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Correspondence and requests for materials should be addressed to M.L.C. (mlcayuela@cebas.csic.es & marialuz.cayuela@gmail.com)

Biochar and denitrification in soils: when, how much and why does biochar reduce N_2O emissions?

Maria Luz Cayuela[1,2], Miguel Angel Sánchez-Monedero[1], Asunción Roig[1], Kelly Hanley[2], Akio Enders[2] & Johannes Lehmann[2]

[1]Department of Soil and Water Conservation and Waste Management, CEBAS-CSIC. Campus Universitario de Espinardo. 30100 Murcia. Spain, [2]Department of Crop and Soil Sciences. 918 Bradfield Hall, Cornell University, 14853 Ithaca, NY. USA.

Agricultural soils represent the main source of anthropogenic N_2O emissions. Recently, interactions of black carbon with the nitrogen cycle have been recognized and the use of biochar is being investigated as a means to reduce N_2O emissions. However, the mechanisms of reduction remain unclear. Here we demonstrate the significant impact of biochar on denitrification, with a consistent decrease in N_2O emissions by 10–90% in 14 different agricultural soils. Using the ^{15}N gas-flux method we observed a consistent reduction of the $N_2O/(N_2 + N_2O)$ ratio, which demonstrates that biochar facilitates the last step of denitrification. Biochar acid buffer capacity was identified as an important aspect for mitigation that was not primarily caused by a pH shift in soil. We propose the function of biochar as an "*electron shuttle*" that facilitates the transfer of electrons to soil denitrifying microorganisms, which together with its liming effect would promote the reduction of N_2O to N_2.

In spite of numerous studies on biochar as a strategy to mitigate N_2O emissions from soil[1–15], the factors and mechanisms involved remain elusive. This is the case in part because N_2O can be formed through several distinct but often connected processes in soil[16], which poses challenges to its quantification due to high spatial and temporal variability[17,18].

Although abiotic denitrification has been reported[19], research has shown that most N_2O emitted from soil is produced by three main biotic processes: denitrification[17], nitrification[20] and nitrifier denitrification[21]. These mechanisms may occur simultaneously at different micro-sites of the same soil, but it is generally assumed that most N_2O emitted from agricultural lands is produced through denitrification[16,17,22,23].

Two principally different pathways can lead to lower denitrification N_2O in soil[16]: (i) a decline in the total N denitrified (with less N_2O emitted from soil in the intermediate reaction) or (ii) an enhancement of its further reduction to N_2. The second pathway does not minimize total N losses ($N_2 + N_2O$), but the ratio $N_2O/(N_2 + N_2O)$ decreases and the environmental consequences of N_2O emissions decrease. There are many mechanisms by which biochar might affect these two pathways. Biochar can modify the microbial activity in soil[24], the concentration of available NO_3^- and organic C[25,26], pH[11,27] and soil aeration[28], which are all important factors known to change both the $N_2O/(N_2 + N_2O)$ ratio and the total N denitrified[29]. At present there is no consensus about what makes a biochar able to mitigate N_2O emissions. Most studies found that, in general, slow pyrolysis high-temperature biochars lead to the greatest N_2O reductions[8–14]. However, there are no studies distinguishing between different N_2O production mechanisms or quantifying total denitrification, which makes interpretation and generalization difficult. Therefore, we investigated the causes and the magnitude of denitrification with particular attention to the climate-relevant N_2O after biochar addition to agricultural soils.

Results

Does biochar promote or inhibit abiotic denitrification? We found no N_2O emitted from soil under abiotic conditions both in the presence and absence of biochar (Experiment 1; Table S1).

Does biochar reduce N_2O emissions during denitrification and by which pathway? In an incubation study (Experiment 2) with brush biochar and 15 agricultural soils (Tables S2, S3) we found significantly ($P < 0.001$) lower N_2O emissions when biochar had been added (Fig. S1, Table 1). The intensity of mitigation ranged from 10 to 90%. In 10 out of the 15 measured soils, biochar did not only decrease the ratio $N_2O/(N_2 + N_2O)$ but also the

Table 1 | Total N_2O emissions from 15 agricultural soils un-amended (control) or mixed with 2% biochar (dry weight basis) under denitrification conditions (90% WFPS and 30°C). Soils were spiked with KNO_3 (^{15}N 99% enrichment), which allowed the determination of the total N denitrified ($N_2 + N_2O$) and the ratio $N_2O/(N_2 + N_2O)$ by the ^{15}N gas-flux method

	Total N_2O produced after 12 days of incubation					Fluxes of N_2 and N_2O when the difference between biochar and control N_2O fluxes was maximum**					
	Total cumulative N_2O (mg N_2O-N kg^{-1} soil)		**Total cumulative N_2O respect to initial NO_3^- in soil (%)**			**N_2 (µmol kg^{-1} soil h^{-1})**		**N_2O (µmol kg^{-1} soil h^{-1})**		**$N_2O/(N_2 + N_2O)$**	
Soil	**Control**	**Biochar**	**Control**	**Biochar**	**Mitigation (%)**	**Control**	**Biochar**	**Control**	**Biochar**	**Control**	**Biochar**
Elba	120	108	91.4	82.5	**10**	2.75	3.03	17.39	17.99	0.864	0.856
Lins	0.9	1.3	1.4	1.9	**−38**	0.06	0.06	2.26	0.44	0.972	0.850
Arkport	2.9	1.0	4.3	1.4	**67**	15.77	4.82	0.54	0.09	0.053	0.038
Lentiscosa	2.1	1.5	2.6	1.8	**31**	3.41	23.73	4.45	2.34	0.550	0.159
Tioga	10.3	4.0	17.2	6.6	**61**	0.12	0.14	1.43	0.38	0.820	0.651
Howard	17.0	10.3	23.2	14.1	**39**	1.23	2.08	7.46	9.39	0.793	0.766
Secanos	12.3	3.1	8.5	2.1	**75**	5.23	0.76	3.64	0.61	0.443	0.394
Cabezo	6.5	0.7	8.8	1.0	**89**	10.95	4.29	1.74	0.67	0.295	0.142
Hudson A	0.4	0.3	0.7	0.6	**18**	0.22	0.22	0.36	0.48	0.607	0.291
Madalin	15.1	1.9	14.7	1.8	**88**	0.58	0.97	3.64	0.17	0.795	0.130
Niagara	23.9	6.6	18.6	5.1	**72**	0.41	1.06	18.53	9.10	0.971	0.854
Hudson B	9.0	3.8	15.2	6.4	**58**	3.59	4.04	7.28	4.61	0.643	0.511
Costa	30.6	6.1	2.7	0.5	**80**	7.79	14.25	5.67	2.36	0.458	0.179
Coronela	69.8	7.0	12.9	1.3	**90**	14.49	2.26	9.99	1.47	0.468	0.244
Guarapuava	3.3	0.4	4.2	0.5	**89**	0.17	0.09	1.63	0.14	0.782	0.447

Values are the mean of 4 replicates.

total N denitrified. However, this result was less consistent, with five soils increasing the total N denitrified between 4 and 232%.

In order to discern which soil characteristics influenced the ability of biochar additions to decrease N_2O emissions, we performed a multivariate correlation (redundancy analysis) with the soil characteristics (Table S3) as predictor variables and the changes induced by biochar as response variables (Fig. 1). The first two ordination axes explained 49% of the variance. The first axis alone explained a significant part of the variation of the response variables ($F = 21.9$; $P \leq 0.002$). High predictor-response correlations (first canonical axis: 0.915; second canonical axis: 0.654) revealed a strong relationship between soil characteristics and biochar effectiveness to reduce N_2O emission. Biochar decreased the ratio of $N_2O/(N_2 + N_2O)$ predominantly in fine-textured soils. However, the ability of biochar to decrease total N_2O emissions was independent of soil texture but highly correlated with initial soil NO_3^- concentrations and dissolved organic C.

Why does biochar reduce N_2O emissions? In Experiment 3 we measured N_2O emitted after soil amendment using nine biochars with different C/N ratios (Table S2; Fig. S2) after their pH had been adjusted to the same pH as the soil (pH: 5.6) (Fig. 2.A). Then, we repeated the incubation with the same biochars added without adjusting the pH (Fig. 2.B). The difference between Fig. 2.A and Fig. 2.B corresponds to the effect of biochar pH on total emissions. We found that this difference strongly correlates ($r^2 = 0.809$; $P < 0.01$) with biochar buffer capacity (*i.e.* mmol H_3O^+ per gram of biochar necessary to adjust its pH to the same pH of the soil), but not with pH alone ($r^2 = 0.615$; $P > 0.05$).

Given the known effect of pH on N_2O production, we performed a parallel incubation (Experiment 4) to monitor the shift in soil pH induced by the different biochar additions. However, only dairy manure biochar led to a statistically significant yet low increase of 0.1 pH units (Table S4). We then analyzed N_2O emissions from the same soil where the pH had been experimentally increased using additions of $CaCO_3$ (Fig. S3) and observed no N_2O mitigation (total N_2O emissions after 13 days: 38.7 ± 4.4 for control (pH 5.60), 44.5 ± 3.9 for pH 5.79 and 45.5 ± 5.5 for pH 6.10, respectively).

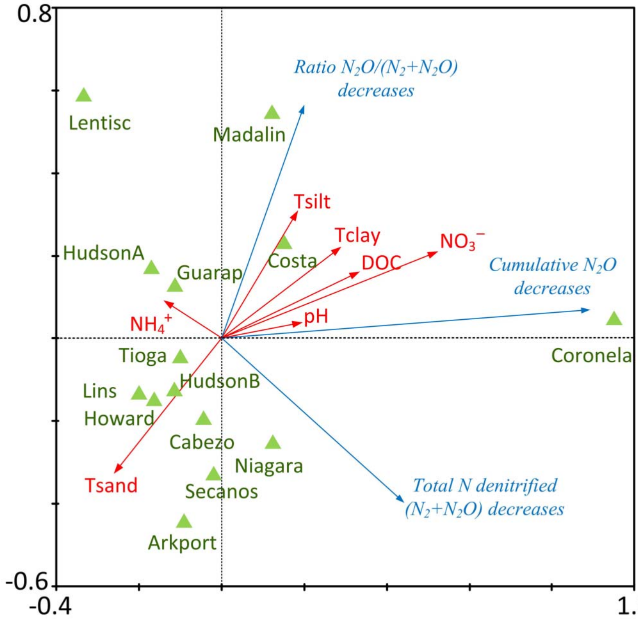

Figure 1 | Correlation triplot based on a redundancy analysis (RDA) depicting the relationship between the main physico-chemical characteristics of the soils (predictor variables) and the differences induced by biochar applications (response in soil) (according to Lepš and Šmilauer[49]). Blue arrows point to maximum shifts produced by the biochar amendment, i.e. a decrease in the total cumulative N_2O, the $N_2O/(N_2 + N_2O)$ ratio, and the flux of total N denitrified ($N_2 + N_2O$). Eigenvalues of the first two axes are 0.343 and 0.161, the sum of all canonical axes is 0.555. "Cumulative N_2O" represents the difference (control-biochar) in total N_2O emitted during the entire incubation period; "ratio" and "Total N denitrified" represent the differences (control-biochar) at the day selected for isotopic gas analysis (see Fig. S1). Tsilt, Tclay and Tsand represent the percentages of soil silt, clay and sand. DOC: dissolved organic C in soil.

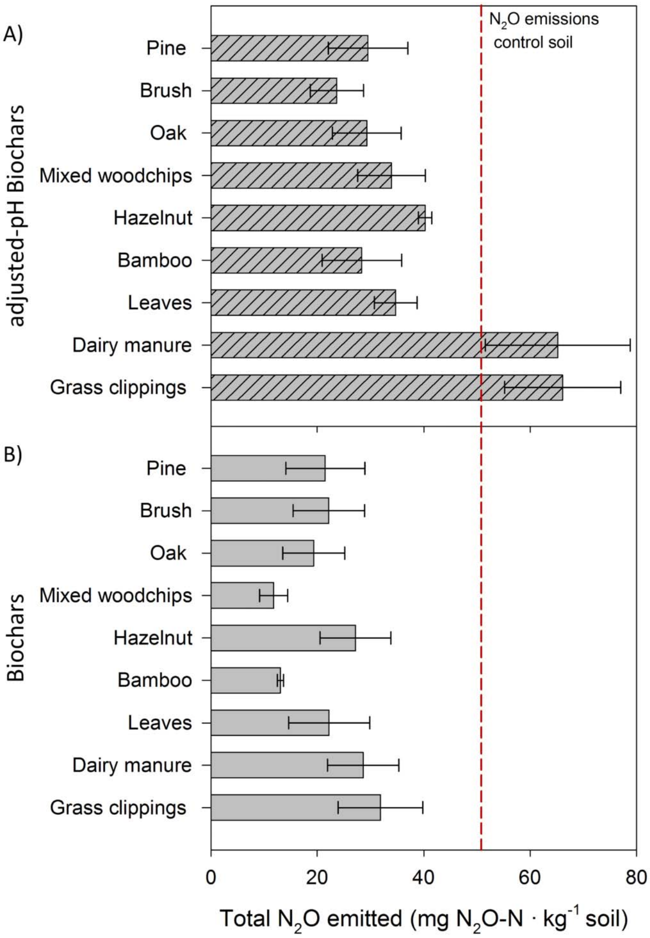

Figure 2 | Total N_2O emissions after 30 days of incubation of a muck soil (Elba) amended with different biochars (2% weight) under denitrification conditions (90% WFPS, 30°C). The dashed line represents emissions from the control soil (unamended). Fig. 2A shows N_2O emissions from soil amended with biochars for which the pH had been adjusted to the pH of the soil (5.6). Fig. 2.B shows N_2O emissions from soil amended with biochars at their actual pH. Biochars are arranged from high to low C/N ratios. Error bars represent standard errors of the mean (n=4).

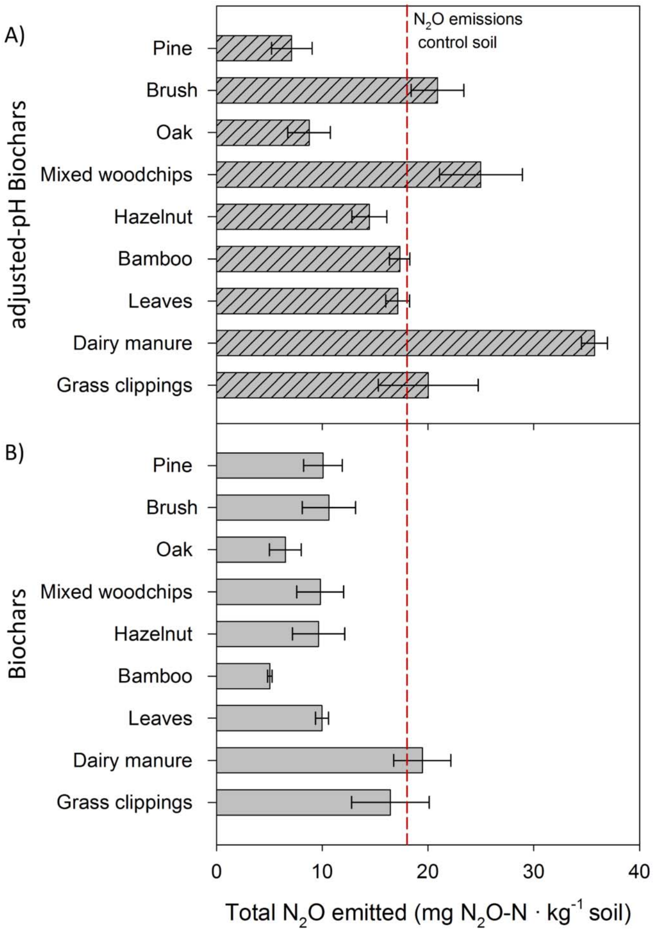

Figure 3 | Total cumulative N_2O emissions produced after 7 days of incubation of a muck soil (Elba) spiked with 100 mg NO_3^- -N and 1 g of glucose-C per kg of soil. The soil had been incubated with different biochars (2% weight) under denitrification conditions (90% WFPS, 30°C) during 1 month prior to N and C addition. The dashed line represents emissions from the control soil (without biochar). Fig. 1A shows N_2O emissions from soil amended with biochars for which the pH had been previously adjusted to the pH of the soil (5.6). Fig. 1.B shows N_2O emissions from soil amended with biochars at their actual pH. Biochars are arranged from high to low C/N ratios. Error bars represent standard errors of the mean (n=4).

Is it a short-term effect? After 30 days of incubation (Experiment 3), N_2O fluxes had leveled off in all treatments (Fig. S2) pointing to a depletion of NO_3^- or available organic C in soil. At day 34 we added 100 mg of NO_3^--N and 1.0 g of glucose-C per kg of soil and production of N_2O increased immediately (Experiment 5; Fig. S4). When pH was adjusted, only two of the biochars (pine and oak) significantly reduced N_2O emissions (Fig. 3A). When pH was not adjusted, however, most biochars decrease the total amount of N_2O emitted (between 41 and 72%), although only additions of biochars made from bamboo and oak had significantly lower emissions compared to the control at $P < 0.05$ (Fig. 3B). It is important to note that the different treatments had undergone 30 days under denitrification conditions, and their initial NO_3^- concentration (before spiking them with NO_3^- and glucose) might significantly differ.

In order to more closely investigate the mechanism of the observed medium-term mitigation we performed a second experiment where three soils (Secanos, Tioga and Elba) were pre-incubated (with and without biochar) for 14 days under denitrification conditions (Experiment 6) and subsequently spiked with a solution of $K^{15}NO_3$. After the pre-incubation period, the soils with added biochar showed far more NO_3^- compared to the soils without added biochar (Table 2). Even after the $^{15}NO_3^-$ addition, the alkaline soil (Secanos) hardly showed any denitrification. The reason may be a lack of available C after the pre-incubation period, since this soil had a markedly lower total organic carbon concentration (8 g C kg^{-1} soil) compared to Tioga (29 g C kg^{-1} soil) or Elba soils (495 g C kg^{-1} soil). Both Tioga and Elba soils emitted N_2O, but without any detectable N_2. Recognizing the different initial NO_3^- concentrations of soils with and without biochar additions, we calculated the N_2O emitted (as a result of the $^{15}NO_3^-$ spike) per unit of NO_3^- in soil and observed a comparable mitigation effect for the Tioga soil and an even larger one for the Elba soil (Table 2) compared to Experiment 2 (Table 1), where soils were not pre-incubated.

Discussion

Although biological processes dominate N_2O production in most environments, chemodenitrification, an abiotic process wherein inorganic N species are reduced to gaseous species has been reported in soils with high concentrations of Fe (II) or humic acid extracts[19]. We did not find N_2O emitted under abiotic conditions in the two soils most susceptible to chemodenitrification used in this study (Elba and Guarapuava), which implies that the N_2O emitted from

Table 2 | Influence of biochar (made from brush at 500°C) on N_2O emissions from three different soils after a preincubation period (2 weeks)

	NO_3^- in soil * ($mg\ N \cdot kg^{-1}$ soil)		Total N_2O respect to NO_3^- in soil ($\mu g\ N_2O\text{-}N \cdot mg^{-1}\ NO_3^-\text{-}N\ kg^{-1}$ soil)			Fluxes of N_2 and N_2O calculated by the ^{15}N gas flux method					
						N_2 ($\mu mol\ kg^{-1}$ soil h^{-1})		N_2O ($\mu mol\ kg^{-1}$ soil h^{-1})		$N_2O/(N_2 + N_2O)$	
Soil	Control	Biochar	Control	Biochar	Mitigation (%)	Control	Biochar	Control	Biochar	Control	Biochar
Secanos	14.7	99.1	2.6	9.1	—	n.d.	n.d.	n.d.	0.06	—	—
Tioga	11.8	14.2	109.4	43.0	61	n.d.	n.d.	1.36	0.11	1.00	1.00
Elba	11.7	48.4	826.9	353.2	57	n.d.	n.d.	12.0	7.8	1.00	1.00

*at the maximum difference in N_2O flux between biochar-control. Calculated by the ^{15}N gas flux method. Measuring the molecular ratios for N_2O of ^{45}R ($^{45}N_2O/^{44}N_2O$ and ^{46}R ($^{46}N_2O/^{44}N_2O$) allows calculation of the enrichment of the source ($^{15}X_N$) of the labeled N_2O[48]. Since we know the amount of added of $NO_3^-{-}^{15}N$ (50 mg, 99% enrichment), we can calculate the amount of $NO_3^{-14}N$ that was in the soil.

n.d.: not detected. According to Stevens and Laughlin[48] the detection limit with this method is 7.5 g $N_2\ ha^{-1}\ d^{-1}$ for an enclosure with a volume to surface ratio of 5 : 1.

the soils was entirely produced through biotically mediated mechanisms. It also shows that biochar did in our study not abiotically induce N_2O formation in soil through previously proposed catalytic reactions with hydroquinones[30,31], metal ions or radicals[19,32], which are abundant in biochars[31,33].

Previous studies on N_2O emissions and biochar are limited to one or two soil types[3,4,7,9,10,12,13,34,35]. Given that these experiments have been conducted under varying environmental conditions and with different biochars, comparison between soil properties is difficult, which enormously limits generalization. Our study revealed that soil texture was closely related to the ability of biochar to decrease the ratio of $N_2O/(N_2 + N_2O)$ at the time of maximum N_2O emissions (Fig. 1). The fact that biochar promoted the last step of denitrification predominantly in fine-textured soils (e.g. Lentiscosa, Madalin, Costa) indicates that the mechanism of reduction is not linked to an increase in soil aeration (if that were the case, biochar would decrease total denitrified N in these soils, instead of promoting the last step of denitrification to N_2). This confirms recent findings[6], which noticed soil aeration to be a negligible factor for N_2O mitigation in soils containing biochar.

The strong correlation between NO_3^- concentration in the soil and total N_2O mitigation by biochar additions (Fig. 1) suggested that biochar might reduce NO_3^- availability. A reduction in NO_3^- availability would indeed decrease the total N denitrified and it would favor the last step of denitrification (decreasing the ratio $N_2O/(N_2 + N_2O)$)[36]. On the other hand, N_2O mitigation was also highly correlated with soil DOC, and less strongly with soil pH, which are known to control the denitrification capacity in soil[37].

In Experiments 3 and 4 we further investigated these possible reasons for the observed reductions in N_2O emissions. We postulated that by using a soil where the addition of biochar does not significantly influence its water filled pore space, the effect of biochar on denitrification N_2O would mostly depend on biochar pH and its C/N ratio. Most biochars used in this study may immobilize NO_3^-, since their C/N ratios are greater than that of the soil (C/N = 18.7). Nitrogen immobilization in soil has often been found to decrease denitrification N_2O[38]. Despite their high C/N ratios, we did not find a correlation between N_2O mitigation and biochar C/N (range of C/N ratios: 11–859) irrespective of whether or not the pH of the biochars was adjusted. The high recalcitrance of high-temperature biochars to microbial degradation[1,8,27] and the lack of correlation between biochar C/N and N_2O mitigation found in our experiment do not suggest microbial immobilization of NO_3^- as the driving mechanism for the observed N_2O reductions.

When the pH was adjusted, additions of high-N biochars led to more emitted N_2O (Fig. 2B), which is probably due to an increase of NO_3^- concentrations in the soil. Although some N from the biochar may be mineralized, this does normally not exceed 10–20% of its total N content[15]. A salting-out effect through an increase of the ionic strength in the soil solution, which has shown to reduce the solubility of N_2O (Setchenow effect) favoring its emission[22], might have played a role for the differences among biochars. The correlation between ash content and N_2O emissions was highly significant ($r^2 = 0.809$; $P = 0.002$). However, the persistence of the reduction in denitrification found in Experiment 6 sheds doubts on this explanation.

A mere shift in soil pH was not the reason for the mitigation of N_2O emissions as demonstrated in Experiment 4 (Fig. S3). Our finding is in agreement with those by Yanai et *al.*[2], who after increasing the soil pH with ash applications (instead of biochar) did not observe any reductions in N_2O emissions. This suggests that the change in soil pH does not by itself induce the N_2O reductions, but rather other properties of biochar intrinsically connected to pH.

Despite its refractory nature, biochar contains abundant redox-reactive organic and inorganic compounds[31]. For example, quinone groups may be reduced during suboxic conditions to hydroquinone or phenols that can subsequently act as electron donors. Such redox systems readily changing from one steady-state to another[31] have been described for other forms of organic matter in soil, such as humic substances extracts[39] or plant and microbial exudates[40], and are known as "*electron shuttles*". The presence of free radicals in some biochars may even increase their reactivity[31].

Under suboxic conditions, facultative anaerobic microorganisms can utilize NO_3^-, Mn (IV) and Fe (III) compounds substituting O_2 as electron acceptor[41]. Biochar contains both Mn and Fe at varying concentrations depending on the feedstock and pyrolysis temperature[27]. For example Enders et al.[27] reported Mn and Fe concentrations of up to 9% (d.w.) in biochars produced at 500°C. Mn (IV) and Fe (III) are known to readily function as electron acceptors at circumneutral pH[42]. Biochar might act as a reducing agent itself and additionally as an *electron shuttle*, facilitating the electron transfer to microorganisms by acting as an electrical conduit. Thus, biochar might compete with NO_3^- as an electron sink, which could explain the lower total N denitrified in many soils. On the other hand, its function as an *electron shuttle* connected to its liming effect might facilitate the activity of N_2O reductase and therefore promote the last step of denitrification[29], both hypotheses that need further testing.

Another controversial aspect of N_2O mitigation with biochar is the debate about its transitory effect. In a recent study Spokas[43] found that three years ageing negated the N_2O mitigation that was originally observed from the fresh biochar in laboratory incubations. Ageing is known to substantially alter biochar surface chemistry and reactivity[31], which may impact the ability of biochars to function as an electron acceptor or shuttle. Experiment 5 showed that N_2O mitigation is effective one month after biochar application and it is still strongly related to pH, albeit not a result of pH changes as indicated above. One of the hypotheses proposed for temporary

N_2O mitigation is that, immediately after application, biochar might have a short-term inhibitory effect, decreasing the microbial activity in soil. For example, Spokas et al.[35] found a correlation between ethylene concentration and N_2O production in soil amended with biochar and proposed the inhibitory impact of ethylene on microbial biomass as a mechanism that could significantly contribute to N_2O mitigation. This mechanism may be significant for biochars produced at low temperatures, with a high concentration of tars, which might temporarily reduce the activity of denitrifier communities in soil. Nonetheless, for biochars produced at 500°C, the contribution of this mechanism appears to be minor. Looking more closely at the mechanism of medium-term mitigation in Experiment 6, the soils pre-incubated with biochar showed far more NO_3^- compared to the control soils (Table 2). These results stand in stark contrast to the hypothesis that decreasing N_2O emissions were caused by the ability of biochar to adsorb NO_3^- in soil[6,13]. Even though we investigated medium-term effects after several weeks separately from effects observed immediately after addition of fresh biochars to soil, aging of biochars over periods of months and years may affect the results[43]. Additional long-term studies on N_2O mitigation with biochar are required to quantify the duration of this effect[31,43].

In summary, under optimum denitrification conditions, biochar consistently reduced N_2O emissions in the investigated agricultural soils. It decreased the ratio of $N_2O/(N_2 + N_2O)$ and in most cases also the total N denitrified. Biochars obtained at 500°C by slow pyrolysis, independently of their original feedstock, were able to decrease N_2O fluxes produced by denitrification. In light of our results, we discard biochar toxicity, NO_3^- immobilization or NO_3^- sorption as relevant mechanisms for N_2O mitigation with high-temperature slow-pyrolysis biochar. The complexity of biotic and abiotic biochar-soil interactions points at several mitigation mechanisms occurring simultaneously. Biochar buffer capacity appears to be fundamental to decreasing N_2O emissions during denitrification, not because of a change of soil pH in itself, but because the mechanism of mitigation is intrinsically connected to pH. We have demonstrated that biochars promote the last step of denitrification, and in two out of three cases it also decreased total N denitrified. We propose as a plausible explanation that biochar is able to facilitate the transfer of electrons to denitrifying microorganisms in soil, thus acting as an *electron shuttle*. This together with its liming effect and high surface area would promote the reduction of N_2O to N_2. Our results are based on short-term laboratory assays that did not take into account other important aspects occurring under field conditions, including biochar ageing or soil-plant-microbe interactions. The results of our study allowed us to formulate a new hypothesis that had not been considered before and that might play an important role explaining reductions in N_2O formation when denitrification is the dominant pathway. This hypothesis requires further experimentation to prove its magnitude and eventually its practical significance.

Methods

Biochars. Nine biochars produced at 500°C by slow pyrolysis as described in Enders et al.[27] were selected with the aim of obtaining a wide range of C/N ratios from 11 to 859 and pH values between 6.4 and 10.7 (Table S2). Biochars were ground and sieved to a particle size between 200–500 μm before soil application. Brush biochar was used in all experiments and selected for those involving different soils (Experiments 1, 2 and 6) because of its neutral pH and also because it can be considered as representative of a generic biochar widely utilized and available worldwide.

Soils. Fifteen agricultural soils from 3 different geographical areas (USA, Spain and Brazil) were selected comprising a wide range of textures and pH values (Table S3). The soils were sampled from a depth of 0–0.25 m of agricultural fields, air-dried and sieved (<2 mm). Agricultural organic soils have been reported to emit high fluxes of N_2O[44]. In experiments involving different biochars (Experiments 3 and 5) we therefore selected a muck soil (drained cultivated Histosol) in order to obtain high N_2O emissions that allowed us to detect the effects of different biochars more sensitively. If an effect (positive or negative) was not observed at high emissions, biochar would not play a role. In addition, this organic soil has a high porosity, which allowed us to assume that biochar additions would not modify its water filled pore space. For practical reasons the collected muck soil was maintained in its field moist condition, sieved (<2 mm) and stored at 4°C until the beginning of the experiment.

Chemical-physical analyses of biochars and soils. Biochars: Proximate analysis was conducted using ASTM D1762-84 Chemical Analysis of Wood Charcoal. Total C and N were determined by Dumas combustion using a PDZ Europa ANCA-GSL elemental analyzer connected to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). pH and electrical conductivity (EC) were determined in 1 : 10 (w : v) water-soluble extracts. NH_4^+ and NO_3^- were extracted with 2.0 M KCl at 1 : 10 (w : v) and quantified colorimetrically using a continuous flow analyzer (Bran and Luebbe Autoanalyzer, SPX, Charlotte, NC). Soils: Soil texture was determined using the pipette method according to Kettler et al.[45]. pH was determined in 1 : 10 (w : v) water extracts. Dissolved organic C (DOC) and total dissolved N (TDN) were determined in 1 : 10 (w : v) water extracts (shaken for 2 h, centrifuged for 10 min at 1500×g and filtered (GF/F Whatman glass filters) with a TOC analyzer (Shimadzu Total Organic Carbon-Visionary Series; TOC-VCSH).

Incubation studies. The incubation experiments were performed with units consisting of 100 g dry soil (control) or 98 g dry soil and 2 g biochar (treatments) in 250 ml (or 500 ml in the case of the muck soil) glass jars at optimum conditions for denitrification: 30°C and a moisture of 90% of the water filled pore space (WFPS). The biochars were thoroughly mixed with the dry soil to obtain a completely homogeneous mixture. Subsequently, water (or a solution containing the appropriate concentration of NO_3^-) was added to attain the required moisture. The jars were covered with a polyethylene sheet that allows gas exchange but minimizes evaporation. Moisture was kept constant by adjusting the water content every other day. The experiments were laid out as a randomized block design with four replicates.

Experiment 1. This experiment was aimed at quantifying any abiotic contribution to N_2O formation (chemodenitrification) from the two soils most susceptible to chemodenitrification in this study (Elba and Guarapuava; total organic C: 495 and 43.5 g kg^{-1}, respectively). 50 g of Elba or 100 g Gurapuava soils were either untreated (controls) or treated with 2% biochar (made from brush), subsequently placed in 250-ml jars at 90% WFPS and doubly autoclaved for sterilisation (103.4 kPa and 121°C for 1 h, incubated 24 h, and autoclaved for an additional 1 h). The samples were cooled down to 30°C overnight and N_2O concentrations were measured by means of gas chromatography equipped with an electron capture detector (ECD) 12 h after sterilization. Since no N_2O was emitted from any of the samples, a sterile solution of KNO_3 was added (100 mg N kg^{-1} soil) and the N_2O accumulated in the headspace during 40 min was immediately measured (Table S1).

Experiment 2. This study was designed to determine the magnitude of N_2O mitigation by biochar in different soils as well as investigating the mechanism involved. Brush biochar was applied at an equal rate (2% dry weight) to 15 different agricultural soils. Moisture was adjusted to 90% WFPS in each jar by adding the required volume of a solution containing KNO_3 (>99% ^{15}N enrichment) at the appropriate concentration to obtain 90% WFPS and exactly 5 mg of NO_3^--^{15}N-per jar (50 mg of NO_3^--^{15}N kg^{-1} soil). Rewetting the soils in this fashion guaranteed a homogenous ^{15}N-NO_3^- pool, which is essential to correctly apply the equations derived by Mulvaney and Boast[46] to calculate total N denitrified. We measured N_2O fluxes from the 15 unamended soils compared to those amended with 2% biochar (Fig. S1).

Experiment 3. The organic soil (Elba) was sampled from a depth of 0–0.25 m of a horticultural field in Genesse County, NY. We assumed that the addition of biochar (at 2%, w : w) to this soil would not modify its aeration at 90% WFPS. With the aim of verifying this assumption we tested its water retention curves (Fig. S6) and observed no significant differences among treatments.

To differentiate the effects of pH values from C/N ratios we carried out two incubations. In the first one the pH of the biochars was adjusted to the same pH of the soil (5.6) prior to application. The pH adjustment was done by shaking each biochar with water (1 : 20, w : v) for 12 hours; pH was measured and adjusted to 5.6 first with a 2 M HCl solution and later with a 0.1 M HCl solution (close to the end point). The volume of HCl solution necessary to adjust the pH was registered in order to calculate the biochars' buffer capacity (i.e. mmol H_3O^+ per gram of biochar necessary to adjust its pH to 5.6). Once the pH had been adjusted, the biochars were shaken for 12 hours and the pH was tested again. Biochars were then dried for 2 days at 80°C and homogenized before their addition to soil. We also checked that the pH adjustment did not modify the amount of water-extractable NO_3^- in biochars. In the second incubation the biochars were applied without any pre-treatment. In both cases biochars were applied at 2% (w : w) and thoroughly mixed with the soil, which was re-packed to the average field bulk density (0.65 g l^{-1}) inside the jars. De-ionized water was added to adjust the moisture to 90% WFPS, equivalent to 2.07 g water per g dry soil. N_2O fluxes were analyzed during one month of incubation (Fig. S2).

Experiment 4. In this experiment we investigated how the manipulation of soil pH influences N_2O emissions. First we evaluated the magnitude of pH changes induced by the biochars in Experiment 2. We carried out a parallel incubation (2 replicates per treatment) under identical environmental conditions where we monitored the pH after 1, 3 and 14 days of incubation (Table S4). Second, we performed an incubation study with the same soil where we modified its pH in the same order of magnitude as was induced by biochars measured in the preceding experiment. We included a control (Elba soil at its natural pH of 5.6) and two treatments where we increased the pH of the soil to 5.79 and 6.10 by adjusting to 90% WFPS with water solutions containing 0.010 and 0.020 g of $CaCO_3$ per 100 g of dry soil, respectively. We compared the N_2O emissions over two weeks (Fig. S3).

*Experiment 5***.** This experiment was an extension of Experiment 3 aimed at studying the temporal dynamics of biochar effects. After 1 month of incubation N_2O fluxes were low in all samples (Fig. S2). At day 34 we applied 5 ml of a solution containing KNO_3 (2 g N L^{-1}) and glucose (20 g C L^{-1}), which is equivalent to 100 mg N and 1 g C kg^{-1} soil and measured the N_2O fluxes resulting from this addition (Fig. S4).

*Experiment 6***.** Two mineral soils with a loamy texture and contrasting pH (Secanos, Tioga) and the organic soil (Elba) were selected for this experiment. Each control soil and its corresponding biochar treatment (2%, w : w) was pre-incubated for 14 days under denitrification conditions (90% WFPS, 30°C). From day 13 to day 16 the samples were left to dry to *ca.* 50% WFPS, which was verified gravimetrically. At day 16, moisture was re-adjusted to 90% WFPS in each jar by adding the required volume of a solution containing KNO_3 ($>99\%^{15}N$ enrichment) at the necessary concentration to obtain 5 mg of NO_3^--^{15}N per jar (similarly to Experiment 2) (Fig. S5).

N_2O sampling and measurements. Gas sampling was conducted by sealing each unit with screw caps for 40 min. 10 ml of the headspace gas was sampled with 25 ml gastight polypropylene syringes and measured within 12 hours by gas chromatography (Shimadzu GC-14A GC equipped with ECD (Ni63) detector (Kyoto, Japan)). Measurements were done daily during the first three days; decreasing subsequently to every other day, three times per week, etc. (see Fig. S1, Fig. S2, Fig. S3 etc. in the supporting information section).

N_2O fluxes were calculated assuming a linear increase during the accumulation (closing) period, a fact that was checked prior to the experiments (every 15 minutes for 1 hour). Cumulative N_2O was calculated assuming linear changes in fluxes between the two closest measurement points.

In experiments with $^{15}NO_3^-$ (Experiments 2 and 6) the ^{15}N gas-flux method[46–48] was used to quantify N_2O and N_2 emissions. The gas sampling for isotopic analysis was made each day preceding the gas sampling for GC-ECD analysis and within an independent accumulation period. Two gas samples were collected using a 12-ml syringe and needle: one immediately after the lid was fitted to the jar ($t = 0$) and the second after 40 min ($t = 40$). The gas samples were transferred to 12-ml vials (Labco) previously purged with He and evacuated. *A posteriori*, gas samples were selected at time points where the difference in N_2O fluxes (measured by GC-ECD) between biochar and control soils were the greatest, which normally corresponded with the peak in N_2O emissions. Selected samples (a total of 320) were analyzed for their isotope ratios of N_2 (29/28 (^{29}R) and 30/28 (^{30}R)) and N_2O (45/44 (^{45}R) and 46/44 (^{46}R)) by automated isotope ratio mass spectroscopy (ThermoFinnigan GasBench & PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany)).

Data calculations and statistics. The molar fraction of ^{15}N-NO_3^- ($^{15}X_N$) in the soil pool was calculated from the $\Delta^{45}R$ and $\Delta^{46}R$ according to Stevens and Laughlin[48]. The flux of N_2 and N_2O was then calculated by the equations given by Mulvaney and Boast[46].

Since data were not normally distributed, they were ln-transformed prior to univariate analysis of variance with SPSS 19.0. The correlation between soil properties and biochar effectiveness in reducing emissions was determined by redundancy analysis (RDA) using CANOCO 4.5 for Windows. The characteristics of the soil (silt, clay, sand, pH, DOC, NO_3^-, NH_4^+) were included as predictor variables and the (i) total N_2O emissions, (ii) flux of total N denitrified, and (iii) $N_2O/(N_2 + N_2O)$ ratios between controls and biochar treatments as the dependent variables. Data were centered and standardized. Significance of the ordination axes was calculated by the Monte-Carlo permutation test; n = 499.

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Authors contributions

M.L.C. and J.L. designed the experiments, M.L.C., K.H. and A.E. performed the experiments, M.L.C., M.A.S. and J.L. analyzed the data, M.L.C. wrote the paper with the help of J.L. and A.R. All authors reviewed the manuscript.

Additional information

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