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Biochar amendment to cattle slurry reduces NH_3 emissions during storage without risk of higher NH_3 emissions after soil application of the solid fraction

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Abstract

Cattle slurry storage is a major source of gaseous N emissions. The aim of this study was to evaluate the effects of biochar, clinoptilolite and elemental sulfur (S°) on (1) NH_3 and greenhouse gas emissions during storage of cattle slurry and (2) after soil application of the enriched solid fractions; and (3) on the agronomic quality of the solid and liquid fractions. In the first phase, biochar was added to the slurry (10 g L^{-1}); subsequently in the second phase, clinoptilolite (50 g L^{-1}), S° (1 g L^{-1}) and 40 g L^{-1} extra biochar were added. Gaseous emissions were monitored by a semi-continuous multi-gas analyzer and the agronomic quality of solid and liquid fractions was assessed after separation. The enriched solid fractions were applied to soil to study the effects on gaseous emissions, N and C mineralization. Amendment of biochar reduced NH_3 emissions during cattle slurry storage by 12% during the first 7 days. Extra amendment of biochar, clinoptilolite and S° in combination with biochar resulted in a decrease of NH_3 emissions of approximately 20%. The N sorbed from the slurry by the biochar was not released as NH_3 during soil application of the solid fractions and was not released as mineral N in the short term (within 28 days). A short-term positive priming effect of biochar on the C mineralization of manure and biochar-manure mixture applied to soil was observed. The biochar-enriched solid fractions contained more C, total and organic N and water-available P with a slow release.

Keywords: biochar; clinoptilolite, elemental sulfur; N sorption; greenhouse gas emissions; NH_3 emissions

1. Introduction

The increase in intensive livestock production has led to severe environmental problems, in particular related to management of large amounts of nutrient-rich slurry (liquid manure). Storage and soil application of cattle slurry are responsible for considerable emissions of greenhouse gases (GHG) and ammonia (NH_3) (Amon et al., 2006), resulting in negative effects on climate change, acid rain, ozone formation in the troposphere (Smithson, 2002) and reduction of the N fertilizer value of the slurry. The EU Directive 2016/2284 requires that starting in 2030, the national NH_3 emission ceilings should be reduced at all stages of animal manure management (10% for feeding, 20% for housing, 40% for storage and 30% for soil application compared with the reference method described in the Ammonia Guidance Document).

The main mitigation measures for reducing the environmental implications of slurries are dietary changes, improved design of the storage tank and stables, covering the slurry storage area, application of additives to the slurry, separation of the slurry into a solid and liquid fraction, and anaerobic digestion (Kupper et al., 2020). Here we focus on addition of sustainable additives during manure storage to reduce N losses at an early stage. Amending the slurry with clinoptilolite, a natural zeolite, has been shown to reduce NH_3 emissions from slurry due to the great affinity of clinoptilolite for NH_4^+-N (Pereira et al., 2020). The use of additives consisting of bio-based products such as biochar (pyrolyzed biomass) may be of great interest as they have shown potential to reduce nutrient losses during manure storage, while also recycling nutrients during biochar production and soil application. Biochar amendment in manure has been proven to reduce NH_3 emissions by 12-77% (Halim et al., 2017; Kalus et al., 2019; Pereira et al., 2020; Rogovska et al., 2011; Taghizadeh-Toosi et al., 2012) via two

mechanisms: formation of a crust (physical barrier for gases) and sorption of NH_3 and NH_4^+-N onto the biochar (Taghizadeh-Toosi et al., 2012; Holly et al., 2017). The latter is strongly dependent on the characteristics of the biochar (mainly determined by feedstock and pyrolysis temperature), and can be predicted by fast screening tests (Viaene et al., 2023). Another strategy used to mitigate gaseous emissions during slurry storage is acidification by adding elemental sulfur (S°) which neutralizes OH^- ions required for the conversion of NH_4^+-N ions to NH_3 gas (Maffia et al., 2020).

After storage, the manure is generally applied to soil as fertilizer (especially the solid fraction after slurry separation). Additionally, the biochar-enriched solid fraction, containing high amounts of stable C, could be beneficial for C sequestration on the long term (Lorenz and Lal, 2014). Obviously, the reduced N losses during slurry storage should not be released after soil application of the solid fraction. There are already several papers studying the effect of biochar addition on soil properties and greenhouse gas emissions in temperate regions (e.g., (Nelissen et al., 2014; 2013)), whereas studies on soil application of biochar-manure mixtures are limited (Abagandura et al., 2022; Khan et al., 2023; Romero et al., 2021). To the best of our knowledge, this is the first study that integrates the measurement of gaseous losses during small-scale manure storage followed by soil application of the biochar-enriched solid fraction. The biochar is thus used more than once: first it helps to reduce emissions during slurry storage, then the biochar-enriched solid fraction is used as a slow-release fertilizer and stable source of C. When used in this way, biochar can support the development of a circular economy by improving nutrient recovery and nutrient use efficiency (Joseph et al., 2021).

The aim of this study was to evaluate the effects of biochar, S° and clinoptilolite on the composition and emission of NH_3, N_2O, CO_2 and CH_4 during the storage of cattle slurry and after soil application of the solid fraction. The following hypotheses were tested:

1) Biochar and clinoptilolite addition and acidification with S° reduce NH_3 losses during cattle slurry storage.
2) The N sorbed on the enriched solid fractions is not released as NH_3 after soil application of the solid fractions.
3) Biochar-enriched solid fraction has a better agronomical quality than the solid fraction without biochar (i.e., higher C input to soil, higher soil microbial activity, more N with a slow release).

2. Materials & Methods

2.1. Storage experiment with cattle slurry

A single bulk sample of cattle slurry was collected from the manure pit at the dairy cattle research barn at ILVO (Melle, Belgium). A small-scale storage experiment was conducted at ILVO by using PVC containers with a removable lid perforated to allow natural ventilation (volume 10 L, diameter 19.5 cm). During phase 1 (7 days), three containers were filled with 5.98 L cattle slurry (20 cm height) as a control, and three containers were filled with 5.98 L cattle slurry mixed with 10% dry weight (DW) (w/w) (10 g L^{-1} slurry) green waste-based biochar (Hasselt University, Hasselt, Belgium). Measurements during the first phase lasted for 7 days until emissions were stabilized (Pereira et al. (2020)). During phase 2 (day 33 - day 48), extra amendments (no amendment, S°, clinoptilolite or biochar) were added to the slurries by stirring in a similar manner in all containers. Per liter slurry, 1 g S° (Aveve NV, Belgium), 50 g clinoptilolite (Orffa Belgium NV, Belgium) or 40 g wood-based biochar (Proininso Inc., Málaga,

Spain) (50 g biochar in total per L slurry) were added. This resulted in six treatments of cattle slurry (CS), indicated in the treatment name with an underscore "_0", "_S", "_B" or "_C" to indicate either no addition or amendment with S°, biochar or clinoptilolite (i.e., "_0_S" indicates no amendment in the first phase and S° amendment in the second). The biochars were chosen because of their high capacity for NH_4^+-N and NH_3 sorption in previous screening tests (Viaene et al., 2023). They were made from different feedstocks and it was tested if they have the same effect on emissions. Biochar characteristics can be found in Table 1. In addition, the gas concentration in the ambient air (background) was measured in an empty container.

2.2. Separation of the slurry into solid and liquid fractions

After the storage experiment, the slurries were separated in a solid and liquid fraction by centrifugation (Avanti® J-26S XPI, Beckman Coulter, CA, USA) for 20 min at 5800 rpm and 6.254 x*g*. Separation efficiency (%) was calculated as the fresh weight of solid/liquid fraction over the total weight. The total separated solid and liquid fractions of the slurry were homogenized and subsamples were retained for analysis.

The liquid fractions were analyzed for total N (Dumas EN 13654-2) and diluted with water (1:5 v/v) for the determination of NH_4^+-N (Skalar San++ flow analyzer, Skalar Analytical B.V., Breda, NL), NO_3^--N (Dionex ICS-3000 ion chromatograph, Dionex, Sunnyvale, CA, USA) and pH-H_2O (EN 13037). The solid fractions were analyzed for pH-H_2O (EN 13037), dry matter (DM, EN 13040) and organic matter (OM, EN 13039). NO_3^--N, Cl^-, SO_4^{2-} and water-available P (P_w) were measured with a Dionex ICS-3000 ion chromatograph (Dionex, Sunnyvale, CA, USA). Total C (TC), organic C (OC), inorganic C (IC) (NBN EN 15936) and total N (Dumas EN 13654-2) were measured with a Primacs SNC100-IC C/N analyzer (Skalar Analytical B.V., Breda, NL) after drying the solid fractions with tartaric acid (300 mL tartaric acid per 250 g solid fraction at

70°C, NEN 7430:1998). C/N ratio was calculated based on OC and total N. NH_4^+-N of the tartaric acid dried solid fractions was also measured (Skalar San++ flow analyzer, Skalar Analytical B.V., Breda, NL). Total concentrations of P, K, Mg, Ca, Na, Fe and Al were determined by 5110 VDV Agilent ICP-OES (Agilent, Santa Clara, CA, USA) in the extract following digestion (120 min at 105 °C) of 0.5 g dried and ground material with 4 mL HNO_3 (p.a. 65%) and 12 mL HCl (p.a. 37%) using a DigiPREP MS 200 Block Digestion System (SCP SCIENCE, Québec, Canada). The cation exchange capacity (CEC) was determined by ammonium acetate at pH 7.0 and KCl, modified from the method by Rajkovich et al. (1992). Five grams of material were extracted in a suspension of 50 mL 1 M ammonium acetate at pH 7.0 and mixed on a shaker table overnight. After mixing, the suspension was transferred to a funnel fitted with filter paper. The volume of the collected filtrate was replenished to 250 mL by slowly pouring 1 M ammonium acetate onto the used filter. After washing the sample on the filter three times with 60% ethanol, the NH_4^+ on the cation exchange sites of the sample was exchanged by K^+ by pouring 250 mL 10% KCl at pH 2.5 in 5 aliquots over the used filter. The NH_4^+ concentration in the filtrate was subsequently determined with a Skalar SAN++ flow analyzer. As an indicator of biological stability, oxygen uptake rate (OUR) was calculated from the oxygen consumption due to microbial activity of the solid fractions (equivalent dose of 2 g OM per flask) and 200 mL buffered nutrient solution in a 1 L Schott flask during five days at 120 rpm at 20 °C based on the method reported in Grigatti et al. (2011). The pressure course in the bottle headspace was continuously recorded by means of the OxiTop device (WTW GmbH, Germany), in which the CO_2 was trapped by Sodalime (Merck). OUR was expressed as mmol kg^{-1} OC h^{-1}.

2.3. Soil application experiment with solid fractions

154 The PVC containers (section 2.1 above) were filled with moistened soil (50% water-filled pore
155 space to optimize soil microbial activity) to a depth of 5 cm. First, the emissions of the soils
156 were measured to exclude excessive variability among the soils. On top of the base layer of
157 soil, a 5 cm layer was added. This layer contained a mixture of soil and different solid fractions
158 to achieve an equal N dose of 340 kg N $ha^{-1'}$ (the double of the maximum allowed N dose from
159 animal manure in Flanders). The experiment included a blank treatment (10 cm soil without
160 solid fraction amendment) to be able to calculate net emissions from the solid fractions. The
161 background gas concentration in the air was measured in an empty container. Each treatment
162 consisted of three replicates. The air temperature in the headspace was measured in six
163 containers (TESTO 175T3). NH_3, N_2O, CO_2 and CH_4, emissions in the headspace were
164 monitored during three periods of 2.24, 2.22 and 4.13 days, respectively (total of 8.6 days).

165 The soil characteristics prior to the experiment were as follows: DM = 88%; pH-KCl = 5.7;
166 electrical conductivity (EC) = 131 µS cm^{-1}; OC = TC = 1.09% dry $soil^{-1}$, mineral N = 26.4 mg kg^{-1}
167 DM, total N = 0.11% dry $soil^{-1}$; P-$CaCl_2$ = 4.6 mg kg^{-1} fresh soil; K-AmLact = 30.1 mg 100 g^{-1} dry
168 soil; Mg-AmLact = 22.2 mg 100 g^{-1} dry soil; Ca-AmLact = 90 mg 100 g^{-1} dry soil; P-AmLact = 32.4
169 mg 100 g^{-1} dry soil. After 28 days, the soil was analyzed for mineral N (ISO 14256-2) and 1 M
170 $CaCl_2$ extractable P (P-$CaCl_2$ according to method described in (Vanden Nest et al., 2014). Net
171 N mineralization and net P-$CaCl_2$ were calculated by subtracting the mineral N and P-$CaCl_2$ of
172 the blank soil.

173 **2.4. Gas measurements**

174 The containers of the storage (2.1) and soil application experiment (2.3) were connected to a
175 CT5200 semi-continuous multi-gas analyzer (Quantum Cascade Laser Emerson, including a
176 Buehler Panel filter AGF-FE-4-I). The containers were connected via Festo push-in fittings and

177 a PFA tube of 7.5 m was attached to the multisampler with a Swagelok fitting. The headspace
178 of each container was measured for NH_3, N_2O, CO_2 and CH_4 in sequence (10 min intervals), at
179 a rate of one measurement per second and with a flow rate of 60 L h^{-1} for phase 1 of the
180 storage experiment and 45 L h^{-1} for phase 2 plus the soil application experiment. The
181 containers were placed in a climate chamber at 18 °C (including two ESCORT iLOG Dataloggers
182 for temperature and relative humidity) with an inlet air tube with a 5 cm diameter. The gas
183 analyzer also measured the ambient air humidity. The detection limits for NH_3 (filter type
184 UA0973), N_2O (filter type UA0985), CO_2 (filter type UA0982) and CH_4 (filter type UA0969) were
185 0.1521, 0.0589, 2.9471 and 0.2864 mg m^{-3}, respectively. Figure 1 shows the experimental
186 setup.

187 To calculate the net dry emissions E_{dry} (g s^{-1}) ('emissions') from the slurries (section 2.1) and
188 solid fractions (section 2.3), the following formula was used:

189 $$E_{dry} = E_{wet} * 100/(100 - \% H_2O))$$

190 with % H_2O = % water in air sample and the net wet emissions calculated as:

191 $$E_{wet} = Q * [(Co - Ci) * 10^{-6}] * M / V_m$$

192 with Q = ventilation rate (1.67E-05 m^3 s^{-1} in phase 1 and 1.25E-05 m^3 s^{-1} in phase 2); C_0 = gas
193 concentration air outlet (ppm); C_i = gas concentration air inlet (ppm) = average of the air
194 treatments (slurry storage) and blank soil treatments (solid fraction experiment); 10^{-6} =
195 conversion ppm (parts per million); M = molar mass gas (g mol^{-1}; CH_4 = 16.042; CO_2 = 44.410;
196 NH_3 = 17.031; N_2O = 44.013); V_m = molar volume gas = $R * T / P_{STP}$, *with* R = 8.314472 10^{-2} m^3
197 hPa K^{-1} mol^{-1}; T = measured temperature in the chamber (K); p_{STP} = standard atmospheric
198 pressure (1013.25 hPa).

2.5. Statistics

During phase 1, gaseous emissions (NH_3, N_2O, CO_2 and CH_4) were calculated per day and the effect of treatment (biochar/no biochar) on the emissions was tested using a Linear Mixed-Effects model with Treatment and Days as fixed effects and Repetition as random effect. The effect of amendments on the separation efficiency, chemical characteristics of the solid and liquid fractions, net N mineralization and net P-$CaCl_2$ of the solid fractions were tested using linear regression. The effect of amendment on net emissions NH_3 and CO_2 during the soil application experiment were tested with ANOVA, with Treatment (amendment) and period (first/second/third) as factors (no interactions), followed by a Post-hoc Tukey's Honest Significant Differences test. A significance level of 5% was used throughout all analyses. All statistical analyses were performed using the open-source software platform R (version 3.6.1; R Core Team, 2019).

3. Results

3.1. NH_3 and greenhouse gas emissions during storage of cattle slurry

The cattle slurry either with or without amendments showed a near absence of N_2O emissions during the whole experiment (Table 2). During the first 7 days (phase 1), NH_3, CO_2 and CH_4 emissions decreased over time. Adding biochar to the slurry decreased the total NH_3 emissions significantly by 12% (p = 0.023), but had no effect on the CO_2 emissions (Table 2). Conversely, the total CH_4 emissions were higher (p = 0.030) for the biochar-amended slurry compared to the slurry without biochar. Furthermore, there was a strong negative correlation between NH_3 and CH_4 emissions (R^2 = 0.90). During phase 2, CS_0_C, CS_B_S and CS_B_B showed approximately 20% lower NH_3 emissions compared to the slurry without amendments (CS_0_0). Remarkably, the addition of S° without biochar (CS_0_S) did not result in a reduction

222 in NH_3 emissions. Small differences in CO_2 and CH_4 emissions were observed across the
223 treatments.

224 **3.2. Manure treatment: separation efficiency and characteristics of the liquid and solid**
225 **fraction**

226 Adding clinoptilolite and a double dose of biochar to the slurry increased the separation
227 efficiency of the solid fraction from 36% to 40% and 49% (Table 3). Adding clinoptilolite
228 (SF_0_C) and biochar (SF_B_0, SF_B_S and especially SF_B_B) to the slurry resulted in a higher
229 N content in the solid fractions. Adding biochar to the slurry resulted in a higher C content of
230 the solid fractions; the C content of SF_B_B was even double that of the control SF_0_0.

231 No significant differences between the chemical characteristics of the liquid fractions with and
232 without amendments were found (Tables 3 and 4). There was a clear negative correlation (R^2
233 = 0.94) between the application of clinoptilolite or biochar and NH_4^+-N content. NO_3^--N
234 contents were below the detection limit.

235 Table 5 and Appendix 1 show the chemical characterization of the different solid fractions.
236 Adding clinoptilolite to the slurry resulted in a drier solid fraction with a much lower C content
237 (OM, TC, OC and IC). The clinoptilolite-enriched solid fraction contained less total and mineral
238 N (resulting in a lower C/N), less total P, Mg and Ca, but a higher N/P and OUR compared to
239 the control solid fraction. The double-biochar-enriched solid fraction had a higher DM and OM
240 content, a lower total N and NH_4^+-N content (resulting in a higher C/N), SO_4^{2-}, total P, Mg
241 content, but a higher N/P and P_w. In contrast to the biochar-enriched solid fraction, the OUR
242 decreased compared to the control after adding a double dose of biochar. The S°-enriched
243 solid fraction contained almost 5 times more SO_4^{2-} and had a much higher CEC (the highest
244 amongst the different solid fractions) than the control solid fraction. The combination of

biochar and S° resulted in a solid fraction with 4 times the SO_4^{2-} content and a higher P_w and CEC as compared to the control.

The biochar-enriched solid fractions (SF_B_0, SF_B_S and SF_B_B) contained a significantly lower total Mg content, NO_3^--N content and NO_3^--N/total N ratio ($p = 0.05$) compared to the solid fractions without biochar (SF_0_0, SF_0_S and SF_0_C). Furthermore, they had a significantly higher P_w ($p = 0.01$).

3.3. NH_3 and greenhouse gas emissions during soil application of solid fractions

Table 6 shows the NH_3 and CO_2 emissions (mg day^{-1}) per container for the different solid fractions after soil application during the three measuring periods. N_2O and CH_4 emissions were negligible and are therefore not discussed further. NH_3 emissions decreased with time and were higher during the first period of 2.24 days than during the last two periods ($p < 0.001$). NH_3 emissions were even below the detection limit during the last two periods. In contrast, CO_2 emissions (expressed based on an equal C input of the SF) were not different during the measuring period. SF_0_C resulted in significantly lower NH_3 emissions than SF_0_S ($p = 0.01$), but no effect from the other additives was observed. SF_B_B showed lower CO_2 emissions ($p < 0.001$) than all other treatments (Table 7).

3.4. N mineralization of solid fractions

After 28 days of soil application, no significant differences in net P-$CaCl_2$ and net N mineralization of the solid fractions in the soil were observed (Table 7). The soil mineral N content varied between 147 and 175 mg kg^{-1} dry soil. More than 94% of the total mineral N was NO_3^--N. No difference in relative soil P release was observed when expressed based on equal P input of the solid fractions.

4. Discussion

4.1. Effect of biochar and clinoptilolite addition and acidification with S° on NH_3 losses during cattle slurry storage

Between 4-7% of the initial N in the manure was lost as NH_3 during cattle slurry storage of 23 days. Amendment of 10 g L^{-1} biochar reduced NH_3 emissions during cattle slurry storage by 12% during the first 7 days. Extra amendment of biochar, clinoptilolite and S° in combination with biochar resulted in a decrease of NH_3 emissions of approximately 20%. Those results are comparable to the study of Pereira et al. (2020), where biochar and clinoptilolite reduced NH_3 emissions by 26% in pig slurry. The reduction in NH_3 emissions is probably related to N sorption onto the biochar and clinoptilolite, supported by the higher N content of the enriched solid fractions (Table 5) and the high capacity for N sorption of those biochars (Viaene et al., 2023). The formation of a thick crust as barrier for NH_3 release (Holly and Larson, 2017) seemed less likely in this experiment, as a thick crust was absent. S° alone did not result in a decrease in NH_3 emissions and there was no pH drop in the liquid and solid fractions due to the addition of biochar or S° (Table 5); therefore, acidification of the slurry by S° (in combination with biochar) seems less likely to cause a reduction in NH_3 emissions. Pereira et al. (2020) even found an increase in the slurry pH (+1) due to biochar amendment. The high IC values of the solid fraction (Table 5) indicate a high acid buffering capacity, probably acting against a drop in pH. Another explanation is that there is not sufficient O_2 and/or oxidizing microorganisms present in the more anaerobic manure to convert S° to sulphuric acid.

During the first days of slurry storage with biochar, slightly higher CH_4 emissions were observed compared to slurry storage without biochar. There was a negative correlation between NH_3 and CH_4 emissions. However, when expressed based on an equal C input of the

290 slurry, the CH_4 and CO_2 emissions were similar, indicating a similar C mineralization. The CH_4
291 and CO_2 emissions were even lower for the highest biochar dose, as related to either sorption
292 of CO_2 onto the biochar or a reduction in the labile C availability (Pereira et al., 2020). When
293 the CO_2 and CH_4 emissions were expressed only based on the C input from the slurry (without
294 taking the stable C coming from biochar into account), the mineralization was on average 6%
295 (first phase) and 15% (second phase) higher for the biochar-enriched slurries. Biochar seems
296 to enhance the decomposition of organic matter of the slurry during storage by stimulating
297 microbial activity. A faster decomposition and lower greenhouse gas emissions were also
298 found when biochar was added at the beginning of the composting process (Vandecasteele et
299 al., 2016). Biochars generally result in short-term positive priming of native soil organic
300 carbon, followed by negative priming and buildup of soil organic carbon on the long term
301 (Chen et al., 2021).

302 In summary, the hypothesis that biochar and clinoptilolite addition reduces NH_3 losses during
303 cattle slurry storage could be confirmed; however, acidification with S° without biochar did
304 not result in a decrease in the NH_3 emissions. In future research, other methods could be
305 tested to acidify biochar and the effects of more acid biochars on NH_3 emissions could be
306 investigated. Furthermore, experiments in real-life settings should be conducted to verify the
307 results.

308 **4.2. The N sorbed on enriched solid fraction is not released as NH_3 after soil application**
309 **of the solid fractions**

310 As it is not desirable to shift N losses from manure storage to soil application, gaseous losses
311 were monitored when (biochar-)enriched solid fractions were applied to soil. It should be
312 noted that gaseous emissions may differ depending on the soil, manure and biochar type. In

this study, the reduced NH_3 emissions during slurry storage with biochar and clinoptilolite (indicating that additional N bound to the biochar or clinoptilolite) was not released as NH_3 during soil application of the enriched solid fractions. Soil application of the clinoptilolite-enriched solid fraction showed the lowest NH_3 emissions, indicating that the N was bound more strongly bound to clinoptilolite than to biochar. The bound N was also not released to the soil as mineral N in the short term (within 28 days), as revealed by a lack of difference in soil N mineralization between the biochar-enriched solid fractions (containing more total N) compared to the solid fractions without biochar.

Regarding C losses during soil application of the enriched solid fractions, this experiment showed that the highest biochar dose resulted in lower CO_2 emissions (expressed based on an equal C input) compared to the other treatments. When expressed based on the C input of the pure solid fraction without taking the C of the biochar into account, the CO_2 emissions seemed to be slightly higher for the biochar-enriched solid fractions compared to those without biochar (32 mg day^{-1} with biochar vs. 27 mg day^{-1} without biochar). This may have been due to either mineralization of the labile biochar C or an increased mineralization of the soil organic matter (Troy et al., 2013).

In summary, N sorbed on enriched solid fractions was not released as NH_3 after soil application of the solid fractions. Moreover, soil application with biochar-enriched solid fraction resulted in lower CO_2 emissions compared to solid fraction without biochar. More research is needed to study the priming effect of biochar regarding to C mineralization of the soil organic matter. The observed effects should be also tested on other soil types.

4.3. Biochar-enriched solid fraction has better agronomical quality than solid fraction without biochar

No significant effect of biochar on the characteristics of the liquid fractions was observed, as most of the nutrients and biochar segregated into the solid fraction. Amendment of clinoptilolite and the highest dose of biochar increased the separation efficiency of the solid fraction up to 13%. Amendment of biochar and S° in combination with biochar resulted in an increase in the total N content, but a decrease in the NO_3^--N content and NO_3^--N / total N ratio of the solid fraction. As there is no effect on NH_4^+-N content, this could indicate that there is more organically bound N in the biochar-amended solid fractions. This could be advantageous, as organic N has a slower release than mineral N. Another advantage of soil amendment of biochar-enriched solid fraction compared to the solid fraction without biochar is the higher C and water-available P input to the soil. There was no difference in soil P-$CaCl_2$ (with equal P input) between solid fraction treatments, indicating slow release of the higher P content in the biochar-enriched solid fractions. S° (in combination with biochar) increased the SO_4^{2-} content of the enriched solid fractions, which is favorable for crops with a high S demand.

The C in biochar is very stable. To assess the stability of the pure manure in the biochar-amended manure, the OUR was expressed relative to the C content of the solid fraction, i.e., without counting the C of the biochar. Based on this calculation, the biological stability (based on OUR) of biochar-enriched solid fractions is higher (on average 44 mmol O_2 kg^{-1} TC) than solid fractions without biochar (on average 32 mmol O_2 kg^{-1} TC). This indicates that soil amendment of biochar-enriched solid fraction would lead to a higher soil microbial activity. This is in accordance with the observed increase in CO_2 mineralization after soil application of the biochar-enriched solid fractions. Total N mineralization/release was calculated by summing NO_3^--N and NH_4^+-N mineralization after 28 days and NH_3 losses extrapolated over 28 days. In general, 92-96% of the total N mineralization/release was mineralized to NO_3^--N, 2-

6% to NH_4^+-N and only 2% was lost as NH_3. There was a significantly lower % NH_3/total N when adding clinoptilolite compared to the solid fraction without any additives (p = 0.01).

In summary, the biochar-enriched solid fraction of cattle manure has better agronomical quality than the solid fraction without biochar, more specifically a higher C and P input to the soil, potentially higher soil microbial activity and more organically bound N with a slow release.

5. Conclusions

The results of this pilot study showed the potential of adding biochar and clinoptilolite to reduce NH_3 emissions in the storage of cattle slurry, at the same time avoiding NH_3 emissions after application of the enriched solid fractions to non-alkaline soil. Furthermore, biochar improved the separation efficiency and the quality of the solid fraction in terms of nutrients (N, P) and C content. Addition of S° during slurry storage was only successful for reducing NH_3 emissions in combination with biochar. A short-term positive priming effect of biochar on the C mineralization of manure and the biochar-manure mixture applied to the soil was observed. To verify this study, more research is recommended with other biochar types (e.g. acid biochars) and other soil types, and the experiments should be scaled up and tested in real-life conditions.

6. Acknowledgements

The Research Foundation - Flanders (FWO) has (co-)financed the research (BASTA, S000119N). We thank Tom Van Gyseghem, Siebert Vierendeels, Koen Van Loo and the other ILVO lab technicians for their technical assistance. We are grateful to Miriam Levenson (ILVO) for English-language editing.

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8. Tables

Table 1 - Characteristics of the two biochars used in the storage experiment. DM = dry matter; OC = organic carbon; CEC = cation exchange capacity.

	Biochar in phase 1	Biochar in phase 2
Pyrolysis conditions	Pilot-scale reactor; 15 min	Kiln reactor; 12–18 h; 0% O_2
Feedstock	Woody fraction of green waste	Oak
Pyrolysis T (°C)	450	650
OC (g kg^{-1} DM)	504	628
pH-H_2O	8.42	9.12
Total N (g kg^{-1} DM)	16.9	7.9
CEC (cmolc kg^{-1} fresh biochar)	64.89	47.05
NH_4^+ -N retention (mg NH_4^+-N g^{-1} fresh biochar)*	1.16	1.04

* determined according to Viaene *et al.* (2023)

Table 2 - Total NH_3, CO_2 and CH_4 emissions during the first (7 days) and second phase (16 days) of the cattle slurry (CS) storage experiment. The underscore in the treatment name indicates the amendment, with "_0", "_S", "_B" or "_C" indicating no amendment, S°, biochar or clinoptilolite amendment, respectively. The first and second underscore plus letter refer to the first and second phases.

	NH_3	CO_2	CH_4
Phase 1: Total emissions (g) over 7 days			
CS_0_0	0.69 ± 0.03	10.1 ± 0.6	3.2 ± 0.2
CS_B_0	0.60 ± 0.02	9.5 ± 0.7	3.7 ± 0.1
Phase 2: Total emissions (g) over 16 days			
CS_0_0	1.0	12.2	2.4
CS_0_S	1.0	13.7	2.1
CS_0_C	0.7	13.2	2.3
CS_B_0	1.0	14.2	3.0
CS_B_S	0.8	14.4	2.7
CS_B_B	0.8	13.6	2.5

457 **Table 3 - Fresh weight of the solid (SF) and liquid fractions (LF) after separation of the slurry, separation**
458 **efficiency, total N and C content. The underscore in the treatment name indicates the amendment, with "_0",**
459 **"_S", "_B" or "_C" indicating no amendment, S°, biochar or clinoptilolite amendment, respectively. The first**
460 **and second underscore plus letter refer to the first and second phases.**

Treatment	Fresh weight	Separation efficiency	N	C
	g	% by fresh weight	g	g
SF_0_0	1835	36	10.7	116.6
SF_0_S	1816	36	10.6	118.0
SF_0_C	2146	40	12.6	116.2
SF_B_0	1912	37	11.7	131.4
SF_B_S	1973	38	12.6	136.4
SF_B_B	2650	49	15.1	266.2
LF_0_0	3280	64	3.1	26.9
LF_0_S	3300	64	2.9	26.8
LF_0_C	3204	60	2.6	23.3
LF_B_0	3257	63	2.8	26.6
LF_B_S	3218	62	2.9	26.8
LF_B_B	2783	51	2.3	23.9

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463 **Table 4 - Chemical characteristics of the liquid fractions (LF). The underscore in the treatment name indicates**
464 **the amendment, with "_0", "_S", "_B" or "_C" indicating no amendment, S°, biochar or clinoptilolite**
465 **amendment, respectively. The first and second underscore plus letter refer to the first and second phases.**

Treatment	pH-H_2O	Total N	NO_3^--N	NH_4^+-N	Total C
	-	g L^{-1} LF	mg L^{-1} LF	g L^{-1} LF	g L^{-1} LF
LF_0_0	8.39	1.0	< 5.0	6.7	8.4
LF_0_S	8.41	0.9	< 5.0	6.5	8.3
LF_0_C	8.34	0.9	< 5.0	5.2	7.7
LF_B_0	8.36	0.9	< 5.0	5.9	8.5
LF_B_S	8.39	1.0	< 5.0	5.7	8.8
LF_B_B	8.33	1.1	< 5.0	5.5	11.0

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Table 5 - Chemical characteristics of the solid fractions (SF). The underscore in the treatment name indicates the amendment, with "_0", "_S", "_B" or "_C" indicating no amendment, S°, biochar or clinoptilolite amendment, respectively. The first and second underscore plus letter refer to the first and second phases.

		SF_0_0	SF_0_S	SF_0_C	SF_B_0	SF_B_S	SF_B_B
pH-H_2O	-	8.62	8.63	8.61	8.71	8.63	8.76
OM	g kg^{-1} DM	657	659	369	635	633	677
DM	g kg^{-1} DM	180	182	259	192	191	217
OC	g kg^{-1} DM	330	334	194	336	340	443
IC	g kg^{-1} DM	23.2	23.2	15.0	21.7	21.4	20.4
Total N	g kg^{-1} DM	32.4	32.2	22.7	31.8	33.5	26.2
C/N	-	10.0	10.1	8.4	10.3	9.9	16.5
NH_4^+-N	mg kg^{-1} DM	6674	6511	5466	6612	5985	5918
NH_4^+-N / total N	%	21	20	24	21	18	23
SO_4^{2-}	mg L^{-1}	195	944	166	123	842	96
Cl^-	mg L^{-1}	1042	1048	979	949	1052	1064
Total P	g kg^{-1} DM	7.50	7.73	4.42	7.16	7.29	5.47
N/P	-	4.3	4.2	5.1	4.4	4.6	4.8
P_w	mg L^{-1}	49.8	51.8	47.1	64.7	57.2	63.1
Total K	g kg^{-1} DM	24.02	22.85	28.68	24.40	22.89	21.28
Total Mg	g kg^{-1} DM	24.44	26.97	16.20	21.64	24.74	17.27
Total Ca	g kg^{-1} DM	51.93	55.03	36.39	47.40	53.87	57.05
OUR	mmol O_2 kg^{-1} OC h^{-1}	34.4	33.9	38.0	36.1	30.9	22.0
CEC	cmolc kg^{-1} DM	85.6	143.0	78.6	109.6	112.1	49.9

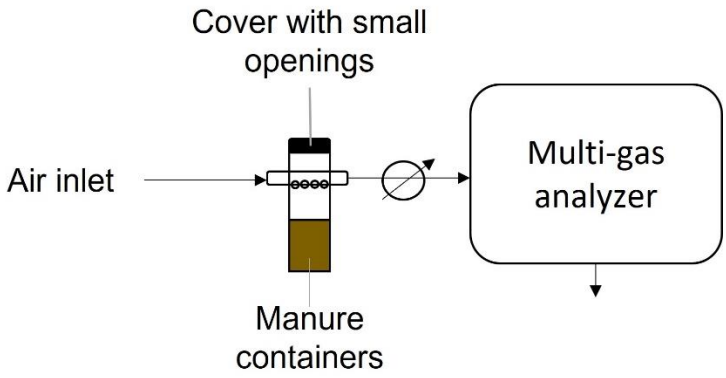
Table 6 - NH_3 and CO_2 emissions after soil application of the solid fractions (SF). The underscore in the treatment name indicates the amendment, with "_0", "_S", "_B" or "_C" indicating no amendment, S°, biochar or clinoptilolite amendment, respectively. The first and second underscore plus letter refer to the first and second phases. SF were applied at the same N dose of 340 kg ha^{-1}, CO_2 emissions were expressed relative to the C content of the SF.

		Emissions (mg day^{-1})					
	Period	SF_0_0	SF_0_S	SF_0_C	SF_B_0	SF_B_S	SF_B_B
NH_3	First	0.72 ± 0.25	0.78 ± 0.13	0.35 ± 0.1	0.51 ± 0.14	0.62 ± 0.22	0.53 ± 0.09
	Second	0.24 ± 0.04	0.3 ± 0.14	0.13 ± 0.01	0.15 ± 0.04	0.21 ± 0.01	0.17 ± 0.06
	Third	0.14 ± 0.05	0.15 ± 0.13	0.07 ± 0.03	0.1 ± 0.02	0.13 ± 0.01	0.1 ± 0.04
	Mean (mg day^{-1})	0.37 ± 0.12	0.41 ± 0.14	0.19 ± 0.05	0.25 ± 0.07	0.32 ± 0.08	0.26 ± 0.06
CO_2/C	First	25.9 ± 2.5	27.7 ± 1.3	29.8 ± 1.9	25.2 ± 0.5	25.4 ± 0.2	15.7 ± 2.6
	Second	28.1 ± 5.4	25.2 ± 5.4	21.8 ± 3	25.1 ± 1.5	23.1 ± 2.2	12.5 ± 2.2
	Third	28.4 ± 3	28.4 ± 4.1	22.2 ± 2.4	24.5 ± 0.2	23.8 ± 3.3	11.1 ± 2.3
	Mean (mg day^{-1})	27.4 ± 3.6	27.1 ± 3.6	24.6 ± 2.4	24.9 ± 0.7	24.1 ± 1.9	13.1 ± 2.4

Table 7 - Net N mineralization (Nmin), NO_3^--N/Nmin and NH_4^+-N/Nmin of the different solid fractions (SF) and net soil P-$CaCl_2$ and P-$CaCl_2$ / P_{input} after 28 days post soil application. The underscore in the treatment name indicates the amendment, with "_0", "_S", "_B" or "_C" indicating no amendment, S°, biochar or clinoptilolite amendment, respectively. The first and second underscore plus letter refer to the first and second phases.

	Nmin	NO_3^--N/Nmin	NH_4^+-N/Nmin	P-$CaCl_2$	P-$CaCl_2$ / P_{input}
	%	%	%	mg kg^{-1} fresh soil	%
SF_0_0	35.9 ± 4.3	94.3 ± 4.5	5.7 ± 4.5	7.6 ± 2.1	3.2 ± 0.9
SF_0_S	34.9 ± 9.7	95.3 ± 4.1	4.7 ± 4.1	7.9 ± 2.5	3.2 ± 1.0
SF_0_C	35.6 ± 5.7	95.7 ± 0.1	4.3 ± 0.1	6.4 ± 0.2	3.2 ± 0.1
SF_B_S	30.1 ± 2.6	95.1 ± 2	4.9 ± 2	6.9 ± 1.0	3.1 ± 0.5
SF_B_0	31.9 ± 0.4	97.1 ± 0	2.9 ± 0	7.7 ± 0.2	3.3 ± 0.1
SF_B_B	35 ± 3.2	95.6 ± 2.5	4.4 ± 2.5	6.1 ± 0.8	2.9 ± 0.4

9. Figures



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488 **Figure 1 - Experimental setup of the gas measurements using the Quantum Cascade Laser Emerson**

489 In a next step, the cumulative emissions were calculated per day. For the CO_2 emissions during the soil
490 application experiment, the emissions were expressed relative to the initial total C content of the solid
491 fractions.

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