# Abiotic mechanisms for biochar effects on soil N₂O emission**

*Chaohui He*[1,2,3]*, Kiril Manevski*[3,4] *, Mathias Neumann Andersen*[3,4]*, Chunsheng Hu*[2]**, Wenxu Dong*[2]*,
and Jiazhen Li*[1,2]

[1]University of Chinese Academy of Sciences, 19(A) Yuquan Road, Shijingshan District, Beijing 100049, China
[2]Key Laboratory of Agricultural Water Resources, Center for Agricultural Resources Research, Institute of Genetics and
Developmental Biology, Chinese Academy of Sciences, 286 Huaizhong Road, Yuhua district, Shijiazhuang 050022, China
[3]Sino-Danish Center for Education and Research, Estern Yanqihu Campus, 380 Huaibeizhuang, Huairou district,
Beijing 101408, China
[4]Aarhus University, Department of Agroecology, Blichers Allé 20, 8830 Tjele, Denmark

Abstract. In this research, sterile soil columns with different contents of biochar made from apple-tree residues (0, 1 and 5% w/w) at three levels of water filled pore space (40, 60, and 80%) were set up in the laboratory to study nitrous oxide diffusion and binding processes. The results indicated that nitrous oxide emission can be effectively mitigated at 5% biochar regardless of soil water content. However, 1% biochar stimulated nitrous oxide diffusion compared to the other biochar treatments, which was opposite to expectations due to the stronger aeration than adsorption effect, while 0% had a suppression effect between 1 and 5%. Nitrous oxide emissions increased with increasing water filled pore space due to concomitantly decreasing biochar tortuousity at high water content. The increase of nitrogen from 1.11 to 1.50% on the biochar surface in the 5% treatment, and from 1.11 to 1.46% in the 100% biochar treatment, suggested that the main abiotic mechanisms for mitigation of nitrous oxide emission is adsorption and subsequent reactions with C=C bonds on apple-tree biochar surfaces since C=O and C-O bonds both increased and C=C/C-C/C-H declined.

Keywords: gas diffusion, mitigation, nitrous oxide, water filled pore space

## INTRODUCTION

The increasing demand for food and application of chemical fertilizers have risen nitrous oxide (N₂O) emissions (Smith *et al.*, 2008), with agriculture contributing as much as 60% of the total anthropogenic N₂O to the atmosphere (Harter *et al.*, 2014). It is well known that N₂O is a potent greenhouse gas with much higher global warming potential than carbon dioxide and methane (IPCC, 2013). The N₂O from agriculture is mainly produced through a series of microbially-driven reactions in the soil, *i.e.* nitrification, denitrification and dissimilatory nitrate reduction to ammonia (Baggs, 2011). However, more than two thirds of the soil N₂O emissions come from nitrification and denitrification (Lassey and Harvey, 2007). Moreover, the relative contribution of N₂O emissions from each of these reactions depends on not only the soil characteristics (soil texture, available carbon source, pH, microbial activity, *etc*.) but also on the environmental conditions (temperature, precipitation, *etc*.). Due to the complexity of the N₂O production reactions and their high spatio-temporal variability, controlling the emissions from agricultural soils is still of great challenge (Venterea *et al.*, 2012). Methods are needed to limit or decrease the N₂O emissions from agriculture without actually compromising food production.

Biochar has been suggested as a promising tool to decrease soil N₂O emissions (Liu *et al.*, 2018). It is a form of charcoal made by the release of energy from plant residues or animal waste during pyrolysis process (<700ºC) under limited oxygen or anoxic conditions, which makes biochar stable and porous (Karhu *et al.*, 2011). Biochar transforms organic into inert carbon (C) not readily available for decomposition by soil microorganisms, thus it sequesters C and alters the soil nitrogen (N) cycle (van Zwieten *et al.*, 2010; Cayuela *et al.*, 2013). In regard to suppressing soil

N$_2$O emissions, the role of biochar is reported as inconsistent in the scientific literature. A recent meta-analysis by Liu *et al.* (2018) used more than 200 peer-reviewed scientific studies and found notable benefits of using biochar such as increased symbiotic biological N$_2$ fixation (63%), improved plant N uptake (11%), decreased soil nitrate leaching (26%) and reduced soil N$_2$O emissions (32%), though up to 4% of the feedstock N could be emitted as N$_2$O if the pyrolytic syngas from biochar production is not purified. Similar suppressing effects of biochar on soil N$_2$O emission are reported by other studies (*e.g.* see the review of Cayuela *et al.*, 2014). However, there is little research on the consumption of N$_2$O after it has been produced in the soil. Lin *et al.* (2014) investigated the microbial contribution to N$_2$O emission following biochar addition to a range of soils and could not attribute the response in produced N$_2$O to a particular microbial group (*e.g.* fungi or bacteria), suggesting the presence of abiotic production or consumption routes in biochar-amended soils. Several studies reported a response of N$_2$O emission to biochar-amended soils due to changes in soil pH, aeration (Case *et al.*, 2012) and cation exchange capacity (Novak *et al.*, 2009). Quin *et al.* (2015) reported only 16% of the total N$_2$O amount injected into a 100% biochar column to be emitted and observed changes in the biochar surface functional groups in relation to the water status, suggesting physico-chemical interactions such as adsorption and reduction-oxidation between N$_2$O and the biochar applied. It is therefore important to investigate the abiotic mechanisms by which biochar amendment to soil may affect soil N$_2$O emission in order to further understand the complex factors governing N$_2$O production and consumption in the soil.

The main aim of the study was to investigate the physico-chemical impact of biochar application on soil N$_2$O emission and the underlying abiotic mechanisms for soils amended with different rates of biochar, relative to a control without biochar. The objectives were to: i) analyze the properties of biochar by Scanning Electron Microscope – Energy Dispersive X-Ray Spectroscopy, ii) conduct X-ray Photoelectron Spectroscopy for a detailed element and functional group analysis, and iii) analyze the actual N$_2$O emission data from the soils and propose abiotic mechanism for soil N$_2$O emission.

## MATERIALS AND METHODS

Soil samples were taken from Luancheng experimental station, Shijiazhuang Prefecture, Hebei Province, China (37°53′N, 114°41′E). The climate is continental and semi-arid, with annual precipitation of 400-550 mm, mostly occurring as rainfall from June to September. The soil is aquic cinnamon according to the Chinese soil classification system, corresponding to silty loam soil according to the USDA soil classification system. The upper 10 cm soil was sampled from a field with auger and roots, stones and plant debris were removed manually. Biochar from

apple-tree residues was purchased from a commercial company (Shaanxi Yixin Biological Energy Technology Development Co. Ltd., the highest treatment temperature is 520ºC), which was sieved to pass through a 2 mm mesh together with the air-dried soil to achieve homogeneous soil/biochar mixtures, which was then stored in a cool and dry place for later use.

Selected physical and chemical characteristics of the soil were measured before conducting the biochar experiment. Soil pH (1:2.5, soil/water, w/w) was determined after 1-2 min stirring by a glass rod and left for 30 min before using a pH meter (Mettler Toledo, USA) and soil organic C was analyzed by the potassium dichromate oxidation method according to Lu (2000). Total C and N contents of the soil were determined by dry combustion analysis using a flash HT elemental analyzer (vario MACRO cube, Elementar, Germany). Bulk density was measured by the cutting-ring method. Soil porosity was obtained from bulk density and soil particle density. The physico-chemical properties of the soil are summarized in Table 1.

The soil samples were grouped in two main treatments, each with four replicates, and amended with biochar (first treatment) at rates of 0, 1 and 5% (dry weight/dry weight), treatments coded as 0% BC, 1% BC and 5% BC. The samples were mixed thoroughly and packed to a bulk density equal to that under field condition (Table 1). The soil and biochar mixtures were packed into 19 cm diameter columns to a depth of 10 cm. After packing, the soil columns and the equipment used in the experiment were autoclaved at 130°C for 1 h to attain abiotic condition and sterilized water was added by a syringe in a UV sterilized biological safety cabinet when columns cooled down to obtain three water content levels (second treatment): low (L), medium (M) and high (H), corresponding to water filled pore space (WFPS) of around 40, 60 and 80% (Table 2). Before autoclaving, the columns were wrapped in tinfoil and tied

**Table 1.** Physico-chemical characteristics of the sampled soil at Luancheng, China

| pH | SOC (%) | TC (%) | TN (%) | C/N | Bulk density (g cm$^{-3}$) | Porosity (%) |
|---|---|---|---|---|---|---|
| 7.61 | 0.93 | 2.93 | 0.10 | 9.30 | 1.30 | 50.94 |

SOC is soil organic carbon, TC and TC are total carbon and total nitrogen, respectively, and C/N is the carbon to nitrogen ratio

**Table 2.** Water filled pore space in the biochar (BC) amendment treatments of the study

| Water filled pore space (%) | 0% BC | 1% BC | 5% BC |
|---|---|---|---|
| Low (L) | 39.97 | 40.70 | 43.91 |
| Medium (M) | 59.99 | 61.04 | 65.91 |
| High (H) | 80.00 | 81.47 | 87.90 |

tightly to avoid diffusion of water vapor inside. The tinfoil-covered columns were weighted by an electronic scale with maximum capacity of 30 kg before and after autoclaving and there was no significant water loss/gain during this process. In addition to the three biochar levels (0, 1 and 5% BC), a 100% biochar level was also included (coded 100% BC) to test for adsorption without altering WFPS.

Each column was placed in a safety cabinet for 60, 90 and 120 min, for low, medium and high WFPS, respectively, when water percolated into the soil/biochar mixtures before N$_2$O injection. Finally, the biochar×WFPS treatments (0, 1 and 5% BC, each at low, medium and high WFPS) and the 100% BC treatment were all injected with N$_2$O using a 100 mL gas mixture of N$_2$O (991 ppm) at the bottom of each column using a gas tight glass syringe directly through an injection port. The N$_2$O concentration in the headspace was measured after injection by a N$_2$O isotope analyzer (Fig. 1).

ten volumes of distilled water (1 g :10 mL) and the soil/biochar mixture suspension was sieved using a 250 μm sieve. Biochar pieces were manually removed from the sieve surface by a tweezer. The isolated biochar samples were then washed several times in a beaker with distilled water, air-dried and stored in a sealing bag at 4°C. The collected biochar was studied by Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDS) and X-ray Photoelectron Spectroscopy (XPS) techniques for describing biochar surface structure, relative content of elements and surface functional group changes. The analysis was conducted by a commercial certified company (Zhong Ke Bai CE, Beijing, China).

Analysis of variance (ANOVA) was conducted on the data and significant differences between treatments were determined using t-test. The statistical analysis was conducted using IBM SPSS Statistics 19.0 for Windows (SPSS Inc., USA).
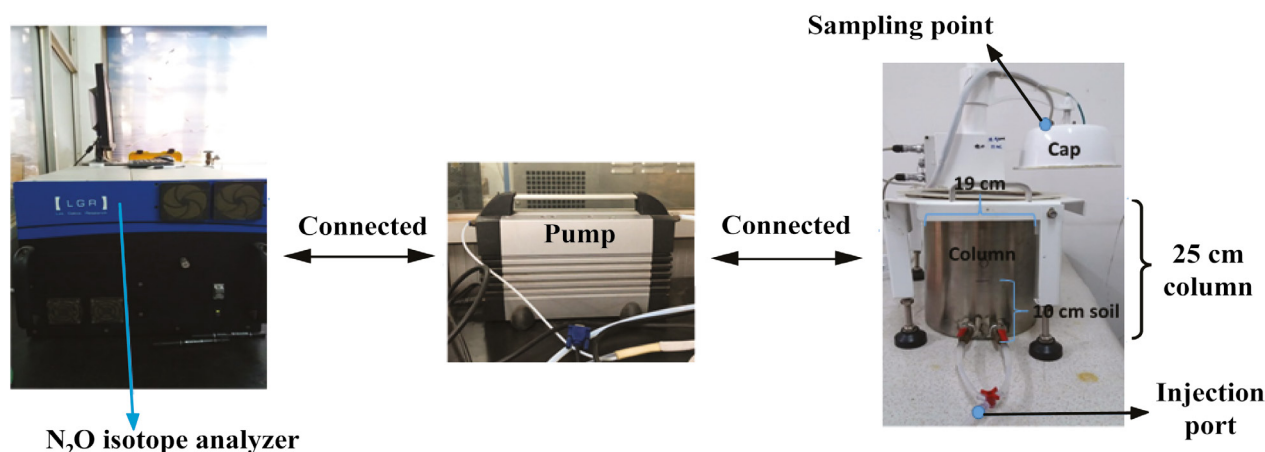


**Fig. 1.** Overall setup of the experiment in the laboratory.

Five g of substrate was collected randomly from the upper surface of the soil columns after autoclaving and at the end of experiment within the safety cabinet and were analyzed for microbial activity by 24 h culturing in the medium composed of peptone (10 g L$^{-1}$), yeast (5 g L$^{-1}$), NaCl (10 g L$^{-1}$) and agar (15 g L$^{-1}$). The results showed no microbial colonies present on the Petri dishes after 24-h incubation.

In order to investigate the possible abiotic mechanisms that result in N$_2$O suppression due to biochar amendment, the soil and the biochar from the 5% BC treatment at low, medium and high WFPS with N$_2$O injection were separated. The treatment without N$_2$O injection, *i.e.*, a "conventional" (CK) treatment soil with 5% biochar amendment and without N$_2$O injection, intended to contrast the surface elemental and functional groups analysis. The separation method followed the procedure of Joseph *et al.* (2010) and Lin *et al.* (2012). Briefly, the samples were dispersed by agitation in

RESULTS

The SEM morphological description of the biochar in the 5%BC treatment pooled for the low, medium and high WFPS samples with injected N$_2$O (LMH 5%BC), as well as of pure biochar before incorporation into the soil are shown in Fig. 2. There were apparent differences between LMH5%BC and biochar before application and a layer of organic molecules and inorganic materials such as minerals could be seen in LMH 5% BC exterior and internal pores, signifying probable interactions between the biochar and the soil. The exterior of a typical woody biochar showed smooth surfaces due to high C content and very few minerals on the surface (Fig. 2d, examined at 1K magnification).

Figure 3 shows the elemental spectra of biochar surface collected from the 5% BC treatment with N$_2$O injection at low, medium and high WFPS. All the sampled biochar had similar patterns of elemental composition, with C making up the largest proportion of the total amount of elements.
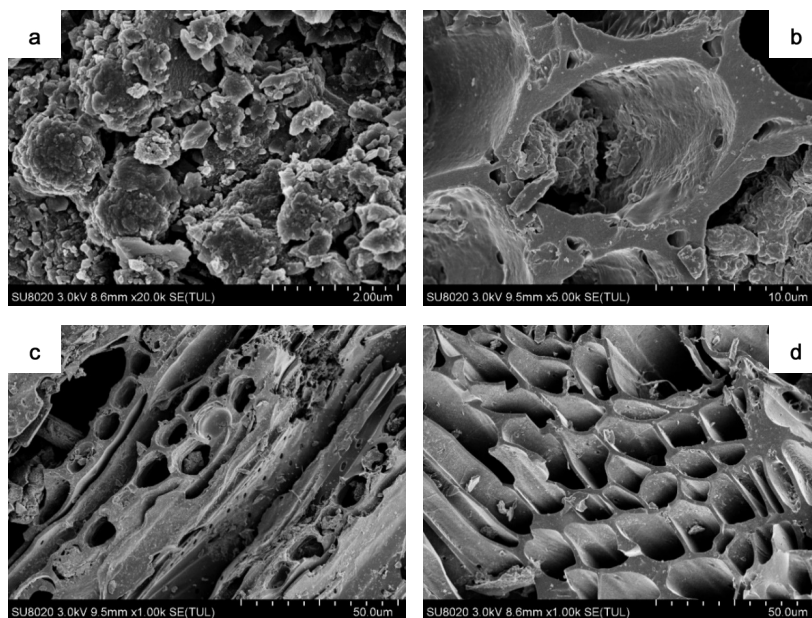
**Fig. 2.** Morphology of biochar amended at 5% to soil at low, medium and high water contents according to the Scanning Electron Microscopy (SEM) for: (a) external surface after interaction with soil, (b) internal surface covered by organo-mineral matters and (c) external surface and pores having reactions with soil and injected $N_2O$, as well as (d) external surface of pure biochar before incorporated into soil. Samples shown on a, b and c were examined at 20K, 5K and 1K magnification, respectively.
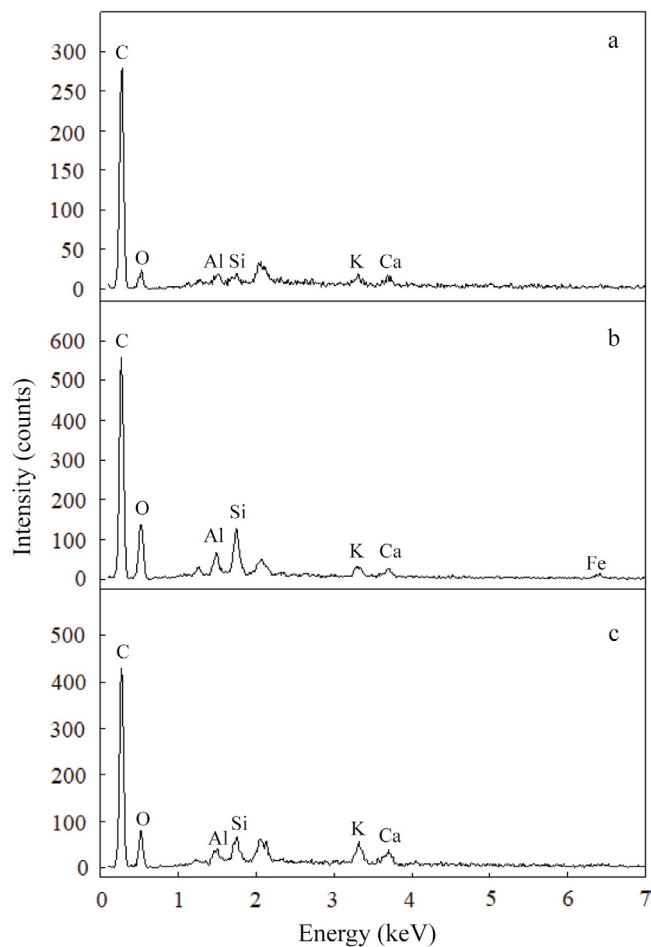


**Fig. 3.** Elemental analysis of biochar separated from 5%-amended soil with injected $N_2O$ under (a) low, (b) medium and (c) high water filled pore space (WFPS).

Other elements included O, Al, Si, K and Ca, whereas Fe, though as a minor part of the total specimen, was observed only at medium water content (Fig. 3b). The few minerals might have come from the indigenous ash contents in the biochar feedstock or from the compounds formed on the biochar surface when it interacted with the soil mineral matter.

Figure 4 shows the elemental spectra of the 5% BC treatment without $N_2O$ injection at low, medium and high WFPS. There were no drastic differences in the elemental composition of the biochar without $N_2O$ injection compared with the biochar with $N_2O$ injection (Fig. 3). Yet, the spectral peaks corresponding to Al and Si decreased (Fig. 4b, c), while those of Ca increased notably (Fig. 4c) compared to the $N_2O$ injection treatment, mineral changes probably due to the soil-biochar-water interactions.

Table 3 shows the results of the XPS analysis for the main elements in the biochar samples collected from 5% BC treatment without $N_2O$ injection (CK treatment), the 5% BC treatment with $N_2O$ injection pooled at the three WFPS (LMH 5% BC) and the 100% biochar with $N_2O$ injection (100% BC). As seen from the table, C was the most abundant element in all examined samples and had an atomic percentage range of 82-85%, which was in line with the EDS results. The abundance of O was around 14% for all samples, whereas that of N was low and ranged from 1.11% in CK to 1.50% in LMH 5% BC. However, the Fe and Al minerals had the lowest abundance of less than 1%. Among the treatments, most of the elements present with the highest abundance were observed in the LMH 5% BC treatment.

Additional XPS analysis results of the bonding states and the biochar surface functional groups are presented in Table 4. Only the functional groups including C and N were detected. The concentration of aliphatics (C=C/C-C/C-H), which are the major components in all biochars, was notably higher in CK than LMH 5% BC and BC 100% treatments. On the contrary, C in oxidation states (C-O, C=O) increased, indicating surface C change from ordinary bonding states to oxidation states due to oxidation reactions or adsorption of soil organic matter or both. Regarding the N groups, C=N increased dramatically to 77.38% in LMH 5% BC compared to 51.51% in 100% BC and 58.43% in CK, possibly due to the consumption of $N_2O$.
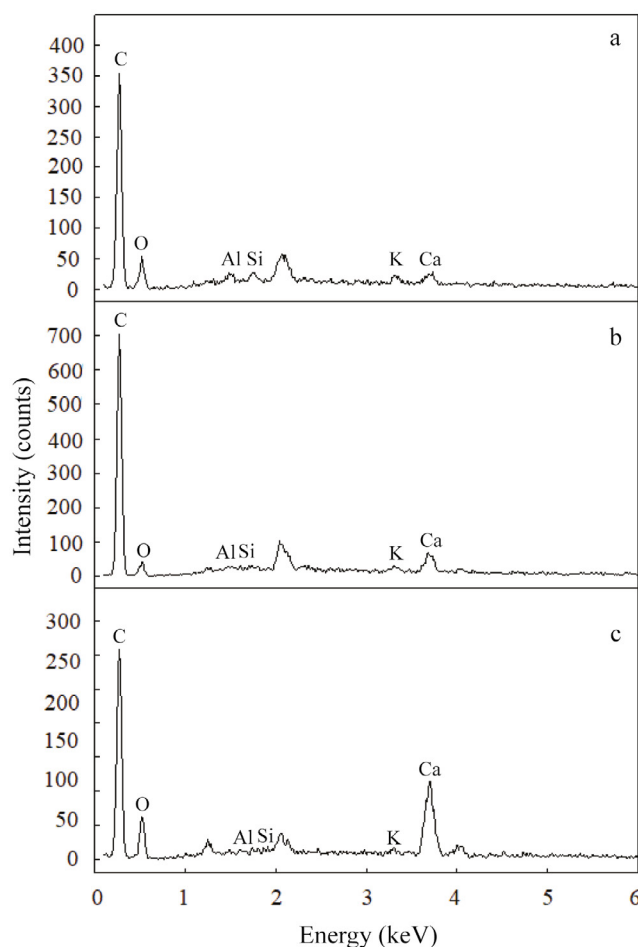


**Fig. 4.** Elemental analysis of biochar separated from 5%-amended soil without injected $N_2O$ under (a) low, (b) medium and (c) high water filled pore space (WFPS).

**Table 3.** X-ray Photoelectron Spectroscopy analysis of the main elements in the biochar samples showing peak binding energy (BE, eV) and atomic percentage (%)

| Element | CK | | LMH 5% BC | | 100% BC | |
|---|---|---|---|---|---|---|
| | Peak BE (eV) | Atomic % | Peak BE (eV) | Atomic % | Peak BE (eV) | Atomic % |
| Al 2p | 74.28 | 0.68 | 74.88 | 0.86 | 74.79 | 0.60 |
| C 1s | 284.78 | 84.49 | 284.79 | 83.10 | 284.82 | 82.72 |
| N 1s | 400.42 | 1.11 | 400.39 | 1.50 | 400.06 | 1.46 |
| O 1s | 532.53 | 13.41 | 532.66 | 14.15 | 532.26 | 14.92 |
| Fe 2p | 719.51 | 0.31 | 721.22 | 0.40 | 722.00 | 0.33 |

CK is conventional treatment of 5% biochar-amended soil without $N_2O$ injection, LMH 5% BC is 5% biochar-amended soil with $N_2O$ injection at low, medium and high water contents, and 100% BC is 100% biochar treatment with $N_2O$ injection.

**Table 4.** C 1s and N 1s binding energy (BE) and their relative atomic percentage on biochar surface

| Element | Functional groups | CK | | LMH 5% BC | | 100% BC | |
|---|---|---|---|---|---|---|---|
| | | Peak BE (eV) | Atomic % | Peak BE (eV) | Atomic % | Peak BE (eV) | Atomic % |
| C 1s A | C=C/C-C/C-H | 284.58 | 82.50 | 284.79 | 71.02 | 284.79 | 72.88 |
| C 1s B | C-O&C-NH$_2$ | 285.84 | 10.00 | 286.14 | 19.65 | 285.96 | 21.00 |
| C 1s C | C=O&C=N | 288.47 | 7.50 | 288.93 | 9.18 | 289.16 | 5.43 |
| N 1s A | C-NH$_2$ | 399.46 | 41.57 | 399.16 | 22.62 | 399.42 | 48.49 |
| N 1s B | C=N | 400.60 | 58.43 | 400.50 | 77.38 | 400.64 | 51.51 |

CK is conventional treatment of 5% biochar to the soil without nitrous oxide ($N_2O$) injection pooled for the three WFPS, LMH 5% BC is 5% amended soil with biochar with pooled samples at low, medium and high water filled pore spaces and with $N_2O$ injection, and 100% BC is 100% biochar treatment with $N_2O$ injection.

The net increase of $N_2O$ as measured in the headspace of the columns relative to the total injected $N_2O$ ($\Delta N_2O$/injected $N_2O$) at the time when it reached the peak concentration is presented in Fig. 5. It is apparent from the figure that $\Delta N_2O$/injected $N_2O$ was markedly lower for the 5% BC treatment at the three soil water contents. However, opposite to the expectations, 1% BC appears to have stimulated $N_2O$ emissions, whereas the $N_2O$ suppression effect for 0% BC was in between that of 1% BC and 5% BC. It can also be seen on the figure that the $\Delta N_2O$/injected $N_2O$ increased with increasing WFPS and this was especially evident for the lower biochar amendments. Since the biochar in the 100% BC treatment was dry and without water additions, $\Delta N_2O$/injected $N_2O$ was constant between the different WFPS, but was lower than that for 0 and 1% BC and higher than that for 5% BC. Moreover, only a slight increase in $\Delta N_2O$/injected $N_2O$ could be seen for the 5% BC between the three WFPS levels.

In addition to $\Delta N_2O$/injected $N_2O$, the absolute value of the net $N_2O$ increase in soil water and air was also computed and the results are shown in Table 5. The dissolved $N_2O$ increased with increasing WFPS for all biochar treatments. The highest value of dissolved $N_2O$ in the water solution
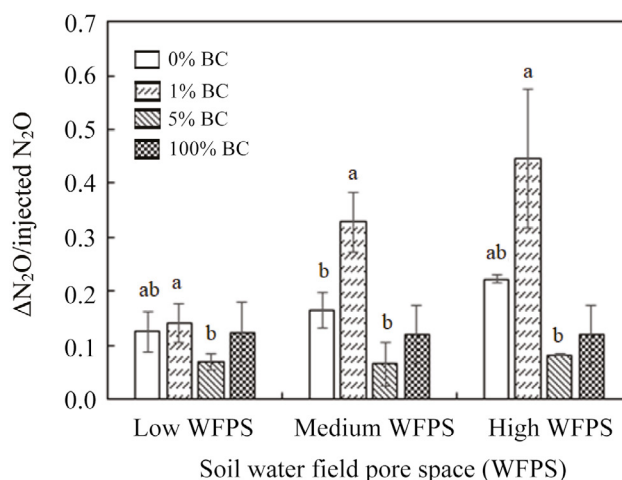


**Fig. 5.** Net increase of $N_2O$ in air relative to total injected $N_2O$ at peak concentration ($\Delta N_2O$/injected $N_2O$) at different soil water contents and biochar amendments. Error bars denote the standard error of the mean (n = 4), lower case letters indicate significant differences between the treatments at 95% confidence level.

**Table 5.** Dissolved nitrous oxide (N$_2$O) in soil water and net increase of N$_2$O in air. 0%BC, 1%BC and 5%BC denote treatments with 0%, 1% and 5% biochar amendment in the soil. Numbers in brackets are standard error of the mean (n = 4) and lowercase letters indicate significant differences between the treatments at 95% confidence level

| Dissolved in water (μmol) (WFPS) | 0% BC | 1% BC | 5% BC |
|---|---|---|---|
| Low | 0.03 (±0.01)[a] | 0.03 (±0.01)[a] | 0.02 (±0.00)[a] |
| Medium | 0.05 (±0.01)[b] | 0.10 (±0.02)[a] | 0.02 (±0.01)[b] |
| High | 0.09 (±0.00)[ab] | 0.20 (±0.06)[a] | 0.04 (±0.00)[b] |
| Net increase in air (μmol) (WFPS) | 0% BC | 1% BC | 5% BC |
| Low | 0.52 (±0.16)[ab] | 0.59 (±0.15)[a] | 0.29 (±0.06)[b] |
| Medium | 0.69 (±0.13)[b] | 1.36 (±0.23)[a] | 0.27 (±0.17)[b] |
| High | 0.92 (±0.03)[ab] | 1.85 (±0.53)[a] | 0.34 (±0.01)[b] |

WFPS – soil water filled pore space.

was 0.20 μmol for 1% BC, whereas the lowest value was 0.02 μmol for 5% BC. Despite the high solubility of N$_2$O in water, the values for net N$_2$O increase in air were much larger, with the highest values of 1.36 and 1.85 μmol for, respectively, medium and high WFPS in the 1% BC treatment. In the experiment, the total injected N$_2$O was around 4 μmol, hence there was a large amount of N$_2$O sorbed on the biochar surface according to the small contributions in both water and headspace.

As shown in Fig. 6, the 5% BC treatment had a suppression effect, whereas the 1% BC treatment appears to have had a stimulating effect on N$_2$O emissions, consistent with the findings in Fig. 5. N$_2$O peak concentration varied from 1000 ppb to 6000 ppb in biochar-amended soil samples and the largest N$_2$O peak concentration was seen for 1% BC, followed by 0, 100 and 5% BC. For all treatments, N$_2$O increased sharply so that within 5 min the concentration reached its highest values after which it declined gradually. However, 5% BC at low WFPS took almost 3 h to peak, illustrating a more complicated system.

## DISCUSSION

The N$_2$O emissions were significantly affected by the WFPS and overall increased from low to high WFPS, though the net N$_2$O increase was the lowest at 40% WFPS but rose with increasing water content (Figs 5, 6). The water content regulated the trend of N$_2$O emissions and had the greatest impact on 1% BC treatment compared to 0 and 5% BC treatments. Similar responses of N$_2$O emissions to WFPS have been found in other studies based on data collected in field or laboratory experiments (Yanai *et al.*, 2007; Chintala *et al.*, 2015; Chang *et al.*, 2016). Most of these studies include microbial participation in nitrification
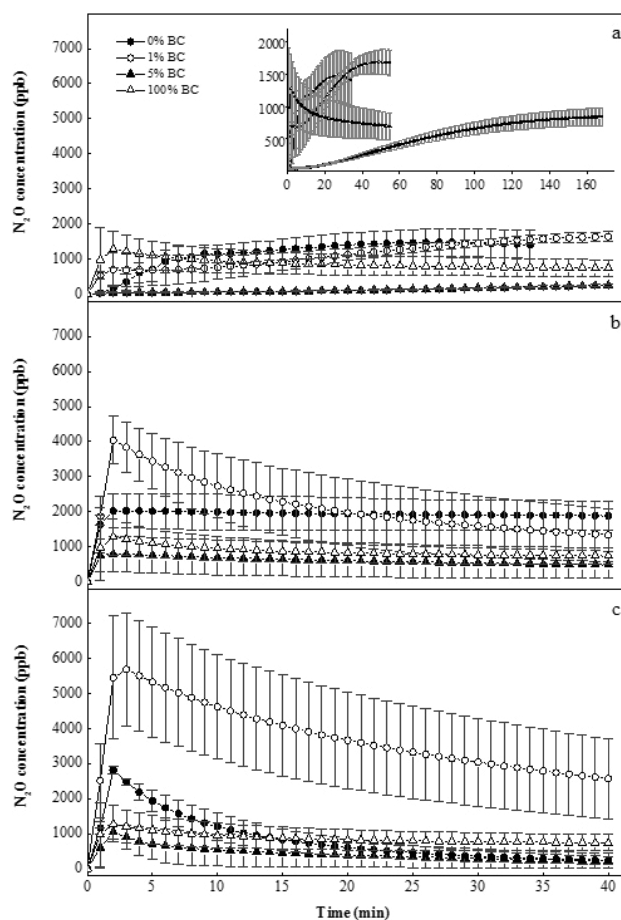


**Fig. 6.** The impact of biochar amendments on N$_2$O emissions at (a) low, (b) medium and (c) high water filled pore space (WFPS). Error bars denote the standard error of the mean (n = 4).
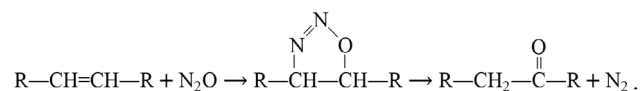
and denitrification processes. The soils used in the present study were autoclaved in order to eliminate any microbial impacts. Autoclaving effectively kills most of the microbes, expect for some thermophiles not relevant for N$_2$O related processes, and this was supported by the negative tests for sterility for nitrifying and denitrifying bacteria and for chemical indicators in the column cores. Hence, the significant increase in N$_2$O emissions with increasing WFPS is due to abiotic mechanisms, such as altered tortuosity and diffusion pathway. Stark and Firestone (1995) argued that diffusion path lengths become more tortuous and water films coating surfaces thinner at low water content. When soil is amended with (porous) biochar, the tortuosity of the gas flow paths is increased, providing better conditions for adsorption. These reactions at low WFPS could have been responsible for the observed low $\Delta$N$_2$O/injected N$_2$O in the 5% BC treatment (Fig. 5), whereas at high WFPS (thus less tortuous paths) the N$_2$O emissions were higher (Figs 5, 6). Comparison between 1 and 5% BC (Fig. 6) demonstrated tortuosity can be easily altered by increasing the WFPS in the 1% BC treatment.

There was a clear response of the observed $N_2O$ concentrations to the apple-tree biochar amendment rate and, compared to the CK treatment, $\Delta N_2O$ in air/injected $N_2O$ was reduced to 5%, corroborating the results of other laboratory and short-term field investigations (Rogovska *et al.*, 2011; Case *et al.*, 2012; Wang *et al.*, 2013; Lan *et al.*, 2015; He *et al.*, 2016; Grutzmacher *et al.*, 2018). Although the previous studies observed a suppression of $N_2O$ by biochar in the soil, the underlying abiotic mechanism remained poorly understood. While soil pH relates well to biotic mechanisms, redox potential (Eh) is a parameter that could explain (or be involved in the explanation of) abiotic mechanisms of $N_2O$ change in biochar-amended soils; yet Eh is characterized by high variability in space and time, irreversibility of redox reactions at the surface of the electrodes, chemical disequilibrium in soils and polarisation of and/or leakage from electrodes (Whitfield, 1974; Grundl, 1994; Thomas *et al.*, 2009; Joseph *et al.*, 2015). Alternatively, the present study performed elemental and functional group analysis for 5% apple-tree biochar application (that is to say, for the most "effective" dosage) under different WFPS treatments with and without $N_2O$ injection, which is more straightforward and accurate than measuring soil Eh and pH.
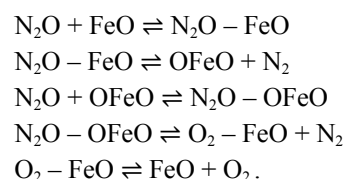
Two main potential mechanisms for the mitigation effect of the 5% BC treatment for $N_2O$ emission pointed by this study are adsorption and subsequent chemical reactions. Biochar surfaces are complex and there will be preferential sites for adsorption and reduction of $N_2O$. Previous studies showed that charred materials have strong sorption capacity of $N_2O$, even better than that of peat, uncharred wood and metal oxides (Cornelissen *et al.*, 2013). Therefore, it is highly probable that $N_2O$ was sorbed on the apple-tree biochar surface, especially in the 5% BC treatment with the highest specific surface area compared to 1% BC treatment. In fact, slightly less $N_2O$ was dissolved in water (Table 5), indicating a large amount of $N_2O$ that was adsorbed on the surface or further reduced by other pathways. Moreover, the XPS analysis (Table 3) showed an increase of N from 1.11 to 1.50% on biochar surface in the 5% BC treatment, and also from 1.11 to 1.46% in the 100% BC treatment. However, the 1% BC treatment promoted $N_2O$ emissions regardless of WFPS (Figs 5, 6), which is in contrast to expectations and previous studies. This is probably due to improved soil aeration by the biochar incorporated in the soil, which causes faster gas diffusion owing to the porous structure.

Sorbed $N_2O$ can also be reduced by organic molecules of biochar or soil organic compounds on the biochar surface (van Zwieten *et al.*, 2009). Avdeev *et al.* (2005) reported a range of aromatic and aliphatic compounds oxidized by $N_2O$ and their calculations indicated that an O atom is transferred through the 1,3-dipolar cycloaddition of $N_2O$ to the C=C bond. Subsequently, the resulting intermediate decomposed to a ketone and released $N_2$ to the gas phase. The schematic diagram of chemical reaction is shown below:

$$R-CH=CH-R + N_2O \rightarrow R-\overset{\overset{\displaystyle N}{\underset{\displaystyle O}{\|}}}{CH}-\overset{}{CH}-R \rightarrow R-CH_2-\overset{\overset{\displaystyle O}{\|}}{C}-R + N_2.$$

Biochar contains a large number of aromatic and aliphatic groups on the surface. As the XPS analysis showed (Table 4), C = O and C-O both increased and C=C/C-C/C-H decreased in the 5% BC, indicating that these compounds may be partly responsible for the reduction in $N_2O$ emissions observed in this treatment. Alternatively, studies have shown that reductive metals such as Fe can be catalytically active for the decomposition of $N_2O$ (Moraghan and Buresh, 1977; Carabineiro *et al.*, 2008) and Fe has already been reported as central for orchestrating the N-transformations in biochar-amended soils (Hans *et al.*, 1996; Alowitz and Scherer, 2002). Sang *et al.* (2005) proposed the following reaction mechanism as being the most likely to fit their experimental data for the reduction of $N_2O$:

$$N_2O + FeO \rightleftharpoons N_2O - FeO$$
$$N_2O - FeO \rightleftharpoons OFeO + N_2$$
$$N_2O + OFeO \rightleftharpoons N_2O - OFeO$$
$$N_2O - OFeO \rightleftharpoons O_2 - FeO + N_2$$
$$O_2 - FeO \rightleftharpoons FeO + O_2.$$

Studies have also demonstrated that up to 84% of $N_2O$ could be rapidly reduced to $N_2$ in alkaline conditions (pH = 8) by ferrous iron (Moraghan and Buresh, 1977). Considering the observed Fe in the element analysis (Fig. 3b), the adsorbed $N_2O$ on apple-tree biochar surface and the alkaline environment, the above reactions might have taken place in the present experiment. Even though the amount of $N_2O$ in air and water can be computed, how to distinguish the effect of adsorption and chemical reaction is not straightforward because adsorption is temporal and might be accompanied by desorption. Finally, it should be mentioned that the soil columns stood 60 to 120 min prior $N_2O$ injection, giving insights on a short-time scale interactions, therefore, future studies may investigate the same reactions occurring after longer time in the organomineral layers of more aged bioachar (*e.g.* Quin *et al.*, 2015).

## CONCLUSIONS

This study performed elemental and functional group analysis of soil amended with different rates of apple-tree biochar application under varying WFPS treatments with and without $N_2O$ injection in order to investigate the abiotic mechanisms of soil $N_2O$ emissions. The results can be summarized as follows:

1. The $N_2O$ emissions were significantly lowered by apple-tree biochar in the 5% BC treatment regardless of water filled pore space, though the effect was more evident at low than at high WFPS.

2. The observed sharp increase in N$_2$O emissions at high WFPS might be due to the changed tortuosity and diffusion pathway of soil amended with porous biochar, which in turn provides suitable conditions for adsorption.

3. Indeed, the biochar surface elemental and functional groups analysis revealed adsorption and subsequent chemical reactions as the most possible abiotic mechanisms for N$_2$O consumption.

**Conflict of interest:** The Authors do not declare conflict of interest.

## REFERENCES

**Alowitz M.J. and Scherer M.M., 2002.** Kinetics of nitrate, nitrite, and Cr(VI) reduction by iron metal. Environ. Sci. Technol., 36(3), 299-306. https://doi.org/10.1021/es011000h

**Avdeev V.I., Ruzankin S.F., and Zhidomirov G.M., 2005.** Molecular mechanism of direct alkene oxidation with nitrous oxide: DFT analysis. Kinetics and Catalysis, 46(2), 177-188. https://doi.org/10.1007/s10975-005-0066-z

**Baggs E.M., 2011.** Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. Current Opinion in Environmental Sustainability, 3(5), 321-327. https://doi.org/10.1016/j.cosust.2011.08.011

**Carabineiro S.A., Fernandes F.B., Silva R.J.C., Vital J.S., Ramos A.M., and Fonseca I.M., 2008.** N$_2$O reduction by activated carbon over iron bimetallic catalysts. Catalysis Today, 133-135, 441-447. https://doi.org/10.1016/j.cattod.2007.11.019

**Case S.D.C., McNamara N.P., Reay D.S., and Whitaker J., 2012.** The effect of biochar addition on N$_2$O and CO$_2$ emissions from a sandy loam soil – The role of soil aeration. Soil Biol. Biochem., 51, 125-134. https://doi.org/10.1016/j.soilbio.2012.03.017

**Cayuela M.L., Sanchez-Monedero M.A., Roig A., Hanley K., Enders A., and Lehmann J., 2013.** Biochar and denitrification in soils: when, how much and why does biochar reduce N$_2$O emissions? Scientific Reports 3, 1732. https://doi.org/10.1038/srep01732

**Cayuela M.L., van Zwieten L., Singh B.P., and Jeffery S., Roig A., and Sánchez-Monedero M.A., 2014.** Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. Agric. Ecosys. Environ., 191, 5-16. https://doi.org/10.1016/j.agee.2013.10.009

**Chang J., Clay D.E., Clay S.A., Chintala R., Miller J.M., and Schumacher T., 2016.** Biochar reduced nitrous oxide and carbon dioxide emissions from soil with different water and temperature cycles. Agronomy J., 108(6), 2214-2221. https://doi.org/10.2134/agronj2016.02.0100

**Chintala R., Owen R.K., Schumacher T.E., Spokas K.A., McDonald L.M., Kumar S., Clay D.E., Malo D.D., and Bleakley B., 2015.** Denitrification kinetics in biomass-and biochar-amended soils of different landscape positions. Environ. Sci. Pollution Res. Int., 22(7), 5152-5163. https://doi.org/10.1007/s11356-014-3762-2

**Cornelissen G., Rutherford D.W., Arp H.P., Dorsch P., Kelly C.N., and Rostad C.E., 2013.** Sorption of pure N$_2$O to biochars and other organic and inorganic materials under anhydrous conditions. Environ. Sci. Technol., 47(14), 7704-7712. https://doi.org/10.1021/es400676q

**Grundl T., 1994.** A review of the current understanding of redox capacity in natural, disequilibrium systems. Chemosphere, 28(3), 613-626. https://doi.org/10.1016/0045-6535(94)90303-4

**Grutzmacher P., Puga A.P., Bibar M.P.S., Coscione A.R., Packer A.P., and de Andrade C.A., 2018.** Carbon stability and mitigation of fertilizer induced N$_2$O emissions in soil amended with biochar. Sci. Total Environ., 625, 1459-1466. https://doi.org/10.1016/j.scitotenv.2017.12.196

**Hans C.B.H., Christian B.K., Hanne N.K., Ole K.B., and Jan S., 1996.** Abiotic nitrate reduction to ammonium: key role of green rust. Environ. Sci. Technol., 30(6), 2053-2056.

**Harter J., Krause H.M., Schuettler S., Ruser R., Fromme M., Scholten T., Kappler A., and Behrens S., 2014.** Linking N$_2$O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. ISME J., 8(3), 660-674. https://doi.org/10.1038/ismej.2013.160

**He L.L., Zhao X., Wang S.Q., and Xing G.X., 2016.** The effects of rice-straw biochar addition on nitrification activity and nitrous oxide emissions in two Oxisols. Soil Till. Res., 164, 52-62. https://doi.org/10.1016/j.still.2016.05.006

IPCC, **2013.** Climate Change 2013: The Physical Science Basis. Cambridge University Press, Cambridge, UK and New York, USA.

**Joseph S., Husson O., Graber E., van Zwieten L., Taherymoosavi S., Thomas T., Nielsen S., Ye J., Pan G., Chia C., Munroe P., Allen J., Lin Y., Fan X., and Donne S., 2015.** The electrochemical properties of biochars and how they affect soil redox properties and processes. Agronomy, 5(3), 322-340. https://doi.org/10.3390/agronomy5030322

**Joseph S.D., Camps-Arbestain M., Lin Y., Munroe P., Chia C.H., Hook J., Van Zwieten L., Kimber S., Cowie A., Singh B.P., Lehmann J., Foidl N., Smernik R.J., and Amonette J.E., 2010.** An investigation into the reactions of biochar in soil. Soil Res., 48, 501-515. https://doi.org/10.1071/sr10009

**Karhu K., Mattila T., Bergström I., and Regina K., 2011.** Biochar addition to agricultural soil increased CH$_4$ uptake and water holding capacity – Results from a short-term pilot field study. Agric., Ecosys. Environ., 140(1-2), 309-313. https://doi.org/10.1016/j.agee.2010.12.005

**Lan Y., Meng J., Yang X., Jiang L.L., Liu Z.Q., Liu S.N., and Chen W.F., 2015.** Effects of different straw incorporation ways on N$_2$O emission and soil physicochemical properties of brown soil (in Chinese). Chinese J. Ecol., 34(3), 790-796.

**Lassey K. and Harvey M., 2007.** Nitrous oxide: the serious side of laughing gas. Water Atmosphere, 15(2), 10-11.

**Lin X., Spokas K., Venterea R., Zhang R., Baker J., and Feyereisen G., 2014.** Assessing microbial contributions to N$_2$O impacts following biochar additions. Agronomy, 4(4), 478-496. https://doi.org/10.3390/agronomy4040478

**Lin Y., Munroe P., Joseph S., Kimber S., and Van Zwieten L., 2012.** Nanoscale organo-mineral reactions of biochars in ferrosol: an investigation using microscopy. Plant Soil, 357(1-2), 369-380. https://doi.org/10.1007/s11104-012-1169-8

**Liu Q., Zhang Y., Liu B., Amonette J.E., Lin Z., Liu G., Ambus P., and Xie Z., 2018.** How does biochar influence soil N cycle? A meta-analysis. Plant and Soil, 426(1), 211-225. https://doi.org/10.1007/s11104-018-3619-4

**Lu R.K., 2000.** Methods of Soil and Agro-Chemical Analysis (in Chinese). China Agric. Sci. Technol. Press, Beijing, China.

Moraghan J.T. and Buresh R.J., 1977. Chemical reduction of nitrite and nitrous oxide by ferrous iron. Soil Sci. Soc. Am. J., 41, 47-50.
https://doi.org/10.2136/sssaj1977.03615995004100010017x

Novak J.M., Busscher W.J., Laird D.L., Ahmedna M., Watts D.W., and Niandou M.A.S., 2009. Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Sci., 174(2),105-112.https://doi.org/10.1097/ss.0b013e3181981d9a

Quin P., Joseph S., Husson O., Donne S., Mitchell D., Munroe P., Phelan D., Cowie A., and Van Zwieten L., 2015. Lowering $N_2O$ emissions from soils using eucalypt biochar: the importance of redox reactions. Scientific Reports, 5, 16773.
https://doi.org/10.1038/srep16773

Rogovska N., Laird D., Cruse R., Fleming P., Parkin T., and Meek D., 2011. Impact of biochar on manure carbon stabilization and greenhouse gas emissions. Soil Sci. Soc. Am. J., 75(3), 871-879. https://doi.org/10.2136/sssaj2010.0270

Sang C., Kim B.H., and Lund C.R.F., 2005. Effect of NO upon $N_2O$ decomposition over Fe/ZSM-5 with low iron loading. J. Physical Chem. B, 109(6), 2295-2301.
https://doi.org/10.1021/jp048884m

Smith P., Martino D., Cai Z., Gwary D., Janzen H., Kumar P., McCarl B., Ogle S., O'Mara F., Rice C., Scholes B., Sirotenko O., Howden M., McAllister T., Pan G., Romanenkov V., Schneider U., Towprayoon S., Wattenbach M., and Smith J., 2008. Greenhouse gas mitigation in agriculture. Phil. Trans. R. Soc. B, Biological Sci., 363(1492), 789-813. https://doi.org/10.1098/rstb.2007.2184

Stark J.M. and Firestone M.K., 1995. Mechanisms for soil-moisture effects on the activity of nitrifying bacteria. Appl. Environ. Microbiol., 61(1), 218-221.

Thomas C.R., Miao S.L., and Sindhoj E., 2009. Environmental factors affecting temporal and spatial patterns of soil redox potential in Florida Everglades Wetlands. Wetlands, 29(4), 1133-1145. https://doi.org/10.1672/08-234.1

van Zwieten L., Kimber S., Morris S., Downie A., Berger E., Rust J., and Scheer C., 2010. Influence of biochars on flux of $N_2O$ and $CO_2$ from ferrosol. Australian J. Soil Res., 48(6), 555-568. https://doi.org/10.1071/sr10004

van Zwieten L., Singh B., Joseph S., Kimber S., Cowie A., and Chan Y.K., 2009. Biochar and emissions of non-$CO_2$ greenhouse gases from soil. In: Biochar for Environmental Management Science and Technology (Eds J. Lehmann, S. Joseph). Earthscan Press, UK.

Venterea R.T., Halvorson A.D., Kitchen N., Liebig M.A., Cavigelli M.A., Grosso S.J.D., Motavalli P.P., Nelson K.A., Spokas K.A., Singh B.P., Stewart C.E., Ranaivoson A., Strock J., and Collins H., 2012. Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. Frontiers in Ecology the Environment, 10(10), 562-570. https://doi.org/10.1890/120062

Wang Z.Y., Zheng H., Luo Y., Deng X., Herbert S., and Xing B.S., 2013. Characterization and influence of biochars on nitrous oxide emission from agricultural soil. Environ. Pollution, 174, 289-296. https://doi.org/10.1016/j.envpol.2012.12.003

Whitfield M., 1974. Thermodynamic limitations on the use of the platinum electrode in Eh measurements. Limnology and Oceanography, 19(5), 857-865.
https://doi.org/10.4319/lo.1974.19.5.0857

Yanai Y., Toyota K., and Okazaki M., 2007. Effects of charcoal addition on $N_2O$ emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. Soil Sci. Plant Nutr., 53(2), 181-188.
https://doi.org/10.1111/ j.1747-0765.2007.00123.x