RESEARCH ARTICLE

Effects of Biochar Addition on CO₂ and N₂O Emissions following Fertilizer Application to a Cultivated Grassland Soil

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Abstract

Carbon (C) sequestration potential of biochar should be considered together with emission of greenhouse gases when applied to soils. In this study, we investigated CO₂ and N₂O emissions following the application of rice husk biochars to cultivated grassland soils and related gas emissions to soil C and nitrogen (N) dynamics. Treatments included biochar addition (CHAR, NO CHAR) and amendment (COMPOST, UREA, NO FERT). The biochar application rate was 0.3% by weight. The temporal pattern of CO₂ emissions differed according to biochar addition and amendments. CO₂ emissions from the COMPOST soils were significantly higher than those from the UREA and NO FERT soils and less CO₂ emission was observed when biochar and compost were applied together during the summer. Overall N₂O emission was significantly influenced by the interaction between biochar and amendments. In UREA soil, biochar addition increased N₂O emission by 49% compared to the control, while in the COMPOST and NO FERT soils, biochar did not have an effect on N₂O emission. Two possible mechanisms were proposed to explain the higher N₂O emissions upon biochar addition to UREA soil than other soils. Labile C in the biochar may have stimulated microbial N mineralization in the C-limited soil used in our study, resulting in an increase in N₂O emission. Biochar may also have provided the soil with the ability to retain mineral N, leading to increased N₂O emission. The overall results imply that biochar addition can increase C sequestration when applied together with compost, and might stimulate N₂O emission when applied to soil amended with urea.

Introduction

Biochar application to agricultural soils is a promising management practice that has the potential to mitigate climate change and increase soil quality [1–5]. However, it is not yet widely used in agricultural fields as a common practice, because the effects of biochar appear to be dependent on the characteristics of the soil and the biochar [6–9].

If biochar is a completely inert material that does not interact with soil components, only the C sequestration potential of biochar needs to be considered, and changes in the physical,
chemical, and biological properties of the soil upon biochar addition do not need to be considered. However, biochar is not completely inert, and some portions of biochar, especially the surface, contain significant amounts of bioavailable nutrients [10–12]. Therefore, the addition of biochar to soils can affect the physical, chemical, and biological aspects of the soil, thereby influencing C and N cycles in the soil [10–14].

Changes in soil C and N dynamics will result in changes in CO₂ and N₂O emissions. Soil CO₂ emissions have been reported to increase [15–17], decrease [6, 9, 18], and remain unchanged [19] by biochar amendment. These widely varying observations can largely be explained by different amounts of volatile organic matter content in the biochar, which generally increases with decreasing pyrolysis temperature. Volatile matter content is widely used as an indicator of the amount of labile C in biochar [20, 21]. Changes in CO₂ emission are also related to the application rate of biochar. Cumulative CO₂ production was reported to be significantly higher than the control at 1% and 2% biochar application rates [22,23], while there was no change in CO₂ emission from soil with 5% and 10% biochar application rates. Effect of biochar also depended on the condition of the soil to which it was applied; addition of biochar to soil with a high C content did not result in any additional change in CO₂ emission [15].

Effects of biochar addition on N₂O emission are even more inconsistent, because the process of N₂O emission is very complicated, involving denitrification, autotrophic nitrification, and heterotrophic nitrification, among other processes [24,25]. N₂O emissions are widely known to be influenced by soil water status, available C content, oxygen content, pH, N availability, and so on [26–28]. Reduced N₂O evolution was reported from rice paddy soil amended with biochar when the soil was relatively wet, while the opposite trend was observed when the soil was drier [29]. A reduction in N₂O emission by biochar amendment was also reported by [30]. These observations have been explained by enhanced soil aeration [31], increased pH [27], and microbial immobilization of soil NO₃⁻ by biochar addition [22]. Biochar has also been reported to have opposite effects on N₂O emission. Higher N₂O emission was observed from rice paddy soil amended with biochar made from swine manure by [32–34]; the changes in N₂O emission were attributed to the size of the available inorganic N pool [32–34].

In this study, we monitored changes in CO₂ and N₂O emissions from cultivated grassland soil in response to biochar amendment. Although the area of cultivated grassland in South Korea is not large, the government plans to extend this area because of increased demand for domestic forage. Grassland in Korea is also important because soil C storage in this ecosystem ranges from 3847 gC m⁻² to 9568 gC m⁻², which is approximately twice as large as that in Korean rice paddy systems [32]. Conventional management of grassland soil includes application of compost and/or N fertilizer. Compost application generally increases soil CO₂ evolution, while the application of N fertilizer is closely related to an increase in N₂O emission [35–37]. We investigated changes in CO₂ and N₂O evolution from soil treated both with biochar and other amendments. Two different types of amendments (compost and urea) were chosen because these are commonly used in current grassland management in Korea. To the best of our knowledge, this is the first field site experiment in South Korea to investigate the effects of biochar on the soil ecosystem. We related changes in CO₂ and N₂O emissions to soil C and N dynamics and evaluated whether application of biochar is a sustainable management practice to simultaneously control greenhouse gas emissions and soil N availability.

Materials and Methods
Experimental site and basic physicochemical properties of biochar and soil
The field experiment was established on cultivated grassland located in Chunan-Si, Chungcheongnam-Do, Korea (East longitude 127°, North latitude 36°), where the average annual
temperature and precipitation are 12.5°C and 1226.5 mm, respectively (Korea Meteorological Administration). The field site was set up on Oct. 25, 2011 and the permission for the location was issued and managed by National Institute for Animal Science (NIAS), Korea. The mix-seeding rates were tall fescue (*Festuca arundinacea*) 8 kg ha⁻¹, orchard grass (*Dactylis glomerata*) 15 kg ha⁻¹, perennial ryegrass (*Lolium perenne*) 5 kg ha⁻¹, and white clover (*Trifolium repens*) 2 kg ha⁻¹.

The biochar used was a commercial product sold by the Farmers’ Association in Gangjin-gun, Korea. Biochar was produced by pyrolyzing rice husks at 500–600°C on a small-scale pyrolysis reactor (DCH-400, 1.4 m×5.2 m×5 m (L×W×H) from Daewon GSI Co., Korea). The amount of rice husk biochar processed was 400 kg h⁻¹. The residence time was 110–120 min and the overall yield was 43%. The particle size of rice husks was 5mm or less before pyrolysis.

Biochar and soil pH were determined at a 1:5 ratio of air-dried biochar or soil to deionized water (w/v). Biochar surface area was measured by the BET method with N₂ gas using mill-ground biochar. Soil texture was determined by a hydrometer and the cation exchange capacity (CEC) of soil was measured using an unbuffered salt extraction method [38]. Total carbon (TC) content in the soil and biochar were analyzed by combustion analysis using a Carlo Erba NS 1500 C/N analyzer (Carlo Erba, Milan, Italy). NH₄⁺-N and NO₃⁻-N concentrations in the biochar and soil were determined through 2M potassium chloride (KCl) extraction and colorimetric methods [39]. Metal content was analyzed by a spectroscopic method using ICP MS (Perkin-Elmer ICP-OES OPTIMA5300DV) to check whether the biochar contained metals that might be toxic to the soil ecosystem. The physicochemical properties of the biochar and soil are shown in Table 1.

**Experimental design and treatments**

Treatments included biochar addition (NO CHAR, CHAR) and amendment (COMPOST, UREA, NO FERT). We used factorial design for six treatment combinations: NO CHAR/COMPOST, NO CHAR/UREA, NO CHAR/NO FERT, CHAR/COMPOST, CHAR/UREA, and CHAR/NO FERT. As we performed three replicates of each type, eighteen plots were completely randomized to the 22 m×37 m experimental field. The size of each plot was 5m×7m and individual plots were separated by protection rows that were 1m in width.

Biochar was applied once a year in fall after harvest (Oct. 25, 2011 and Sep. 21, 2012). The form of biochar applied was the particle of < 3 mm. The application rate of biochar was 0.3% by weight, which is equivalent to 3.3 ton/ha when calculated based on a 10-cm depth field application. Biochar was incorporated into the soil profile down to a depth of 10 cm using shovels. The non-biochar amended plots were also mixed with a shovel. Soil amendment was conducted twice per year in fall and spring (Oct 25, 2011, March 21, 2012, Sep 21, 2012, March 21, 2013). Amendment rates were 140 kg ha⁻¹ yr⁻¹ of UREA and 1500 kg ha⁻¹ yr⁻¹ of COMPOST, which are equivalent to 51.8 kg N ha⁻¹ yr⁻¹ and 11.3 kg N ha⁻¹ yr⁻¹, respectively.

**Gas sampling and analysis**

Gas samples were taken every month from 10/31/2011 to 05/01/2013 except over winter using a chamber method [29]. Two chambers (20-cm diameter, 25-cm height) were inserted 5 cm deep into the soil for each plot. On sampling dates, the chambers were closed with airtight lids for 40 minutes and gas samples were withdrawn from the headspace of the closed chamber using a 10-ml three-way syringe (BD Luer-LokTip).

Gas samples were analyzed using a gas chromatograph (Agilent 7890A, USA) equipped with two detectors. CO₂ was detected using a thermal conductivity detector (TCD) and N₂O was detected using an electron capture detector (ECD).
Table 1. Physicochemical properties of the soil and biochar.

| Texture       | pH   | CEC   | Bulk density | Total C | Total N | NH$_4^+$ | NO$_3^-$ |
|---------------|------|-------|--------------|---------|---------|----------|----------|
| Sandy loam    | 7.20 | 1.57  | 1.27         | 4.23    | 0.58    | 10.22    | 30.13    |

Biochar

| pH    | Surface Area (BET) | Total C | Total N | HWC$^*$ | NH$_4^+$ | NO$_3^-$ | Al | Ca | Fe | Mg | K | Na | P |
|-------|---------------------|---------|---------|---------|----------|----------|-----|----|----|----|----|----|----|
| 10.30 | 27.76               | 429.00  | 11.00   | 2.94    | 1.49     | 22.25    | 0.52| 2.07 | 0.60 | 0.65 | 15.95 | 0.33 | 1.52 |

$^*$HWC stands for hot water extractable C

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Gas fluxes were calculated from the changes in headspace concentration over the measured period using the following equation [34]:

\[
\text{Flux} = \frac{d\text{Gas}}{dt} \times \frac{V \times P \times 100 \times \text{MW} \times 273}{A \times R \times 273 + T} \tag{1}
\]

where, \(d\text{Gas}/dt\) is the difference in gas concentrations between the initial and end time points, \(V\) is the volume of the chamber, \(A\) is the surface area which the chamber covers, \(P\) is the atmospheric pressure, \(\text{MW}\) is the molecular weight of the gas, \(R\) is a gas constant, 8314 J mol\(^{-1}\) K\(^{-1}\), and \(T\) is the absolute temperature.

**Soil sampling and analysis**

On the same dates as gas sampling, soil samples were collected from each plot from a depth of 0–15 cm using a soil core sampler (4.9-cm i.d., Forest supplier, USA). Samples were sealed in marked plastic bags and taken to the laboratory after sampling.

For soil temperature measurements, a mini thermometer probe (Testo, 905-T1) was inserted to a depth of 10 cm. To measure soil gravimetric water content, approximately 15 g of soil was taken from each plastic bag and dried in the oven at 105°C for 24 h. Soil bulk density was determined by taking a soil core (4.9-cm diameter, 15-cm depth) and drying the soil at 105°C for 24 h to determine the soil dry weight contained in the known volume. Soil bulk density and gravimetric water content were used to determine the water-filled pore space (WFPS) of each soil core [39].

Temporal soil samples were passed through a 2-mm sieve and air-dried for 2 weeks before analysis. Labile organic C content of biochar was measured by the amount of hot water extractable C (HWC) following the method described in [40]. To investigate N mineralization in soil, we measured NH\(_4^+\)-N and NO\(_3^-\)-N. Soil NH\(_4^+\)-N and NO\(_3^-\)-N concentrations were determined through 2M potassium chloride (KCl) extraction and colorimetric methods [41]. Soil microbial activity was evaluated with the fluorescein diacetate (FDA) hydrolysis method [42]. We analyzed the changes in soil pH, CEC, microbial biomass C and water holding capacity (WHC) for the last soil samples which was taken on 05/22/2013. Microbial analysis was measured by the CHCl\(_3\) fumigation extraction method [43, 44] and the WHC in the soil was determined using modification of the method described in [45].

To further examine the effect of biochar on soil N dynamics in the UREA soil, we set up a postulated balance of added urea N. The balance started with the amount of urea N applied which was 5180 mgN m\(^{-2}\). We assumed that 40% of applied urea N would be recovered in the plant biomass and soil organic N pool and this amount was not affected by biochar amendment [46]. We also assumed that 20% of applied urea were lost via NH\(_3\) volatilization process [47] and the amount of volatilization loss was not affected by biochar amendment. The remaining of the urea N was divided N\(_2\)O emission, soil mineral N content (NH\(_4^+\) + NO\(_3^-\)), and N loss from leaching. We calculated the amount of N leaching by subtracting measured N\(_2\)O emission and soil mineral N content from the remaining amount of the urea N.

\[
\text{Total amount of urea N} = \text{plant biomass N} + \text{soil organic N} + \text{NH}_4\text{, volatilization} + \text{N}_2\text{O emission} + \text{soil mineral N} (\text{NH}_4^+ + \text{NO}_3^-) + \text{N leaching} \tag{2}
\]

**Statistical analyses**

Analysis of variance (ANOVA) was performed using the MIXED procedure of SAS 9.2 [48] on CO\(_2\) emissions, N\(_2\)O emissions, TC contents, bulk density, water filled pore space, microbial activity (FDA activity), HWC content, soil NH\(_4^+\) and NO\(_3^-\) contents. Biochar treatment, urea and compost amendment, and date were fixed effects. The ANOVA was performed separately.
on the soil pH, CEC, microbial biomass C, and WHC for the final soil sample and for this ANOVA, date was not considered as a fixed effect because the measurement was conducted only once. Least square means of the parameters were used to compare date, fertilization, and biochar effects. Pearson’s correlation coefficients among the CO₂ emissions, N₂O emissions, soil temperature, soil water content, and HWC content were calculated using the CORR procedure of SAS [48]. Statistical significance was set to the 5% probability level for all analyses.

Results and Discussion
Carbon dioxide emission and soil C dynamics

The overall CO₂ emission pattern was positively correlated with soil temperature (Table 2, $r = 0.610^{***}$), which has been reported by many researchers [22, 49, 50]. In contrast, the CO₂ pattern was not correlated with soil water content, most likely because the gravimetric soil water content on our sampling dates was very low (range, 3.5–25.5%) (Fig 1A).

An average throughout the 19 months of the field experiment revealed that overall CO₂ emission from the COMPOST soil was significantly greater than that from the UREA and NO FERT soils (Table 3, Fig 1C). Greater CO₂ emission from compost-treated soil than untreated soil has been reported previously by several researchers [51, 52]. Soluble C measured by hot water extractable C (HWC) from compost was hypothesized to stimulate the microbial community, resulting in higher CO₂ evolution, and extractable organic C showed a high correlation with CO₂ evolution (Table 3, $r = 0.586^{***}$). However, unlike the consistent trend in HWC with CO₂ emission, the patterns in FDA activity and MBC were not consistent with that of CO₂ emission (Fig 2) and the correlations of them with CO₂ emission rate were not significant. We attributed this inconsistency to the lower sensitivity of FDA activity and MBC to the treatment. It was widely reported that FDA activity and MBC are highly correlated [53] and they both represent “potential” microbial activity because FDA activity is measured after sufficient substrate is added and MBC includes dead biomass of microbes. Hence, we would say that “actual” microbial activity in the COMPOST soil might have been stimulated but it was not detected by these parameters.

The temporal pattern of CO₂ emissions differed according to soil amendment type and biochar addition (Table 3I). Addition of biochar did not significantly affect CO₂ emissions from any of the amended or control soils at the start of the experiment until 06/01/2012 (Table 3, Fig 1C). During Jul 2012, when the soil temperature and CO₂ emission level were both very high, biochar addition had a negative effect on CO₂ flux in the COMPOST soil (Fig 1B). From Aug 2012 to May 2013, we again did not observe any effects of biochar addition on CO₂ emission from any of the soils. The reason why the biochar treatment only had an effect on CO₂

| Table 2. Pearson correlation coefficients among CO₂ emission rate, N₂O emission rate, soil temperature, gravimetric water content, and hot water extractable C. |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| CO₂ emission rate                               | N₂O emission rate                               | Soil Temperature                                | Soil water content                              | Hot water extractable C                          |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| CO₂ emission rate                               | 1                                               | 0.247* (0.0379)                                 | 0.610*** (<0.001)                               | 0.586*** (<0.0001)                              |
| N₂O emission rate                               | 0.216* (0.077)                                  | 1                                               | -0.004 (0.975)                                 | 0.069 (0.568)                                   |
| Soil Temperature                                | 0.610*** (<0.001)                               | -0.004 (0.975)                                 | 1                                               | -0.060 (0.614)                                 |
| Soil water content                              |                                                | -0.004 (0.975)                                 | -0.060 (0.614)                                 | 0.626*** (<0.0001)                              |
| Hot water extractable C                         |                                                |                                                |                                                | 1                                               |

Numbers in parentheses are the probability to reject the null hypothesis.

*, **, *** significant at the P = 0.1, P = 0.05, P = 0.001 probability levels, respectively.

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emission when the temperature was high may in part be due to temporal changes in HWC (Table 4). The HWC content on 07/22/2012 was the highest measured, and on the same day, it was higher in the CHAR soils than in the NO CHAR soils. This result implies that labile C contained in the COMPOST soil was respired more freely in the NO CHAR soil than the CHAR soil. The suppression of CO2 emission by biochar was also reported in [19]; these authors attributed this to the high adsorptive affinity of biochar for existing organic C. We also argue that the higher content of labile C in the COMPOST soil protected from mineralization by biochar, resulting in a low CO2 evolution rate in this treatment.

The pattern of soil CO2 emission was consistent with that of total C content (Fig 3). On average, soils with char addition had a 139.28% soil C content in the 19 months after biochar was first applied. In soils with biochar addition, total C was slightly higher in Nov 2012 than May 2013. The reason is that soil samples collected in Nov 2012 contained a lot of newly
Table 3. Results of analysis of variance by date, biochar and amendments.

| Source | Soil analysis | Gas analysis |
|--------|--------------|--------------|
|        | TC | BD | WFPS | FDA | NH₄⁺ | NO₃⁻ | HWC | CO₂ | N₂O |
| Date   | <.0001 *** | <.0001 *** | <.0001 *** | <.0001 *** | <.0001 *** | <.0001 *** | <.0001 *** | <.0001 *** | <.0001 *** |
| CHAR   | <.0001 *** | 0.0421 ** | 0.0426 * | 0.0506 * | 0.0004 *** | 0.4635 | 0.0016 *** | 0.4517 | 0.0214 * |
| Date*CHAR | 0.1771 | 0.3206 | 0.0001 *** | 0.0495 ** | <.0001 *** | 0.9901 | 0.0310 ** | 0.961 | 0.9008 |
| AMEND* | 0.0383 ** | 0.0061 *** | 0.0008 *** | 0.1733 | 0.0049 *** | 0.0021 *** | 0.0026 *** | <.0001 *** | <.0001 *** |
| Date*AMEND | 0.6207 | 0.864 | 0.0065 *** | 0.8512 | 0.1946 | 0.0165 ** | 0.0143 ** | <.0001 *** | <.0001 *** |
| CHAR*AMEND | 0.0996 * | 0.8321 | 0.9562 | 0.957 | 0.0006 *** | 0.9644 | 0.8550 | 0.5731 | 0.0332 ** |
| Date*CHAR*AMEND | 0.7042 | 0.598 | 0.0473 ** | 0.2287 | 0.0079 *** | 0.9692 | 0.2813 | 0.0001 *** | 0.0001 *** |

| Source | Soil analysis | WHC | pH | CEC | MBC |
|--------|--------------|-----|----|-----|-----|
| CHAR   | 0.0499 ** | 0.8788 | 0.5902 | 0.0006 *** |
| AMEND  | 0.2696 | 0.4064 | 0.8467 | 0.0023 *** |
| CHAR*AMEND | 0.0906 | 0.8010 | 0.5358 | <.0001 *** |

* *** significant at the P = 0.1, P = 0.05, P = 0.001 probability levels, respectively.
* AMEND means application of the compost and urea; CHAR means biochar addition treatment; BD, soil bulk density; WFPS, soil water filled pore spaces; FDA, fluorescein diacetate hydrolysis activity; HWC, hot water extractable carbon; WHC, water holding capacity; CEC, cation exchange capacity; MBC, microbial biomass carbon.
incorporated biochar particles, because we applied biochar in Sep 2012 for the second time, therefore there was not enough time for the biochar to become completely mixed with the soil. In May 2013, the soil C content was the highest in the COMPOST soil with biochar, and this result is consistent with low CO₂ emission from the COMPOST soil amended with biochar.

Fig 2. Interactive effect between biochar and amendments on a) microbial activity and b) microbial biomass C in the soil. Bars with different letters indicate significant differences in the average values of sampling dates among treatments at a 5% probably level.

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during the hot summer days. Overall, the data imply that when biochar is applied together with compost, higher C sequestration can be expected, probably because decomposition of compost C is lowered by adsorption of labile C by biochar.

Nitrous oxide emissions and soil available N dynamics

The overall pattern of N₂O emission showed a correlation with soil temperature (Table 2, \( r = 0.216^* \)), but no correlation with soil water content. Soil water status has been reported to have a strong effect on N₂O emission by several research groups [23, 39, 54, 55]. However, because the soil water content on our sampling dates was very low (less than 25% gravimetric water content) (Fig 1A), we did not observe a significant correlation between soil water content and N₂O emission.

![Graph showing temporal change in total C contents influenced by biochar and amendments. Bars with different letters indicate significant differences among treatments at a 5% probability level.](doi:10.1371/journal.pone.0126841.g003)

**Table 4. Seasonal change in the soil hot water extractable C (HWC) concentrations by biochar and amendments.** Comparison was made within the column by biochar and amendments within one date.

| Source | 03/29/2012 | 07/22/2012 | 10/28/2012 | 05/01/2013 |
|--------|------------|------------|------------|------------|
| COMPOST NO CHAR | 0.19a | 0.33b | 0.29a | 0.24a |
| CHAR | 0.20a | 0.49c | 0.28a | 0.21a |
| UREA NO CHAR | 0.20a | 0.25a | 0.22a | 0.18a |
| CHAR | 0.21a | 0.31b | 0.27a | 0.24a |
| NO FERT NO CHAR | 0.19a | 0.28a | 0.20a | 0.18a |
| CHAR | 0.23a | 0.36b | 0.23a | 0.21a |

Values followed by the same letter are not significantly different at a 5% probability level.

**Fig 3. Temporal change in total C contents influenced by biochar and amendments.** Bars with different letters indicate significant differences among treatments at a 5% probability level.

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N$_2$O emission was significantly influenced by the interaction between amendment and biochar treatment (Fig 4A and 4B). In the COMPOST and NO FERT soils, biochar amendment did not change N$_2$O emission at any point during the experiment. In contrast, biochar addition to UREA soil significantly stimulated N$_2$O emission by 49% on average compared to soil without biochar. This result is consistent with the results reported in [56], namely that N$_2$O emission was significantly increased by biochar addition, especially when added together with mineral fertilizer N. Other researchers have also reported that biochar treatment of fertilized soil results in higher N$_2$O emission than from the fertilized soil alone [32, 39, 57]. However, contrary to our results, it was reported that biochar treatment significantly reduced N$_2$O emission from rice paddy soils with no N fertilization [19, 29]. In the mechanism-based research of [29], 11 of 15 agricultural soils were found to emit a lower level of N$_2$O upon biochar amendment, and these authors discussed the interaction between black C and N dynamics. However,
no change in N$_2$O emission in response to biochar addition was also noted when urea N was added [20]. These inconsistent results indicate that the effect of biochar on soil N$_2$O emission is dependent on water status, pH, C status, and N fertilizer management of soils [4, 33, 58].

In our study, we assumed that most of the N$_2$O produced was from autotrophic and heterotrophic nitrification, because our WFPS ranged from 7–57%, which we assumed meant that the soil was aerobic. It has been reported that all of N$_2$O emitted over 70% WFPS was produced during denitrification, but that at 35–60% WFPS, nitrification was the main process producing N$_2$O [26]. Apart from the autotrophic nitrification process, heterotrophic nitrification has also been reported to be an important mechanism to remove NH$_4^+$ under aerobic conditions [59]. If we assume that heterotrophic nitrification is the main process resulting in N$_2$O emission from the UREA soil, the higher N$_2$O emission resulting from CHAR treatment of this soil can be explained as follows. In the CHAR treatment of UREA soil, consistently higher amounts of HWC and mineral N were observed (Table 4, Fig 5). Hence, the combination of a higher amount of labile C and mineral N can explain the higher rate of N$_2$O emission from UREA soil with CHAR treatment compared to UREA soil with NO CHAR treatment due to stimulation of heterotrophic nitrification. This interaction was especially important at our field site because our soil was severely C limited due to a very low organic C content (0.4% weight basis). We did not consider biochar-derived mineral N as making a direct contribution to the higher mineral N content in the UREA soil because the mineral N content in the biochar has been reported to be very low [60] and was not increased by biochar addition to the COMPOST and NO FERT soils. This argument is further supported by the microbial FDA activity and microbial biomass C results (Fig 2). Higher FDA activity and microbial biomass C in the UREA soil with biochar
addition indirectly indicated higher microbial autotrophic and heterotrophic nitrification in this soil.

Another possible mechanism to explain the higher N$_2$O emission from the soil amended with both urea and biochar is as follows. Theoretical distribution of urea-derived mineral N in the UREA soil revealed that in the biochar-amended soil, N loss through N$_2$O emission was higher by 48.89% and the soil mineral N content was higher by 28.13% than in soil without biochar. In contrast, N loss via leaching was significantly lower in CHAR soil than in the NO CHAR soil (Fig 6). This result is consistent with [61], which observed that soil with biochar addition showed less NO$_3^-$ leaching than soil without biochar. They proposed that the soil with biochar had better water-holding capacity due to the enhanced volume provided by soil mesoaggregates. In this study, we also observed higher WHC in the UREA and NO FERT soils with biochar addition (Fig 7), further supporting that N loss through leaching is reduced in biochar-treated soil.

In summary, C-poor soil treated with urea fertilizer and biochar was able to retain a higher amount of mineral N than untreated soil. This could supply sufficient available N for plant growth and explain the reported increases in plant yield in response to biochar addition.
However, the higher retention of mineral N within the soil might cause greater N loss through N$_2$O emissions, which is a strong greenhouse gas. Therefore, when applying biochar and urea to C-poor soils, the growth pattern of plants should be considered to determine the best timing to maximize the plant’s utilization and minimize N$_2$O loss.

Conclusions

As grassland ecosystems have the potential to sequester significant amounts of C, development of good management practices for these systems is needed to enhance their potential to mitigate climate change by maximizing C sequestration and minimizing greenhouse gas emissions. When both compost and biochar were applied to soil, less CO$_2$ evolved from the soil during the summer, probably due to adsorption of labile C by the biochar. Soil C sequestration was highest in the COMPOST soil with biochar addition, consistent with the pattern of CO$_2$ evolution.

Soil N$_2$O emissions were significantly influenced by interactions between the biochar and amendment materials. Higher N$_2$O emission was observed in UREA soil with biochar than without it, while in the COMPOST and NO FERT soils, there was no difference in N$_2$O emissions between soils with and without biochar. We proposed two possible mechanisms to explain the higher N$_2$O emission from UREA soil amended with biochar. The first mechanism was enhanced autotrophic and heterotrophic nitrification due to interaction between labile C from the biochar and mineral N. When soil is C limited, as was the soil in our study, the addition of biochar could provide the soil with labile C, resulting in enhanced N mineralization (ammonification) and heterotrophic nitrification, finally leading to an increase in N$_2$O emission. The second mechanism was enhanced retention of mineral N content by the biochar. Mineral N could easily be leached out if there was no holding mechanism in the soil. Biochar
addition reduced N loss due to leaching in the UREA soil, leading to greater retention of available N, which resulted in higher N$_2$O emissions from the soil. The overall N$_2$O emission results indicate that care should be taken when applying biochar together with urea fertilizer. To develop a sustainable management strategy for fertilizer and biochar application, the timing of N fertilizer and biochar application should take into consideration of N loss through both N$_2$O emission and leaching. In future studies, we intend to trace fertilizer N using an isotope to gain a mechanistic understanding of the effects of biochar on soil N dynamics.

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Author Contributions

Conceived and designed the experiments: GY. Performed the experiments: HK JC GY. Analyzed the data: JC GY. Contributed reagents/materials/analysis tools: HK JC GY. Wrote the paper: JC GY. Designed the experimental site: GY. Management of the field site: GY.

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