Nitrogen availability, water-filled pore space, and N_2O-N fluxes after biochar application and nitrogen fertilization

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Abstract – The objective of this work was to investigate the impact of the application of wood biochar, combined with N fertilizations, on N_2O-N fluxes, nitrogen availability, and water-filled pore space (WFPS) of a clayey Oxisol under rice (wet season) and common bean (dry season) succession. Manual static chambers were used to quantify N_2O-N fluxes from soil immediately after a single application of wood biochar (32 Mg ha^{-1}) and after four crop seasons with N applications (90 kg ha^{-1} N). Soil ammonium (N-NH_4^+) and nitrate (N-NO_3^-) availability, as well as WFPS, was measured together with N_2O-N fluxes. There was no interaction between biochar and N fertilization regarding N_2O-N fluxes in any of the four seasons monitored, although these fluxes were clearly enhanced by N applications. At 1.5 and 2.5 years after biochar application, the WFPS decreased. In addition, in the seasons characterized by low WFPS, N_2O-N fluxes and soil N-NO_3^- and N-NH_4^+ availability were enhanced after N applications. Long-term experiments in the field are important to quantify the impacts of biochar on N_2O-N fluxes and to determine the dynamics of these fluxes on soil-related variables.

Index terms: cropping systems, gas fluxes, greenhouse gases, nitrate and ammonium, soil amendment, soil porosity.

Disponibilidade de nitrogênio, espaço poroso preenchido por água e fluxos de N_2O-N após aplicação de biochar e fertilização nitrogenada

Resumo – O objetivo deste trabalho foi investigar o impacto da aplicação de biochar de madeira, combinada com fertilizações nitrogenadas, nos fluxos de N_2O-N, na disponibilidade de nitrogênio e no espaço poroso preenchido por água (EPPA), em um Latossolo argiloso sob sucessão com arroz (época chuvosa) e feijão (época seca). Câmaras estáticas manuais foram utilizadas para quantificar os fluxos de N_2O-N no solo logo após uma única aplicação de biochar (32 Mg ha^{-1}) e após quatro épocas de cultivo com aplicações de N (90 kg ha^{-1} de N). A disponibilidade de amônio (N-NH_4^+) e de nitrato (N-NO_3^-) no solo, bem como o EPPA, foi medida juntamente com os fluxos de N_2O-N. Não houve interação entre biochar e fertilização nitrogenada quanto aos fluxos de N_2O-N, em nenhuma das quatro épocas monitoradas, apesar de esses fluxos terem aumentado com as aplicações de N. Aos 1,5 e 2,5 anos após a aplicação do biochar, o EPPA diminuiu. Além disso, nas épocas caracterizadas por reduzido EPPA, os fluxos de N_2O-N e a disponibilidade de N-NO_3^- e N-NH_4^+ no solo aumentaram após as aplicações de N. Experimentos em campo de longa duração são importantes para quantificar o impacto do uso de biochar sobre os fluxos de N_2O-N e para determinar a dinâmica desses fluxos sobre as variáveis relacionadas ao solo.

Termos para indexação: sistemas de cultivo, fluxo de gases, gases de efeito estufa, nitrato e amônio, condicionador de solo, porosidade do solo.

Introduction

Biochar is the charred by-product of biomass pyrolysis (Sohi et al., 2010). Wood biochars are generally alkaline and rich in micropores, characteristics that, in theory, can contribute to increase ammonium absorption and soil water retention, enhancing their availability to plants and lowering potential nitrous

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oxide (N_2O) emissions from cropping systems (Clough & Condron, 2010). N_2O is a powerful greenhouse gas with a global warming potential nearly 310 times higher than that of carbon dioxide (CO_2). It is also an important component of gas emissions coming from agricultural lands, accounting for around 14% of the total anthropogenic CO_2-equivalent emitted globally (Bernstein et al., 2007). The N_2O from agricultural fields is primarily a direct consequence of mineral and organic fertilization. In Brazil, agricultural fields are the source of 88% of the total anthropogenic N_2O (Brasil, 2013).

Although a number of studies have shown that biochar can reduce N_2O emissions (Lehmann et al., 2006; Spokas et al., 2009; Atkinson, 2010; Cayuela et al., 2010; Sohi et al., 2010; Zhang et al., 2010), others have not confirmed this effect (Karhu et al., 2011; Scheer et al., 2011). In a recent metanalysis, Cayuela et al. (2013) found that biochar reduces, on average, 54% of soil N_2O emissions; however, of the 30 studies analyzed by these authors, only 5 were field trials (Scheer et al., 2011; Taghizadeh-Toosi et al., 2011; Liu et al., 2012; Zhang et al., 2012a, 2012b). In short-term studies under pasture conditions, for example, Scheer et al. (2011) observed no effect of biochar application, whereas Taghizadeh-Toosi et al. (2011) found that it decreased N_2O-N fluxes. In a two-year field trial on a Chinese rice paddy system, Liu et al. (2012) and Zhang et al. (2012a) reported a decrease in N_2O-N fluxes when N fertilization was combined with biochar amendment. Conversely, in another two-year field trial, Verhoeven & Six (2014) found no effect of biochar in reducing N_2O-N fluxes from a commercial wine grape vineyard. Clearly, the effects of biochar and of its combination with N fertilization on N_2O emissions, in field studies, are the result of complex interactions and need to be more thoroughly explored. Furthermore, the assessment of soil-related variables, such as soil nitrate and ammonium availability, as well as of the water-filled pore space, are fundamental to elucidate the origin of N_2O-N fluxes in a cropping system.

Mukherjee & Lal (2014) reported contradictory results for laboratory and field observations, calling for a more careful extrapolation of laboratory data to field conditions. In this context, temporal dynamics of N_2O emissions are of particular relevance for biochar studies. Cayuela et al. (2013) also showed that reductions in N_2O emission are directly proportional to the amount of biochar applied. It should be noted that, very often, the amount of biochar applied in laboratory studies is much higher than what is feasible under field conditions. For example, in an incubation experiment, Spokas et al. (2009) only found a significant decrease in N_2O emissions with a biochar amendment rate higher than 20% (w/w), which is improbable under field conditions. Therefore, the over-presence of laboratory studies and the lack of long-term field studies on the effects of realistic biochar rates on N_2O emissions is a problem. In the present study, a by-product of charcoal production from a timber plantation was tested as a soil amendment for cropping systems. This type of biochar is potentially available in large quantities in the Cerrado (Brazilian savanna) region, but its value for agriculture is still unclear.

The objective of this work was to investigate the impact of the application of wood biochar, combined with N fertilizations, on N_2O-N fluxes, nitrogen availability, and water-filled pore space of a clayey Oxisol under rice (wet season) and common bean (dry season) succession.

Materials and Methods

The field trial was established in June 9, 2009, on a clayey Rhodic Oxisol, at the Capivara farm, belonging to Embrapa Arroz e Feijão, located in the municipality of Santo Antônio de Goiás, in the state of Goiás, in the Central West region of Brazil (16°29'17"S, 49°17'57"W). The trial was conducted under center-pivot irrigation. Since 2001, the area had been cultivated under no-tillage with an intercrop between corn (*Zea mays* L.) and forage [*Urochloa ruziziensis* (R.Germ. & C.M.Evrard) Morrone & Zuloaga] throughout the wet season, from November to March, followed by irrigated common bean (*Phaseolus vulgaris* L.) during the dry season, from June to August. Immediately after the establishment of the field trial, irrigated common bean was cultivated as the first crop throughout the dry season, followed by rice (*Oryza sativa* L.) during the wet season.

For the establishment of the field trial, the soil was ploughed twice to a depth of 20 cm in order to incorporate crop residues. Biochar was milled to pass through a 2-mm sieve, broadcasted manually over the soil surface, and incorporated to a 10–15-cm soil depth using a harrow. Chemical properties of the biochar and the experimental design are presented in Carvalho et al. (2013a). The evaluated treatment was the effect of the biochar rate of 32 Mg ha^{-1}, with or without the application of 90 kg ha^{-1} N, on N_2O-N fluxes and on soil-related variables, at four cropping seasons (S) after biochar application: from June 16 to September 21, 2009; from November 3 to February 22, 2010; from November 8 to February 21, 2011; and from November 28 to March 19, 2012. These seasons were equivalent to the periods: S0.0, immediately after biochar application to the soil; and S0.5, S1.5, and S2.5, after 0.5, 1.5, and 2.5 years from the application, respectively. At sowing, all plots received the same rate of P_2O_5-K_2O (kg ha^{-1}) according to the demand of each cropping system, as follows: 15–20 in S0.0, 120–60 in S0.5, 60–30 in S1.5, and 30–30 in S2.5. Mineral N (urea) was divided into two or three applications, at sowing and around crop flowering. In S0.0, 5 and 85 kg ha^{-1} N were applied at sowing and 30 days after sowing (DAS), respectively. In S0.5, 45 kg ha^{-1} N were applied at sowing and then at 40 DAS. In S1.5, 45 kg ha^{-1} N were applied at sowing and 22.5 kg ha^{-1} N at 30 and 50 DAS. Finally, in S2.5, 36 kg ha^{-1} N were applied at sowing and 27 kg ha^{-1} N at 30 and 50 DAS.

Along the dry season, in S0.0, 10 mm of water were applied at every three days throughout the growing season, from June 17 to September 9, 2009, resulting in a total amount of ~573 mm of water supplied via irrigation and rainfall (316 and 257 mm, respectively). During the wet season, irrigation was applied only after more than six days of dry weather, in order to avoid crop failure. In S0.5, the amount of irrigation was 78 mm, with a total amount of water of ~966 mm supplied via irrigation and rainfall. In S1.5 the total amount of water supplied via irrigation and rainfall was of ~1,040 mm, whereas, in S2.5, it was of ~1,022 mm.

Measurements of N_2O-N fluxes were taken in 16 plots using manual static chambers. One static chamber per plot was used in S0.0 and S0.5, and two static chambers per plot in S1.5 and S2.5. The manual static chamber consisted of a metal base (0.38-m width x 0.58-m length) covering a soil area of 0.22 m^2 and of a plastic cap (0.1-m height) fixed on the metal base, similar to the chambers used by Carvalho et al. (2013b). In S2.5, the plastic covers were substituted by metal ones with the same dimensions previously described. The cover was always protected with an insulation foil in order to keep temperature inside the chamber as stable as possible at the sampling moment. When closed, the chamber had 19.8-L volume. Fluxes were measured weekly, in three to six consecutive DAS and N fertilization events. Gas samples were taken between 9:00 and 11:00 a.m., as recommended by Alves et al. (2012). Gases accumulated in the static chamber in 30 min were collected using a manual vacuum pump. Gas samples were then analyzed via gas chromatography with an electron capture detector (ECD), model Auto system XL GC (PerkinElmer do Brasil Ltda., São Paulo, SP, Brazil) calibrated with certified N_2O standards of 350 and 1,000 ppb (White Martins Gases Industriais Ltda., Brasília, DF, Brazil). The air temperature was measured simultaneously with N_2O-N flux sampling. Fluxes of N_2O-N (μg m^2 per hour) were calculated according to Rochette et al. (2004).

Soil moisture and ammonium (N-NH_4^+) and nitrate (N-NO_3^-) concentrations were determined from 100-g soil samples, collected within the 0–10-cm soil depth, simultaneously with N_2O-N sampling. Around 10 g of soil were weighed before and after drying for 24 hours at 105ºC. The water-filled pore space (WFPS) was calculated by considering the soil moisture (g g^{-1}) at the moment of N_2O-N sampling, soil bulk density (g cm^{-3}), and mineral particle density (g cm^{-3}). Mineral particle density (2.53 g cm^{-3}) was determined once, prior to the establishment of the field trial. Soil bulk density was obtained for each plot at every growing season and was calculated from the soil dry matter mass of undisturbed samples collected with a metal ring of known volume. The WFPS was determined according to Paul & Clark (1996). The available ammonium and nitrate were extracted from soil samples by shaking 20 g of soil with 60 mL of 1 mol L^{-1} KCl, for 60 min, according to Mulvaney (1996). Extraction was followed by determination through flow injection analysis (Ocean Optics, Inc., Dunedin, FL, USA); the final result was given in mg L^{-1}. To estimate mineral N (mg kg^{-1}), soil moisture at the moment of sampling was taken into account.

The effect of N fertilization, biochar application, and their interaction on N_2O-N fluxes and on soil-related variables (N-NH_4^+, N-NO_3^-, and WFPS) was assessed using linear mixed modelling. The averaged fluxes for the pre-established periods within each season, for three to six consecutive days after N fertilizations –

fertilization 1, at sowing, and fertilizations 2 and 3, at the top dressings performed around flowering – were compared through contrasts. The plot was considered as a random effect, in order to account for the correlations among measurements taken in the same plot during the cropping seasons and periods after N fertilization, which characterizes a data set of repeated measures. The analysis for each cropping season was performed separately. The model applied to analyze the data is described as: $y_{ijk} = \mu + N_i + Char_j + N_i \times Char_j + u_{ijk} + e_{ijk}$, in which μ is the overall mean; y_{ijk} is the observation of the response variable y corresponding to the i-th level of N fertilization (i = 0, 90 kg ha^{-1} N) and to the j-th level of biochar amendment (j = 0, 32 Mg ha^{-1}) of replicate k (k = 1, 2, 3, 4); N_i is the effect of the i-th N level; $Char_j$ is the effect of the j-th biochar level; $N_i \times Char_j$ is the interaction effect between the i-th N level and the j-th biochar level; $u_{ijk} \sim N(0, \Sigma)$ is the random effect to account for potential correlations among repeated measurements taken within the same plot (ijk = 1, …, 16); and $e_{ijk} \sim N(0, \sigma^2)$ is the random error associated with each observation y_{ij}.

Whenever the F-tests indicated significant interaction effects, at 5% probability, the F-tests for biochar effects were performed within N treatments. However, significant effects were not found for the interaction; therefore, only F-tests for the main effects were presented. Correlations between measured fluxes and soil-related variables within each season were determined with Pearson's correlation coefficient. Analyses were performed using the linear mixed model procedure (Proc Mixed) and the correlation procedure (Proc Corr) of the SAS software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The N_2O-N fluxes and soil-related variables were not significantly affected by the interaction between biochar and N fertilizer, in any of the monitored crop seasons (Table 1). The most significant effects on N_2O-N fluxes and soil-related variables were due to N application. In general, N application significantly enhanced N_2O-N fluxes, except in the S0.0 and S1.5 (Figure 1) seasons. Soil N-NH_4^+ and N-NO_3^- availability increased significantly with N application, in most of the cropping seasons, except in S0.0, when only soil N-NO_3^- availability was significantly increased by N fertilization (Figure 2).

The WFPS was around 50–70% in S0.0 and around 50–60% in S0.5 (Figure 3). In S1.5, the WFPS was around 60–80%, being significantly reduced by N application or increased by biochar amendment; however, when considering the entire season, the

Table 1. F-test p-values for the effects of N fertilization, of biochar application, and of their interaction on N_2O-N fluxes and on soil-related variables of a clayey Oxisol, along four cropping seasons, in the Cerrado (Brazilian savanna) region[(1)].

Source of variation	Fertilization 1				Fertilization 2				Fertilization 3				Season			
	N_2O-N	N-NH_4^+	N-NO_3^-	WFPS	N_2O-N	N-NH_4^+	N-NO_3^-	WFPS	N_2O-N	N-NH_4^+	N-NO_3^-	WFPS	N_2O-N	N-NH_4^+	N-NO_3^-	WFPS
									S0.0							
N fertilization	0.9087	0.2479	0.5963	0.1727	0.6394	0.4300	0.0046	0.2565	nd	nd	nd	nd	0.4605	0.2075	0.0081	0.9362
Biochar	0.5813	0.3273	0.3088	0.4835	0.4383	0.2726	0.5798	0.9048	nd	nd	nd	nd	0.7876	0.8772	0.4548	0.5487
Interaction	0.6425	0.9343	0.8552	0.6426	0.7083	0.5769	0.4076	0.2565	nd	nd	nd	nd	0.1159	0.6985	0.5054	0.3153
									S0.5							
N fertilization	0.4773	0.0007	0.0170	0.5552	0.0218	0.0044	<0.0001	0.7477	nd	nd	nd	nd	0.0408	<0.0001	0.0001	0.2685
Biochar	0.9592	0.8899	0.8990	0.8186	0.2008	0.6315	0.6908	0.6043	nd	nd	nd	nd	0.4012	0.7191	0.8314	0.4633
Interaction	0.3916	0.7724	0.6937	0.9235	0.7540	0.8237	0.6167	0.7477	nd	nd	nd	nd	0.3256	0.8515	0.5461	0.9359
									S1.5							
N fertilization	0.4767	<0.0001	<0.0001	0.0030	0.1329	<0.0001	<0.0001	0.0004	0.1741	0.0015	<0.0001	<0.0001	0.0791	<0.0001	<0.0001	<0.0001
Biochar	0.9015	0.8901	0.8644	0.2843	0.7048	0.4220	0.2983	0.0301	0.5419	0.4708	0.1138	0.0004	0.0804	0.1898	0.6637	<0.0001
Interaction	0.3073	0.9461	0.5452	0.9862	0.5959	0.5081	0.7271	0.3881	0.8376	0.4844	0.1748	0.1131	0.5707	0.5212	0.6818	0.5093
									S2.5							
N fertilization	0.0031	<0.0001	<0.0001	0.0007	0.0024	0.0020	<0.0001	<0.0001	0.2201	<0.0001	<0.0001	0.0006	0.0024	<0.0001	<0.0001	<0.0001
Biochar	0.9555	0.2117	0.0829	0.0004	0.4029	0.4343	0.3147	<0.0001	0.6484	0.8876	0.5078	0.0003	0.9767	0.1898	0.6637	<0.0001
Interaction	0.3044	0.4318	0.2056	0.4364	0.9297	0.9153	0.1218	0.8887	0.7271	0.9285	0.7994	0.9018	0.3098	0.5212	0.6818	0.5093

[(1)]N_2O-N, nitrous oxide fluxes (μg m^{-2} per hour); N-NH_4^+, available soil ammonium (mg kg^{-1}); N-NO_3^-, available soil nitrate (mg kg^{-1}); WFPS, soil water-filled pore space (%); S0.0, immediately after biochar application; S0.5, after 0.5 year from the application; S1.5, after 1.5 year; and S2.5, after 2.5 years. nd, not determined.

WFPS was significantly reduced by both biochar and N applications. Finally, in S2.5, the WFPS was around 50–70%, significantly lower in the treatments with N application. The inherent field capacity of a clayey Oxisol in the Cerrado region is around 40% of the WFPS (Andrade & Stone, 2011). The positive correlation between N_2O-N fluxes and the WFPS in N application treatments in S1.5 ($R^2 = 0.23$, $p \leq 0.011$) and, to a lesser extent, in S0.0 ($R^2 = 0.27$, $p \leq 0.099$), indicates that the WFPS is a relevant soil variable related to N_2O emission. Additionally, due to the highest WFPS observed in S1.5 (around 80%) and to the continuous pivot irrigation in S0.0, denitrification was probably the dominant process of N transformations in these specific seasons.

The WFPS in S1.5 was up to two times higher than the inherent field capacity of the evaluated Oxisol. Under these conditions, mostly anaerobic, the N lost to the atmosphere due to mineral N fertilization is likely to occur through N_2, resulting in a reduced probability to detect N_2O-N fluxes. As observed in the present study, no effect of the N fertilizer on N_2O-N fluxes was detected in S1.5. Furthermore, in a well-structured Oxisol, aggregation favors aeration but can also create permanent anaerobic hot spots intra-aggregate, where the reduction of N_2O into N_2 can occur, as shown by Leffelaar (1986). The formation of these hot spots can

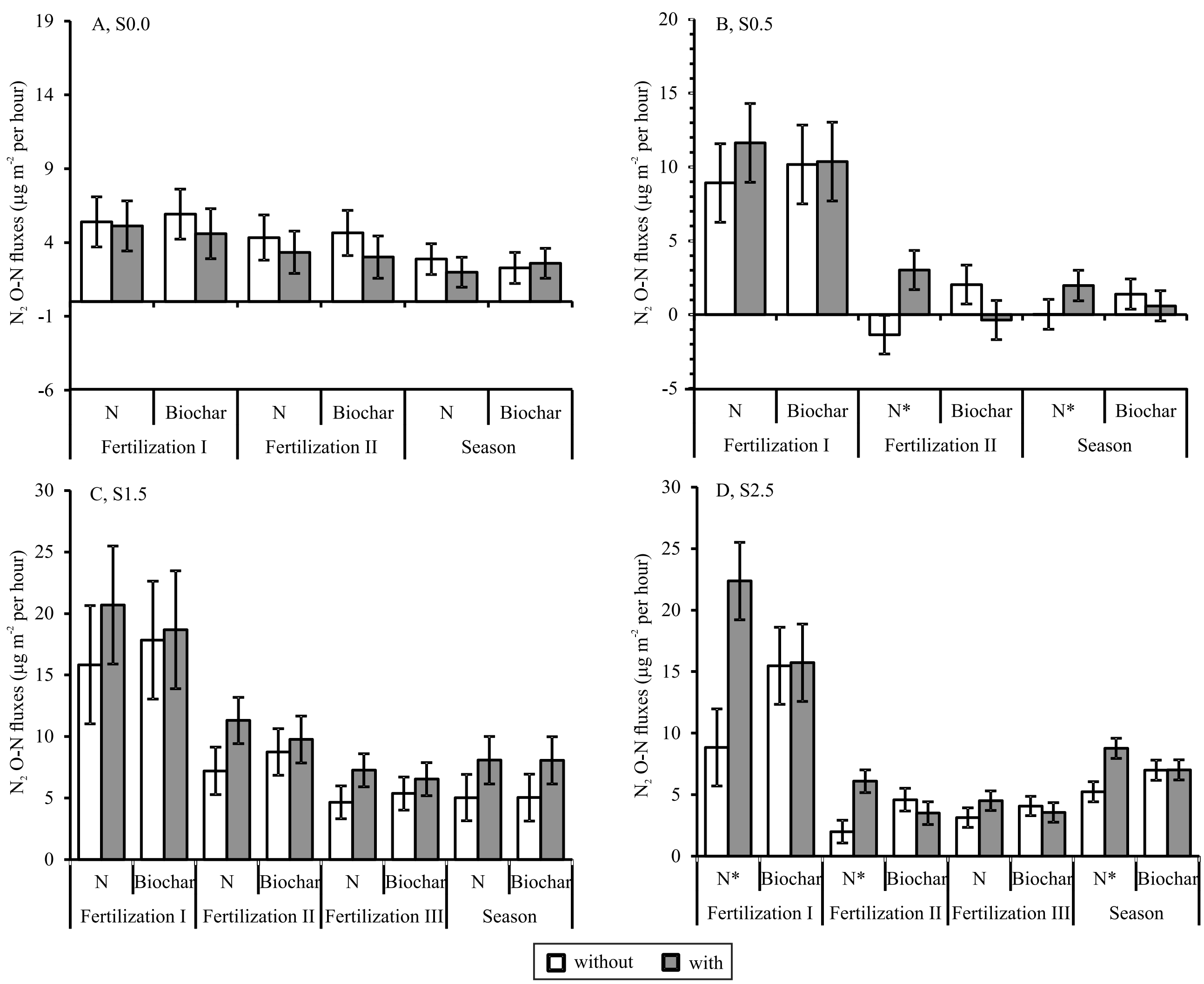

Figure 1. N_2O-N fluxes of a clayey Oxisol treated or not with 32 Mg ha^{-1} biochar and 90 kg ha^{-1} N (N), along three to six consecutive days after N fertilizations (Fertilization 1, at sowing; and Fertilizations 2 and 3, around flowering) and along the entire cropping season (Season), in the Cerrado (Brazilian savanna) region. A, immediately after biochar application (S0.0); and after B, 0.5 (S0.5); C, 1.5 (S1.5); and D, 2.5 (S2.5) years. Columns represent averaged fluxes, and error bars represent the standard error (n=4). *Significant effects of N and Char treatments, as in Table 1.

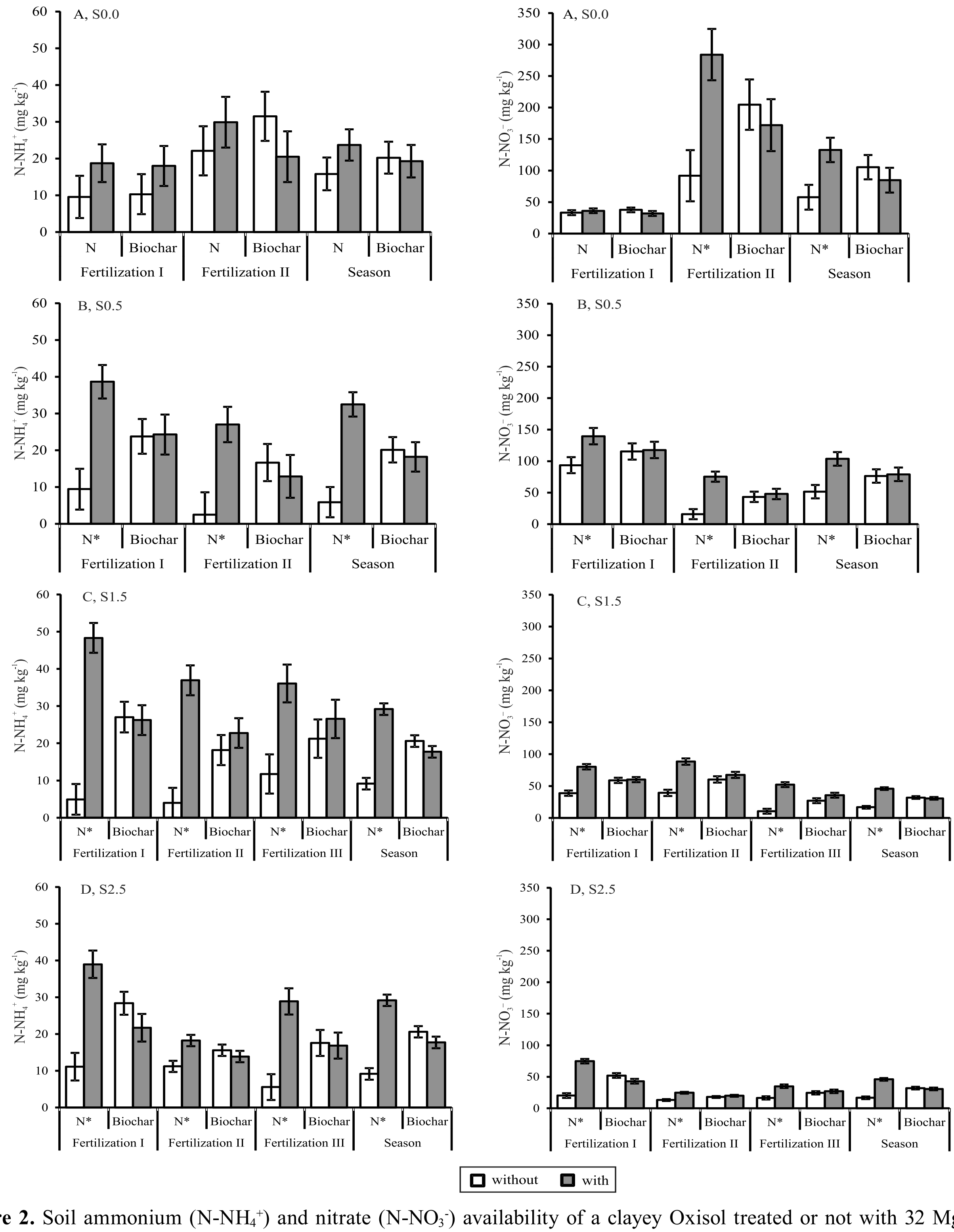

Figure 2. Soil ammonium ($N\text{-}NH_4^+$) and nitrate ($N\text{-}NO_3^-$) availability of a clayey Oxisol treated or not with 32 Mg ha^{-1} biochar and 90 kg ha^{-1} N (N), along three to six consecutive days after N fertilizations (Fertilization 1, at sowing; and Fertilizations 2 and 3, around flowering) and along the entire cropping season (Season), in the Cerrado (Brazilian savanna) region. A, immediately after biochar application (S0.0); and after B, 0.5 (S0.5); C, 1.5 (S1.5); and D, 2.5 (S2.5) years. Columns represent averaged fluxes, and error bars represent the standard error (n=4). *Significant effects of N and Char treatments, as in Table 1.

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explain the lack of a significant effect of N application on N_2O-N fluxes in S0.0, which was conducted under intermittent pivot irrigation. Moreover, if soil pH increases immediately after the application of biochar, as reported by Carvalho et al. (2013a), then the activity of denitrifiers was favored in S0.0, regardless of N fertilization. In the presence of biochar, denitrifiers can increase the reduction of N_2O into N_2, as reported by Taghizadeh-Toosi et al. (2011) and Mukherjee et al. (2014).

The lowest magnitude of the WFPS over the seasons was observed in S0.5 (Figure 3). Moreover, in S2.5, the WFPS was significantly lower and the N_2O-N fluxes were positively correlated with soil N-NO_3^- availability, in the treatments with N application (Table 2). Only in S2.5, were the N_2O-N fluxes in the treatments without biochar positively correlated with N-NH_4^+ availability (R^2 = 0.15, $p \leq 0.08$). It should be highlighted that N-NH_4^+ is an important substrate for the nitrification process in the soil. Apart from the higher soil mineral N availability due to N application (Figure 2), the obtained results indicate that the enhancement of N_2O-N fluxes is probably attributed to the predominant aerobic-soil conditions. Contrary to what was observed in the S0.0 and S1.5 seasons, in S0.5 and S2.5 increased nitrification processes are most likely the main causes for significant N_2O emissions in the treatments with N application.

Regarding treatments with biochar amendment, soil N-NO_3^- was the soil-related variable that positively correlated with N_2O-N fluxes, in all three rainfed cropping seasons – S0.5 (R^2 = 0.53, $p \leq 0.0001$), S1.5 (R^2 = 0.31, $p \leq 0.0005$), and S2.5 (R^2 = 0.35, $p \leq 0.0001$) – and, to a lesser extent, in the irrigated season – S0.0 (R^2 = 0.30, $p \leq 0.07$) (Table 2). The strongest Pearson's correlation coefficient was observed in S0.5, when the WFPS was relatively the lowest over all seasons. In addition, biochar significantly reduced the WFPS in

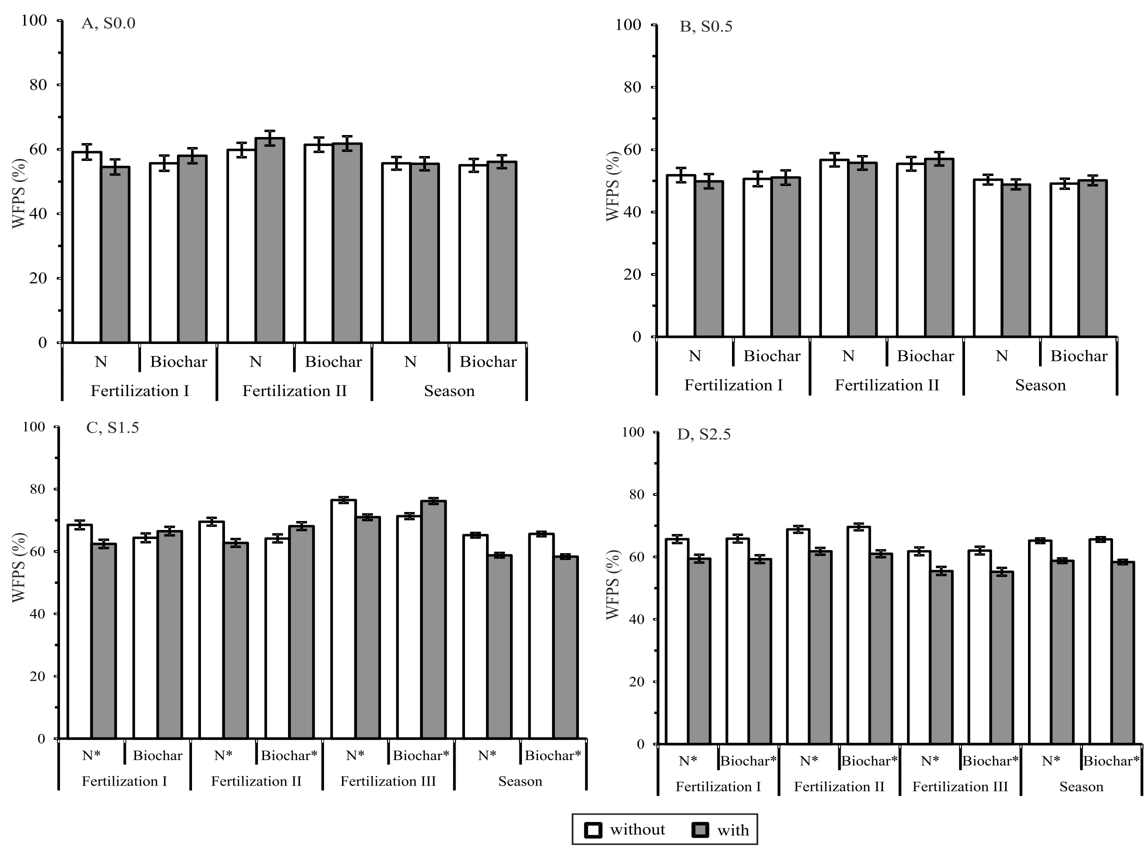

Figure 3. Soil water-filled pore space (WFPS) of a clayey Oxisol treated or not with 32 Mg ha^{-1} biochar and 90 kg ha^{-1} N (N), along three to six consecutive days after N fertilizations (Fertilization 1, at sowing; and Fertilizations 2 and 3, around flowering) and along the entire cropping season (Season), in the Cerrado (Brazilian savanna) region. A, immediately after biochar application (S0.0); and after B, 0.5 (S0.5); C, 1.5 (S1.5); and D, 2.5 (S2.5) years. Columns represent averaged fluxes, and error bars represent the standard error (n=4). *Significant effects of N and Char treatments, as in Table 1.

Table 2. Pearson's correlation coefficients for the relation between N_2O-N fluxes and soil-related variables of a clayey Oxisol treated or not with 32 Mg ha^{-1} biochar and 90 kg ha^{-1} N, along four cropping seasons, in the Cerrado (Brazilian savanna) region[(1)].

Biochar	S0.0			S0.5			S1.5			S2.5		
	$N-NH_4^+$	$N-NO_3^-$	WFPS	$N-NH_4^+$	$N-NO_3^-$	WFPS	$N-NH_4^+$	$N-NO_3^-$	WFPS	$N-NH_4^+$	$N-NO_3^-$	WFPS
N Fertilization												
Without	-0.12	-0.13	0.16	0.05	0.33*	-0.18	-0.23*	0.10	0.15	0.04	-0.01	0.24**
With	0.23	0.04	0.27	0.02	0.18	-0.21	0.14	0.26*	0.23*	0.09	0.26**	0.12
Biochar application												
Without	-0.06	-0.15	0.21	0.06	0.09	-0.18	0.13	0.14	0.18	0.20*	0.23**	0.14
With	0.28	0.02	0.30	0.18	0.53**	-0.24	0.14	0.31**	0.10	0.15	0.35**	0.01
Observations	40≥ n ≥30			48≥ n ≥29			120≥ n ≥109			136≥ n ≥129		

[(1)]S0.0, immediately after biochar application; S0.5, after 0.5 year from the application; S1.5, after 1.5 year; and S2.5, after 2.5 years. * and **Significant by the t-test at 5 and 1% probability, respectively.

S1.5 and S2.5 (Figure 3). In soils where nitrification is the main pathway for N_2O production, such as under aerobic conditions, the presence of biochar can even intensify the process of nitrification, as shown by Sánchez-García et al. (2014).

The use of biochar amendment, however, had no significant effect on N_2O-N fluxes from N fertilizer, under the conditions in the present study. Similarly, Verhoeven & Six (2014) found no reduction in N_2O emission in a sandy clay loam soil treated with 10 Mg ha^{-1} walnut shell and pine chip biochar, in a cropping system under Mediterranean climate. According to these authors, pine chip biochar enhanced N_2O-N fluxes when compared with the treatment with no amendment, during the two growing seasons after biochar application. This result differs from that obtained by Zhang et al. (2012a), who found a reduction of around 51–56% in N_2O-N fluxes from a typical Chinese rice paddy soil. This reduction was observed in the first and second cropping seasons, on a 39% clayey soil, after the application of 40 Mg ha^{-1} wheat straw biochar together with 300 kg ha^{-1} N, in comparison with the application of N fertilization alone. A reduction in N_2O emission from the N fertilizer applied with wheat straw biochar in rice paddy systems was also reported by Liu et al. (2012). This effect is probably related to an increase in soil aeration due to a decrease in soil bulk density with biochar amendment.

The results obtained in the present study show that, regardless of biochar amendment, N fertilization was the major factor associated with N_2O-N fluxes in the cropping system. These findings differ from those of Liu et al. (2014), who observed a decrease in N_2O emission when 300 kg ha^{-1} N fertilizer were combined with 40 Mg ha^{-1} wheat straw biochar, in a five-year irrigated maize-wheat cropping system on a Chinese calcareous soil.

Conclusions

1. Wood biochar amendment (1.6% w/w) does not interact with N fertilization and does not affect N_2O-N fluxes, up to 2.5 years after its application on a clayey Oxisol in the Cerrado (Brazilian savanna) region, under aerobic conditions.

2. Mineral N application enhances N_2O-N fluxes, as well as soil $N-NH_4^+$ and $N-NO_3^-$ availability, especially during seasons characterized by lower water-filled pore space (WFPS).

3. Long-term studies in the field are important to quantify the impacts of biochar on N_2O-N fluxes and to determine the dynamics of these fluxes on soil-related variables, such as WFPS.

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