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Mitigation of GHG Emissions from Soils Fertilized with Livestock Chain Residues

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Abstract: Using livestock residues as alternative fertilizers is a sustainable practice which recycles nutrients that would otherwise be lost. However, organic fertilizers may have a large impact on N_2O emissions, offsetting the beneficial effects of C sequestration. After four years from biochar application, greenhouse gas fluxes were monitored for two years from a Maize field fertilized with digestate, slurry, or urea, with and without biochar. The objectives of the present study were to assess (i) the climate feedback of using residues from the livestock chain as alternative fertilizers and (ii) the contribution of biochar in mitigating GHGs emissions, while increasing the organic C in soil. Digestate was shown to have the highest impact on CO_2 and N_2O emissions from soil, with respect to mineral fertilization (+29 and +142%), more than slurry (+21 and −5%), whereas both residues positively affected CH_4 uptake (+5 and +14%, respectively). The maximum N_2O peaks occurred between 7–20 days after fertilization, accounting for 61% of total emissions, on average. Biochar was effective in reducing N_2O emissions derived from mineral fertilization and digestate (−54% and −17%, respectively). An excess of labile organic matter and N induced the highest CO_2 emissions and N_2O peaks, independent of—or even triggered by—biochar. Mitigation of GHG emissions, from soils fertilized with livestock chain residue, can be obtained using biochar, but with limitations dependent on (i) the quantity of organic matter added, (ii) its quality, and (iii) the time from application: those aspects that deserve further investigations.

Keywords: biochar; digestate; slurry; sustainable management; climate change mitigation

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1. Introduction

Currently, the agriculture sector accounts for 30–35% of global Greenhouse Gas (GHG) emissions, and it is the largest contributor to anthropogenic non-CO_2 GHGs [1,2], largely due to methane (CH_4) emissions from livestock and rice cultivation, and nitrous oxide (N_2O) emissions from fertilized soils. It is estimated that approximately 60% of the global anthropogenic N_2O emissions is from cultivated soil [3,4], either as a direct consequence of synthetic N fertilizers, crop residues and manure, or indirectly from leached N compounds, such as nitrates [5–8]. Given its high global warming potential, N_2O emissions represent the highest risk of negating the beneficial effects of increasing organic matter (OM) inputs to enhance C sequestration [9].

The use of N-fertilizers directly influences the amount of NH_4^+ or NO_3^- available in soil, which, in turn, affects nitrification [10–12] and denitrification [13–15] activities. It has also been shown that the intensity of N_2O emissions is related to N fertilizer rate [16]; soil N excess, such as N amounts not available to plants, lead to increasing N_2O emissions [17].

In the vision of circular economy, using crop or livestock residues is a sustainable strategy, improving soil organic matter and recycling nutrients that otherwise would be lost from the soil system. Organic fertilization includes different types of organic matter, such as animal manures, slurry, crop residues, green manures, and compost [18]. Depending on the type of residue, organic fertilizers generally have a large impact on N_2O emissions, even when compared to mineral fertilizers [19]. To predict N_2O emission, the quality and quantity of crop residues being applied to agricultural fields should be linked to soil properties and pedo-climatic conditions [20–22]. The application method is significant as well, with the highest emissions occurring with surface application, in comparison to techniques that rely upon incorporation further into the soil [19,23].

Anaerobic digestion of agricultural materials and sludges is a promising strategy for generating products that can be applied to enhance the productivity of agricultural soils, being rich in plant available nutrients [24], but only few relevant studies have been conducted [25–27]. Overall, a short-term increase in N_2O emissions is reported, depending on soil texture, moisture, and temperature [28], as well as digestate composition, soil type, and NH_4^+ availability [27]. The increase can be significantly reduced by adjusting the timing of application, controlling pH, and combining digestate with other amendments, such as biochar [29].

Biochar addition to soil has been proposed as a method to increase soil C storage and reduce N_2O emissions from soil on a global scale [30–32]. Biochar has been reported to decrease the denitrification process [33,34], while positively affecting nitrification [35]. Several possible mechanisms driving reduction in N_2O emissions from soil after biochar application have been proposed, including: (i) change of soil structure, improving soil aeration, and/or reducing soil moisture, which inhibit denitrification through enhancing O_2 concentration [33,35,36]; (ii) sorption of inorganic-N substrates no longer available for nitrification and denitrification processes [37,38]; (iii) increase in soil pH that may stimulate the N_2O reductase enzyme activity, increasing N_2 production and N_2/N_2O ratio [36,39,40]; (iv) the capacity of biochar to act as electron acceptor through Mn(IV) and Fe(III) surface sites, therefore promoting the last step of denitrification pathway [33,41]. However, large gaps of knowledge exist on the real mitigation capacity, depending on the type of applied fertilizers and the processes involved.

Actually, there is a good understanding regarding the effect of specific soil management strategies on either C sequestration or non-CO_2 GHG fluxes [42]. Nevertheless, integrated and comprehensive investigations, considering the trade-off between C sequestration and the outputs, including the three GHGs, are still scarce [43]. The objectives of the present study were to assess (i) the climate feedback of using residues from livestock chain (slurry and digestate) as alternative fertilizers, (ii) the contribution of biochar in mitigating GHGs emissions, while increasing the organic C in the soil. With our approach, we intend to cover knowledge gaps on the mitigation potential of the interactions between livestock chain residues and biochar, assessing the dynamics of mineral N lost into the groundwater or in the atmosphere.

2. Materials and Methods

2.1. Experimental Site

The experimental site was established in 2018 at Cascina Baroncina (Lodi, Po Valley, Italy; 45°17′25′′ N−9°29′43′′ E−81.5 m asl) on a sandy loam soil with a sub-acid reaction. Main soil characteristics are reported in Table S1. The Lodi area lies in a "nitrate vulnerable zone" (according to the 91/676/EEC Directive against pollution caused by nitrate from agricultural sources) in which a maximum of 170 kg ha^{-1} of N, available for plants from livestock effluents, is allowed. The daily air temperature (mean, max, and min values), rainfall, and irrigation interventions during the 2020 and 2021 growing seasons are reported in Figure S1. The crop rotation system is represented by silage maize–Italian ryegrass characterizing the intensive dairy cattle farming regions of the Po Valley (Figure S2). The Pioneer hybrid 1547 class 600 was sown in June (Table 1) and harvested after 90 and 99 days in 2020 and 2021, respectively. The Italian ryegrass cv. Star was sown at the beginning of October and harvested in May.

Table 1. Crop management, GHGs monitoring, and soil sampling events during the maize growing seasons 2020 and 2021.

	2020	2021
Fertilization (33% urea; 100% organic) and ploughing	23 June	7 June
Harrowing and sowing	24 June	8 June
Pre-emergency weeding	25 June	9 June
Static chambers installation	29 June	10 June
Gas monitoring	17 events	17 events
N mineral sampling	6 events	6 events
Irrigation	3 events	6 events
Post emergency weeding	17 July	6 July
In-season (top-dress) urea fertilization (67%)	15 July	7 July
End of gas sampling	31 August	5 August

The treatments investigated in the trial involved the application of biochar from pyrolysis processes in 2018, at a dose of 20 t ha^{-1} of dry matter (DM), and incorporated it into the soil by ploughing 30 cm depth. Biochar main characteristics are reported in Table S2.

Starting from 2018, and for the following 4 years, digestate and slurry were spread in springtime each year and incorporated into the soil by ploughing within 24 h. Main characteristics are reported in Table 2. Urea distribution was split, with one third at sowing and the remaining part at one in-season application (Table 1). The amount of fertilizer supplied was calculated to provide 170 kg ha^{-1} N available for a plant, considering 100% of urea and of the N-NH_4 fraction of the organic fertilizers, as well as 50% of the organic N fraction (Table 2).

Table 2. Characteristics, added doses, and N inputs of digestate (solid + liquid fractions) and slurry for the 2020 and 2021 growing seasons. N added is the sum of N-NH_4 + N org. Effective N is the sum of N-NH_4 + 0.5 N org.

	Digestate		Slurry	
	2020	2021	2020	2021
Moisture content (%)	92.1	93.1	94.6	94.7
Norg content (g kg^{-1})	2.43	1.61	4.32	1.64
N-NH_4 content (g kg^{-1})	2.57	1.48	0.98	0.46
Effective N (%)	0.38	0.23	0.31	0.13
Added dose (Mg ha^{-1})	54	74	45	133
N added (Kg ha^{-1})	270	229	239	279
Effective N added (Kg ha^{-1})	205	170	140	173

In the case of mineral fertilization, 80 kg ha^{-1} P_2O_5 and 180 kg ha^{-1} K_2O were also distributed at sowing. The Italian ryegrass was grown without fertilization to highlight possible residual nutrients present into the soil after the maize cropping.

Within the field, 2 blocks were arranged in strips, having received the same agronomical treatment for 20 years. In each block, six plots were established, considering mineral fertilization, digestate, and slurry with and without biochar. In each plot, 2 chambers were installed as sub-replicates, for a total of 24 chambers (2 blocks $\times$ 2 sub−replicates $\times$ 3 fertilization treatments $\times$ with and without biochar).

2.2. Crop and Soil Analyses

Crop yield has been assessed at harvest, manually cutting a subsample of 5 m^2 (about 30 plants) for each plot, which was chopped and dried at 60 °C to measure the dry matter percentage of silage.

Post-harvest soil samples were taken from each individual parcel (0–30 cm deep), dried at 40 °C in a ventilated oven, and sieved at 2 mm. Total organic C was determined on a homogenized fine fraction (500 µm), with an elemental analyzer (Flash Smart NC Soil, Thermo Fisher, Waltham, MA, USA), after subtracting inorganic C from total C.

Extractable mineral N was determined after collecting the samples at a 0–30 cm depth and transporting them to the laboratory in a refrigerated container. Samples have been collected in the first six GHGs monitoring events of 2020 and in the first 6 weeks in 2021. The NH_4 and NO_3 fractions of mineral N were determined by extraction with 2M KCl and subsequent distillation (UDK 132, Velp Scientific, Usmate Velate, Italy), with the addition of Devarda for the determination of nitric N. Samples moisture, which was also determined to express the data on dry matter.

Cumulated NH_4 and NO_3 values were assessed by means of ion-exchange resin lysimeters and were assembled using pure resin PMB 101-3 (with an ion exchange capacity of 1.3 eq/L min). Additionally, 15 g of resin were enclosed in a nylon bag with sufficient fine mesh to prevent material from escaping. The nylon bag was placed between two layers of glass beads (2–3 mm diameter) and enclosed in a polyvinyl chloride pipe section (5.3 cm diameter and 3 cm height), all enclosed in a mesh net. In 2020, at sowing, in each plot, a lysimeter was placed at 40 cm deep and subsequently recovered at harvest. In 2021, two lysimeters for plot (excluding those with slurry) were placed at sowing and recovered at harvest. The collected lysimeters were opened in the laboratory, and N was extracted by washing the resin with 2 M KCl extraction solution (ratio 1:10), in 500-mL Erlenmeyer flasks on an orbital shaker, at 100 rpm for 1 h. Extracts were filtered (Whatman no. 42 filters) and analyzed for NO_3 and NH_4 concentration through distillation (UDK 132, Velp Scientific), with the addition of Devarda for the determination of nitric N.

2.3. GHG Fluxes

During each maize growing season, 17 gas samplings were performed (see Table 1 for starting dates) by means of the closed chamber method described in [44], for measuring soil CO_2, CH_4, and N_2O fluxes. Gas sampling was performed between 9.30 and 13.30 to minimize changes in soil CO_2 effluxes associated with diurnal cycles [45]. During each gas sampling event, chambers were closed for 30 min with four gas samplings (at 0, 10, 20, and 30 min). Additionally, 25 mL of headspace gas were collected with air-tight 30 mL propylene syringes and were immediately pressurized into pre-evacuated 12 mL glass Exetainer® vials (Labco Ltd., Buckinghamshire, UK). Soil temperature and moisture were also measured at each time. Soil temperature was measured with a probe (HI 9043 Hand-Held Thermometer, Hanna Instruments, Woonsocket, RI, USA) and volumetric soil moisture was measured with a ML3 ThetaProbe Soil Moisture Sensor (Delta-T Device, Cambridge, UK) at a depth of 5 cm.

Gas samples were analyzed within 4 weeks of collection using a GC-2014 gas chromatograph (Shimadzu Scientific, Kyoto, Japan) with a thermal conductivity detector for CO_2, ^{63}Ni electron capture detector for N_2O and flame ionization detector for CH_4. Chamber gas concentrations were converted to mass per volume unit using the Ideal Gas Law and measured chamber air temperatures and volumes. Fluxes of CO_2, N_2O and CH_4 were calculated using the slope of linear regression of gas concentration vs. chamber closure time and the enclosed soil surface area. Fluxes were set to zero if the change in gas concentration during chamber enclosure fell below the minimum detection limit of GC, and flux values were rejected (i.e., treated as missing data) if they passed the detection test but had a coefficient of determination (R^2) < 0.90. Estimates of cumulative CO_2, N_2O and CH_4 fluxes for each field replicate were based on linear interpolation between sampling dates during each growing season [46].

2.4. Statistical Analysis

Mixed model analysis of variance (ANOVA) module of Statistica package (StatSoft Inc., Tulsa, OK, USA) and Fisher's LSD Post-Hoc test were performed to evaluate the effects of fertilizers and biochar on CO_2, N_2O and CH_4 fluxes, SOIL TOC, crop yield, NH_4 and NO_3 availability in soil in 2020 and 2021. Univariate Tests of Significance for CO_2, N_2O and CH_4, with Sigma-restricted parameterization for each year was performed for cumulative values.

3. Results

3.1. Crop Yield and Soil Organic C

Overall, 11% increase in maize yield was observed in 2021 with respect to the previous year, although it was not significant (Table 3). Among treatments, the mineral fertilization showed the lowest values with respect to all other treatments in 2020, and the highest with respect to mineral fertilization plus biochar in 2021.

Table 3. Crop yield, soil TOC content, and cumulated NH_4 and NO_3 at the end of 2020 and 2021 maize growing seasons. Standard deviation is reported in brackets. Different letters (a, b, c, etc.) indicate significant differences between treatments and years.

		Yield Gg ha^{-1}	TOC Gg ha^{-1}	N-NH$_4$ Kg ha^{-1}	N-NO$_3$ Kg ha^{-1}
2020	Digestate	16.4 (1) ^{a,b,c}	44 (0.4) ^{d,e,f}	6.7 (2.3) ^{b}	5.7 (1.0) ^{a}
	Digestate + biochar	16.9 (1) ^{a,b,c}	49 (2) ^{c,g}	7.1 (0.1) ^{b}	50.1 (47) ^{a}
	Slurry	14.9 (0.2) ^{a,b,c}	39 (0.8) ^{f}	7.3 (0.7) ^{b}	10.2 (6.3) ^{a}
	Slurry + biochar	14.9 (1) ^{a,b,c}	49 (0.6) ^{c,d,g}	7.7 (1.1) ^{b}	3.7 (2.2) ^{a}
	Mineral	13.6 (1) ^{c}	39 (1) ^{f}	6.2 (1.8) ^{b}	11.6 (5.7) ^{a}
	Mineral + biochar	14.2 (0.8) ^{b,c}	56 (3) ^{a,b}	13.0 (2.0) ^{a}	14.4 (4.2) ^{a}
2021	Digestate	16.6 (0.5) ^{a,b,c}	46 (3) ^{d,e,g}	8.6 (0.5) ^{b}	2.9 (1.1) ^{a}
	Digestate + biochar	18.6 (3) ^{a,b}	50 (2) ^{c,g}	10.2 (0.2) ^{a,b}	29.5 (3.6) ^{a}
	Slurry	16.9 (0.2) ^{a,b,c}	46 (0.8) ^{d,e,g}		
	Slurry + biochar	15.6 (3) ^{a,b,c}	59 (3) ^{a}		
	Mineral	19.2 (1) ^{a}	42 (1) ^{e,f}	10.3 (0.7) ^{a,b}	27.6 (19.4) ^{a}
	Mineral + biochar	14.0 (1) ^{c}	54 (1) ^{b,c}	11.2 (1.2) ^{a,b}	21.5 (12.6) ^{a}

Soil TOC (Table 3) remained stable in the 2 years of measurement, with a slight not significant increase in 2021. Among treatments, there was a clear effect of biochar addition in 2020, with all fertilizers, and in 2021, with slurry and mineral fertilization. No significant differences among fertilizers were observed.

3.2. NH_4 and NO_3 Dynamics and Accumulation in Soil

Inorganic N showed slight oscillations in the first 2 weeks of 2020 and in the first month of 2021 growing seasons, with NO_3 values about two times higher than those of NH_4 (Figure 1). Higher NH_4 values were observed with digestate than mineral, 2–3 weeks after fertilization in 2020. Mineral fertilization + biochar showed the highest NH_4 content after the top dress fertilization in 2021 growing season. Digestate + biochar showed higher NO_3 values immediately and 1 month after initial fertilization in 2021. Mineral fertilization + biochar showed lower values of NO_3 in one event of each growing season.

Cumulative NH_4 reached the maximum values with mineral fertilization + biochar, which is significantly different from those without biochar. NO_3 values did not show significant differences, although a peak was observed with digestate + biochar (Table 3).

3.3. GHGs Fluxes

CO_2 emissions (Figure 2) showed a decreasing trend in the two growing seasons, with the maximum emissions observed in the first 10 days after digestate and slurry application, followed by a slow decrease. A significant correlation with temperature ($p < 0.05$) was observed. The increase in CO_2 emissions with digestate was significant in four events in the second half of the 2020 growing season and throughout 2021 (15 out of 17 events).

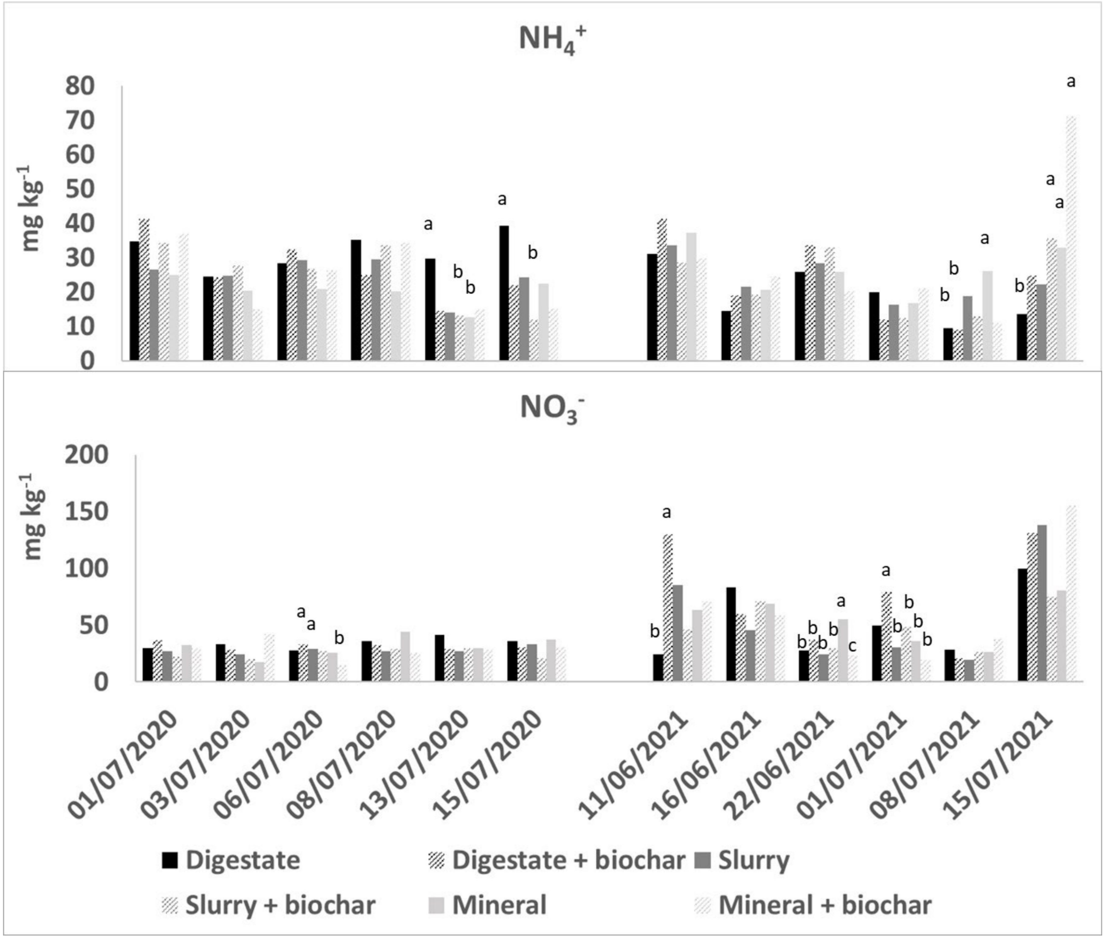

Figure 1. NH_4 (**top**) and NO_3 (**bottom**) content of soil at 0–30 cm depth during the first six monitoring events of 2020 and during the first 6 weeks of 2021 maize growing seasons in the 6 treatments (digestate, slurry, and mineral fertilization with and without biochar). Different letters indicate significant differences between treatments for each sampling event.

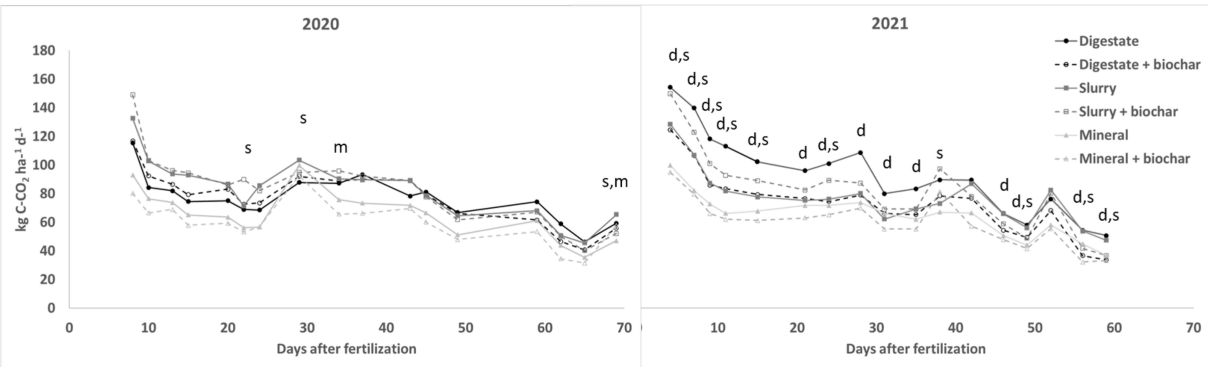

Figure 2. C-CO_2 emissions from soil during 2020 and 2021 maize growing seasons for the 6 treatments (digestate, slurry, and mineral fertilization with and without biochar). Significative biochar effects on emissions are reported for each date from digestate (d), slurry (s), and mineral (m) fertilization.

Slurry induced an increase in CO_2 emissions in seven events during the 2020 growing season and in eight events at the beginning and at the end of the 2021 growing season (Table 4). Biochar significantly reduced CO_2 emissions from soils fertilized with digestate in most of the 2021 growing season. CO_2 emissions from soils fertilized with slurry increased with biochar at the beginning of the 2021 growing season and decreased at the end.

Table 4. Percentage effects of organic fertilizers (digestate or slurry vs. Mineral fertilization) and biochar (in combination with digestate, slurry, and mineral fertilization) on CO_2, CH_4, and N_2O fluxes during 2020 and 2021 maize growing seasons. DAF: Days After Fertilization. Significances of the effects are reported at * $p < 0.05$ (Univariate ANOVA).

2020																	
DAF	**8**	**10**	**13**	**15**	**20**	**22**	**24**	**29**	**34**	**37**	**43**	**45**	**49**	**59**	**62**	**65**	**69**
DAY	1/7	3/7	6/7	8/7	13/7	15/7	17/7	22/7	27/7	30/7	5/8	7/8	11/8	21/8	24/8	27/8	31/8
CO_2																	
Dig. vs Min.	24	10	11	14	18	23	21	12	15 *	27 *	9	22	31 *	22	34	31	26 *
Slu. vs Min.	42	35	27 *	43 *	37 *	27	51 *	4	19 *	22	24 *	18	26	11	16	28	39 *
Bioch-Dig	1	9	6	6	11	5	7	5	2	−4	14	−3	−1	−17	−21	−12	−6
Bioch-Slu	12	0	3	2	−1	26 *	−4	−8 *	6	3	0	−1	−4	−1	−1	−12	−20 *
Bioch-Min	−14	−13	−7	−11	−7	−5	0	−7	−13 *	−10	−3	−10	−6	−13	−21	−11	23 *
CH_4																	
Dig. vs Min.	75	0	31	25	6	34 *	−15	−6	59 *	21	67 *	27	34	28	235 *	115 *	39
Slu. vs Min.	35	−19	37	−3	−26	−2	−28 *	−13	31	0	31	12	68 *	−2	197 *	30	25
Bioch-Dig	4	44	171	309	434	573 *	468 *	1	18	15	−90 *	−84 *	−22	−13	−36	−1	−13
Bioch-Slu	5	22	98	87	−21	18	−19	−23	−13	−151	−38	−42	−22	−19	31	−12	−48
Bioch-Min	−29	−38	−53	−29	−53	−44	−28	−53	−44	−33	−76	−73	−51	−84 *	−65 *	21	−51
N_2O																	
Dig. vs Min.	2701 *	2004	954	322	123	44	20	15	−22	451	193	99	12	−68 *	2	15	−16
Slu. vs Min.	326	148	18	6	71	6	41	16	−43	−71	−84	−84	−56	−79 *	−56 *	−11	−27
Bioch-Dig	4	44	171	309	434	573 *	468 *	1	18	15	−90 *	−84 *	−22	−13	−36	−1	−13
Bioch-Slu	5	22	98	87	−21	18	−19	−23	−13	−151	−38	−42	−22	−19	31	−12	−48
Bioch-Min	−29	−38	−53	−29	−53	−44	−28	−53	−44	−33	−76	−73	−51	−84 *	−65 *	21	−51

2021																	
DAF	**4**	**7**	**9**	**11**	**15**	**21**	**24**	**28**	**31**	**35**	**38**	**42**	**46**	**49**	**52**	**56**	**59**
DAY	11/6	14/6	16/6	18/6	22/6	28/6	1/7	5/7	8/7	12/7	15/7	19/7	23/7	26/7	29/7	2/8	5/8
CO_2																	
Dig. vs Min.	55 *	70 *	62 *	71 *	51 *	34 *	41 *	48 *	21 *	34 *	34	35 *	31 *	32 *	31 *	21	38 *
Slu. vs Min.	29 *	30 *	20 *	23 *	15	5	6	9	−6	11	9	31	30 *	27 *	42 *	20	29 *
Bioch-Dig	−19 *	−24 *	−27 *	−26 *	−22 *	−20 *	−26 *	−27 *	−17 *	−22 *	−13	−14	−18 *	−15 *	−11	−33 *	−34 *
Bioch-Slu	17 *	15 *	15 *	13 *	15 *	10	17 *	9	11	0	33 *	−10	−11	−13 *	−3	−22 *	−23 *
Bioch-Min	−5	−3	−9	−6	−10	−12	−9	−5	−16	−11	21	−14	−6	−6	−4	−28	−10
CH_4																	
Dig. vs Min.	−920	−202	9	19	23	29	−12	−1124	40	62	35	−230	−176	23	1	−13	48
Slu. vs Min.	−130	20	6	77 *	−12	30	−6	−1146 *	5	72 *	25	146	−10	−33	19	7	9
Bioch-Dig	5	−66 *	−70 *	−75 *	−75 *	−71 *	−66 *	−21	−52 *	−52 *	−65	−74	−57	−39	−58	−16	−29
Bioch-Slu	41	164 *	151 *	177 *	150	−11	69	59	42	11	−59 *	−35	−12	77	−16	4	−63
Bioch-Min	−20	−7	−14	−15	77	−50	−16	10	−12	−22	−41	−91 *	−64	−70	−53	−63	−39
N_2O																	
Dig. vs Min.	546 *	914 *	796 *	597 *	259	5	33	−24	10	37 *	28	−70 *	−38	−54 *	−33	−54 *	−52
Slu. vs Min.	271	380	391	208	70	−36	−12	−39	−53 *	−40 *	170 *	−67 *	−45	−63 *	−44	−59 *	−46
Bioch-Dig	5	−66 *	−70 *	−75 *	−75 *	−71 *	−66 *	−21	−52 *	−52 *	−65	−74	−57	−39	−58	−16	−29
Bioch-Slu	41	164 *	151 *	177 *	150	−11	69	59	42	11	−59 *	−35	−12	77	−16	4	−63
Bioch-Min	−20	−7	−14	−15	77	−50	−16	10	−12	−22	−41	−91 *	−64	−70	−53	−63	−39

Overall, cumulative CO_2 emissions (Figure 3 and Table 5) were higher from soil with digestate and slurry than from soil with mineral fertilization in both years, with the largest increase in 2020 with slurry and in 2021 with digestate. The effect of biochar was significant in 2021, with a reduction in CO_2 emissions from soil with digestate and an increase with slurry.

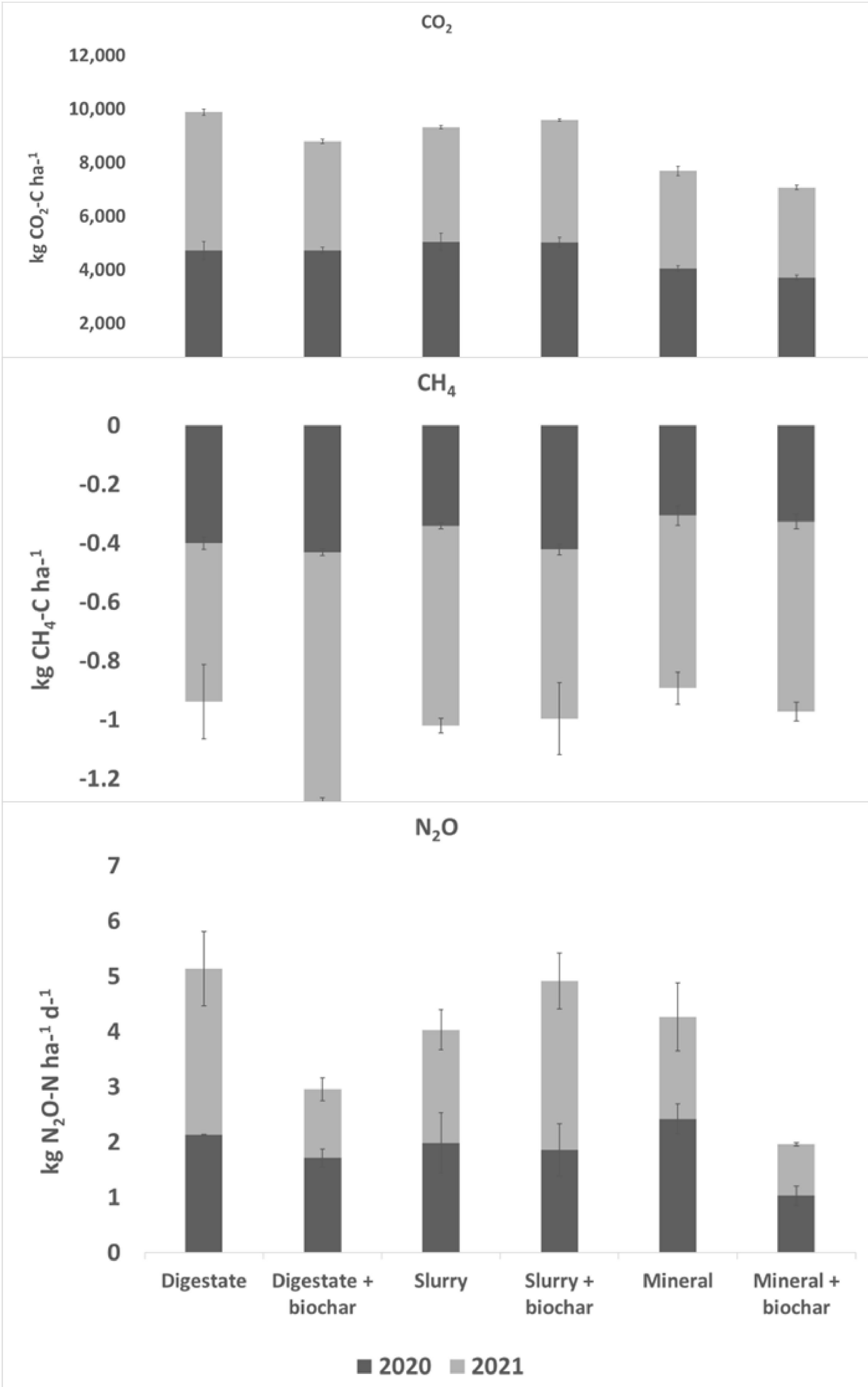

Figure 3. Cumulative fluxes of CO_2 (**top**), CH_4 (**middle**), and N_2O (**bottom**) from soil in 2020 and 2021 maize growing seasons for the six treatments (digestate, slurry and mineral fertilization with and without biochar). Error bars represent standard errors for each year.

Table 5. Percentage effects of organic fertilizers (digestate or slurry vs. Mineral fertilization) and biochar (in combination with digestate, slurry, and mineral fertilization) on cumulative CO_2, CH_4, and N_2O fluxes in 2020 and 2021 maize growing seasons. Significances of the effects are reported at $p < 0.1$ #, $p < 0.05$ *, $p < 0.01$ **, and $p < 0.01$ *** (Univariate Analysis of Variance).

	2020						2021					
	Organic vs. Mineral Fertilizer (%)			Biochar Effect (%)			Organic vs. Mineral Fertilizer (%)			Biochar Effect (%)		
	CO_2	CH_4	N_2O	CO_2	CH_4	N_2O	CO_2	CH_4	N_2O	CO_2	CH_4	N_2O
Digestate	17 #	+31 *	+202 #	0	+8	+24	+42 ***	−8	+63 #	−21 ***	+58 *	−59 *
Slurry	25 *	+11	−18	0	+24 **	−6	+18 *	+16 #	+11	+6 **	−15	+49
Mineral	–	–	–	−8 *	+7	−57 **	-	–	–	−8 #	+10	−50 #

CH_4 uptake was prevalent throughout the 2020 growing season and for most of the monitoring events in 2021. CH_4 emissions were observed in the first two events, after fertilization in digestate and slurry + biochar plots, and in two events in the second half of the growing season with digestate (Figure 4, Table 4). CH_4 uptake increased significantly with digestate, with respect to mineral, in five events in 2020, and with slurry in two events in 2020 and three events in 2021. Biochar increased CH_4 uptake with slurry in four events in 2020, as well as with mineral fertilization in two events in 2020 and one event in 2021. CH_4 emissions decreased with biochar, after fertilization with digestate, and increased after fertilization with slurry in 2021.

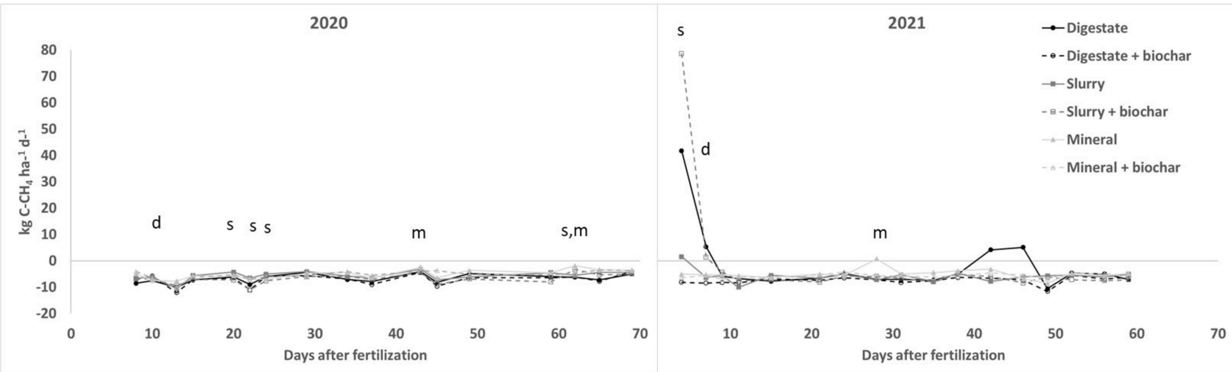

Figure 4. C-CH_4 fluxes from soil during the 2020 and 2021 maize growing seasons for the six treatments (digestate, slurry, and mineral fertilization, with and without biochar). Significative biochar effects on emissions are reported for each date from digestate (d), slurry (s), and mineral (m) fertilization.

Overall, cumulative CH_4 uptake was higher with digestate in 2020 and with slurry in 2021, with respect to mineral fertilization. Conversely, biochar increased CH_4 uptake with slurry in 2021 and with digestate in 2021 (Figure 3).

N_2O emissions showed a decreasing trend in both seasons, with peaks occurring 5–10 days after organic and mineral fertilization events (Figure 5).

In 2020, the highest peaks were observed with digestate, with and without biochar, 6–8 times higher than in 2021. The high heterogeneity in the field plots reduced the significance, and N_2O emissions with digestate were higher than those with mineral fertilization in the first event of 2020 and in the first four events of 2021 (Table 4). After the top dress fertilization in 2021, an inverse trend was observed, with higher emissions from mineral fertilization than digestate or slurry in three and four events, respectively. Biochar + digestate induced an increase in N_2O emissions in the first half of 2020 (two significant events), and a decrease in the second half of 2020 (two significant events) and in eight events of 2021. Biochar + slurry induced a significant increase in three events at the beginning of 2021. Biochar with mineral fertilization significantly reduced N_2O emissions after the top dress fertilization in both years.

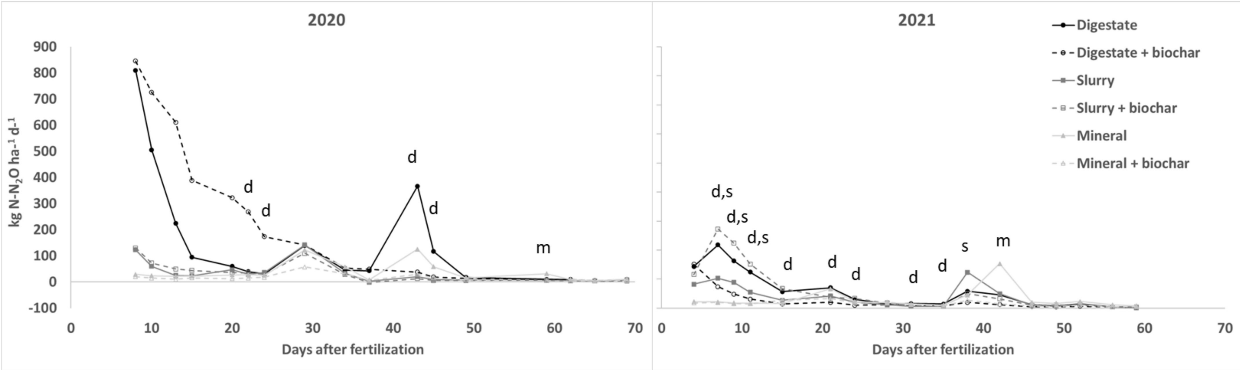

Figure 5. N-N_2O fluxes from soil during 2020 and 2021 maize growing seasons for the six treatments (digestate, slurry, and mineral fertilization, with and without biochar). Significative biochar effects on emissions are reported for each date from digestate (d), slurry (s), and mineral (m) fertilization.

Cumulative N_2O emissions were generally lower in 2021 than 2020, with the exception of those from slurry fertilized soil (Figure 3). Digestate induced a higher increase, with respect to mineral fertilization, in both growing seasons. The reduction in N_2O emissions with biochar was significant with digestate in 2021 and with mineral fertilization in both growing seasons.

N_2O emissions showed a significant linear relation with the effective N (N-NH_4 + ½ N org), added with digestate and slurry (Figure 6 top), and a significant exponential regression with NO_3 availability in soil (Figure 6, bottom).

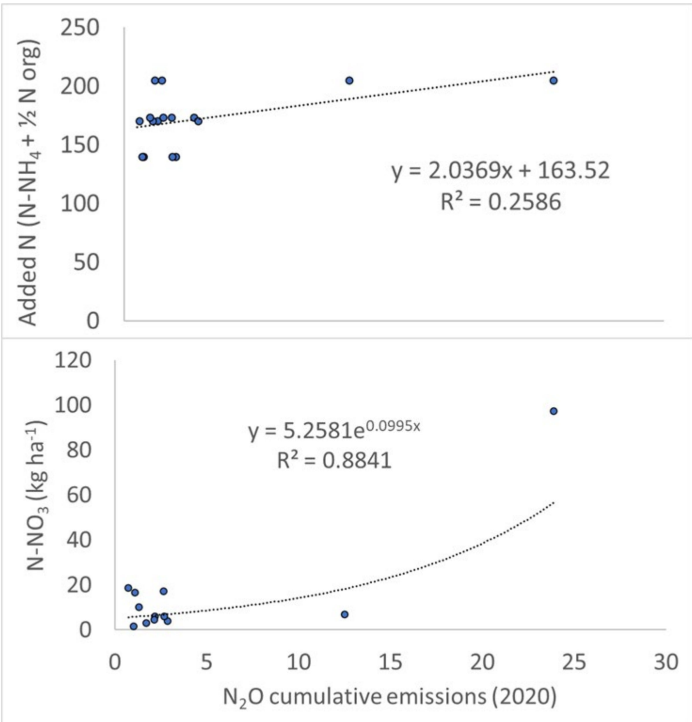

Figure 6. Linear regression between N_2O cumulative emissions and effective N, added by digestate and slurry in the 2020 and 2021 maize growing seasons (**top**), and exponential regression between N_2O cumulative emissions and NO_3 cumulated in the soil in the 2020 maize growing season (**bottom**).

4. Discussion

4.1. Fertilizer Types

Using livestock residues is a sustainable practice, which improves soil organic matter and recycles nutrients that, otherwise, would be lost from the soil system. However, depending on the type of residue, negative feedback to climate may occur, mainly mediated by increased N_2O emissions [19]. The residues used in our study were different in composition between them, being digestate N richer than slurry, and between years. This also influenced the application doses, which varied between years depending on the effective N availability, which, in turn, affected the input of organic matter to soil. The higher NH_4 content of digestate than slurry is commonly reported [47], and it is considered positive in a fertilizer, being immediately available to plants. However, evidence on negative environmental feedbacks through NH_3 [48] and N_2O [49] emissions, as well as N leaching [50], are rising. In both years of the present study, the increase in CO_2 and N_2O emissions from soils fertilized with digestate with respect to mineral fertilization, was higher than that from soils fertilized with slurry. Ambiguous results on the impact of digestate and slurry on N_2O emissions are also reported in literature because of the large variability of residues composition and amounts. Higher [51] and lower [52,53] N_2O emissions from digestate than slurry were found, as well as no effects [54,55]. N_2O emissions negatively correlate with the C/N ratio of residues [56], which also explain the higher rates of N_2O emissions from digestate (N richer) than slurry found in our study. Baral et al. [57] suggested that nitrification in manure hotspots was one of the factors controlling N_2O emissions, either directly or indirectly, supplying substrate for denitrification. The higher initial NH_4 content of digestate can have therefore triggered N_2O emissions, as suggested by [49] and confirmed by the positive correlation with the added effective N (N-NH_4 + $\frac{1}{2}$ N org) we found. Moreover, an increase in NO_3 content in soil due to nitrification might have occurred, which is reported as inhibitory on N_2O reduction during coupled denitrification by several authors, especially when combined with a simultaneous depletion of readily available C [53,58]. This effect was confirmed by the exponential increase in N_2O emissions with cumulated NO_3 found in our study.

The N_2O peaks and the highest distance between organic and mineral fertilizers occurred at the beginning of the growing seasons, within 7 and 24 days from the application, and fluxes gradually decreased afterwards. This pattern was consistent with several other studies, as reported by [53]. After this transient increase, a reduction in N_2O from digestate and slurry with respect to mineral fertilization occurred, although not large enough to compensate the initial increase. This temporal variability might also explain the mixed effects found in literature.

In the first few days after application to soil, digestate, and slurry caused CH_4 emissions too, which were visible in 2021 (4 days after fertilizers application) but not in 2020 (8 days after fertilizers application). As already found in other studies, maximum values of CH_4 emissions were observed immediately after digestate [59] and slurry [54,60] application to the soil, followed by a sharp decrease and then uptake. The rapid and transient increase might be related to (i) fresh and easily decomposable organic substrate with (ii) high water content, which provided optimal conditions for CH_4 emissions. After the initial flush, CH_4 uptake was prevalent and further enhanced by digestate in 2020 and slurry in 2021. In the context of mineral upland environments, agricultural soils play an important role as CH_4 sink [61–63]. Interactive effects of fertilizers on CH_4 emission are complex and sometimes contradictory, depending on the nature of the fertilizer, the quantity applied, and the method of application [64]. Mineral N fertilizer application can also have a negative effect on the CH_4 oxidation capacity of arable soils [65,66]. In addition, the impact of organic fertilizers on C cycling was significant in the short-term. In fact, soil organic C was not affected by organic fertilizer's addition, implying that the external input of organic matter was rapidly decomposed and lost, as CO_2 in the atmosphere or leached dissolved organic matter, as evident from the 17 to 42% increase in CO_2 emissions, with respect to mineral fertilization. Indeed, other studies did not observe effects on TOC after digestate or slurry application to soil [67,68], and a priming effect was hypothesized, after organic residue application [69,70], that explains higher CO_2 losses.

4.2. Biochar Contribution

The positive effect of biochar on C sequestration in soil is widely demonstrated through (i) increased stabilization of soil organic C, (ii) deepening of soil organic C distribution, and (iii) higher crop yield and above/belowground productivity, as reviewed by [71]. After 3 and 4 years from biochar application, a significant increase in soil organic C was found, independent of fertilizer type, even without significant effects on maize yield. The increase seemed, therefore, to be the result of a direct effect of C added with biochar, which was then rapidly stabilized into the soil.

The impact of biochar on GHG fluxes is more uncertain, varying among soil and crop types, biochar feedstock source, and pyrolysis temperature, as synthesized by the meta-analysis of [72]. Additionally, differences among the three gases were found, with several studies reporting an initial increase in CO_2 emissions [73], a decrease in N_2O fluxes [32], and no effects on CH_4 fluxes [72]. These general trends are only partly confirmed by results found in the present study, which were dependent on the type of fertilizer, its quantity, and the time from application. A clear decrease in CO_2 emissions was observed only in the fourth year from biochar application, thus confirming the suppression of mineralization, once the labile C was consumed [73]. In addition, the decrease was observed with digestate, whereas even an increase was observed with slurry. This difference suggests that the larger amount of fresh labile C, supplied with slurry rather than with digestate, might have favored microbial activity and organic C mineralization.

An overall decrease in N_2O emissions with biochar is reported for several climates and soil types [32] and a suppressive effect of biochar on N_2O emissions, induced by N fertilization, was typically reported [74]. However, this effect was variable, depending on the application period and the land-use [32]. A biochar-mediated reduction in N_2O emissions, from soil amended with digestate, was observed by [29], and our study confirmed this decrease, although only after four years from the beginning of application. This temporal variability, coupled with the different applied doses of effective N, being added in 2020, is higher by 20% than that added in 2021. Therefore, biochar seemed more effective if below a certain threshold of N availability. After that, the excess of N not fitting the plant demand triggered N_2O emissions [19,75,76]. The N_2O emissions from soil fertilized with slurry seemed to confirm this pattern. In fact, in 2021, an almost two times higher amount of slurry was added to the soil, with respect to 2020, inducing N_2O peaks, which are even higher with biochar. The effect of biochar was found to be negligible on N_2O emissions in organic C rich soils [32], and excessive N fertilization was found to increase NO_3 content in soil, since biochar has a limited capacity to entrap it after a certain threshold [77]. Therefore, we can hypothesize that, when labile organic matter and N were in excess, as in the case of digestate in 2020 and slurry in 2021, the impact of biochar on N_2O emissions was null or even negative.

Similarly, CH_4 fluxes responded differently to biochar depending on the quantity and quality of livestock residues. Decreased CH_4 emissions from livestock residues, during composting [78,79] and after application to soil [80], were observed by several authors. However, other studies observed no effects of biochar and manure on CH_4 fluxes from aerobic soils [81–83]. In our study, the only event of CH_4 emissions was within the first week from residue application, in 2021, when an increase was observed with slurry + biochar. In that case, the slurry amount added to soil was almost twice that from the previous year, adding a large quantity of fresh and labile organic matter, which was promptly degraded in soils treated with biochar that are supposed to host a more active microbial community [84]. After that unique event, CH_4 uptake increased significantly in soils treated with biochar, suggesting a major role of CH_4 oxidation that is possibly driven by a better soil structure and aeration [35].

5. Conclusions

The hypothesis of higher N_2O emissions from livestock residues compared to mineral fertilization was confirmed by our study after four annual applications. Digestate induced higher N_2O emissions than slurry because of a larger N content, while it showed a higher CH_4 uptake. The highest N_2O peaks occurred between 1 and 3 weeks after fertilization, in concomitance with not limiting moisture and optimal NO_3 availability.

We provided evidence that biochar was effective in reducing the environmental impact of organic fertilizers from the livestock chain, which even resulted in a synergy with the positive effect on C sequestration. However, the effect on CO_2 and N_2O emissions were dependent on (i) the quantity of organic matter added, (ii) its quality, and (iii) the time from application. An excess of labile organic matter and N induced higher CO_2 emissions and peaks of N_2O, independent of—or even triggered by—biochar. Indeed, the mitigation effect of biochar was evident below a certain threshold of organic fertilizer. Above that, no effect (digestate), or even an initial priming effect (slurry), was observed.

The results of the present study may contribute to policy recommendations at the EU level, which are at the base of sustainable agriculture improvement in the near-future, including climate change mitigation and water pollution reduction. We also highlighted the needs of identifying specific threshold for the quantity of livestock residues applied to soil to limit N losses.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071593/s1, Figure S1. Mean and maximum temperature (left axis), precipitation and irrigation (right axis) during 2020 and 2021 maize growing seasons; Table S1. Main soil characteristics of the experimental field at the beginning of the trial in 2018. Figure S2. Aerial image of the experimental trial at Cascina Baroncina (45°17′25″ N–9°29′43″ E). Source: Google Earth. Table S2. Biochar characterization according to Italian (ICH-AR, Italian Biochar Association), EU (EBC, European Biochar Certificate) and international (IBI, International Biochar Initiative) prescriptions.

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