

Denitrification kinetics in biomass- and biochar-amended soils of different landscape positions

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Abstract Knowledge of how biochar impacts soil denitrification kinetics as well as the mechanisms of interactions is essential in order to better predict the nitrous oxide (N₂O) mitigation capacity of biochar additions. This study had multiple experiments in which the effect of three biochar materials produced from corn stover (*Zea mays* L.), ponderosa pine wood residue (*Pinus ponderosa* Douglas ex Lawson and C. Lawson), switchgrass (*Panicum virgatum* L.), and their corresponding biomass materials (corn stover, ponderosa pine wood residue, and switchgrass) on cumulative N₂O emissions and total denitrification in soils from two different landscape positions (crest and footslope) were studied under varying water-filled pore space (40, 70, and 90 % WFPS). Cumulative N₂O emissions were reduced by 30 to 70 % in both crest and footslope soils. The effect of biochars and biomass treatments on cumulative N₂O emissions and total denitrification were only observed at ≥40 % WFPS. The denitrification enzyme activity (DEA) kinetic parameters, K_s (half-saturation

constant), and V_{max} (maximum DEA rate) were both significantly reduced by biochar treatments, with reductions of 70–80 % in footslope soil and 80–90 % in the crest soil. The activation energy (E_a) and enthalpy of activation of DEA (ΔH) were both increased with biochar application. The trends in DEA rate constants (K_s and V_{max}) were correlated by the trends of thermodynamic parameters (activation energy E_a and enthalpy of activation ΔH) for denitrifying enzyme activity (DEA). The rate constant V_{max}/K_s evaluated the capacity of biochars to mitigate the denitrification process. Denitrifying enzyme kinetic parameters can be useful in evaluating the ability of biochars to mitigate N₂O gas losses from soil.

Keywords Nitrification · Michaelis-Menten · Activation energy · Enthalpy · Nitrous oxide · Arrhenius

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Introduction

Current agricultural practices are responsible for terrestrial production of nitrous oxide (N₂O) which has 320 times the global warming potential of CO₂ on a mass basis (Cayuela et al. 2013; IPCC 2007; Paustian 2004; Ravishankara et al. 2009). A majority of N₂O emissions originate from agriculture due to the incorporation of nitrogen (N)-based agricultural inputs including fertilizers, manures, and crop residues into soils (Mosier et al. 1998; Williams et al. 2011). Farming operations in the Midwestern US region account for 25 to 33 % of N₂O emissions in the USA every year (Li et al. 1996; Mummey et al. 1998).

Nitrous oxide (N₂O) is produced by multiple biological pathways including microbial nitrification, denitrification, and nitrifier denitrification which can happen simultaneously in the complex soil environment (Wrage et al. 2005; Prasad 2014). For a majority of soils, microbial denitrification is

believed to be the principle pathway of N_2O production, which is favored in anaerobic conditions (Cavigelli and Robertson 2001; Hoffmann et al. 2000) and is regulated by several factors: climate (Kessel et al. 2013), soil pH (Yamamoto et al. 2014; Zaman and Nguyen 2010), soil texture (Gu et al. 2013), organic residue quality (i.e., C/N ratio) (Huang et al. 2004), crop type (Dalal et al. 2003), soil water content (Klemmedtsson et al. 1988), O_2 partial pressure (Ball et al. 2008; Beare et al. 2009; Hernandez-Ramirez et al. 2009), as well as the type and application rate of N-based agricultural inputs (Jarecki et al. 2009; Thornton et al. 1996; Venterea et al. 2005).

Most recent investigations have observed that biochar could be an effective soil amendment to mitigate N_2O emissions (e.g., Van Zwieten et al. 2010; Yanai et al. 2007). Biochars have been found to influence the soil pH (Enders et al. 2012), availability of nitrate (Cayuela et al. 2013), organic carbon (Pendergast-Miller et al. 2011), soil aeration (Kinney et al. 2012), and microbial activity (Lehmann et al. 2011) which could ultimately impact the enzymatic pathways that produce N_2O in soil. The effect of biochar on N_2O emissions may also be influenced by highly heterogeneous soil properties including spatial and temporal variability in microbial communities and anaerobic microsites (Ball 2013; Robertson and Tiedje 1987). There is high variability in properties and behavior of biochars in soil which depend on the parameters of their production process, aging (surface oxidation/alteration), and feedstock type (Spokas 2013; Spokas and Reicosky 2009; Zheng et al. 2013). The spatial variability of soils combined with the complex behavior of biochar materials obscure the understanding of biochar's interference with the denitrification process and its potential to be optimized as agricultural soil amendment (Lehmann et al. 2011).

The study of biochar's influence on denitrification kinetics would help to predict their long-term impact and provide the basis to design biochar as an effective N_2O mitigation tool. However, there have been limited studies into the impact of biochar on the fundamental enzymatic reactions in soil (Cayuela et al. 2013; Lehmann et al. 2011). In this context, this study focused on three objectives: (1) quantify the effect of four biochars (activated corn stover biochar, non-activated corn stover biochar, non-activated ponderosa wood residue biochar, and non-activated switchgrass biochar) and their corresponding biomass feedstocks on cumulative nitrous oxide (N_2O) emissions in two soils from contrasting landscape positions; (2) quantify the effect of water-filled pore space on cumulative N_2O emissions and total denitrification of soils with incorporated biochars and biomass; and (3) determine the kinetic and thermodynamic parameters including half saturation constant (K_s), denitrification enzyme activity (DEA) rate (V_{max}), activation energy (E_a), and enthalpy (ΔH) for the denitrification

enzyme activity in selected soils amended with both biochars and original biomass feedstocks.

Materials and methods

Biomass, biochars, and soils used for incubation studies

Biomass materials (corn stover (CS), ponderosa pine wood residue (WC), and switchgrass (SG)) widely varied by C/N values which ranged from 445 to 61 (Chintala et al. 2014) (Table S1). Biochars were created from corn stover (*Zea mays* L.; designated as CSB), ponderosa pine wood chips (*Pinus ponderosa* Douglas ex Lawson and C. Lawson; WCB), and switchgrass (*Panicum virgatum* L.; SGB). Biochars were produced using carbon optimized gasification, which is a two-stage continuous patent pending process in which the reactor temperature ramps from 150 to 850 °C with residence time of 4 h (Biochar Solutions, Inc, Carbondale, CO, USA). These biochars were highly alkaline (pH ranged from 9.3 to 10.8; water to solid ratio 1:10) with high ash content (ranged from 379 to 652 g kg⁻¹) and C/N values (ranged from 110 to 167) (Table S2) (Chintala et al. 2014). In addition, corn stover biochar was chemically activated by heating at 200 °C the solution of (1:1) biochar with concentrated (12.1 N) hydrochloric acid (Chintala et al. 2013). All biochar materials were homogenized and ground to pass through a 2-mm sieve before use.

Composite soil surface samples (0–15 cm) were collected from the crest and footslope positions of an agricultural landscape (under corn-soybean rotation) which was located near Brookings, SD, USA (44° 12' 36" N and 96° 44' 23.9" W). Soil from the crest position represented the Maddock soil series (sandy, mixed, frigid Entic Hapludolls) and the footslope position represented the Brookings soil series (fine-silty, mixed, superactive, frigid Pachic Hapludolls) (USDA 2005). The footslope soil was a clay loam with pH 6.1, organic matter 34 g kg⁻¹ soil, and nitrate—N 13 mg kg⁻¹ soil. Crest soil was a silt loam with an acidic pH of 5.2, organic matter 16 g kg⁻¹ soil, and nitrate—N 17 mg kg⁻¹ soils (Table S3) (Chintala et al. 2014). These soils were air-dried, crushed, and passed through a 2-mm sieve, then stored at room temperature (25 °C) until used for incubation studies.

Incubations—cumulative N_2O emissions

A soil incubation study was conducted to determine the effect of three biomass feedstocks (corn stover (CS), Ponderosa pine wood chips (WC), and switchgrass (SG)) and their corresponding biochars (activated corn stover biochar (ACB), non-activated corn stover biochar (CSB), non-activated ponderosa pine wood residue biochar (WCB), and non-activated switchgrass biochar (SGB)) at different application

rates on cumulative N_2O emissions over a period of 112 days. The air-dried soil of 40 g was placed into 125-mL serum vials (Wheaton Science Products, Millville, NJ, USA) and sealed with butyl rubber septa. Amendments (biochars and biomass) were applied based on equivalent carbon basis at three rates including 10, 30, and 50 g kg^{-1} . All the serum vials with soil and amendment were pre-incubated for 2 weeks at 60 % water-filled pore space (WFPS) for reactivation of the soil microbial populations. The bulk density of the soils in the serum vials was approximately 1.2 $Mg\ m^{-3}$. These incubations were conducted under aerobic conditions to simulate the actual surface soil conditions in the field. After 2 weeks, an appropriate volume of potassium nitrate solution was added at the rate of 150 mg N kg^{-1} and also to achieve 90 % WFPS in soil-amendment mixture. All the treatments were maintained at 90 % WFPS and temperature of $30 \pm 1\ ^\circ C$ during incubation. Oxygen levels were monitored and did not drop below 15 % in serum vials during the incubation period (Spokas and Reicosky 2009). All the treatments had three replications. Gas samples of 5 mL were taken from the headspace of serum vials on 1, 2, 3, 4, 5, 6, 7, 21, 28, 42, 56, 70, 84, 98, and 112 days after addition of potassium nitrate to soil-amendment mixtures. The gas samples were collected with gas-tight syringes and transferred to helium-flushed and pre-evacuated 20-mL headspace vials. Gas samples were analyzed for nitrous oxide (N_2O) concentration within a week using a gas chromatograph (Schimadzu GC-2014, Japan) with ^{63}Ni -electron capture detector, which was calibrated daily with appropriate traceable gas standards (Scotty Analyzed Gases, Air Liquide America Specialty Gases LLC, Plumsteadville, PA, USA). Inorganic N (NO_3^- -N and NH_4^+ -N) was determined in the soils before and after incubation study by extracting 20 g of soil with 1 M KCl (solid and solution ratio 1:2) using flow injection analysis (Quick Chem FIA+, 8000 series, Lachat Instruments, USA) (Gillam et al. 2008).

Incubations—abiotic N_2O emissions

This experiment was conducted to determine the production of N_2O due to chemo-denitrification (abiotic process). Forty grams of soils of different landscape positions (footslope and crest soils) were placed into triplicate 125-mL serum bottles. These soils were added with appropriate volume of double-distilled water to achieve 70 % WFPS. Biochar (ACB, CSB, WCB, and SGB) and biomass (CS, WC, and SG) were applied to the soil at a rate of 20 g kg^{-1} soil. Serum bottles with treatments were autoclaved twice for 1 h at 121 $^\circ C$ and 103 kPa (Cayuela et al. 2013). The autoclaved serum bottles were cooled overnight. An appropriate volume of sterilized (autoclaved) potassium nitrate solution was injected at 75 mg N kg^{-1} soil and to attain 90 % WFPS. Sterilized serum vials were incubated at $30 \pm 1\ ^\circ C$ for 1 h with oxygen levels $\geq 15\ %$ (Cayuela et al. 2013). The gas samples were collected

from headspace of serum vials to analyze for N_2O concentration using gas chromatography described previously.

Effect of water-filled pore space on cumulative N_2O emissions and total denitrification

To determine the impact of soil water-filled pore space (WFPS) and its interaction effect with amendments on cumulative N_2O emissions and total denitrification potential, the amendments (four biochars and three biomass types) were applied at the rate of 20 g kg^{-1} (2 % w/w air-dried basis) to the soils (40 g) in triplicate 125-mL serum vials. The bulk density of soil and amendment mixtures in serum vials was approximately 1.2 $Mg\ m^{-3}$. The soil-amendment mixtures were pre-incubated for 2 weeks at 40, 70, and 90 % WFPS, temperature of $30 \pm 1\ ^\circ C$, and oxygen levels $\geq 15\ %$ to reduce random greenhouse gas production pulses (Muhr et al. 2008). Following this pre-incubation, potassium nitrate solution was added to serum vials at 100 mg N kg^{-1} soil. Additional distilled water was added to achieve 40, 70, and 90 % water-filled pore space (WFPS) in soil-amendment mixtures. All these serum vials were kept airtight with butyl rubber septa and incubated for a week (oxygen levels $\geq 15\ %$) at temperature of $30 \pm 1\ ^\circ C$ either with or without acetylene (10 % v/v headspace of serum vial). Acetylene (C_2H_2) was added to one set of serum vials with treatments to block the last step of denitrification (conversion of N_2O to N_2) (Weier et al. 1993; Wrage et al. 2001). After 1 week (168 h), gas samples were collected from the incubations for determining the N_2O concentrations. Nitrous oxide (N_2O) concentrations from serum bottles without C_2H_2 treatment were considered as cumulative N_2O emissions. The N_2O concentrations in the C_2H_2 treatments provided the total denitrification potential (i.e., $N_2O + N_2$) (Ryden et al. 1979; Gillam et al. 2008). All the treatments of this study were conducted with three replications.

Effect of biochars and biomass additions on denitrification enzyme activity kinetics

Soils (40 g) were placed into 125-mL serum vials and mixed separately with treatments of biochar (ACB, CSB, WCB, and SGB) and biomass (CS, WC, and SG) at the application rate of 20 g kg^{-1} soil. Soil-amendment mixtures were incubated in serum bottles (oxygen levels $\geq 15\ %$) treated with C_2H_2 (10 % v/v) at 90 % WFPS and $30 \pm 1\ ^\circ C$. Glucose (5 mL of 2 mM glucose solution) and nitrogen (three application rates— KNO_3 solution at 50, 100, and 150 mg N kg^{-1} soil) were added to serum vials. Gas samples were collected for measuring N_2O concentrations (denitrification) from the headspace of serum vials at 0, 24, 48, 64, 88, 112, and 136 h after application of potassium nitrate solution. The kinetic parameters (K_s and V_{max}) for the denitrification enzyme activity (DEA) were determined by linearized plots of Michaelis-

Menten equation (Eq. 1).

$$\text{Michaelis-Menten equation : } V = V_{\max} \times S / (K_s + S) \quad (1)$$

where V =DEA rate, S =nitrate concentration, V_{\max} =DEA rate at saturating levels of S (maximum denitrification potential), and K_s =half saturation constant at $V_{\max}/2$. Lineweaver-Burk, Eadie-Hofstee, and Hanes-Woolf plots were used to model the DEA versus time (Halwachs 1978).

Thermodynamic parameters (activation energy (E_a) and enthalpy of activation (ΔH)) for DEA were calculated at temperatures varying from 10 to 30 °C using the Arrhenius equation (Eq. 2) (Cuhel et al. 2010; Laidler 1987).

$$\text{Arrhenius equation : } k = A \exp(-E_a / RT) \quad (2)$$

$$\text{Enthalpy of activation : } \Delta H = E_a - RT \quad (3)$$

where k =reaction rate constant, A =Arrhenius constant, E_a =activation energy, R =universal gas constant, and T =absolute temperature.

Statistical analyses

Analysis of variance (ANOVA) was performed using the SAS Statistical Package (version 9.2, Raleigh, NC, USA) to

determine statistically significant effects of treatments on denitrification parameters. The significance of treatments was assessed at 95 % confidence level ($\alpha=0.05$). The separation of means was calculated between treatments by a Holm-adjusted Fisher’s LSD test (Holm 1979). Spearman’s rank correlation coefficients were used to determine the relationship between V_{\max}/K_s and DEA.

Results and discussion

Effect of biochars and biomass additions on cumulative N₂O emissions

Application of biochars (ACB, CSB, WCB, and SGB) and their corresponding biomass feedstocks (CS, WC, and SG) at application rates of 10, 30, and 50 g kg⁻¹ soil significantly affected the cumulative N₂O emissions in soils of different landscape positions during the incubation period of 112 days at 90 % WFPS and 30±1 °C (Table 1). The response of cumulative N₂O emissions to the type and application rate of added amendments was varied with soil type. Cumulative N₂O emissions were relatively lower in footslope soil compared to the crest soil. This is not surprising, since inorganic N levels (as primary denitrification regulator) also followed this trend (Table S1). However, previous studies have attributed this enhancement of N₂O emissions due to increased activity of heterotrophic denitrifying bacteria as the availability of organic C and nitrogen substrates increased in soil (Aulakh and Rennie 1987; Weier et al. 1993; Gillam et al. 2008). But in

Table 1 Effect of biochars and their corresponding biomass feedstocks on cumulative N₂O emissions (mg N₂O-N kg⁻¹ soil) and mitigation values (%) in footslope (Brookings) and crest (Maddock) soils for incubation period of 112 days (at 90 % WFPS and 30±1 °C)

	Footslope soil						Crest soil					
	Application rate (g kg ⁻¹ soil)						Application rate (g kg ⁻¹ soil)					
	10		30		50		10		30		50	
	N ₂ O	%	N ₂ O	%	N ₂ O	%	N ₂ O	%	N ₂ O	%	N ₂ O	%
Control	192±8.1 ^a						307±19 ^a					
ACB	137±7.6 ^{dA}	-28	90±2.6 ^{cD}	-53	88±4.3 ^{bD}	-53	227±15 ^{dB}	-26	127±10 ^{dC}	-58	123±5.1 ^{dC}	-59
CSB	102±8.8 ^{fB}	-46	69±1.6 ^{fC}	-64	71±4.1 ^{cC}	-63	144±11 ^{fA}	-52	102±9.1 ^{eB}	-66	106±2.3 ^{eB}	-65
WCB	126±10 ^{eC}	-34	82±7.7 ^{dD}	-57	89±7.2 ^{bD}	-53	218±13 ^{eA}	-28	133±6.5 ^{eB}	-56	124±4.5 ^{dC}	-59
SGB	106±1.4 ^{fB}	-44	73±2.9 ^{eD}	-62	65±8.8 ^{dE}	-65	141±11 ^{fA}	-54	96±5.7 ^{fC}	-68	100±4.0 ^{fC}	-67
CS	186±1.5 ^{bC}	-3	128±8.4 ^{bD}	-33	129±2.7 ^{aD}	-32	260±12 ^{bA}	-15	199±6.8 ^{aB}	-35	202±2.1 ^{bB}	-34
WC	180±2.7 ^{cD}	-6	139±9.6 ^{aE}	-27	135±6.1 ^{aE}	-29	247±14 ^{cA}	-19	187±3.0 ^{bC}	-38	192±3.7 ^{eB}	-37
SG	188±3.2 ^{bC}	-2	135±5.3 ^{aD}	-29	132±1.4 ^{aD}	-31	269±14 ^{bA}	-12	191±6.9 ^{bC}	-37	211±6.2 ^{aB}	-31

Each value is mean of triplicate incubations shown with standard error. Significant differences by Holm-adjusted Fisher’s LSD test at $\alpha=0.05$ within a row are represented by uppercase letters, and lowercase letters indicate the within in a column

ACB activated corn stover biochar, CSB corn stover biochar, WCB ponderosa pine wood biochar, SGB switchgrass biochar, CS corn stover, WC ponderosa pine wood residue, SG switchgrass

this study, the low pH of crest soil (5.20) may also reduce the activity of N_2O -reductase and increase N_2O emissions (Flessa et al. 1998). Moreover, the fine texture (clay loam) and higher organic matter of footslope soil may have higher surface interaction with N substrates and also affect the diffusivity of N_2O in the soil.

Incorporation of biochars and their biomass feedstocks reduced the cumulative N_2O emissions in both soils (Table 1). In the footslope position, the biochars mitigated the cumulative N_2O emissions to an extent ranging from 28 to 65 % compared to non-treated soils at all application rates. The incorporation of biomass feedstocks also had a significant mitigation effect (ranging from 27 to 33 %) at application rates of 30 and 50 g kg^{-1} soil. In the crest soil, the incorporation of biochars reduced the cumulative N_2O emissions ranging from 26 to 68 %. Whereas the incorporation of biomass feedstocks mitigated the N_2O emissions ranging from 12 to 38 % of the observed N_2O emissions. However, there were no significant difference between application rates of both biochars and biomass feedstocks. The chemically activated corn stover biochar (ACB) was less effective in reducing N_2O production than the non-activated biochars (CSB, WCB, and SGB). This could be attributed to the oxidation of the surface moieties during the acid activation. However, since the biochar was activated with HCl, would have resulted with an acidic biochar which suggested that pH alterations alone do not fully explain biochar's N_2O mitigation potential.

At the start of the incubation study, the inorganic N content (NO_3^- -N and NH_4^+ -N) was significantly higher with biochar and biomass treatments in both footslope and crest soils (Figure S1). The ratio of final inorganic N concentrations per total initial inorganic N (soil inorganic N+added inorganic N due to amendment) varied significantly at the end of incubation study (after 112 days) with different application rates of amendments (Fig. 1). In footslope soil, the application of biochar and biomass significantly increased the ratio of final inorganic N per total initial inorganic N. There was the suggestion of increased soil inorganic N and lower N_2O production rates higher amendment rates, but this trend was not consistent as the application rate increased above 10 g kg^{-1} soil. Whereas in crest soil, the ratio of final inorganic N per total initial inorganic N was significantly enhanced with the incorporation of biochars and biomass to soil. The final inorganic N that remained in crest soil was correlated to the amendment rate. Overall, biochar treatments showed a higher ratio of final inorganic N per total initial inorganic N compared to biomass treatments in both soils.

Several previous studies have documented the ability of various biochars to offset N_2O emissions from soils (Case et al. 2012; Cayuela et al. 2013; Rondon et al. 2007; Scheer et al. 2011; Spokas and Reicosky 2009; Yanai et al. 2007). In this study, the potential mitigation of cumulative N_2O emissions with the incorporation of these high pyrolytic

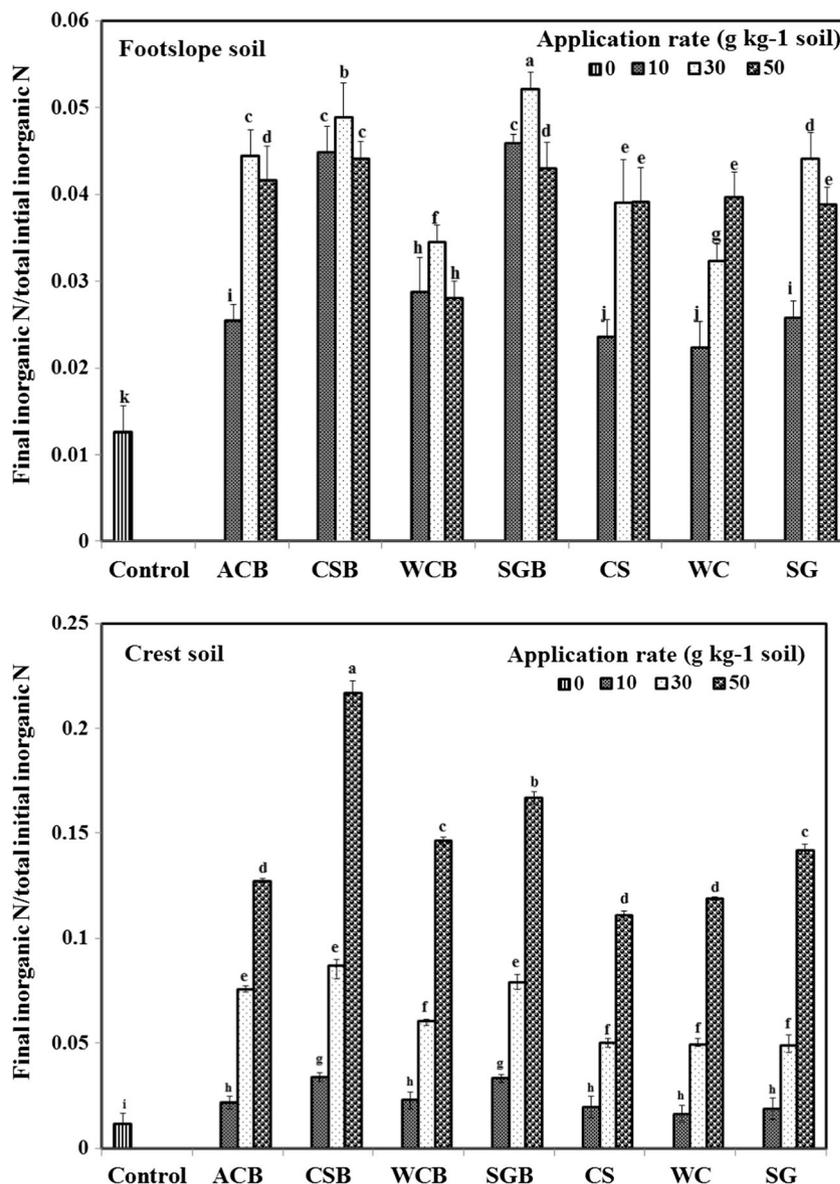
temperature biochars could be due to an increase of pH (liming effect) in the vicinity of biochar particles and therefore improving the function of N_2O reductase independent of soil pH (Cayuela et al. 2013; Firestone et al. 1980). Previously, these biochars showed negative priming effects on the microbial activity and mineralization of C and N (Chintala et al. 2014). Biochar particles can also exhibit strong competition to sequester the redox couples including the inorganic N substrates (electron sink) and labile soil C (electron donor) which may affect the heterotrophic microbial activity and denitrification reaction in soil (Cayuela et al. 2013). Biochars in this study exhibited higher nitrate removal, potentially due to their large variable charge potential (Chintala et al. 2013).

The addition of biomass feedstocks to soils also showed the reduced effect on N_2O emissions in this study which could be due to their ability to immobilize inorganic N and reduce mineralization (Baggs et al. 2000; De Hao et al. 2001; Velthof et al. 2002; Rahn et al. 2003; De Neve et al. 2004). The mitigation effects of biochars and biomass feedstocks were higher in the crest soil compared to the footslope. Landscape differences could be attributed to the reduction of competition to retain redox couples from native soil organic C and less vulnerable to microbial reduction. In this study, the production of N_2O was only due to biotic processes mediated by microbial functional groups (Table S4). Overall, the incorporation of biochars had relatively higher mitigation effects on cumulative N_2O emissions in both soils compared to biomass additions. The mitigation effect of biochars also varied as the soil characteristics (texture, organic C, and inorganic N) changed due to spatial variability of erosional processes across the landscape.

Effect of water-filled pore space on cumulative N_2O emissions and total denitrification

Cumulative N_2O emissions and total denitrification (during 168 h of incubation) were significantly affected by as the water-filled pore space (WFPS) (soil water content) increased from 40 to 90 % in soils of both slope positions (Fig. 2). Cumulative N_2O emissions and total denitrification were very low at 40 and 70 % WFPS. In the crest soil, cumulative N_2O emissions and total denitrification were relatively higher compared to the footslope soil at 90 % WFPS. This might be due to relatively higher availability of inorganic N for denitrification process as the supply of organic C is optimized despite the differences in topographic position. There was no significant effect of biochars (ACB, CB, WB, and SB) and biomass additions (CS, WC, and SG) on cumulative N_2O emissions ($P < 0.05$) and total denitrification at 40 % WFPS at both positions, suggesting a soil moisture potential limitation to microbial activity (Aulakh et al. 1991; Weier et al. 1993). On the other hand, incorporation of biochars reduced cumulative N_2O emissions and total denitrification at 70 and 90 % WFPS.

Fig. 1 Inorganic N per total initial inorganic N in crest soil and footslope soil with biochar and biomass treatments at the end of the incubation study for 112 days at 90 % WFPS and 30±1 °C. Each value is mean of triplicate incubations shown with standard error. Significant differences by Holm-adjusted Fisher’s LSD test at $\alpha=0.05$. Letters indicate the significant differences between the mean values



The biomass additions at 90 % WFPS showed a small increase in cumulative N₂O emissions and total denitrification but not significantly at 70 % WFPS in both soils. Soil water content (WFPS) regulated the trends of cumulative N₂O emissions and total denitrification irrespective of treatments (biochar and biomass) and soil locations in this study. The significant increase in cumulative N₂O emissions and total denitrification may be due to diffusion of dissolved organic C and inorganic N as substrates for anaerobic microbial activity as the WFPS (reduced O₂ partial pressure) increased (Stark and Firestone 1995; Dobbie and Smith 2001; Porporato et al. 2003; Rivett et al. 2008; Jahangir et al. 2012). The reduction in partial pressure of O₂ may also increase the demand for inorganic N substrates as a terminal electron acceptor (De Klein and Van Logtestijn 1996). The WFPS regulates the trends for cumulative N₂O emission, but it is dependent on both treatments,

with more pore pronounced effect with biochars, and also on soil type, where a bigger effect was observed for footslope soil.

The ratio of N₂O to (N₂O+N₂) was also significantly increased with WFPS in footslope soil, but these ratios were not significantly different at 40 and 70 % WFPS in the crest soils (Fig. 2). The N₂O/(N₂O+N₂) ratios were reduced by biochar treatments at 70 and 90 % WFPS in both soils. The supply of electron acceptors may be diminished in the presence of biochar, which alters the activity of microbial denitrifiers. Alternatively, the presence of biochar may have helped shuttle electrons more effectively to denitrifiers, fostering the complete reduction of nitrate to dinitrogen (Cayuela et al. 2013). The liming effect of biochar treatments may also lead to reduced N₂O/(N₂O+N₂) ratios due to changes in the denitrification rates (Arah, and Smith 1991; Stevens and Laughlin

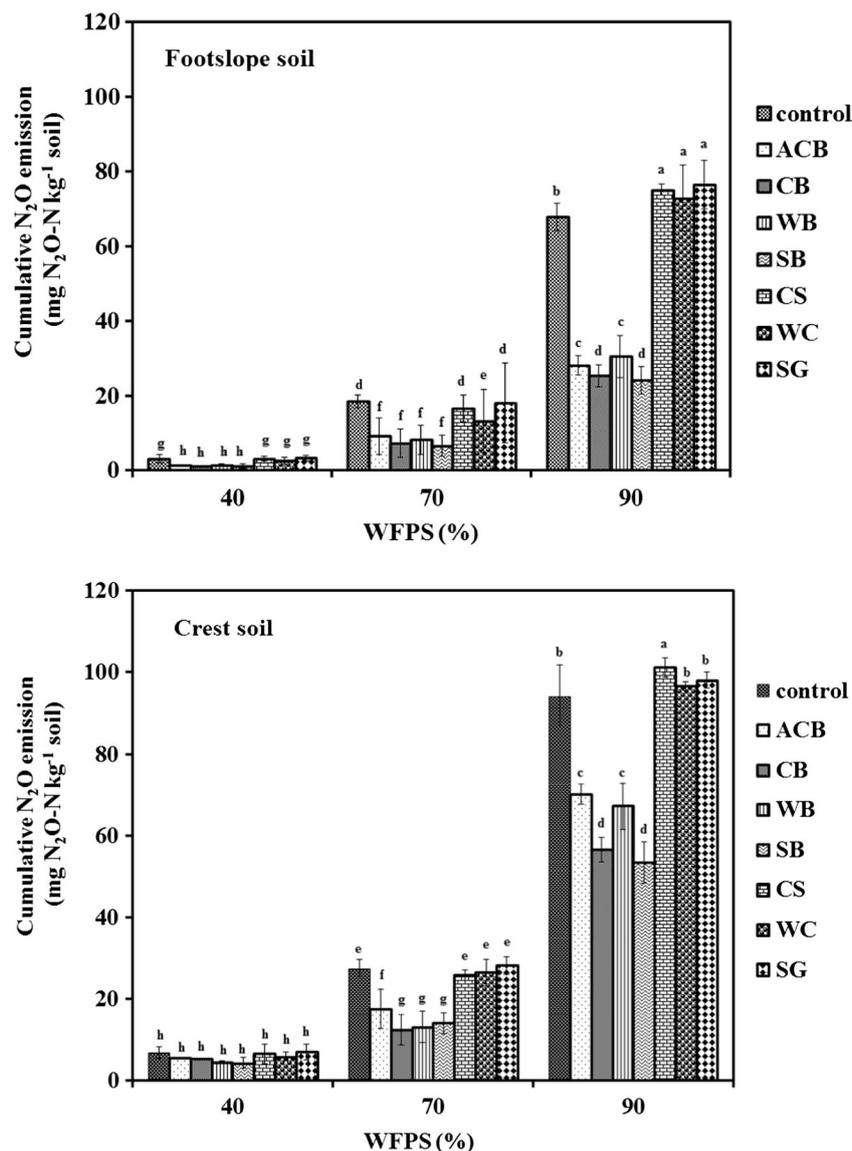


Fig. 2 Effect of water-filled pore space (WFPS) on cumulative N_2O emissions, total denitrification, and $N_2O/(N_2O+N_2)$ in footslope soil and crest soil amended with biochar (ACB, CSB, WCB, and SGB) and biomasses (CS, WC, and SG) during incubation period of 168 h at $30 \pm$

$1^\circ C$. Each value is mean of triplicate incubations shown with standard error. Significant differences by Holm-adjusted Fisher's LSD test at $\alpha=0.05$. Letters indicate the significant differences between the mean values

1998; Cuhel et al. 2010; Van Zwieten et al. 2010). On the other hand, biomass additions increased the $N_2O/(N_2O+N_2)$ ratios at 70 and 90 % WFPS in both soils but not significantly when compared to control. In this study, the $N_2O/(N_2O+N_2)$ ratios were ≥ 0.7 at 70 and 90 % WFPS suggesting N_2O was the dominant product of the denitrification process (Ruser et al. 2006; Burton et al. 2008). The denitrification becomes the dominant process over nitrification at higher WFPS (Wrage et al. 2001; Bateman and Baggs 2005). The $N_2O/(N_2O+N_2)$ ratios were also influenced by soil location, biochar type, and biomass additions during these laboratory incubations ($P < 0.05$).

Influence of biochars and biomass additions on denitrification enzyme kinetics

The effect of soil type and amendments (biochars and biomass additions) on denitrification enzyme activity (DEA) kinetics was reported for $30 \pm 1^\circ C$ and 90 % WFPS during an incubation period of 168 h (Table 2). These measured rate constants represent the DEA rates expected in surface soil environment (at oxygen levels $\geq 15\%$) in which both aerobic and anaerobic processes can occur at the same time as a function of anaerobic microsite distribution (Sextone et al. 1985). Denitrification enzyme activity (DEA) was increased as the application rate of N increased from 50 to 100 mg N kg^{-1} in all

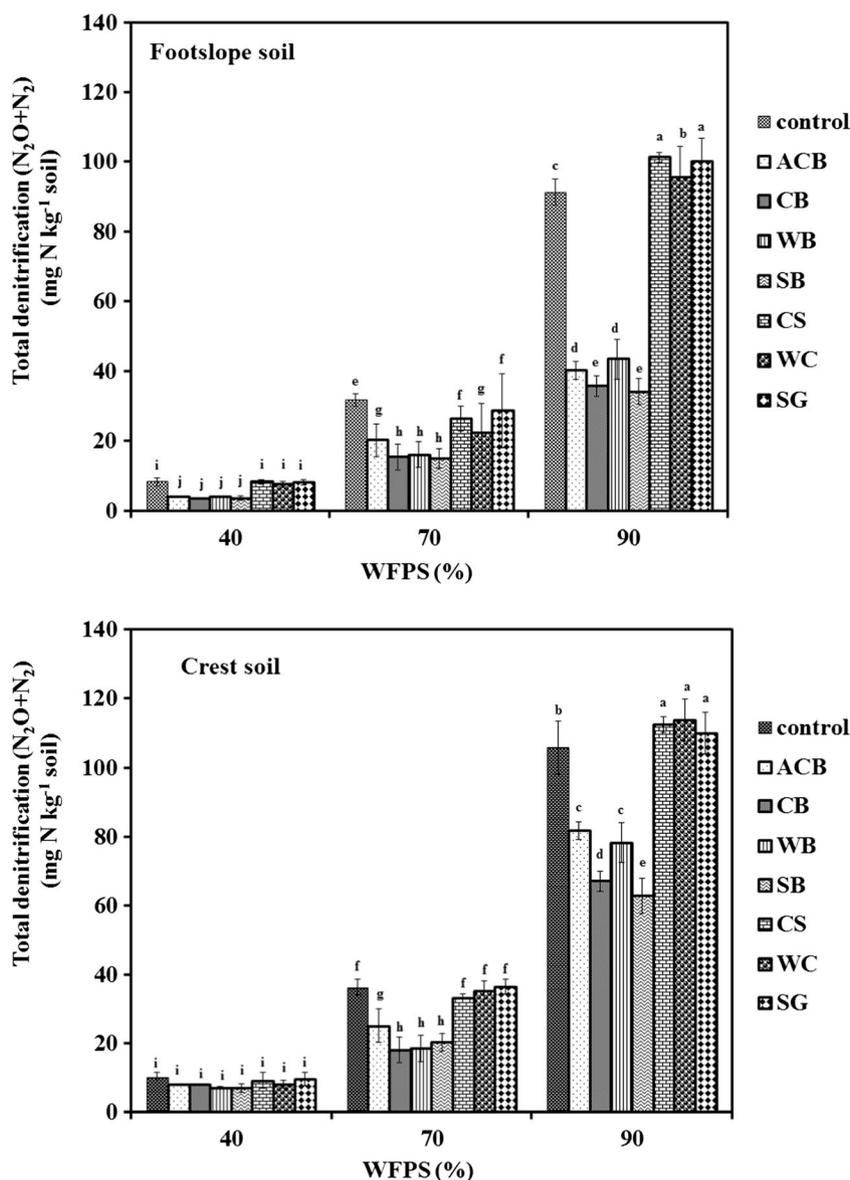


Fig. 2 continued.

treatments. But there was no significant difference between 100 and 150 mg N kg⁻¹. The values of K_s and V_{max} of the DEA were higher in the footslope soil than the crest soil, which may be related to the abundance of labile C at the footslope which can induce the positive priming effect in relatively short time to support the growth of the denitrifier community. The incorporation of biochars (ACB, CSB, WCB, and SGB) significantly reduced the K_s and V_{max} values in soils of both landscape positions. The biomass additions also reduced DEA rates, but not as great as the biochars. The low K_s in the biochar treatments suggest that there is a higher chemical affinity for inorganic-N substrate and enzymatic inhibition which may reduce the DEA rate (V_{max}). These patterns of rate constants were also supported by the trends in thermodynamic parameters (activation energy (E_a) and

enthalpy of activation (ΔH) of DEA which were calculated based on its positive response as the soil temperature increased from 10 to 30 °C at 90 % WFPS. The activation energy (E_a) and enthalpy (ΔH) of DEA were increased with the incorporation of biochars. On the other hand, the addition of biomass materials did not show any significant effect on E_a and ΔH of DEA in soils (Table 2). The increase in E_a and ΔH values of soils amended with biochars indicated the increase in amount of energy required for the denitrification process. In other words, it takes more energy for the microbes to produce N₂O in biochar-amended soil suggesting a decrease in the nitrate availability, which was observed in the final extraction values. The rate constants (K_s and V_{max}) were inversely correlated to the energies of reactions (E_a and ΔH) as a function of soil and biochar type.

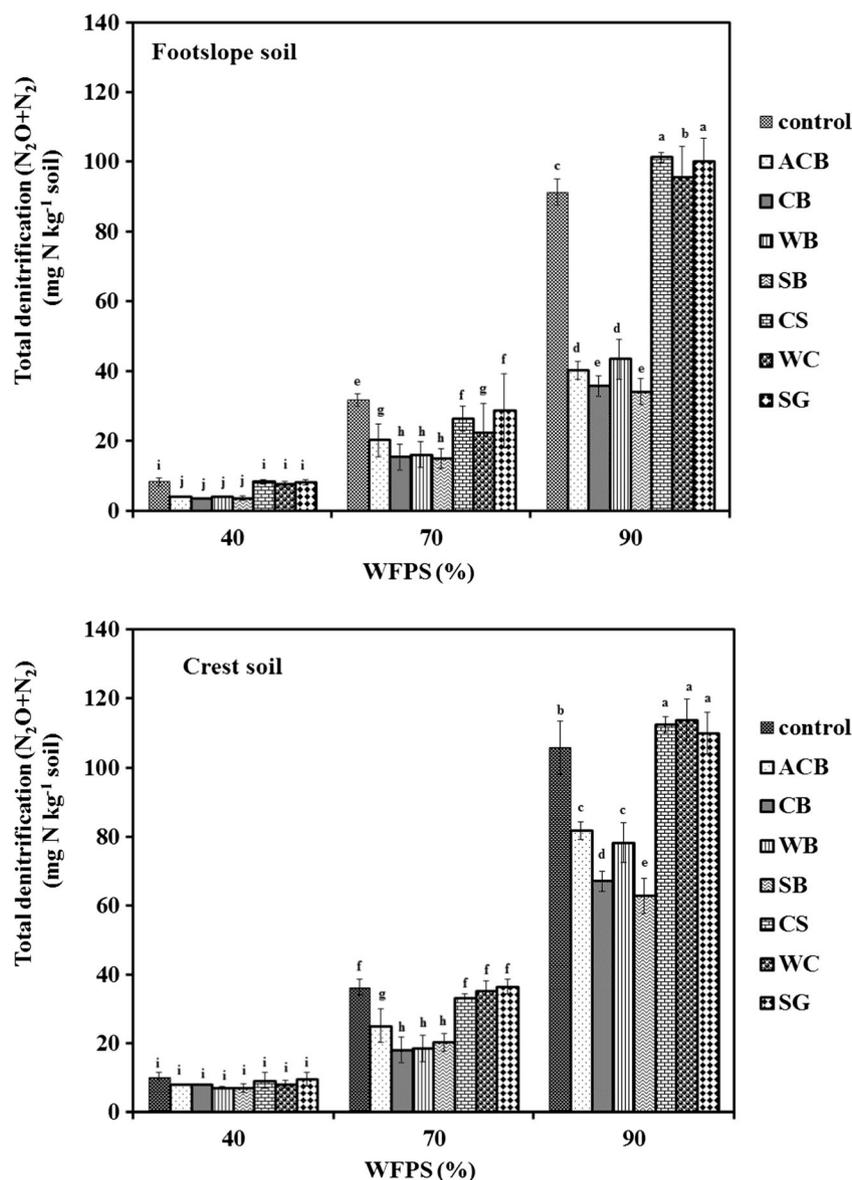


Fig. 2 continued.

The rate constant V_{\max}/K_s values were plotted against DEA of biochar and biomass treatments in both soils (Fig. 3). There was a significant negative correlation ($P < 0.05$) between the ratio of the rate constants (V_{\max}/K_s) and DEA values ($r_s = -0.76$). Therefore, the V_{\max}/K_s values of amendments (biochars and biomass additions) indicate their mitigating capacity by governing the DEA kinetics. Higher V_{\max}/K_s values of the amendments indicate a higher capacity to reduce the rate of denitrification process. The V_{\max}/K_s values of biochar and biomass treatments were given by soil position in Fig. 4. The incorporation of biochars yielded relatively higher V_{\max}/K_s values of DEA in the crest position compared to footslope which was correlated with the previously observed higher mitigation effect on cumulative denitrification. This observation indicates higher mitigation effect of biochars on

denitrification rate in the crest soil compared to footslope soil. The V_{\max}/K_s values of DEA in biomass treatments (CS, WC, and SG) were inconsistent across locations and also did not possess significant differences with the corresponding control treatments. Herbaceous biochars (CSB and SGB) showed relatively higher V_{\max}/K_s values of DEA compared to wood species biochar (WCB) in the crest soil, but it was not significant in the footslope soil.

Conclusions

Cumulative laboratory N_2O emissions were significantly reduced by the incorporation of biochars and biomass to soils

Table 2 Half-saturation constant (K_s) and maximum rate (V_{max}), activation energy (E_a), and enthalpy of activation (ΔH) for denitrification enzyme activity (DEA) in footslope (Brookings) and crest (Maddock) soils amended with biochars and corresponding biomass feedstocks

	Footslope soil				Crest soil			
	K_s	V_{max}	E_a	ΔH	K_s	V_{max}	E_a	ΔH
Control	29.1±5 ^a	1.30±0.02 ^a	93.1±4 ^b	90.7±5 ^c	28.3±4 ^a	1.30±0.04 ^a	87.2±3 ^d	84.8±4 ^d
ACB	7.90±0.8 ^d	0.42±0.006 ^c	94.5±3 ^b	92.1±3 ^b	5.21±1 ^c	0.30±0.001 ^c	93.5±5 ^c	91.1±3 ^c
CSB	5.70±0.3 ^f	0.30±0.005 ^d	97.6±5 ^a	95.3±3 ^a	3.06±0.7 ^f	0.28±0.003 ^c	103±8 ^b	101±4 ^b
WCB	6.10±0.5 ^e	0.31±0.002 ^d	94.1±5 ^b	91.8±4 ^b	3.73±0.3 ^f	0.26±0.002 ^c	118±5 ^a	115±6 ^a
SGB	5.20±0.3 ^f	0.28±0.002 ^d	97.7±4 ^a	95.4±5 ^a	3.51±0.4 ^f	0.26±0.002 ^c	103±6 ^b	101±5 ^b
CS	22.7±5 ^b	0.96±0.008 ^b	92.0±3 ^b	89.6±2 ^d	19.1±3 ^d	0.86±0.005 ^b	88.1±4 ^d	85.7±3 ^d
WC	20.1±3 ^c	0.91±0.005 ^b	91.6±4 ^b	89.2±2 ^d	21.5±3 ^b	0.97±0.004 ^a	86.3±5 ^d	83.9±2 ^d
SG	21.8±2 ^b	0.96±0.004 ^b	92.4±6 ^b	90.5±4 ^c	17.9±2 ^c	0.82±0.005 ^b	88.5±2 ^d	86.1±2 ^d

Letters indicate the statistical significance

Units: K_s mg N L⁻¹, V_{max} μg N cm⁻³ h⁻¹, E_a kJ mole⁻¹, ΔH kJ mole⁻¹. K_s and V_{max} were calculated at 30±1 °C and 90 % WFPS. E_a and ΔH were calculated for denitrifying enzyme activity (DEA) as it responded to the increase of soil temperature from 10 to 30 °C at 90 % WFPS and application rate of 50 mg N kg⁻¹

ACB amended com stover biochar, CSB corn stover biochar, WCB ponderosa pine wood biochar, SGB switchgrass biochar, CS corn stover, WC ponderosa pine wood residue, SG switchgrass biochar

collected from different landscape positions. The biochar mitigating effect could be due to their ability to interfere with soil N transformation pathways; in particular, the results here suggested the increased activation energy is one key mechanism. Water-filled pore space (WFPS) was found to have a significant impact on the ratio of cumulative N₂O emissions and total denitrification which was also observed by several studies in the past. The higher ratios of cumulative N₂O emissions and total denitrification were observed at 90 % WFPS in both soils which might be due to instigation of heterotrophic microbial activity in presence of increased availability of redox couples (labile organic C and inorganic N) for nitrification and denitrification processes. Denitrification enzyme activity rate (kinetic rate constants, K_s and V_{max}) was reduced by biochar additions. Kinetic rate constants of

DEA were relatively higher in the footslope soil compared to the crest soil which was reverse of the trend observed in cumulative N₂O emissions and total denitrification activity, which suggests the differences in efficiency of the enzymatic processes as a function of landscape position. The trends in DEA were supported by the similar trends of thermodynamic parameters (E_a and ΔH) which were calculated based on the response of DEA to soil temperature. The rate constant V_{max}/K_s appeared to indicate the capacity of biochars to mitigate the denitrification process. These denitrification kinetic parameters determined for amendments (biochars and biomass additions in this study) in short-term studies indicate a change in the rates of the underlying

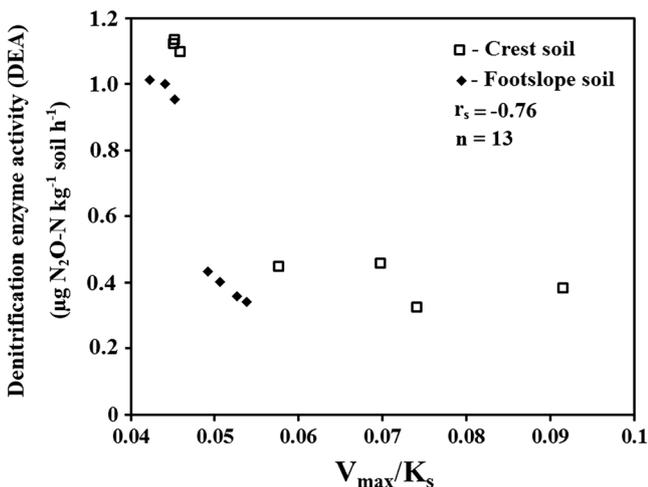


Fig. 3 Relationship between DEA and kinetic rate constant V_{max}/K_s in footslope and crest soils amended with biochar and biomasses. Each value is mean of triplicate incubations

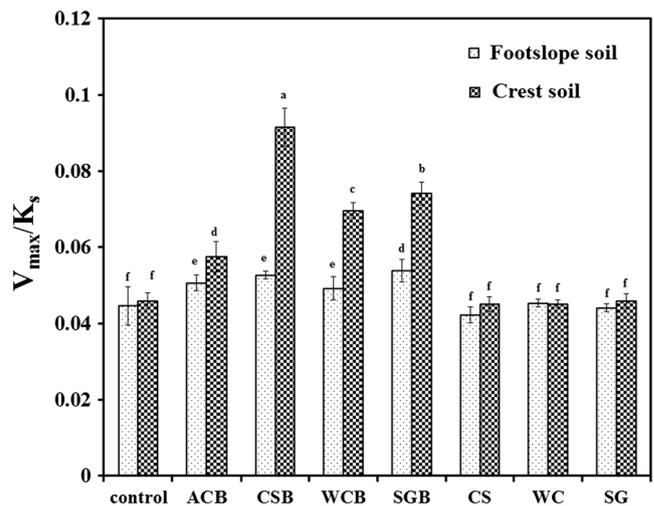


Fig. 4 Denitrification enzyme activity (DEA) rate constants of biochars and biomass treatments in footslope and crest soils at 30±1 °C and 90 % WFPS. Each value is mean of triplicate incubations shown with standard error. Significant differences by Holm-adjusted Fisher’s LSD test at $\alpha=0.05$. Letters indicate the significant differences between the mean values

mechanisms. Additional kinetic studies of the biochar materials are vital to assess their economic and environmental feasibility as soil amendments and also help to design their functionality through synthetic pathways.

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References

- Arah JRM, Smith KA (1991) Nitrous oxide production and denitrification in Scottish arable soils. *J Soil Sci* 42:351–367
- Aulakh MS, Rennie DA (1987) Effect of wheat straw incorporation on denitrification of N under anaerobic and aerobic conditions. *Can J Soil Sci* 67:825–834
- Aulakh MS, Doran JW, Walters DT, Power JF (1991) Legume residue and soil water effects on denitrification in soils of different textures. *Soil Biol Biochem* 23:1161–1167
- Baggs EM, Rees RM, Smith KA, Vinten AJA (2000) Nitrous oxide emission from soils after incorporating crop residues. *Soil Use Manag* 16:82–87
- Ball BC (2013) Soil structure and greenhouse gas emissions: a synthesis of 20 years of experimentation. *Eur J Soil Sci* 64:357–373
- Ball BC, Crichton I, Horgan GW (2008) Dynamics of upward and downward N₂O and CO₂ fluxes in ploughed or no-tilled soils in relation to water filled pore space, compaction and crop presence. *Soil Tillage Res* 101:20–30
- Bateman EJ, Baggs EM (2005) Contributions of nitrification and denitrification to N₂O emissions from soils at different water-filled pore space. *Biol Fertil Soils* 41:379–388
- Beare MH, Gregorich EG, St-Georges P (2009) Compaction effects on CO₂ and N₂O production during drying and rewetting of soil. *Soil Biol Biochem* 41:611–621
- Burton DL, Zebarth BJ, Gillam KM, Macleod JA (2008) Effect of split application of fertilizer nitrogen on N₂O emissions from potatoes. *Can J Soil Sci* 88:229–239
- Case SDC, McNamara NP, Reay DS, Whitaker J (2012) The effect of biochar addition on N₂O and CO₂ emissions from a sandy loam soil—the role soil aeration. *Soil Biol Biochem* 51:125–134
- Cavigelli MA, Robertson GP (2001) Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. *Soil Biol Biochem* 33:297–310
- Cayuela ML, Sanchez-Monedero MA, Roig A, Hanley K, Enders A, Lehmann J (2013) Biochar and denitrification in soils: when, how much and why does biochar reduce N₂O emissions? *Nat Sci Rep* 3(1732)
- Chintala R, Mollinedo J, Schumacher TE, Malo DD, Papiernik S, Clay DE, Kumar S, Gulbrandson W (2013) Nitrate sorption and desorption by biochars produced from microwave pyrolysis. *Microporous Mesoporous Mater* 179:250–257
- Chintala R, Schumacher TE, Kumar S, Malo DD, Rice J, Bleakley B, Chilom G, Papiernik S, Julson JL, Clay DE, Gu ZR (2014) Molecular characterization of biochar materials and their influence on microbiological properties of soil. *J Hazard Mater* 279:244–256
- Cuhel J, Simek M, Laughlin RJ, Bru D, Cheneby D, Watson CJ, Phillippot L (2010) Insights into the effect of soil pH on N₂O and N₂ emissions and denitrifier community size and activity. *Appl Environ Microbiol* 76:1870–1878
- Dalal RC, Wang WJ, Robertson GP, Parton WJ (2003) Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. *Aust J Soil Res* 41:165–195
- De Klein CAM, Van Logtestijn RSP (1996) Denitrification in grassland soils in the Netherlands in relation to irrigation, N-application rate, soil water content and soil temperature. *Soil Biol Biochem* 28:231–237
- De Neve SSGS, Chaves Daguilar B, Hofman G (2004) Managing N release from high-N crop residues using on- and off-farm organic materials. *Soil Biol Biochem* 36:127–134
- Dobbie KE, Smith KA (2001) The effects of temperature, water-filled pore space and land use on N₂O emissions from an imperfectly drained gleysol. *Eur J Soil Sci* 52:667–673
- Enders A, Hanley K, Whitman T, Joseph S, Lehmann J (2012) Characterization of biochars to evaluate recalcitrance and agronomic performance. *Bioresour Technol* 114:644–653
- Firestone MK, Firestone RB, Tiedje JM (1980) Nitrous oxide from soil denitrification: factors controlling its biological production. *Science* 208:749–751
- Flessa H, Wild U, Klemisch M, Pfadenhauer J (1998) Nitrous oxide and methane fluxes from organic soils under agriculture. *Eur J Soil Sci* 49:327–335
- Gillam KM, Zebarth BJ, Burton DL (2008) Nitrous oxide emissions from denitrification and the partitioning of gaseous losses as affected by nitrate and carbon addition and soil aeration. *Can J Soil Sci* 88:133–143
- Gu J, Nicoulaud B, Rochette P, Grossel A, Hénault C, Cellier P, Richard G (2013) A regional experiment suggests that soil texture is a major control of N₂O emissions from tile-drained winter wheat fields during the fertilization period. *Soil Biol Biochem* 60:134–141
- Halwachs W (1978) KM and Vmax from only one experiment. *Biotechnol Bioeng* 20:281–285
- Hao X, Chang C, Carefoot JM, Janzen HH, Ellert BH (2001) Nitrous oxide emissions from an irrigated soil as affected by fertilizer and straw management. *Nutr Cycl Agroecosyst* 60:1–8
- Hernandez-Ramirez G, Brouder SM, Smith DR, Van Scoyoc GE, Michalski G (2009) Nitrous oxide production in an eastern corn belt soil: sources and redox range. *Soil Sci Soc Am J* 73:1182–1191
- Hoffmann CC, Rysgaard S, Berg P (2000) Denitrification rates predicted by nitrogen-15 labeled nitrate microcosm studies, in-situ measurements, and modelling. *J Environ Qual* 29:2020–2028
- Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Stat* 6:65–70
- Huang Y, Zou J, Zheng X, Wang Y, Xu X (2004) Nitrous oxide emissions as influenced by amendment of plant residues with different C:N ratios. *Soil Biol Biochem* 36:973–981
- IPCC (2007) 2006 IPCC guidelines for national greenhouse gas inventories, National Greenhouse Gas Inventories Programme, Hayama, Japan
- Jahangir MMR, Khalil MI, Johnston P, Cardenas LM, Hatch DJ, Butler M, Barrett M, O’Flaherty V, Richards KG (2012) Denitrification potential in subsoils: a mechanism to reduce nitrate leaching to groundwater. *Agric Ecosyst Environ* 147:13–23
- Jarecki MK, Parkin TB, Chan ASK, Kaspar TC, Moonman TB, Singer JW, Kerr BJ, Hatfield JL, Jones R (2009) Cover crop effects on nitrous oxide emission from a manure-treated Mollisol. *Agric Ecosyst Environ* 134:29–35
- Kessel C, Venterea R, Six J, Adviento-Borbe MA, Linquist B, Groenigen KJ (2013) Climate, duration, and N placement determine N₂O emissions in reduced tillage systems: a meta-analysis. *Glob Chang Biol* 19:33–44
- Kinney TJ, Masiello CA, Dugana B, Hockaday WC, Deana MR, Zygourakis K, Barnes RT (2012) Hydrologic properties of biochars produced from different temperatures. *Biomass Bioenergy* 41:34–43
- Klemmedtsson L, Svensson BH, Rosswall T (1988) Relationships between soil moisture content and nitrous oxide production during nitrification and denitrification. *Biol Fertil Soils* 6:106–111

- Laidler KJ (1987) Chemical kinetics. Harper & Row, New York
- Lehmann J, Rillig MC, Thies J, Masiello CA, Hockaday WC, Crowley D (2011) Biochar effects on soil biota—a review. *Soil Biol Biochem* 43:1812–1836
- Li C, Narayanan V, Hariss R (1996) Model estimates of nitrous oxide emissions from agricultural lands in the United States. *Glob Biogeochem Cycles* 10:297–306
- Moraghan JT, Buresh RJ (1977) Chemical reduction of nitrite and nitrous oxide by ferrous iron. *Soil Sci Soc Am J* 41:47–50
- Mosier AR, Kroeze C, Nevison C, Oenema O, Seitzinger SP, van Cleemput O (1998) Closing the global N₂O budget: nitrous oxide emissions through the agricultural nitrogen cycle. *Nutr Cycl Agroecosyst* 52:225–248
- Muhr J, Goldberg SD, Borken W, Gebauer G (2008) Repeated drying-rewetting cycles and their effects on the emission of CO₂, N₂O, and CH₄ in a forest soil. *J Plant Nut Soil Sci* 171:719–728
- Mummey D, Smith J, Bluhm G (1998) Assessment of alternative soil management practices on N₂O emissions from U.S. agriculture. *Agric Ecosyst Environ* 70:79–87
- Paustian K (2004) Agricultural mitigation of greenhouse gases: science and policy options. In: Council on Agricultural Science and Technology (CAST) p120
- Pendegast-Miller MT, Duvall M, Sohi SP (2011) Localization of nitrate in the rhizosphere of biochar-amended soils. *Soil Biol Biochem* 43:2243–2246
- Peterson SO, Regina K, Pollinger A, Rigler E, Valli L, Yamulki S, Esala M, Fabbri C, Syvasalo E, Vinther FP (2006) Nitrous oxide emissions from organic and conventional crop rotations in five European countries. *Agric Ecosyst Environ* 112:200–206
- Porporato A, Odorico PD, Laio F, Rodriguez-Iturbe I (2003) Hydrologic controls on soil carbon and nitrogen cycles. I. Modelling scheme. *Adv Water Resour* 26:45–58
- Prasad R (2014) Using nitrogen and phosphorus budgets as effective tools for assessing nitrogen and phosphorus losses from agricultural systems. Ph.D. Diss., Univ. of Florida, Gainesville
- Rahn C, Bending GD, Turner MK, Lillywhite R (2003) Management of N mineralization from crop residues of high N content using amendment materials of varying quality. *Soil Use Manag* 19:193–200
- Ravishankara AR, John SD, Robert WP (2009) Nitrous oxide (N₂O): the dominant ozone-depleting substance emitted in the 21st century. *Science* 326:123–125
- Rivett MO, Buss SR, Morgan P, Smith JWN, Bemment CD (2008) Nitrate attenuation in groundwater: a review of biogeochemical controlling processes. *Adv Water Resour* 42:4215–4232
- Robertson GP, Tiedje JM (1987) Nitrous oxide sources in aerobic soils: nitrification, denitrification and other biological processes. *Soil Biol Biochem* 19:187–193
- Rondon MA, Lehmann J, Ramirez J, Hurtado M (2007) Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol Fertil Soils* 43:699–708
- Ruser R, Flessa H, Schilling R, Beese F, Munch J (2006) Effect of crop-specific field management and N fertilization on N₂O emissions from fine-loamy soil. *Soil Biol Biochem* 38:263–274
- Ryden JC, Lund LJ, Focht DD (1979) Direct measurement of denitrification losses from soils: I. Laboratory evaluation of acetylene inhibition of nitrous oxide reduction. *Soil Sci Soc Am J* 43:104–110
- Samarkin VA, Madigan MT, Bowles MW, Casciotti KL, Priscu JC, McKay CP, Joye SB (2010) Abiotic nitrous oxide emission from the hypersaline Don Juan Pond in Antarctica. *Nat Geosci* 3:341–344
- Scheer C, Grace P, Rowlings D, Kimber S, Van Zwieten L (2011) Effect of biochar amendment on the soil-atmosphere exchange of greenhouse gases from an intensive subtropical pasture in northern New South Wales, Australia. *Plant Soil* 345:47–58
- Sextone AJ, Revsbech NP, Parkin TB, Tiedje JM (1985) Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Sci Soc Am J* 49:645–651
- Spokas KA (2013) Impact of biochar field aging on laboratory greenhouse gas production potentials. *GCB Bioenergy* 5:165–176
- Spokas KA, Reicosky DC (2009) Impacts of sixteen different biochars on soil greenhouse gas production. *Ann Environ Sci* 3:179–193
- Stark JM, Firestone MK (1995) Mechanisms for soil-moisture effects on the activity of nitrifying bacteria. *Appl Environ Microbiol* 61:218–220
- Stevens RJ, Laughlin RJ (1998) Measurement of nitrous oxide and dinitrogen emissions from agricultural soils. *Nutr Cycl Agroecosyst* 52:131–139
- Thomton FC, Bock BR, Tyler DD (1996) Soil emissions of nitric oxide and nitrous oxide from injected anhydrous ammonia and urea. *Soil Sci Soc Am J* 25:1378–1384
- U.S. Department of Agriculture (2005) Natural Resources Conservation Service (2005) National Soil Survey Handbook, Title 430 - VI, U.S. Department of Agriculture, Natural Resources Conservation Service, Washington, DC
- Van Zwieten L, Kimber S, Morris S, Downie A, Berger E, Rust J, Sheer C (2010) Influence of biochars on flux of N₂O and CO₂ from ferrosol. *Aust J Soil Res* 48:555–568
- Velthof GL, Kuikman P, Oenema O (2002) Nitrous oxide emission from soils amended with crop residues. *Nutr Cycl Agroecosyst* 62:249–261
- Venterea RT, Burger M, Spokas KA (2005) Nitrogen oxide and methane emissions under varying tillage and fertilizer management. *J Environ Qual* 34:1467–1477
- Weier KL, Doran JW, Power JF, Walters DT (1993) Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Sci Soc Am J* 57:66–72
- Williams JD, Long DS, Wuest SB (2011) Capture of plateau runoff by global positioning system-guided seed drill operation. *J Soil Water Conserv* 66:355–361
- Wrage N, Velthof GL, van Beusichem ML, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol Biochem* 33:1723–1732
- Wrage N, van Groeningen JW, Oenema O, Baggs EM (2005) Distinguishing between soil sources of N₂O using a new 15 N- and 18O-enrichment method. *Rapid Commun Mass Spectrom* 19:3298–3306
- Yamamoto A, Akiyama H, Naokawa T, Miyazaki Y, Honda Y, Sano Y, Nakajima Y, Yagi K, K. (2014) Lime-nitrogen application affects nitrification, denitrification, and N₂O emission in an acidic tea soil. *Biol Fertility Soils*, In press:
- Yanai Y, Toyota K, Okazaki M (2007) Effects of charcoal addition on N₂O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Sci Plant Nutr* 53:181–188
- Zaman M, Nguyen ML (2010) Effect of lime or zeolite on N₂O and N₂ emissions from a pastoral soil treated with urine or nitrate-N fertilizer under field conditions. *Agric Ecosyst Environ* 136:254–261
- Zheng H, Wang Z, Deng X, Zhao J, Luo Y, Novak JM, Herbert S, Xing B (2013) Characteristics and nutrient values of biochars produced from giant reed at different temperatures. *Bioresour Technol* 130:463–471