzed using LightCycler®480 SW Software (v. 1.5.1; Roche, Germany). Standard curves were generated using a series of ten-fold dilutions over concentrations from 10^2 to 10^8 copies per assay except 16S rRNA genes (10^5 to 10^{10} copies per assay). Amplification efficiencies ranged from 88% to 102% with R^2 values of 0.99.

2.5. Statistical analysis

Two-way analysis if variance (treatments and sampling zones) followed by the least significant difference (LSD) was carried out to check the difference. $P < 0.05$ was considered as statistically significant. Multiple comparisons (treatments, sampling zones, sampling times and plant growth) with LSD was performed to check for the abundance of N-related functional genes. Principal component analyses (PCA) and redundancy analysis (RDA) were performed by CANOCO v.4.5 for windows. Heatmaps of functional genes were conducted in R 3.6.2.

3. Results

3.1. N_2O emissions

Where no plants were grown, all three biochar treatments decreased N_2O emissions with the lowest emission observed in the BC treatment (Fig. 1). There was no significant difference between BC and BE, while WB showed a moderate effect on the reduction of N_2O emissions relative to BC and BE. N_2O emissions were much higher and reached up to 94.9 and 77.3 mg m^{-2} from the near- and

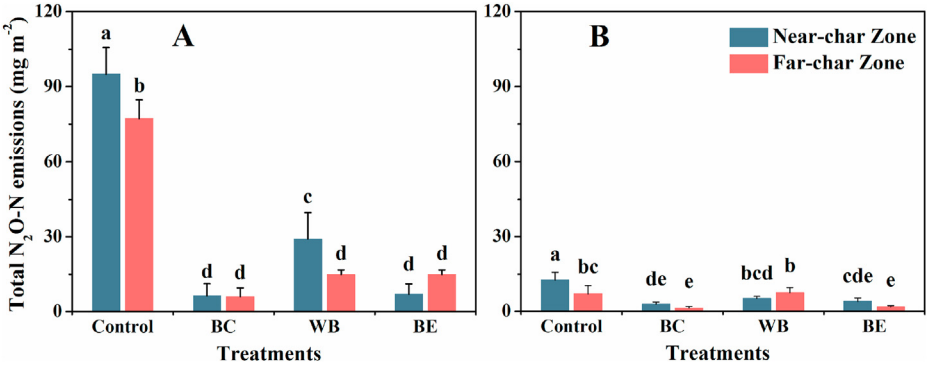

Fig. 1. Total N_2O emissions in the soils without (A) or with (B) rice plants. Error bars indicate the standard deviation of triplicate analyses. Different letters indicate significant differences between treatments ($p < 0.05$).

far-char soil zones of the unamended control, respectively (Fig. 1A). In rice-planting treatments, total emissions of N_2O were < 17 mg m^{-2} in all treatments since plant growth took up substantial amounts of N (Fig. 1B). Irrespective of plant growth, N_2O emissions were significantly greater from the near-char zone than other zones.

3.2. N concentration in the plants

At the maturity stage, total N of rice roots differed between near- and far-char zones in three biochar treatments, especially BC and BE treatments (Fig. 2A). Both BC and BE increased the total N in near-char zones significantly, which were almost double that in far-char zone. Total N contents in far-char zones were a little lower with three biochar treatments than control. The highest total N at 10.4 g kg^{-1} was found in near-char zone with BC. A similar result was observed at tillering stage, but the contents decreased form tillering stage to maturity stage (Fig. S2A). As for total N of grains, they were not markedly different among treatments. However, total N contents were still higher in near-char zones than those in the far-char zones with three biochar treatments, showing an inverse relationship to C/N ratio in grains (Fig. 2B).

3.3. Soil chemical properties

The addition of biochars increased soil pH values by up to 0.53 units at the tillering stage and by up to 0.35 units at the maturity stage with the average pH increment being 0.26 units higher at the tillering stage compare to control (Tables 2–3). The pH increments were also higher in the near-char than in the far-char zone, especially with the BC treatment. The pH differences between the near- and far-char zones could reach up to 0.35 (without rice) and 0.39 (with rice) at the tillering stage. On average, the pH was 0.15 unit lower in the presence of plants than without plants.

The dissolved organic C only increased in BC and BE treatments while the increasing value was not significant in WB relative to control (Tables 2–3). The average dissolved organic C was 14.9 mg kg^{-1} higher in near-char zone than in far-char zone with three biochar treatments. Growth stages and the presence of plants did not show significant influence on the concentration. On the contrary, total organic N showed little difference among treatments, but the concentration was 0.11 g kg^{-1} lower with rice growth than without rice presence.

The highest concentration of NH_4^+-N was founded in BE treatment at tillering stage, which was significantly higher than those in other treatments (Tables 2–3). NH_4^+-N was utilized more by rice growth which led to 78.5 mg kg^{-1} differences of NH_4^+-N concentration on average between soils without or with rice. NO_3^--N levels were very low (< 4 mg kg^{-1}) for all treatments and growth stages (Tables 2–3).

All three biochar treatments (especially BC and BE) increased available P in near-char zones with the average concentration being 69.2 mg kg^{-1} higher in near-char zone than in far-char zone (Tables 2–3). There was no obvious elevation being observed in far-char zone.

3.4. Microbial biomass C and 16S rRNA

At the maturity stage, all treatments with rice resulted in increased soil microbial biomass C compared to control for both

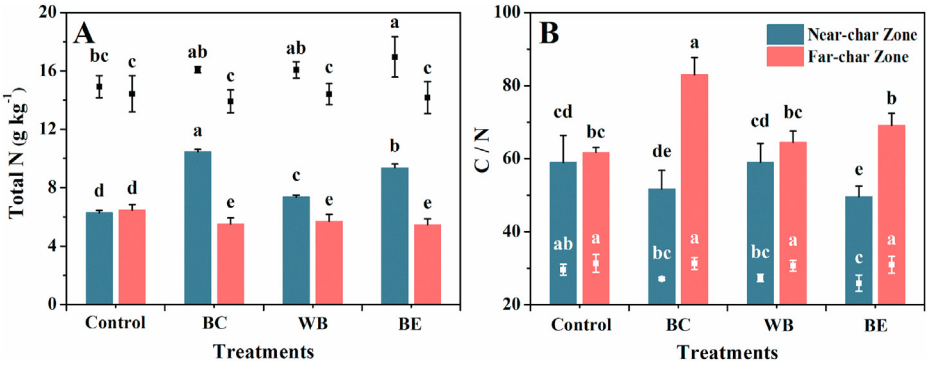

Fig. 2. The total N (A) and C/N ratio (B) in roots (histograms) and grains (square) at the maturity stage. Error bars indicate the standard deviation of triplicate analyses. Different letters indicate significant differences between treatments ($p < 0.05$).

Table 2
Chemical properties of the soils at the tillering stage. The values with a common lower-case letter or a common italic lower-case letter within the same column indicates no significant difference at $p < 0.05$ without or with rice growth, respectively.

		Treatment	pH	DOC (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	TON (g kg^{-1})	AP (mg kg^{-1})
Without Rice	**NC**	Control	5.04d	101d	69.0b	0.68c	0.66ab	64.3bc
		BC	5.57a	149a	87.2b	3.04a	0.64ab	82.5b
		WB	5.35abc	110cd	90.9b	2.43ab	0.67a	78.9b
		BE	5.54a	137ab	131.6a	2.81ab	0.73a	141.5a
	FC	Control	5.14cd	101d	89.1b	1.59abc	0.69a	54.0bc
		BC	5.22bcd	110cd	99.2ab	2.14abc	0.65ab	48.5bc
		WB	5.40ab	97d	72.5b	1.37bc	0.68a	31.7c
		BE	5.41ab	120bc	136.1a	1.97abc	0.57b	52.3bc
With Rice	**NC**	Control	5.11cd	96*d*	6.3cd	1.43*ab*	0.59*ab*	20.6*c*
		BC	5.40*a*	143*a*	13.4*c*	1.96*a*	0.52*bc*	142.6*a*
		WB	5.27*b*	107cd	6.1cd	1.58*ab*	0.51*c*	62.0*b*
		BE	5.47*a*	129*ab*	43.9*a*	1.92*a*	0.61*a*	127.1*a*
	FC	Control	5.05*d*	99*d*	1.9*d*	0.67*b*	0.56*abc*	23.7*c*
		BC	5.01*d*	125*abc*	12.9*c*	0.81*b*	0.52*c*	29.6*c*
		WB	5.24*b*	97*d*	4.1cd	1.48 *ab*	0.51*c*	35.8*bc*
		BE	5.22*bc*	110bcd	30.3*b*	1.28 *ab*	0.57*abc*	28.3*c*

*BC, intact biochar; WB, washed biochar; BE, washed biochar.
*NC, near-char zone; FC, far-char zone; DOC, dissolved organic C; TON, total organic N; AP, available P.

Table 3
Chemical properties of the soils at the maturity stage. The values with a common lower-case letter or common italic lower-case letter within the same column indicates no significant difference at $p < 0.05$ without or with rice growth, respectively.

		Treatment	pH	DOC (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	TON (g kg^{-1})	AP (mg kg^{-1})
Without Rice	**NC**	Control	5.13cd	102bc	76.2a	2.11ab	0.62bc	45.0c
		BC	5.48a	119a	80.1a	1.49b	0.59bc	139.4ab
		WB	5.23bc	108abc	77.4a	2.16a	0.73a	79.6 ab
		BE	5.32b	121a	90.8a	1.23b	0.67ab	153.8a
	FC	Control	5.04d	94c	85.1a	1.68ab	0.67ab	53.9bc
		BC	5.34b	116ab	73.4a	2.59a	0.56c	41.1c
		WB	5.10d	106abc	84.3a	2.58a	0.59bc	60.3abc
		BE	5.04d	112ab	73.8a	2.49a	0.59bc	66.9bc
With Rice	**NC**	Control	4.99*bc*	107*ab*	5.2*bc*	2.63*a*	0.53*bc*	21.5*c*
		BC	5.29*a*	132*a*	2.9*d*	1.81*a*	0.49*d*	131.5*a*
		WB	5.07*b*	112*ab*	4.7bcd	2.20*a*	0.51bcd	51.9*b*
		BE	5.01*bc*	130*a*	3.9cd	1.97*a*	0.55*b*	95.8*b*
	FC	Control	4.98*bc*	97*b*	5.9*abc*	1.57*a*	0.60*a*	22.6*c*
		BC	5.06*b*	110*ab*	4.3cd	1.96*a*	0.51cd	23.2*c*
		WB	4.95*bc*	102*b*	6.7*ab*	2.51*a*	0.48*d*	19.4*c*
		BE	4.88*c*	112*ab*	7.9*a*	2.56*a*	0.50cd	19.8*c*

*BC, intact biochar; WB, washed biochar; BE, washed biochar.
*NC, near-char zone; FC, far-char zone; DOC, dissolved organic C; TON, total organic N; AP, available P.

near- and far-char zones (Fig. 3A). The peak microbial biomass C content reached was 148.5 mg kg^{-1} soil with BC, followed by 131.9, 102.5 and 59.1 mg kg^{-1} with WB, BE and control in near-char zone, respectively. The content of microbial biomass C was much higher in near-char zone than in far-char zone with BC and WB. A similar trend was observed at tillering stage, but average content was 42 mg kg^{-1} lower than at the maturity stage (Fig. S4).

Compared to control, total bacterial abundance of soil with rice increased with three biochar treatments in near-char zone and only BC in far-char zone at maturity stage (Fig. 3B). The copy numbers reached a maximum of 2.7 × 10^{10} copies g^{-1} dry soil with BC in near-char zone, which was higher than in far-char zone. The 16S rRNA gene copy number in soil with rice was averagely 1.5 × 10^{10} copies g^{-1} dry soil lower than soil without rice. There was no

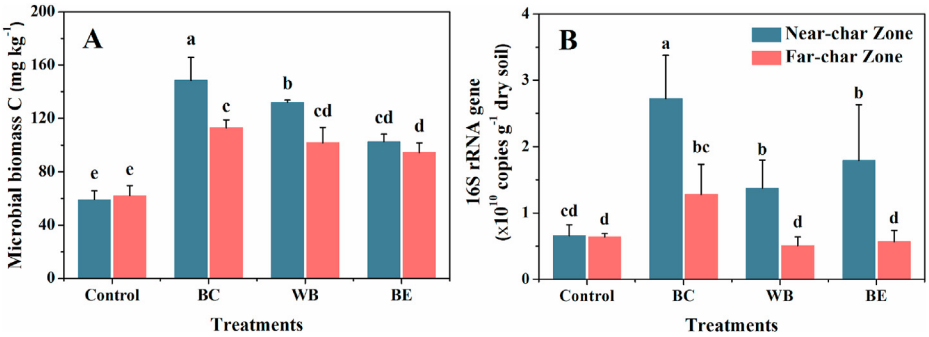

Fig. 3. Microbial biomass C (A) and the abundance of 16S rRNA gene (B) in the soils with rice plants at the maturity stage. Error bars indicate the standard deviation of triplicate analyses. Different letters indicate significant differences between treatments ($p < 0.05$).

significant difference among treatments at tillering stage, but the abundance was 1.5 × 10^9 copies g^{-1} dry soil higher than at maturity stage (Fig. S5).

3.5. N-related functional genes

The abundance of bacterial *amoA* was highest with BC amendment in the near-char zone, whereas no difference was observed between BC and control with rice growth at the maturity stage (Fig. 4). Only BE increased the abundances of bacterial *amoA* gene in both near- and far-char zones with rice plants at the maturing stage (Fig. 4D). The presence of plants reduced the abundance of bacterial *amoA* gene in all the treatments (Fig. S6). All three biochar treatments had 1.1 × 10^6 copies g^{-1} dry soil lower copy numbers of archaeal *amoA* gene than the unamended control on average (Fig. 4). No significant difference was found between the near- and far-char zones for all treatments. The copy numbers of *narG* at the maturity stage increased only in the BC but not in WB and BE treatments (Fig. 4). The abundance of *nirS* in soils without plant growth was higher with BE than in the control, BC and WB treatments, and higher at the tillering than the maturity stage (Fig. S9). Unlike *nirS*, copy numbers of *nirK* gene were comparable between all four treatments and ranged from 1.71 × 10^6 and 2.09 × 10^7 copies g^{-1} dry soil (Fig. 4). A significantly higher copy number was measured in soils with WB but no plants than with the other three treatments at the tillering stage. Similar to *nirK*, the abundance of *nosZ* gene did not differ between the treatments or between the near- and far-char zones (Table 4). At the maturity stage, BC increased the *nifH* abundance in the near-char zone, while WB and BE increased its abundance only in the soils with plant growth. The difference between near- and far-char zones was significant (Table 4).

Principal component analysis (PCA) was applied to access how N-related functional genes varied among the treatments. The first two principal components explained 78.6%, 82.4%, 78.7% and 89.8% of the total variation among treatments for the soils without and with rice plants at the tillering stage, and without and with rice plants at the maturity stage, respectively (Fig. 5 and S14). The separation among the treatments was more obvious for the soils with rice growth than without rice. At the tillering stage, the BC and BE were separated from the control and WB treatments, whereas at the maturity stage, the BC and WB were clearly distinct from the control and BE treatments (Fig. 5). The separation between the near- and far-char zones was not apparent, except the BC treatment without plants at both stages (Fig. S14).

4. Discussion

4.1. Effects of biochar on N distribution in soils and plants

The complete separation of biochar and soil enabled us to investigate the spatial variability in soil properties at different

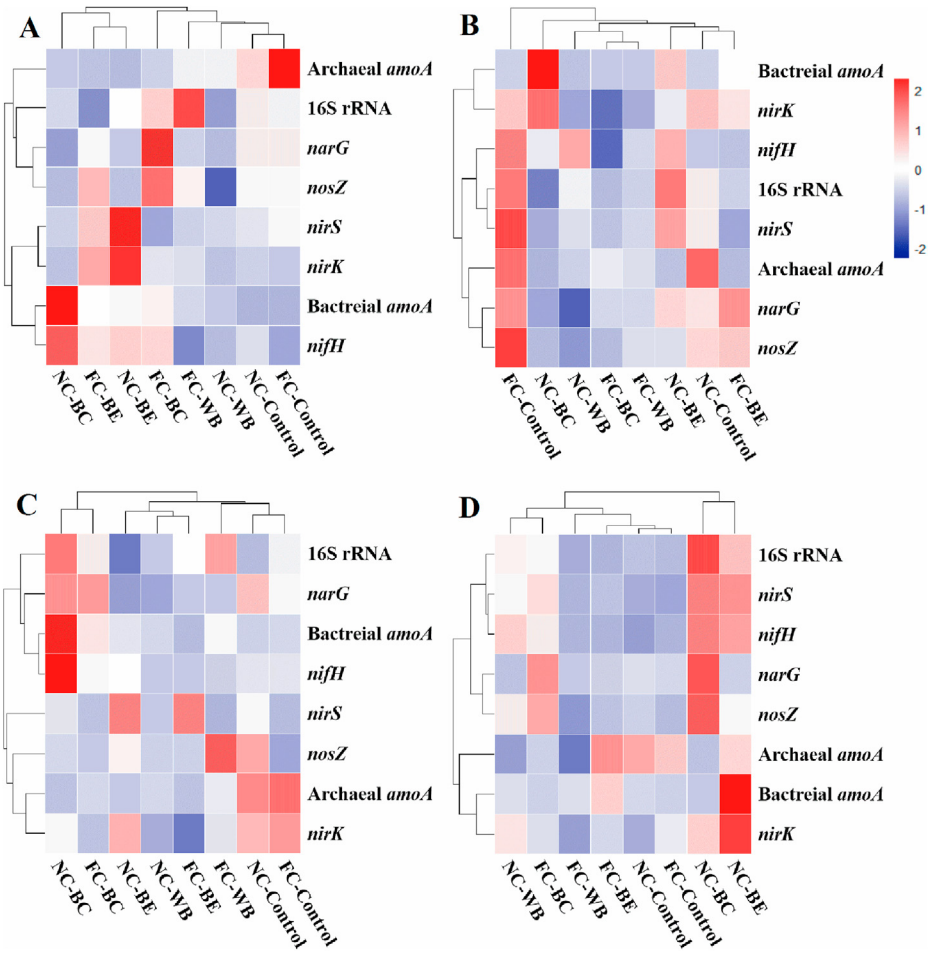

Fig. 4. Heatmaps of N-related functional gene copy numbers in the soils without (A, C) or with rice plants (B, D) at the tillering (A, B) or maturity (C, D) stage. NC, near-char zone; FC, far-char zone. Data expressed as Z-scores, with red or blue colors indicating a higher or lower Z-score (relative abundance compared with the mean for that gene category).

Table 4
Results from multiple comparisons showing the importance of sampling times, rice, sampling zones and biochar treatments on the abundance of N-related functional genes.

	Bacterial *amoA*	Archaeal *amoA*	*narG*	*nirS*	*nirK*	*nosZ*	*nifH*
Sampling time	< 0.01	> 0.05	0.01	< 0.01	0.01	< 0.01	> 0.05
With or without rice	< 0.01	< 0.01	< 0.01	0.02	< 0.01	< 0.01	> 0.05
Sampling zone	< 0.01	0.03	> 0.05	> 0.05	> 0.05	> 0.05	< 0.01
Treatment	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	> 0.05	< 0.01

*Values shown are the probability levels (p) from multiple comparisons with least significant difference (LSD).

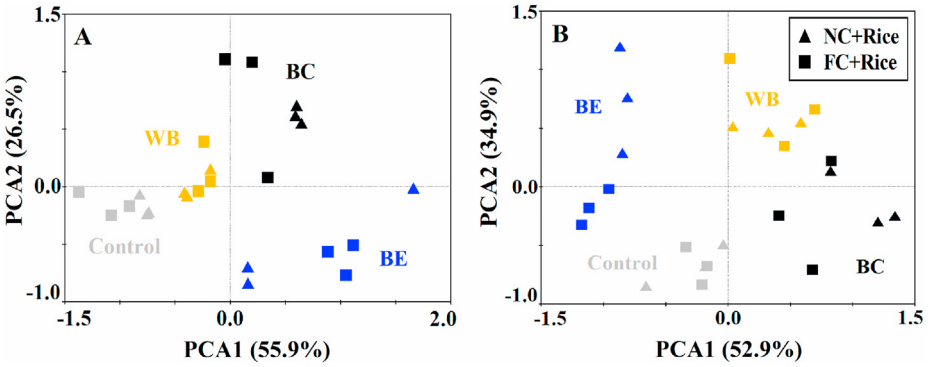

Fig. 5. Principal component analysis (PCA) of N-related functional gene copy numbers in the soils with rice plants at the tillering (A) or maturity (B) stage. Axes show the percentage of total variation explained by the first two factors. NC, near-char zone; FC, far-char zone.

distance intervals from the biochar. However, the higher N_2O emission in the near-char zone than far-char zone in the control was somewhat unexpected (Fig. 1). Except N_2O emission, the concentrations of NH_4^+-N, NO_3^--N, total organic N and rice traits did not show significant differences between the two sampling zones in the control (Tables 2–3). Therefore, the difference in N_2O emission had mainly resulted from the quartz sand in the central compartment. The density and porosity of quartz sand in the central compartment was different from soil and biochar. Samson et al. (1990) reported that the entrapment of N gases in soils led to the inaccurate quantitation of $^{15}N_2$+$^{15}N_2O$. The compaction stress of soil in the near-char zone might release more N_2O from entrapped N gases (Liu et al., 2017).

Three types of biochar only slightly decreased N_2O emissions in the soils with rice growth (Fig. 1B). Compared to soils without rice growth, the reduction was negligible, indicating that considerable amount of N was utilized by the plants. This was also supported by the lower total organic N and inorganic N, especially NH_4^+-N, in soils with plants (Tables 2–3). NH_4^+-N and NO_3^--N are the two major N forms for plant uptake, with rice preferring ammonium as the N-source (Zhang et al., 2020). Many previous studies have indicated that biochar could decrease soil N_2O emissions in agricultural soils with multiple plant types, such as maize (Edwards et al., 2018) and rice (Borchard et al., 2019). However, N was mostly utilized by rice plants instead of being transformed into N_2O in this study.

Two factors which are related to N uptake by plants are N availability and root traits (Huang et al., 2018). The higher content of total N in rice roots and grains with biochar treatments in near-char zone was mainly because of the following three mechanisms. First, the addition of biochar especially the biochar extract released NH_4^+-N and NO_3^--N into soil, as evidence by the increasing concentration of NH_4^+-N and NO_3^--N in the BE treatments (Table 2). Therefore, the potential N availability was higher in the near-char zone than far-char zone. Second, biochar improved soil P availability by directly releasing PO_4^{3-}. Pratiwi et al. (2016) reported that rice husk biochar provided more available P to soil as a net P source. Also, a meta-analysis showed that biochar application significantly increased P availability for plants especially in acid soils by decreasing P sorption on Al and Fe oxides (Glaser and Lehr, 2019). It coincides with the higher available P concentration in near-char zone (Table 2). Liu et al. (2019a) found that biochar could increase the shoot N:P of *Lolium multiflorum* with P fertilization. Since plants are likely to assimilate the most limiting nutrient to keep stoichiometric homeostasis, accumulated P exerts a stronger effect on rice N uptake to keep the balance in plant N and P content (Wei et al., 2017). Therefore, the increasing uptake of N by rice plants was partly due to the higher P availability. Third, biochar application could improve soil properties to create a more suitable environment to alter root traits then influence rice growth. Xiang et al. (2017) reported a meta-analysis showing that mixing biochar with soil could increase root biomass, volume and surface area. Although the soil physical properties may not change significantly since the biochar was separated from rice roots in our present study, the improved soil fertility could promote the rice root traits to enlarge the rhizosphere effect (Zhang et al., 2020). Meanwhile, the optimum pH for rice growth is around 5.0–6.5 (McCall, 1976). Biochar raised the soil pH, which led to higher aboveground biomass (Fig. S3). Higher biomass in near-char zone also indicates that rice in this zone was better able to access nutrients, resulting in lower N content of rice plants in the far-char zone.

4.2. The relationship between soil properties and N-related functional genes

The distribution of N-related genes was clearly divided into three groups, indicating a significant change in the abundance of N-related functional genes under BC amendment with considerable difference between near- and far-char zones (Fig. 6). Plant N uptake can decrease soil N availability, triggering the competition with microorganisms for N and thus resulting in a decrease of nitrifier abundance and activity (Liu et al., 2016). In this study, the abundance of *amoA* gene was higher in soils without rice growth (Figs. S6–7). The positive correlation existed between bacterial *amoA* abundance and NH_4^+-N concentration in soils amended with biochar, whereas the abundance of archaeal *amoA* gene was negatively correlated with dissolved organic C and P availability but

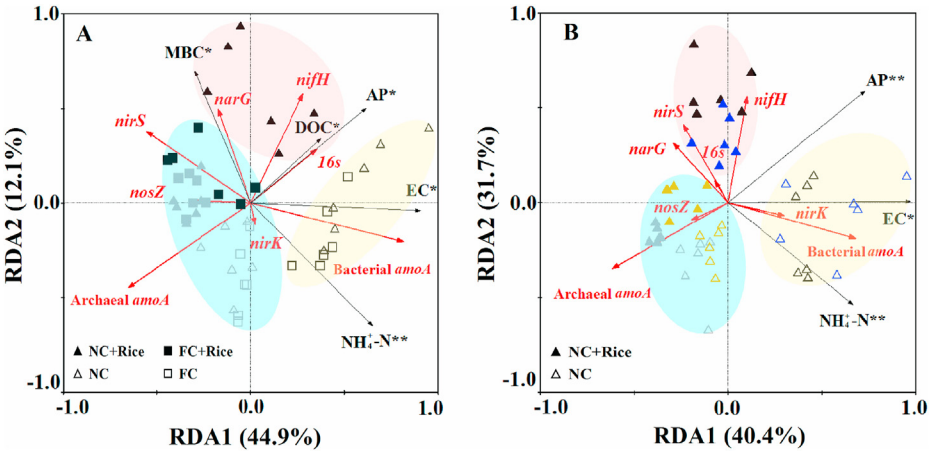

Fig. 6. Redundancy analysis (RDA) ordination plots analyzing the correlation between the abundance of N-related functional genes and soil properties amended without (Control, Grey) and with intact biochar (Black), washed biochar (Yellow) and washed biochar + extract (Blue) at the tillering and maturity stages without or with rice plants (+Rice). NC, near-char zone; FC, far-char zone; MBC, Microbial biomass C; AP, Available P; EC, electrical conductivity. * and ** represent significance at $p < 0.05$ and $p < 0.01$, respectively.

not correlated with NH_4^+-N or microbial biomass C (Fig. 6). A meta-analysis showed that ammonia-oxidizing bacteria (AOB) responded more strongly and had greater potential for nitrification than ammonia-oxidizing archaea (AOA) after additions of N (Carey et al., 2016). AOA has been reported to better adapt oligotrophic conditions because it could develop specific ecological niches in poor nutrient conditions (Zhang et al., 2012). Therefore, the NH_4^+-N concentration was irrelevant to AOA growth in this study. Moreover, Xiao et al. (2019) concluded that the effect of biochar application on AOB copy number was over 1.3 times higher than that of AOA based on 122 reported experiments, indicating that AOB was more dynamic than AOA to biochar amendment. Biochar increased the carbon availability and nutrients to soil microorganisms. The increasing bacterial *amoA* abundance in biochar treatment indicated a shift of the predominate ammonia oxidizer from AOA to AOB (Figs. S6–7). Our previous study demonstrated that AOB was more dependent on proximity to the biochar because of the high pH niche and abundant C source (Yu et al., 2019), which was consistent in this study. Since soil was not mixed with biochar during incubation, the pH elevation was not as high as soil and biochar mixture (Afzal et al., 2019). Therefore, the relationship between pH and *amoA* gene was not significant here.

The genes for denitrification were much more stable than ammonia oxidizers under different biochar treatments. Bai et al. (2015) demonstrated that biochar only decreased *narG* gene abundance but did not influence other denitrifiers. On the contrary, we found that *narG* was stimulated by the biochar, correlated with microbial biomass C and NH_4^+-N concentration (Fig. 6A). The higher *narG* encoded nitrate reductase indicated the strong activity of nitrate reduction upon biochar addition. However, since the MBC and 16S rRNA increased with BC as well, the *narG* abundance relative to 16S rRNA was actually lower compared with control (Fig. S13). This may explain that many previous studies reported that biochar increased the absolute abundance of denitrifiers (Xiao et al., 2019) but decreased the N_2O emissions (Edwards et al., 2018). The relative abundances of *nirS* and *nirK* genes to 16S rRNA were higher with BE treatments, while *nosZ* copy numbers did not show any significant difference from other treatments. Our previous work proposed that there may exist a shift of *nosZ*/*nirX* from near charosphere to far charosphere, presenting a reduced capacity of complete denitrification in near "charosphere" (Yu et al., 2019). The lower *nosZ*/*nirX* ratio with BE in this present study confirmed that it was mainly because of the water-soluble component of biochar (Nelissen et al., 2014).

A positive correlation was also found between *nifH* gene and dissolved organic C or available P. The supply of labile C, such as dissolved organic C from organic fertilizers, has been shown to increase *nifH* abundance and make diazotroph more energy-efficient (Gonzalez Perez et al., 2014). More recently, Liu et al. (2019b) has demonstrated that soil available P concentration was an important factor affecting diazotrophic communities after biochar addition. Thus, high soil P and C availability provided optimal conditions for diazotroph growth (Hu et al., 2018). Although the abundance of *nifH* gene was higher with BC at maturity stage, the content of total organic C was lower with biochar (Table 3). The opposite result was because of the demand of available N after the large C input. The N-mineralization was still stronger that N-fixing despite the higher *nifH* copy numbers after biochar addition, which indicated that biochar increased the net N mineralization (Xu et al., 2016).

4.3. Impact of dissolved organic C from biochar on soil N transformation

The extract of biochar played an important role in N transformation. The separation between WB and BE for N-related functional genes was observed both at the tillering and maturity stages, while the impact of WB was similar to the unamended control at the tillering stage but made a difference from the control at the maturity stage (Fig. 5). The data indicate that the influence of biochar at the tillering stage had resulted from the initial release of dissolved substances, so that the WB did not play any role in soil properties at the beginning. At maturity stage, the residual effect of WB started to make a difference from control. The influence of BE on soil properties was similar to the comparable zones of BC in both near- and far-char zones, while WB showed a different pattern from BE at tillering stage (Table 2). This demonstrates that the direct effect of biochar on soil properties were due to the diffusion of dissolved substances instead of the directly contact between soil and biochar particles. The initial effect of biochar, caused by the intrinsic addition of labile C and nutrients, is likely to diminish over time. After the quick release of dissolved substances, the residual effects of biochar differed from the initial effects of soluble component in biochar. The base cations and dissolved organic C were two major groups in the biochar extract that affected N transformation. However, the soil pH with BE did not increase compared to WB, demonstrating that the base cations did not play an important role in the extract. Dissolved organic matter of biochar contained carboxyl, ester, quinone moieties (Fu et al., 2016)

and aromatic C from low-molecular weight polycyclic aromatic hydrocarbons, which provided soil microbes with available C source (Dong et al., 2014). The total concentration of 16 PAHs was about 106 μg L^{-1} (Table S2). Most PAHs were less than 2 μg L^{-1} except some low-molecular-weight PAHs, such as anthracene and pyrene. Joseph and Taylor (2014) reported that biochars pyrolyzed at low temperatures contained higher oxygenated functional groups and dissolved organic compounds that can stimulate microbial growth and systemic resistance, which was a beneficial response to low-dose toxin. The return of these dissolved organic C with extractions addition in BE could increase the nitrogen demands and then regulate the functional microbes (Mukherjee and Zimmerman, 2013). The extend of influence of BE on *amoA* genes was between BC and WB, while WB had greater impacts on *nirS*. Many previous studies demonstrated that fresh biochar with a variety of labile C could stimulate the denitrifier activities. He et al. (2019b) found that the aged biochar changed the relative ratio of denitrification functional genes, which showed that the ability of N_2O mitigation decreased with biochar aging process. However, WB in this study still largely decreased N_2O emissions, while the reduction with BE did not show great difference from WB, indicating that dissolved organic matter was not significantly related with N_2O emissions (Fig. 1). Residual biochar could still mitigate N_2O emissions with little dissolved organic matter.

5. Conclusion

To our knowledge, the present work is the first study to assess the effects of biochar and its released dissolved organic matter on N transformation with respect to spatial variability, which is an innovative aspect of this study. The total N of rice grains and roots were much higher in near-char zone than in the far-char zone because of the higher N demands from rice and N availability in near-char zone. Meanwhile, the addition of biochar decreased N_2O emissions. However, the reduction of N_2O emissions did not result from biochar extract, but rather the effect of residual biochar. Moreover, the N-functional microorganisms responded to the biochar, its extract and residual biochar differently, highlighting the need to consider biochar aging and the spatial heterogeneity in the future to improve understanding on how biochar affects N_2O emissions from agricultural land.

CRediT authorship contribution statement

Mengjie Yu: Investigation, Data curation, Writing - original draft and, Writing - review & editing. **Wei-qin Su:** Investigation and, Writing - review & editing. **Sanjai J. Parikh:** Supervision and, Writing - review & editing. **Yong Li:** Writing - review & editing. **Caixian Tang:** Writing - review & editing. **Jianming Xu:** Conceptualization, Writing - review & editing, Supervision and, Funding acquisition.

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.jclepro.2021.126259.

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