Biochar amendment reduces paddy soil nitrogen leaching but increases net global warming potential in Ningxia irrigation, China

Yongsheng Wang¹, Yansui Liu¹, Ruliang Liu², Aiping Zhang³,⁴, Shiqi Yang³, Hongyuan Liu¹, Yang Zhou¹ & Zhengli Yang³

The efficacy of biochar as an environmentally friendly agent for non-point source and climate change mitigation remains uncertain. Our goal was to test the impact of biochar amendment on paddy rice nitrogen (N) uptake, soil N leaching, and soil CH₄ and N₂O fluxes in northwest China. Biochar was applied at four rates (0, 4.5, 9 and 13.5 t ha⁻¹ yr⁻¹). Biochar amendment significantly increased rice N uptake, soil total N concentration and the abundance of soil ammonia-oxidizing archaea (AOA), but it significantly reduced the soil NO₃⁻ -N concentration and soil bulk density. Biochar significantly reduced NO₃⁻ -N and NH₄⁺ -N leaching. The C2 and C3 treatments significantly increased the soil CH₄ flux and reduced the soil N₂O flux, leading to significantly increased net global warming potential (GWP). Soil NO₃⁻ -N rather than NH₄⁺ -N was the key integrator of the soil CH₄ and N₂O fluxes. Our results indicate that a shift in abundance of the AOA community and increased rice N uptake are closely linked to the reduced soil NO₃⁻ -N concentration under biochar amendment. Furthermore, soil NO₃⁻ -N availability plays an important role in regulating soil inorganic N leaching and net GWP in rice paddies in northwest China.

Synthetic nitrogen (N) fertilizer is currently the largest source of anthropogenic reactive N worldwide¹ and has enabled the doubling of world food production in the past four decades². However, excessive fertilizer N intended for crops results in environmental pollution problems, such as greenhouse gas (GHG) emissions and surface runoff and leaching³. The three main greenhouse gases (i.e., CO₂, CH₄ and N₂O) in combination contribute to more than 90% of anthropogenic climate warming⁴. N leaching may deplete soil fertility, accelerate soil acidification and reduce crop yields⁵.

Global rice paddies occupied more than 1.61 × 10⁸ ha of land and produced 4.82 × 10⁸ T yr⁻¹ of grain in 2015–2016, creating a major challenge for N leaching and greenhouse gas emission mitigation⁶,⁷. In China, 23% of the nation’s croplands are used for rice production, accounting for approximately 20% of the world’s total⁸. A meta-analysis showed lower N use efficiency of 28.1% during the period 2000–2005 for rice in China⁹, compared with 52% in America and 68% in Europe¹⁰. The average total N leaching rate was 2.2% in paddy fields¹¹. The total amounts of CH₄ and N₂O emissions from China’s rice paddies are estimated to be 7.7–8.0 Tg CH₄ yr⁻¹ and 88.0–98.1 Gg N₂O-N yr⁻¹, respectively¹²,¹³. Judicious methods are needed to reduce GHG emissions and N leaching losses to achieve lower agricultural environmental costs¹⁴, while not impairing the capacity of ecosystems to ensure food security.

Biochar is a solid carbon-rich organic material generated by pyrolysis or gasification of biomass residues in the absence of oxygen at a relatively low temperature. Biochar application to agricultural soils has the potential to

¹Key Laboratory of Regional Sustainable Development Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, 100101, China. ²Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan, 750000, China. ³Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences/Key Laboratory of Agro-Environment and Climate Change, China Ministry of Agriculture, Beijing, 10081, China. ⁴Present address: Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences (CAAS), 12 Zhongguancun South Street, Haidian District, Beijing, 100081, China. Correspondence and requests for materials should be addressed to A.Z. (email: apzhang0601@126.com)
slow carbon and N release via the high content of recalcitrant organic carbon in the biochar and concomitant changes in soil properties, which affect microbial activity. Recent reviews have highlighted biochar as a possible method to decrease soil CH4 and N2O emissions and reduce N leaching. The effects of biochar on paddy soil CH4 emissions remain controversial depending on the biochar type, climatic conditions and soil properties. A laboratory incubation study showed that amendment with bamboo biochar and rice-straw biochar decreased paddy soil CH4 emissions by up to 51% and 91%, respectively, while wheat-straw biochar amendment increased soil CH4 emission by 37%. In addition, no significant difference in soil CH4 flux was found between a biochar plot and a control plot in Germany. Biochar application can affect N transformation and N fate in soil. The soil N2O flux increased significantly in some studies, but substantially decreased or remained unchanged in others. These contrasting results emphasize the need for further studies to assess the role of biochar in mitigating paddy soil CH4 and N2O fluxes. Moreover, the mechanisms of action are not well understood, which has impeded the adoption of biochar in a wide range of rice ecosystems.

Nitrification, through which microorganisms oxidize ammonium (NH4+) to create nitrate (NO3−), releasing N2O as a by product, has long been a concern of scientists in paddy soils. Many studies found lower N leaching after biochar amendment in laboratory and field experiments. However, the underlying mechanisms are still controversial. Recent studies have demonstrated that increased water-holding capacity, enhanced microbial biomass and altered bacterial community structure in soils may contribute to the reduction of N leaching. Other studies suggest that reduced N leaching may result from improved plant N use and decreased N immobilization. NH3 oxidation to NO3−, the first and rate-limiting step of nitrification, is catalyzed by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB). Due to the homology of methane monooxygenase and ammonia monooxygenase enzymes, the same habitats, and the variety of analog substrates, CH4 in the soil can be simultaneously oxidized by both methanotrophs and ammonia oxidizers. It is essential to explore the links between ammonia oxidizers and the soil CH4 and N2O fluxes in the field under biochar amendment.

In the upper reaches of the Yellow River basin of China, preliminary studies revealed that biochar amendment significantly improved N use efficiency and reduced total inorganic N leaching. Consequently, we hypothesized that biochar amendment decreases soil N2O emission and reduces NO3−-N and NH4+-N leaching. We also hypothesized that biochar amendment increases soil CH4 emissions due to the labile carbon input and the positive priming effects of biochar and also increases crop productivity. The aim of the present study was to provide insight into the effects of biochar amendment on paddy soil NO3−-N and NH4+-N leaching and soil CH4 and N2O emissions throughout the entire growth period. By considering the net global warming potential (GWP) of the soil CH4 and N2O fluxes and the NO3−-N and NH4+-N leaching under different treatments, it should be possible to determine the optimal amount of biochar application for China’s rice paddies.

Materials and Methods

Study site. This experiment was conducted at Yesheng Town (106°11′35″ E, 38°07′26″ N) in Wuzhong City, China. The temperate continental monsoon climate dominates the region, with a mean temperature of 9.4 °C and a mean annual precipitation of 192.9 mm. The soil is classified as anthropogenic alluvial soil, with a soil texture of 28.0% clay, 53.76% silt, and 27.99% sand. The top soil (0–20 cm) organic matter is 16.1 g kg−1, the total N is 1.08 g kg−1, and the soil bulk density is 1.33 g cm−3.

Experimental design and rice management. Biochar was applied to the field plots at rates of 0 (C0), 4.5 (C1), 9.0 (C2) and 13.5 t ha−1 yr−1 (C3). Each treatment was performed in triplicate. A total of 12 plots (30 m × 20 m) were established, and each was separated by plastic film to 130 cm in depth, preventing water interchange between adjacent plots. Each plot was irrigated with an equal amount of water (approximately 14500 m3 ha−1 yr−1). The biochar was produced by pyrolysis of wheat straw at 350–550 °C by the Sanli New Energy Company, Henan Province, China. The biochar had C, N, P and K contents of 65.7%, 0.49%, 0.1% and 38.25%, respectively, of which 50% was applied as a base fertilizer before transplanting (26 May, 2014). 30% was applied at the tillering stage (6 June, 2014), and the remaining 20% was applied at the elongation stage (25 June, 2014). Double superphosphate and KCL were also applied as basal fertilizers before transplanting at rates of 90 kg P2O5 ha−1 and 90 kg K2O ha−1, respectively. Biochar and fertilizers were broadcast on the soil surface and incorporated into the soil by plowing to a depth of approximately 13 cm in May 2014. To maintain consistency, plowing was also performed for the plots without biochar. Rice (Oryza sativa L., cv. 96D10) was sown in a nursery bed on 1 May. Rice seedlings were transplanted on 28 May and harvested on 12 October 2014. Crop management was consistent across plots.

Measurement of the soil CH4 and N2O fluxes. The soil CH4 and N2O fluxes were measured using a static opaque chamber and gas chromatograph, as described by Wang et al. Sampling of emitted gases was conducted between 8:00 and 10:00 in the morning. Fluxes were measured twice a week after irrigation and fertilization before the booting stage. Afterwards, the measurement frequency decreased to three times a month during the rice booting, filling and maturity stages and to two times a month during the fallow period. The gas fluxes were measured on 21 occasions during the observation period. The concentrations of CH4 and N2O in the gas samples were simultaneously analyzed within 24 h using a gas chromatograph (Agilent 7890A, USA) equipped with a flame ionization detector (FID) and an electron capture detector (ECD). High-purity N2 and H2 were used as the carrier gas and fuel gas, respectively. The ECD and FID were heated to 350 °C and 200 °C, respectively, and the column oven was kept at 55 °C. The CH4 and N2O fluxes were calculated based on the rate of change in concentration within the chamber, which was estimated as the linear or nonlinear regression slope between concentration and time.
Soil sampling and analysis. Soil samples (0–20 cm) were collected three times: tillering stage (16 June), filling stage (10 August) and harvest stage (12 October). Five soils from two diagonal lines through each plot were collected and pooled into one composite sample. Soils were sieved to 2-mm mesh size in the field and were then transported to the lab in a biological refrigerator. Soil samples were stored at −80°C before analysis. Soil NH$_4^+$-N and NO$_3^-$-N were determined using a continuous-flow auto analyzer (Seal AA3, Germany). Soil bulk density was measured using a 100 cm$^3$ cylinder. The total N (TN) contents in the bulk soil were determined by dry combustion using the Kjeldahl method.

 Soil DNA was extracted from 0.5 g of soil using the Fast DNA® SPIN Kit (Qbiogen Inc., Carlsbad, CA, USA) for soil following the manufacturer's instructions. The extracted DNA was checked on 1% agarose gel, and the DNA concentration was assessed using a Nanodrop® D-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Tenfold diluted DNA was used in the PCR analysis. Primer pairs Arch-amoAF/Arch-amoAR and amoA1F/amoA2R were used for the qPCR of AOA and AOB amoA genes, as described by Wang et al. Product specificity was checked by melt curve analysis at the end of the PCR runs and by visualization via agarose gel electrophoresis. A known copy number of plasmid DNA for AOA or AOB was used to generate a standard curve. For all assays, the PCR efficiency was 90–100% and $r^2$ was 0.95–0.99.

Soil leachate sampling and analysis. Soil water samples used for the leaching calculations were collected from lysimeters, as described by Riley et al. Four PPR (polypropylene random) equilibrium-tension lysimeters (ETLs) (0.19 m$^2$) were installed at the desired depth (20, 60 and 100 cm) below the soil surface for each treatment condition. Soil leachate samples were collected using 100 ml plastic syringes and were transferred to a plastic tube and stored at 4°C before analysis. Samples were taken on days 1, 3, 5, 7, and 9 after transplanting and topdressing; subsequent sampling was conducted at 10-day intervals. Soil leachate samples were collected 14 times during the observation period. The NH$_4^+$-N and NO$_3^-$-N leaching losses were calculated by multiplying the N concentration by the leachate volume.

Rice yield and N uptake. At rice maturity, rice aboveground biomass was estimated by manually harvesting three 0.5 m$^2$ areas. Rice straw and grain were oven-dried to a constant weight at 80°C, weighed, finely ground, sieved, and analyzed for total N using the Kjeldahl method. Total N uptake was calculated from the sum of the N mass in the straw and grain harvested from each plot.

Statistical analyses. GWP is an index of the cumulative radiative forcing between the present and some chosen later time by a unit mass of gas emitted under specific conditions. To compare net GWP after biochar amendment to soil, we calculated the CO$_2$ equivalents for CH$_4$ and N$_2$O for a time horizon of 100 yr (assuming a GWP of 25 for CH$_4$ and 298 for N$_2$O) using the following equation.

$$GWP = 25 \times F_{CH_4} + 298 \times F_{N_2O}$$

Repeated measures of analysis of variance (ANOVA) with the least significant difference (LSD) test were applied to examine the differences in N leaching, soil CH$_4$ and N$_2$O fluxes, and soil microbial amoA gene copy numbers among the different treatments. Biochar amendment was set as a between-subjects factor, and the measurement period was selected as a within-subjects variable. We performed one-way ANOVA with an LSD test to evaluate the effects of biochar amendment on the soil properties, rice yield and N uptake. Linear regression analyses were used to examine the relationships among the soil CH$_4$ and N$_2$O fluxes, the microbial amoA gene copy numbers and the soil inorganic N concentrations. All statistical analyses were conducted using the SPSS software (version 16.0), and differences were considered significant at $P < 0.05$, unless otherwise stated. All figures were drawn using SigmaPlot software (version 10.0).

Results

Soil properties, rice yield and N uptake. In the C0 treatment, the average concentration of soil NO$_3^-$-N was 26.52 mg kg$^{-1}$ during the whole observation period (Table 1). The soil NO$_3^-$-N concentration was reduced significantly by 15.10%, 32.13% and 51.02% in the C1, C2 and C3 treatments (Table 1). However, the soil NH$_4^+$-N concentration was only significantly decreased in the C1 treatment (Table 1). Biochar amendment significantly increased soil TN by 10.01–22.22% and significantly decreased bulk density by 4.51–7.81% compared with the treatment without biochar (Table 1). Biochar amendment tended to decrease soil pH (Table 1, $P > 0.05$).

In the control, the rice yield and grain N uptake were 8357 kg ha$^{-1}$ and 87.2 kg ha$^{-1}$, respectively. Biochar amendment significantly increased the rice yield and grain N uptake compared with the C0 treatment for the C2 and C3 treatments (Fig. 1a,b). Straw N uptake increased with increasing biochar application rate. Moreover, the

| Treatment | NO$_3^-$-N (mg kg$^{-1}$) | NH$_4^+$-N (mg kg$^{-1}$) | TN (g kg$^{-1}$) | Bulk density (g cm$^{-3}$) | Soil pH value |
|-----------|--------------------------|--------------------------|-----------------|--------------------------|---------------|
| C0        | 26.52 ± 3.03a            | 9.81 ± 0.62a             | 1.08 ± 0.01c    | 1.33 ± 0.01a             | 8.62 ± 0.02a  |
| C1        | 19.14 ± 0.23b            | 8.44 ± 0.40b             | 1.15 ± 0.01b    | 1.28 ± 0.02b             | 8.58 ± 0.04a  |
| C2        | 15.30 ± 0.97bc           | 8.72 ± 0.18ab            | 1.20 ± 0.02b    | 1.28 ± 0.02b             | 8.56 ± 0.03a  |
| C3        | 12.99 ± 0.39c            | 9.25 ± 0.32ab            | 1.32 ± 0.02a    | 1.27 ± 0.01b             | 8.56 ± 0.03a  |

Table 1. Soil inorganic N, soil TN, bulk density and soil pH value under the four experimental treatments. Data are mean ± SE. Lowercase letter in the same column represents significant differences among experimental treatments at the level of 0.05.
The relative increase induced by biochar amendment on straw N uptake was 5.62%, 10.06% and 12.87% for the C1, C2 and C3 treatments, respectively (Fig. 1c). In addition, total N uptake was increased by 5.43%, 10.53% and 12.61% in C1, C2 and C3, respectively, compared with C0 (Fig. 1d).

**Soil NO$_3^-$-N and NH$_4^+$-N leaching.** There was a clear seasonal variation in NO$_3^-$-N and NH$_4^+$-N leaching at various soil depths (Fig. 2, Table 2, P < 0.001). Supplementary fertilizer N application during the tillering and elongation stages induced NO$_3^-$-N and NH$_4^+$-N leaching peaks. At greater depth, the NO$_3^-$-N and NH$_4^+$-N leaching peaks were dampened. There were no major changes in the NO$_3^-$-N and NH$_4^+$-N leaching during the final two months (Fig. 2). The average NO$_3^-$-N leaching increased with soil depth, whereas NH$_4^+$-N leaching decreased with soil depth (Table 2). Biochar application significantly reduced the mean NO$_3^-$-N and NH$_4^+$-N leaching (Table 2). C1 had significantly decreased NO$_3^-$-N leaching at a depth of 100 cm and NH$_4^+$-N leaching at depths of 60 cm and 100 cm, while C2 and C3 showed significantly decreased NO$_3^-$-N and NH$_4^+$-N leaching throughout the soil profile (Table 2). Furthermore, significant interactions were found between the observation period and biochar treatment, except for the NO$_3^-$-N leaching at 100 cm (Table 2).

**Soil CH$_4$ and N$_2$O emissions**

The soil CH$_4$ flux significantly varied among growth periods, with the maximum occurring in the booting and filling stages (Fig. 3a, Table 3, P < 0.001). In C0, the soil CH$_4$ flux ranged from −9.60 µg C m$^{-2}$ h$^{-1}$ to 6688.37 µg C m$^{-2}$ h$^{-1}$, with an average of 1264.54 µg C m$^{-2}$ h$^{-1}$ (Fig. 3a). The cumulative annual soil CH$_4$ emission was 110.77 kg C ha$^{-1}$ (Table 3). Biochar amendment significantly increased cumulative soil CH$_4$ emissions (Table 3, P = 0.008). C2 and C3 showed significantly increased cumulative soil CH$_4$ emissions (by 35.16% and 40.62%, respectively) compared with C0 (Table 3). Furthermore, there was a significant interaction for soil CH$_4$ flux between observation period and biochar treatment (Table 3, P < 0.001).

Soil N$_2$O flux showed an obvious variation among growth stages (Fig. 3b, Table 3, P < 0.001). The maximum soil N$_2$O emission occurred during the rice tillering stage (Fig. 3b). Soil N$_2$O flux fluctuated from 2.55 µg N m$^{-2}$ h$^{-1}$ to 72.70 µg N m$^{-2}$ h$^{-1}$ in C0, with an average of 63.88 µg N m$^{-2}$ h$^{-1}$ (Fig. 3b). This rate translated into a cumulative annual soil N$_2$O emission of 1.87 kg N ha$^{-1}$ (Table 3). Biochar amendment significantly reduced soil N$_2$O emissions (Table 3, P = 0.039), and the interaction between observation period and treatment was also significant (Table 3, P < 0.001). C2 and C3 showed significantly decreased annual cumulative N$_2$O emissions (by 25.13% and 28.88%, respectively, relative to C0) (Table 3). However, the C1 treatment decreased soil N$_2$O emissions (Table 3).

Biochar amendment consistently increased the net GWP, and C2 and C3 showed the largest increases (29.01% and 25.13%, respectively) (Table 3). However, the increase in net GWP elicited by the C1 treatment was only 3.24% (Table 3).

**Abundance of soil AOA and AOB communities.** Soil AOA amoA copy numbers exhibited a slight seasonal variation (Fig. 4a, Table 3, P = 0.074), while soil AOB amoA copy numbers showed a clear pattern of seasonal changes (Fig. 4b, Table 3, P = 0.011). Biochar amendment significantly increased soil AOA amoA.

---

**Figure 1.** Rice yield (a), grain N uptake (b), straw N uptake (c) and total N uptake (d) under the four experimental treatments. Data are shown as means with standard errors. Different letters in the same subfigure indicate significant differences of different treatment according to the LSD test (P < 0.05).
copy numbers (Table 3, \( P < 0.001 \)). The highest AOA \( amoA \) gene copy numbers were observed in the C3 treatment (12.32% greater than that of C0) (Table 3). Conversely, there was no significant difference in soil AOB \( amoA \) copy numbers among the four treatments (Table 3, \( P = 0.349 \)).

**Relationships between soil fluxes and ammonia-oxidizer abundances and soil inorganic N concentrations.** The soil \( CH_4 \) fluxes were negatively linearly correlated with the soil \( NO_3^-\) -N concentration (Table 4). The soil \( N_2O \) fluxes were negatively correlated with the soil AOA abundance and positively correlated with the soil \( NO_3^-\) -N concentration (Table 4). The soil AOA abundance was negatively correlated with the soil

![Figure 2](https://www.nature.com/scientificreports/) Variation of soil \( NO_3^-\) -N (a–c) and \( NH_4^+\) -N (d–f) leaching under the four experimental treatments. Data are shown as means with standard errors.

| Treatments | \( NO_3^-\) -N (mg L\(^{-1}\)) | \( NH_4^+\) -N (mg L\(^{-1}\)) |
|------------|-----------------|-----------------|
|            | 20 cm | 60 cm | 100 cm | 20 cm | 60 cm | 100 cm |
| C0         | 12.49 ± 0.45a | 14.87 ± 0.61a | 18.60 ± 0.12a | 11.84 ± 0.46a | 6.29 ± 0.13a | 4.40 ± 0.20a |
| C1         | 11.56 ± 0.06a | 15.73 ± 0.19a | 15.21 ± 0.29b | 12.10 ± 0.38a | 4.76 ± 0.24b | 3.15 ± 0.03b |
| C2         | 7.26 ± 0.48b  | 13.01 ± 0.82b | 15.16 ± 1.14b | 10.12 ± 0.27b | 3.89 ± 0.20c | 2.55 ± 0.11c |
| C3         | 6.87 ± 0.33b  | 11.25 ± 0.56b | 13.76 ± 0.17b | 9.77 ± 0.46b  | 3.54 ± 0.28c | 2.11 ± 0.19c |

ANOVA results
- Period: \(<0.001\) \(<0.001\) \(<0.001\) \(<0.001\) \(<0.001\) \(<0.001\)
- Treatment: \(<0.001\) 0.003 0.003 0.007 \(<0.001\) \(<0.001\)
- Period \(\times\) Treatment: \(<0.001\) 0.843 0.001 0.001 0.001 0.001

Table 2. Soil \( NO_3^-\) -N and \( NH_4^+\) -N leaching at various soil depths affected by different treatments over the entire experimental period. Data are mean ± SE. Lowercase letter in the same column represents significant differences among experimental treatments at the level of 0.05.
NO₃⁻-N concentration, whereas the soil AOB abundance was not correlated with any of the measured soil fluxes or inorganic N concentrations (Table 4).
to CH4 because methanotrophs can utilize a variety of substrates50. Therefore, biochar amendment reduced the

correlations, n = 12. (+), positive relationship; (−), negative relationship.

Table 4. Correlation coefficients (R2) for the relationships among soil CH4 and N2O fluxes, soil ammonia-

|                  | CH4  | N2O  | AOA | AOB |
|------------------|------|------|-----|-----|
| AOA              | 0.24 | 0.45 |     |     |
| AOB              | 0.04 | 0.06 |     |     |
| NO3−-N           | 0.45 | 0.65 |     |     |
| NH4+-N           | 0.04 | 0.18 |     |     |

Figure 5. Potential mechanisms of paddy soil N leaching and total GWP in response to biochar amendment.

Discussion

Effects of biochar amendment on soil NO3−-N and NH4+-N leaching. In our study, significant

toxicizers, soil NO3−-N and NH4+-N concentrations. Note: Significance: *P < 0.05; **P < 0.01. For all

correlations, n = 12. (+), positive relationship; (−), negative relationship.

Effects of biochar amendment on the soil CH4 and N2O fluxes. The net CH4 flux from soil is the sum

of production and oxidation. The effects of biochar amendment on the CH4 flux were thus unclear. In agreement

with previous studies24, 41, the application of biochar at rates of 9 t ha−1 yr−1 and 13.5 t ha−1 yr−1 significantly

increased the soil CH4 flux by 35.16% and 40.62%, respectively (Table 3). The promotion of the soil CH4 flux was

comparable to that of the Tai Lake plain in China for biochar amendment at rates of 10 t ha−1 and 40 t kg ha−123.

This was attributed to the following three aspects. First, the soil NH4+-N accumulation decreased soil CH4 oxidation

by altering the activity and composition of the methanotrophic community48. However, biochar amendment

did not significantly change the soil NH4+-N accumulation (Table 1), and there was no significant relationship

between the soil CH4 flux and soil NH4+-N concentration (Table 4). Wang et al.49 found that soil NO3−-N accumu-

lation could significantly promote soil CH4 uptake. The lower soil CH4 uptake was due to the decreased soil

NO3−-N concentration under biochar amendment (Table 1), which increased the soil CH4 emissions in our study

(Table 3, Fig. 5). The significant negative relationship between the soil NO3−-N concentration and the soil CH4

flux reflected this prediction (Table 4). Second, methanotrophs use the sorbed organic compounds in addition

to CH4 because methanotrophs can utilize a variety of substrates50. Therefore, biochar amendment reduced the

net soil CH4 oxidation51. Third, Knoblauch et al.52 reported that the labile components of biochar increase the

substrate supply and create a favorable environment for methanogens53. The lower pH in our biochar plots may

have promoted methanogenic archaea, which have an optimal pH of 754. Thus, a larger archaeal population may

be the main cause of the reduction in NO3−-N and NH4+-N leaching (Fig. 5). In addition, the increased soil

water holding capacity due to the reduced soil bulk density (Table 1) may have also reduced NO3−-N and NH4+-N

leaching48.

Suppression of soil N2O emissions following biochar amendment has been observed both under laboratory

conditions25, 26 and in the field23, 47. Enhanced soil aeration2, altered ammonia-oxidizer and denitrifier activ-

ity28, sorption of NH4+-N or NO3−-N by biochar29 and the presence of inhibitory compounds such as ethylene30

have been suggested as mechanisms to explain the reduction in N2O flux with biochar amendment. In anaerobic
paddy soil, N₂O production from denitrification is thought to be the dominant source. Baggs et al. suggested that decreased total N denitrification and enhanced reduction of N₂O to N₂ can lead to lower N₂O denitrification in soil. A reduction in NO₃⁻-N availability would decrease the total denitrified N and would reduce the ratio of NO₃⁻/(NO₃⁻+N₂O) [17]. In our study, biochar amendment reduced the soil NO₃⁻-N concentration by accelerating ammonia oxidation (Fig. 4, Table 3) and promoting total N uptake in rice (Fig. 1). The significant negative correlation between the soil NO₃⁻-N concentration and soil AOA abundance provided direct evidence for this result (Table 4). Soil NO₃⁻-N availability was positively correlated with soil N₂O flux (Table 4), which could partially explain the reduction in soil N₂O flux in the studied paddy soil (Fig. 5). In addition, evidence for the decreased N₂O denitrification was provided by the decreased soil bulk density after biochar amendment (Table 1). Soil pH was not significantly changed after biochar amendment at our study site (Table 1). However, soil Eh was not monitored when the gas samples were taken in situ. Mechanisms associated with the oxidation and reduction of nitrogen species need to be studied under the alternating redox conditions on the surface of biochar.

However, our flux results were drawn from relatively few measurements. The gas fluxes were measured on 19 occasions during 150 days of rice growth, with an average sampling interval of 8 days. This frequency was slightly lower than the average 6.5 day interval of the reviewed rice paddy studies in China with single biochar amendment and biochar combined with NPK compound fertilizer (Supplementary Table S1). The effects of biochar amendment on the soil CH₄ flux are contradictory, including positive, negative, and neutral effects. Moreover, biochar amendment is mainly reported to decrease soil N₂O flux. Increased soil CH₄ flux and decreased soil N₂O flux were observed in our study. This evidence suggests that our conclusions can be drawn from relatively few measurements.

Biochar is considered to be an effective tool to mitigate GWP via carbon sequestration in soil and to influence carbon mineralization through priming effects. In the present study, the changes in net GWP were not significant between C1 and C0 treatments, while biochar amendment significantly increased soil CH₄ emissions and decreased N₂O emissions in the C2 and C3 treatments, leading to a significantly increased net GWP (Table 3). This result is in line with that of a rice paddy from Tai Lake plain, China [21]. Our results indicate that higher amounts of biochar amendment are associated with a risk of increased paddy net GWP in northwest China (Fig. 5). The stimulation effects of biochar on native soil organic carbon mineralization decreases with time due to the depletion of labile SOC from the initial positive priming and stabilization of native SOC via biochar-induced organ-mineral interactions during 5-year laboratory incubations [59]. In another 120-day incubation study, the time effects showed an initial increase followed by a decrease and stabilization, resulting in a significantly increased sequestration of carbon in the soil over the long term compared with conventional biowaste amendments [60]. In our study, improved yield and reduced soil nitrous oxide emissions contributed to the mitigation potential of biochar amendment. However, the response of native soil organic carbon mineralization to biochar amendment remains uncertain. Relative priming effects and cumulative CO₂ emissions studies are needed to evaluate the GWP of biochar amendment in our paddy soil.

Conclusions

The effects of biochar amendment on soil N leaching and soil CH₄ and N₂O fluxes were investigated in paddy soil in northwest China. We found that biochar amendment significantly decreased soil NO₃⁻-N and NH₄⁺-N leaching, but that the C2 and C3 treatments significantly increased soil CH₄ emissions and reduced N₂O emissions, leading to significantly increased net GWP. Biochar amendment significantly increased soil AOA abundance and rice N uptake. Soil NO₃⁻-N availability can explain the responses of soil N leaching and soil CH₄ and N₂O fluxes to biochar amendment. Our results indicated a commended dose of biochar amendment of 4.5 t ha⁻¹ yr⁻¹ with conventional N application in the study area. The responses of the soil CH₄ and N₂O fluxes to biochar amendment were also influenced by the interannual variability in climate, temperature and precipitation. The long-term effects of biochar amendment on N leaching and net GWP in rice production required further investigation to identify the most cost-effective and environmentally friendly management practices for rice culture.

References

1. Fowler, D. et al. The global nitrogen cycle in the twenty-first century. Philos T R Soc B 368, doi:10.1098/rstb.2013.0164 (2013).
2. Tilman, D. Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. Proceedings of the National Academy of Sciences 96, 5985–6000, doi:10.1073/pnas.96.11.5985 (1999).
3. Vitousek, P. M. et al. Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7, 737–750 (1997).
4. IPCC. Climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge, United Kingdom and New York: Cambridge University Press (2013).
5. Laird, D., Fleming, P., Wang, B. Q., Horton, R. & Karlen, D. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158, 436–442, doi:10.1016/j.geoderma.2010.05.012 (2010).
6. Ju, X. T. et al. Reducing environmental risk by improving N management in intensive Chinese agricultural systems. P Natl Acad Sci USA 106, doi:10.1073/pnas.0902655106 (2009).
7. Chauhan, B. S., Jabran, K. & Mahajan, G. Rice production worldwide. Cham, Switzerland: Springer International Publishing (2017).
8. Frolking, S. et al. Combining remote sensing and ground census data to develop new maps of the distribution of rice agriculture in China. Global Biogeochem Cy 16 (2002).
9. Zhang, F. S. et al. Nutrient use efficiencies of major cereal crops in China and measures for improvement. Acta Pedologica Sinica 45, 915–924 (2008).
10. Ladha, J. K., Pathak, H., Krupnik, T. I., Six, J. & van Kessel, C. Efficiency of fertilizer nitrogen in cereal production: Retrospects and prospects. Advances in Agronomy 87, 85–156, doi:10.1016/S0065-2113(05)78003-8 (2005).
11. Hu, Y. T., Liao, Q. J. H., Wang, J. W. & Yan, X. Y. Statistical analysis and estimation of N leaching from agricultural fields in China. Soils 43, 19–25 (2011).
12. Yan, X. Y., Cai, Z. C., Ohara, T. & Akimoto, H. Methane emission from rice fields in mainland China: amount and seasonal and spatial distribution. J Geophys Res Atmos 108, doi:10.1029/2002jd003182 (2003).
Acknowledgements
This project was supported by the National Water Pollution and Treatment Science and Technology Major Project (No. 201ZX07201-009), Project of the National Natural Science Foundation of China (No. 41130748, 31660957, 41471143), Special Foundation for Basic Scientific Research of Central Public Welfare Institute (No. BSRF201306), and Priority Funds for Ningxia Scientific and Technological Innovation (No. NKYJ-15-05).

Author Contributions
Y.W. and A.Z. conceived the study. R.L., S.Y., Y.Z. and H.L. sampled and analyzed the samples. Y.W. analyzed the data and drafted the manuscript. Y.L., S.Y., and Z.Y. contributed to discussing the results and editing the manuscript. All authors reviewed the manuscript.

Additional Information
Supplementary information accompanies this paper at doi:10.1038/s41598-017-01173-w

Competing Interests: The authors declare that they have no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2017