Pellets from Biogas Digestates: A Substantial Source of N₂O Emissions

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Abstract

The field application of pellets from biogas residues resulted in high N₂O emissions which could not yet be parametrized through soil drivers. Therefore, the aim of this study was to determine potential N₂O production from pellets themselves. N₂O and CO₂ release from the pure pellet body (in form of intact, crushed or finely ground pellets produced from biogas digestates) were measured during the first seven days after pellet wetting under constant laboratory conditions. Three pellet water contents were examined: 47, 62 and 72% water of the total fresh pellet weight. Additional replicates of similarly wetted intact pellets were used to determine NH₄⁺, NO₃[−] and DOC contents on days 0, 1 and 4 of incubation. Two further treatments of wet intact pellets (62% moisture) were sterilized prior or after moistening to investigate the emissions' origin. N₂O release was found to increase with decreasing pellet size fraction. A maximum of N₂O fluxes within all three fractions was determined at 62% moisture, whereas lowest fluxes were measured at 72% moisture. The cumulative N₂O emissions over seven days ranged between 1 µg N₂O–N g^{−1} pellet (intact pellets at 72% moisture) and 166 µg N₂O–N g^{−1} pellet (finely ground pellets at 62% moisture). In general, our findings indicate that denitrification was the main factor for N₂O emissions, driven by indigenous microbial communities already present in the pellets. The results show that the N₂O emissions released by the pellets themselves can explain a major portion of the N₂O fluxes measured in situ.

Graphic Abstract

Keywords Nitrous oxide · Pelleted biogas digestate · Pellet fraction · Indigenous microbial activity · Denitrification

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Statement of Novelty

Pellets produced from biogas residues are a valuable organic fertilizer but trigger a relatively high amount of climate relevant N₂O emissions when applied on soils. Up to now it was not possible to explain the N₂O flux rates after application with soil drivers like mineral N or soil moisture. Therefore, the present study investigates the effect of pellet moisture

and pellet size fraction on the release of N_2O from the pellets themselves. Furthermore, it tackles the question if the autochthonous microflora in the pellets contributes significantly to the N_2O release from the pellets.

Introduction

Anaerobic digestion primarily aims at the production of biogas but also generates digestate, a valuable organic fertilizer, as a by-product. In Germany, the local field application of digested effluent often causes nutrient surpluses in the affected regions due to irregular geographical distribution of livestock and biogas plants [1–3]. As regulated by the current amendment of the fertilizer ordinance in Germany, total amount of organic fertilizer, as well as application time frames are limited. Consequently, large storage facilities are required to enable utilization of digestates in accordance with the legal regulations [4, 5]. Exemplarily, German operators of biogas plants have to provide facilities, which are able to store digestates between six to nine months depending on size of the owned cropland. A possible strategy for solving these problems is the transportation of the digestate over long distances which, in turn, is costly and non-economical due to the large water to nutrient ratio [6, 7].

Processing techniques for reducing the water content of digestates facilitate the handling and also generate marketable organic fertilizers. A common technique is the mechanical separation of digested effluent into a solid and a liquid fraction. Based on the total fresh matter (FM) of the original material, the separated solid and liquid split at a ratio of 22.5 to 77.5% [8, 9]. However, from an economical point of view the transportation of these products is limited as well, since the water content is still relatively high [10]. Möller et al. [9] noted that the feasible transport distance strongly depends on the dry matter (DM) content of the digestate and accounts for a maximum of 8.5 km for the solid and 7.5 km for the liquid after separation. Another important aspect is the gaseous loss during storage and handling of these fractions. In the case of the solid digestate, Hansen et al. [11] measured significant gaseous N losses, especially N_2 , NH_3 and N_2O , on the surface of storage heaps. Consequently, the nutrient availability in this fraction decreases [12] and the fertilizer value is reduced.

A suitable managing approach is the subsequent processing of the separated solid. Established techniques for further treating of solid digestate are drying and pelletizing. Drawbacks of both processes are high energy demands and costs [13]. Consequently, only about 1% of biogas plants in Germany implemented drying of solid digestate [8]. During a study targeting stakeholders' interests, farmers expressed the willingness to adopt pelleted organic fertilizers. However, in the same study, a substantial lack of knowledge concerning

chemical properties of such kind of fertilizers and their effect on soil organic matter dynamics was revealed [14]. Pelletizing reduces the volume of the raw material through compressing and simultaneously increases bulk density and durability of the product [15–18]. As a result, handling and transportation are facilitated and the required storage volume is minimized [15]. Additionally, the substrate is homogenized and nutrients are concentrated, which ultimately leads to improved fertilizing and amending properties [14, 17, 19]. Pelletizing of composted organic material for fertilizing purposes, such as manures [13, 17–19], crop residues [13, 17, 18], butchery wastes and wastepaper [20] is widely applied. By using “co-formulates” or bulking agents, such as biochar or wood chips, storage and transportation properties, as well as field spreading characteristics of pellets can be further improved [17, 18].

During the pelletizing process, agglomerations are formed in the channels of the die. The high pressure and increased friction force between biomass and channel wall lead to a considerable rise in temperature (70–100 °C depending on biomass and pelletizing process) of the solid [1, 7, 15, 21]. Due to the high temperature, a change in the physical state of several components, e.g. lignin and extractives like waxes, occurs in the solid. According to previous studies, lignin is the most recalcitrant component of lignocellulosic biomass and thus particularly difficult to decompose during anaerobic digestion [22–25]. At low compaction pressures and a temperature in the range of its glass transition temperature, lignin undergoes plastic deformation (i.e. “softening”) [25, 26]. For corn stover, one of the main biomass feedstocks used to produce the respective pellets, the glass transition temperature generally ranges between 50 and 113 °C [27]. The softened natural binders, such as lignin, in combination with water contained in the solid formed the outer layer of the pellet [28, 29]. In addition to a stabilization effect, this layer could help to create and keep a constant inner milieu including certain microsites.

In the case of pelleted manure, Alemi et al. [19] reported a slow release of N and P after pelletizing which generally reduced leaching and improved the nutrient uptake by plants. The availability of nutrients was also influenced by pellet diameter and application method [30]. However, information about the environmental effect of pellet application is still rare. Within a measuring period of one month, Cabrera et al. [31, 32] reported nitrous oxide (N_2O) emissions from soils after pellet utilization between 0.2 and 3.9% of applied N. The N_2O flux rates depended on the soil water regime and on physical characteristics of the pellets. Pampuro et al. [33] measured N_2O emissions between 0.05 and 0.12% of applied N, depending on pellet size and application method. For CH_4 and NH_3 , negligible release was reported [33].

Nitrous oxide is a climate relevant trace gas with absorption bands in the IR spectrum thus reducing the atmospheric

transparency to thermal radiation from the earth's surface [34]. Since pre-industrial times, the atmospheric N_2O concentration has increased by approximately 21% to 328 ppb in 2015 (0.73 ppb per year) [35]. Nitrous oxide contributes 7.4% of the total anthropogenic radiative forcing [36]. Besides that, N_2O is also involved in stratospheric ozone depletion [37, 38]. More than 60% of the anthropogenic N_2O emission originates from agricultural soils [36]. It is generally accepted that biological denitrification and nitrification are the main sources for the production and release of N_2O in soils [39]. Especially the application of stable isotopes in environmental studies opened new insights in sink and sources of N_2O . This initiated an intense discussion about the contribution of other process, such as nitrifier-denitrification, to the total release of N_2O from soils [40, 41].

Concerning the N_2O emissions after pellet application to soils, Hayakawa et al. [42] observed a higher N_2O release after fertilization with pellets than with the original digestate. In an own field study with pellets and other processed biogas digestates, we could confirm this result: highest N_2O emission was measured in the pellet treatment. The reason for that emission remained unclear because the main drivers for N_2O production in soil such as mineral N (N_{min}) or moisture did not correlate with the N_2O fluxes in the pellet treatment (data not shown). In contrast to our results, the positive relationship between CO_2 and N_2O fluxes as well as increasing NO_3^- content in soil measured in the study of Hayakawa et al. [42] indicated nitrification and denitrification as emission drivers. In accordance with this conclusion, Yamane [43] stated various denitrifying activities directly in the pellets as a reason for the high N_2O release.

Therefore, our main aim was to test the assumption that the autochthonous microflora in the pellets produces and releases a substantial amount of N_2O . Another major point of interest was to study the effect of pellet moisture and pellet size on N_2O emissions.

Materials and Methods

Pellet Manufacturing and Composition

The pellets used in this study were obtained from a biogas plant located in South Germany. Feedstocks were pig slurry and cattle manure, energy crops like silage maize and sunflower (400 g kg^{-1} total fresh matter, FM), pomace and grape marc (200 g kg^{-1} FM) and poultry manure (50 g kg^{-1} FM). In this biogas plant, raw digestate was separated with the help of a screw press, equipped with a tubular slit screen (0.5 mm pore size). The liquid and smaller particles passed the screen, while the retentate exited by a rotating screw [1, 44]. Afterwards, the separated solid was dewatered to about 80% DM content in a solar greenhouse drier, equipped

with an electric mole (THERMO-SYSTEM, Industrie- & Trocknungstechnik GmbH, Germany). During the consecutive pelletizing, pan grinder rollers forced the dried substrate through a die (5–10 mm channels diameter). During the compressing, a part of the residual water is evaporated and a tough outer layer is formed. After exiting the channels, the solid is cut by a share blade to small cylinders with a diameter of 6 mm and an average length of 14 mm. Table 1 shows the main physical and chemical properties of the pellets.

Dry matter content was measured by drying at 105 °C until constant weight. Total C (C_t) was investigated by elemental analysis (vario MAX CN, Elemental Analysensysteme, Hanau). Carbonate content was determined with a Scheibler apparatus according to DIN EN ISO 10693. Organic C was calculated as difference between total C and inorganic C. Total N (N_t) and NH_4^+-N contents of the pellets were determined according to Kjeldahl [45] and by steam distillation with titration [46], respectively. The pH value was measured with a glass electrode (Schott, Lab 850) in a 0.01 mol L^{-1} CaCl_2 solution at a ratio of 1:10 (w w $^{-1}$) [47]. Furthermore, the fiber fractions 'amylase-treated neutral detergent fiber' (aNDF), 'acid detergent fiber' (ADF) and 'acid detergent lignin' (ADL) were analyzed according to van Soest and Wine [48].

Further Pellet Analysis

Prior to the experimental set-up, the amount of easily degradable C and N was quantified in intact, manually crushed and finely ground pellets (the latter processed by an agate disc swing mill, Siebtechnik, Mülheim an der Ruhr, Germany).

Table 1 Dry matter (DM), total carbon (C_t), organic carbon (C_{org}), total nitrogen (N_t), ammonium-N (NH_4^+-N), nitrate-N (NO_3^--N), $\text{NH}_4^+-\text{N}:\text{N}_t$ ratio, C:N ratio, pH, neutral detergent fiber (aNDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of the pellets

Parameter	Value
DM (g kg^{-1} FM)	851
C_t (g kg^{-1} DM)	447
C_{org} (g kg^{-1} DM)	445
N_t (g kg^{-1} DM)	34.9
NH_4^+-N (g kg^{-1} DM)	2.4
NO_3^--N (g kg^{-1} DM)	2.7
$\text{NH}_4^+-\text{N}:\text{N}_t$ ratio	0.07
C:N ratio	12.8
pH ^a	7.9
aNDF (%)	58.3
ADF (%)	52.0
ADL (%)	31.6

All parameters were determined according to VDLUFA [46], no replicates

^a10 $^{-2}$ M CaCl_2 solution

In this way, the effect of different total surface area on water absorption, mineralization and resulting C and N release was studied. The crushed pellet fraction consisted of particles in the ranges of > 5 mm, 2.5–< 5 mm, 1–< 2.5 mm and < 1 mm with a distribution of 64%, 14%, 10% and 12%, respectively. In finely ground pellets, particles sizes of > 1 mm, 0.5–< 1 mm, 0.25–< 0.5 mm, 0.1–< 0.25 mm and < 0.1 mm were distributed as follows 3%, 14%, 26%, 26% and 31%. To determine the C and N amounts of the pellet fractions, a hot water extraction was performed according to Leinweber et al. [49] with slight modifications [50]. In brief, samples of 20 g air-dried pellets were boiled in 100 ml distilled water for 1 h. After adding 5 drops of 2 mol L⁻¹ MgSO₄ solution, the pellet-water mixture was centrifuged for 10 min at 2600 rpm under room temperature. The supernatant was collected and filtered through a syringe filter (0.45 µm pore size). Hot water extractable carbon (C_{hws}) and nitrogen (N_{hws}) contents of the extracts were analyzed with a C/N-analyzer for liquid samples (Multi N/C 2001 S, Jenoptik, Germany). This analysis was done in triplicate.

Experimental Set-Up

To assess the effect of particle size and water content of pellet on N₂O and CO₂ emissions, a laboratory incubation experiment was conducted. It was built up as a randomized block design, consisting of 14 treatments and four replicates in total. To investigate the effect of particle size on gaseous losses, the treatments included intact, crushed and finely ground pellets. Three different pellet water contents were examined: 47, 62 and 72% of the total fresh pellet weight. We chose 47 and 72% water content as lower and upper boundary because the gravimetric water contents of the pellets in the field varied in the same order of magnitude. The third moisture level (62% water content) was approximately the moisture in the period with highest N₂O fluxes in our field experiment. For each trial, 0.5 g pellet was placed in a 20 ml vial and wetted with distilled water. Prior to each gas measurement, the vials were closed with butyl stoppers and crimped. Following each gas sampling, the vials were re-opened, covered with a gas-permeable and liquid-tight PE-LD sheet and incubated at 20 °C in the dark. To compensate for vapor losses from the pellet body during incubation, the vials were weighed daily and, where necessary, the water content was re-adjusted manually.

Two control treatments with intact pellets wetted to 62% were assessed to control the efficiency of microbial inactivation. For that purpose, those pellets were autoclaved (30 min at 120 °C and 2000 hPa) and moistened (i) before (in the following text marked as “sterile 1”) or (ii) after (“sterile 2”) the sterilization procedure. Utilized gas vials and butyl stoppers were also autoclaved. All samples were incubated as stated before.

Trace Gas Measurements and Flux Rate Calculation

The N₂O and CO₂ release from the pellets was measured on days 0, 1, 2, 4 and 7 after pellet moistening. Gas fluxes were determined by taking three gas samples in intervals of six hours after crimping the vials.

Trace gas analysis was done with a gas chromatograph (GC 450, Bruker Daltonik, Bremen, Germany), coupled to an autosampler (GX-281, Gilson, Germany). The latter was equipped with a magnetic valve connected to a N₂ line (ECD quality) regulated to a pressure of 2000 hPa using a reduction valve. The overpressure in the vials was used to transfer the sample to the sample loops of the GC. Since the pressure in the vials before the addition of overpressure was 1000 hPa, samples were diluted 1:1. Although overpressure addition works reliable, additional gas vials with trace gas standards were included in the sample schedule in order to verify the dilution factor. The GC was equipped with a ⁶³Ni electron capture detector (ECD) for N₂O and CO₂ analysis after separation on a Haysep D 80/100 column.

The calculation of the flux rates took into account CO₂ and N₂O concentrations of three gas samples (µL L⁻¹ or nL L⁻¹, respectively), dilution factor through overpressure addition, air temperature (°C), weight of the air-dried pellet (g) and volume (L) of the headspace inside the vial. The flux rates were calculated using the linear slope of the trace gas concentrations in the headspace inside the vial over time [51]. Cumulative emissions were calculated by linear interpolation and numerical integration between sampling events.

Further Laboratory Measurements

In addition to the gas measurements, dissolved organic carbon (DOC) and mineral N (NH₄⁺-N and NO₃⁻-N) of intact moistened pellets were determined. For that purpose, four additional replicates were used. They were prepared and incubated identically to the intact pellet treatments with the three above-mentioned water contents. These samples were extracted on days 0, 1 and 4 of incubation with 15 mL of 0.5 mol L⁻¹ K₂SO₄ solution. The mixture was filtered (filter paper MN 619 eh ¼, 2–4 µm pore size, MACHEREY-NAGEL, Germany) prior to analysis. On day 7, the extraction was carried out with the actual intact pellet treatments immediately after the last gas sampling. Mineral N concentrations in the extracts were determined with a flow injection analyzer (3 QuAatro.AQ2.AACE, SEAL Analytical, UK). Total N and DOC were measured using a C/N-analyzer for liquid samples (Multi N/C 2001 S, Jenoptik, Germany). Dissolved organic nitrogen (DON) was then calculated by subtracting mineral N from the total N in the liquid samples.

Statistical Analysis

Prior to the statistical analysis, the experimental data were tested for normality and homogeneity of variance. If these criteria were not given, they were transformed by a $\log_{10}$ or a square root function. The change in N_2O and CO_2 flux rates with time was evaluated using a two-way repeated measures ANOVA. Here, the pellet fraction (intact, crushed or finely ground) and adjusted water content acted as main independent variables while the measurement day described the repeated statement. The effect of the independent variables on the cumulative emissions was tested using a two-way ANOVA. A one-way ANOVA including the previous repeated statement was performed to study the temporal change in DOC , NH_4^+-N and NO_3^--N contents of intact pellets with three different water contents. The same model was run to analyze the influence of pellet sterilization on greenhouse gas emissions during the observation period. Significant differences between the treatments for each of the performed statistical models were calculated using a Tukey-HSD test ($p \leq 0.05$).

Results

Hot Water Extractable C and N as Affected by Pellet Fraction

The results of the hot water extraction are shown in Table 2. It was observed that crushing pellets resulted in a significant lower amount of extractable C than finely grinding ($p = 0.006$) or even without treating ($p = 0.019$). In the crushed fraction C_{hws} accounted for 10.7% of C_{t} . The corresponding values for finely ground and intact pellets were 11.9% and 12.5%. There was no statistically significant difference in N_{hws} between the three pellet fractions. It ranged between 42.6% (crushed) and 47.1% (finely ground pellet) of N_{t} content.

Table 2 Mean hot water extractable C (C_{hws}) and N (N_{hws}) contents of three different pellet fractions ($n=3 \pm$ standard error); Means followed by a common letter are not significantly different by the Tukey HSD test at the 5% level of significance

Pellet fraction	C_{hws} mg C g ⁻¹ pellet	N_{hws} mg N g ⁻¹ pellet
Intact	45.6 ± 1.0 a	13.4 ± 0.8 a
Crushed	41.5 ± 1.0 b	12.6 ± 0.2 a
Finely ground	46.8 ± 0.5 a	14.0 ± 0.2 a

N_2O and CO_2 Flux Rates

The N_2O fluxes ranged between -0.05 (intact pellets 72%, day 2) and $1.9 \mu\text{g N}_2\text{O-N g}^{-1}$ pellet h⁻¹ (finely ground pellets 62%, day 4) (Fig. 1a–c). We found a significant interaction ($p < 0.05$) between pellet fraction (intact, crushed and finely ground pellet) and water content (47%, 62% and 72%). On day 0, the N_2O flux rate from finely ground pellets moistened to 47% was significantly lower than those wetted to 62% or 72%. During the following three days increasing flux rates were observed in all pellet fractions wetted to 47% and 62%, while in those with 72% moisture a slight decrease was measured. In contrast to the other pellet fractions, the flux rate from intact pellets wetted to 62% dropped on day 4 from 0.9 to $0.3 \mu\text{g N}_2\text{O-N g}^{-1}$ pellet h⁻¹ (Fig. 1b). It was approximately fourfold lower than in the treatment with crushed pellets and sixfold lower than in finely ground pellets with the latter difference being statistically significant ($p < 0.001$). Between day 4 and the end of the measurements, the N_2O fluxes decreased in all treatments.

Concerning the CO_2 release, the flux rates increased significantly ($p \leq 0.01$) in all treatments within the first four days of incubation (Fig. 1d–f). The lowest CO_2 flux rate was measured on day 0 in intact pellet fraction moistened to 72% ($0.03 \text{ mg CO}_2\text{-C g}^{-1}$ pellet h⁻¹) (Fig. 1f). In contrast, the highest one ($0.14 \text{ mg CO}_2\text{-C g}^{-1}$ pellet h⁻¹, day 4) was determined in finely ground pellets wetted to 47% (Fig. 1d). Towards the end of the measurement, the CO_2 fluxes decreased in all treatments. Generally, the treatments with the highest moisture (72%) showed lower CO_2 flux rates than those with less water addition.

Cumulative N_2O and CO_2 Emissions

The cumulative N_2O and CO_2 emissions over the experimental period of seven days are shown in Fig. 2. The highest cumulative N_2O emission of $166 \mu\text{g N}_2\text{O-N g}^{-1}$ pellet (corresponded to 0.6% of N_{t}) was measured in the treatment with finely ground pellets wetted to 62% (Fig. 2a). For the moisture treatments 47% and 62%, cumulative N_2O emissions increased with decreasing particle size of pellets in the order intact < crushed < finely ground. Besides that, the moisture significantly ($p < 0.001$) affected the N_2O emissions in the order 62% > 47% > 72% regardless of the pellet fraction. The N_2O emission of the treatment with the highest moisture was distinctively lower than those from the treatments with 47% and 62% moisture (by a factor of 7–21 and 31–59, respectively).

Regardless of the pellet fraction, increasing moisture of the pellets reduced the CO_2 release (Fig. 2b). Here, a strong positive correlation was found ($r^2 = 0.52$, $p < 0.001$). The CO_2 emissions measured at the highest moisture (72%) were significantly lower ($p < 0.01$) than in the other treatments.

Fig. 1 Mean N_2O and CO_2 flux rates ($n=4 \pm$ standard error) as affected by pellet fraction (intact, crushed, finely ground) and water amount (a, d: 47%; b, e: 62%; c, f: 72%) during the incubation;

Means followed by a common letter are not significantly different by the Tukey HSD test at the 5% level of significance. Missing letters indicate means without significance

Correspondingly to the cumulative N_2O emissions, the lowest CO_2 release was determined in the intact pellet treatment wetted to 72% ($11.7 \text{ mg CO}_2\text{-C g}^{-1} \text{ pellet}$). Similarly, the finely ground pellets wetted to 47% showed the highest average value ($18.1 \text{ mg CO}_2\text{-C g}^{-1} \text{ pellet}$). Within the 62% water content treatments, CO_2 emission from the crushed pellets was significantly lower ($p < 0.05$) than those from intact and finely ground.

Temporal DOC, DON and N_{min} -Dynamics in Intact Pellets

On day 0 and 1 of incubation, the amount of water added to the pellets had no significant effect on DOC (Fig. 3a). However, a slight (but statistically not significant) decrease in DOC was observed within this time period in all moisture treatments. During the following three days, DOC

Fig. 2 Mean cumulative N_2O and CO_2 emissions ($n=4 \pm$ standard error) as affected by pellet fraction (intact, crushed, finely ground) and water amount during the incubation; Means followed by a common letter are not significantly different by the Tukey HSD test at the 5% level of significance. Capital letters depict differences between the pellet fractions, lowercase letters depict differences between moisture treatments

concentrations in treatments with 47% and 62% moisture further declined while that in the highest moisture increased. Between day 4 and the end of the experiment, increasing DOC concentrations were observed in all treatments. The highest concentration (22.7 mg C g^{-1} pellet) was measured at 72% moisture on day 7. In this treatment, the high DOC concentrations determined on day 4 and 7 differed statistically significant from the treatments with 47% and 62% moisture ($p < 0.05$).

Nitrate concentrations measured in pellets with 62% and 72% moisture decreased steadily, except for a short-term increase on day 1 (Fig. 3b). Here only in the case of 62% moisture, it was statistically significant ($p = 0.035$) when compared to the NO_3^- concentration observed in the same treatment on day 0. In the treatment with the highest moisture, the NO_3^- pool was completely depleted on day 7. In contrast to the other treatments, NO_3^- concentration at 47% moisture remained rather constant.

Ammonium concentrations in the intact pellets increased in all treatments one day after incubation start (Fig. 3c). Here, the pellets with 72% moisture showed the highest

Fig. 3 Temporal changes of mean DOC, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations ($n=4 \pm$ standard error) as affected by moisture of intact pellets; Means followed by a common letter are not significantly different by the Tukey HSD test at the 5% level of significance, ns: no statistically significant difference

extractable NH_4^+ concentrations over all sampling dates. On day 4 in this treatment, the highest mean value ($8.5 \text{ mg NH}_4^+\text{-N g}^{-1}$ pellet) was determined. Lowest NH_4^+ concentrations were measured in the treatment with 47% moisture where this pool was nearly depleted on day 7.

The calculated DON values (total N in extracts – mineral N in extracts) are reported in Table S1. The highest DON content (14.9 mg N g^{-1} intact pellet) was determined

immediately after pellet moistening to 72% which, afterwards, decreased continuously toward the end of observation. In contrast, pellets wetted to 47% showed almost constant DON content with a slight increase on the last measurement day. For the 62% treatment, fluctuating values (3.2 to 10.5 mg N g^{-1} intact pellet) were noticed during the whole experimental period.

Effect of Sterilization Method on N_2O and CO_2 Fluxes from Intact Pellets

The N_2O and CO_2 flux rates in the sterilization experiment showed similar patterns as the flux rates in the main experiment. Nitrous oxide fluxes ranged between -0.005 (sterile 2, day 7) and $2.3 \text{ } \mu\text{g N}_2\text{O-N g}^{-1}$ pellet (intact, day 1) (Fig. 4). The highest CO_2 flux rates were in the order of magnitude of those observed in intact pellets at 62%

moisture during the main experiment. In contrast, N_2O flux rates after sterilization were approximately two times higher than those in the main trial.

Non-sterile pellets and those wetted after sterilization (sterile 2) showed highest N_2O and CO_2 flux rates on day 1 (Fig. 4). Throughout the whole experiment, neither N_2O nor CO_2 fluxes from these two treatments differed statistically significant, indicating the failure of sterilization of a dry pellet. In contrast, the sterilization performed after pellet moistening (sterile 1) significantly decreased N_2O ($p < 0.001$) and CO_2 ($p < 0.001$) release during the first two days. This indicates that the sterilization in this treatment was successful on a short-term. In this treatment, the fluxes increased with delay between day 2 and 4. Toward the end of the measurement, they remained significantly higher than those of the other treatments.

Discussion

General Aspects

Regardless of moisture and pellet size fraction, all tested treatments released N_2O and CO_2 which indicates the ability of pellets to serve as a trace gas source in the field. Obviously, pellet indigenous microflora was provided with sufficient substrate for N_2O and CO_2 production. In this context, Flessa et al. [52] investigated the effect of grass mulch on trace gas fluxes under laboratory conditions. They showed that the treatment with mulch applied on sand induced 69% of the N_2O emissions and 72% of the CO_2 emissions when compared to its application on soil surface. Using acetylene inhibition, they could also conclude that denitrification was the main source for N_2O released during the initial phase (approximately 14 days) of their experiment. In our study, CO_2 release and its temporal dynamics clearly hints at C-heterotrophic microbial activity as main source rather than carbonate dissolution. Yamane [43] determined nitrite reductase genes (*nirK* and *nirS*) in manure compost pellets which were sampled three days and 26 days after field application. The author could show that the *nirK* and *nirS* clones in the pellets were related to the clones of several denitrifying bacteria. Concerning the plants' capability to produce N_2O , Hakata et al. [53] reported that maize, which was also a component of our pellets, has the enzymatically equipment for NO_3^- reduction and thus to denitrify. For soybean seedlings, Sun et al. [54] found eleven genera of denitrifying bacteria indicating the importance of endophyte-plant co-denitrification for N_2O emissions from plant.

Fig. 4 Mean N_2O and CO_2 flux rates ($n=4 \pm$ standard error) of intact pellets as affected by sterilization method (**sterile 1**: moistening of intact pellet before sterilization or **sterile 2**: moistening of intact pellet after sterilization); Means followed by a common letter are not significantly different by the Tukey HSD test at the 5% level of significance. Missing letters depict sampling dates without statistically significant differences between the treatments

Effect of Sterilization on N₂O and CO₂ Emissions

Autoclaving of intact pellets after moistening resulted in negligible emissions during the initial phase of incubation which can be explained by the high thermal conductivity of water. Hence, the effect of heat sterilization was improved and an inactivation of indigenous microorganisms and spores occurred. The retarded trace gas release indicated a delay in microbial activity and proofed our assumption that N₂O production in the pellets stems from microbial sources. Additionally, cellulose and hemicellulose in the pellets may have been degraded during sterilization [25, 55–57] thus serving as an energy source for reactivated indigenous microorganisms. A consequent ongoing decomposition of organic matter and conversion of C and N pools due to reactivated indigenous microbial activity could be the reason for the subsequent rise in emissions of treatment wetted prior to autoclaving.

In contrast, the CO₂ and N₂O release from pellets moistened after sterilization can be attributed to a failed sterilization. Here, we can assume that the outer, compact layer of pellet might have hindered the heat transport into the pellet and thus protected indigenous microorganisms.

Effect of Pellet Moisture on N₂O Emissions

The release of N₂O strongly depended on moisture of the pellets. Concerning the pellets wetted to 47%, the slight decrease in NH₄⁺ concentrations and simultaneous increase in NO₃[−] concentration between day 4 and 7 indicated a conversion of mineral N. In contrast, an inhibited nitrifying activity was assumed in the treatment with 62% moisture during the same period. There, the rise in NH₄⁺ concentration and the simultaneously decreasing NO₃[−] pool pointed to O₂ deficiency. As reported by Heincke and Kaupenjohann [58], O₂ diffusion in water is lower than in air by a factor of approximately 10^{−4}. Therefore, the lower O₂ diffusion at higher soil moisture and the simultaneous microbial O₂ consumption favored anaerobic conditions and hence denitrification. Increased denitrification and resulting higher N₂O losses with decreasing O₂ partial pressure were often shown for soils (i.e. [59, 60]); however, there is currently no information on O₂ availability in pellets.

The lowest N₂O emission was measured in the pellets with the highest moisture which were nearly water-saturated (Figs. 1c and 4a). Based on the high CO₂ emissions observed at this moisture content, a possible explanation for the low N₂O release could be a complete denitrification. As demonstrated e.g. by Russenes et al. [61], the N₂O concentration in closed vials with soil might decrease under strong anaerobic conditions, thus resulting in negative flux rates. This was also found in our measurements, where the N₂O–N enrichment showed a negative value in at least two out of four

replicates of the same treatment. The depleted NO₃[−] pool on day 7 and the high NH₄⁺ concentrations indicated strong anaerobic conditions which might have led to a complete N₂O reduction to N₂ [62]. Furthermore, the anaerobic conditions expected in this treatment might have induced dissimilatory nitrate reduction to ammonium which was also shown to act as a N₂O source in environments with low O₂ availability [40]. This process might have been favored by the high C:NO₃[−] ratio of the pellets and could also give an explanation for the negative N₂O fluxes measured in the nearly water-saturated treatment.

As already shown for soils [41, 63], the moisture of the pellets can be considered as a strong driver for N₂O emissions.

Effect of Pellet Size Fraction on N₂O Emissions

In addition to the above mentioned effect of moisture on N₂O release, it was observed that the particle size of the pellet was the second major factor which controlled the emissions. Overall, grinding has a negative impact on pellet handling and distribution pattern and is no desirable state for actual field application. The different size fractions were chosen in order to measure the effect of total surface area on C and N release. Nevertheless, the findings reported in this work are relevant for practical uses, as they would mostly also occur if smaller pellets are applied. Regarding the highest N₂O emissions measured in finely ground pellets, two main reasons were assumed: (i) the rough surface of this pellet fraction enabled fast water spreading and penetration due to lower contact angle and greater actual surface of the solid–liquid interface [64]; (ii) the fast water spreading decreased O₂ diffusion and thus enhanced the creation of anaerobic conditions favoring N₂O release from denitrification.

Additionally, the finely grinding increased the total surface area of this fraction in comparison to intact and crushed pellets. As a consequence, microbial growth and therefore the decomposition of organic matter and conversion of N pools were promoted. Although no statistically significant differences were found, the mean CO₂ emission increased with decreasing pellet size fraction in all tested moisture classes as well. For soils, it is well known that physical crushing of aggregates results in increased organic C availability and turnover [65–67]. Navarro-García et al. [68] reported a higher mean microbial biomass determined after the first rewetting (which was equivalent to our moistening procedure of the pellets) in crushed soil aggregates compared to intact ones. Despite the higher metabolic quotient in the intact aggregates, the total CO₂ release in their work was higher in the crushed aggregate samples. Increased C turnover and consequent O₂ consumption induced O₂ limiting environment which favored denitrification and thus N₂O release. Similar results have been observed in the field after

soil tillage. In this context, the studies of Staley et al. [69] and Guzman-Bustamante et al. [70] showed that a mechanical disturbance of soil (caused by tillage) can induce high N_2O fluxes.

Concerning the organic matter decomposition and more specifically the C availability after crushing, the significant lower CO_2 emissions observed in crushed pellets wetted to 62% moisture indicated the presence of additional uncertain factors. The similar trend found in the results of the hot water extraction also remains unclear. A possible explanation could be the inhomogeneity of particle size and texture of the individual pellet pieces which built up this pellet fraction. The crushed pellet fraction represented a mixture of small, medium and large pieces of the original pellet which were not distributed homogeneously. The size of the small ones was comparable with that after finely grinding. Here, the mechanical breaking down of large initial particles coming from the original solid manure might have enabled a faster decomposition of organic matter after water addition. The medium and large pieces, in turn, were still cluster formations which partly retained the original shape of the pellet and consequently consisted of various sizes of solid components. Based on that it is assumed that different pore sizes were present, which might have affected the water absorption and spreading within the larger pieces of the crushed pellet [71]. Furthermore, due to crushing, only a small part of the initial outer layer remained and the inner milieu and microsites were changed. As a consequence, lower CO_2 emissions were determined due to affected decomposition of organic matter and lower C release.

Study Implications Related to Pellet Application in the Field

In order to assess the environmental impact of pellet application on trace gas emissions, the presented cumulative N_2O emissions were compared with those measured after pellet application in an own field experiment (data are part of a separate manuscript currently being processed). Therefore, the following assumptions were made: (i) present cumulative N_2O emissions were calculated for a pellet amount equivalent to an application of $170 \text{ kg total N ha}^{-1}$ and (ii) N_2O fluxes measured in the field and covered the same sampling period (seven days and four field replicates) were cumulated. The N_2O emission in the field accounted for $301 \text{ g N}_2\text{O-N ha}^{-1}$.

The N_2O emissions scaled up from the lab measurements amounted to 144, 405 and $7 \text{ g N}_2\text{O-N ha}^{-1} 7 \text{ day}^{-1}$ after pellet wetting to 47%, 62% and 72% moisture, respectively. Although measured under constant lab conditions, these values were well in accordance with the N_2O emission determined in the field. There, the highest N_2O fluxes were also observed at around 62% pellet moisture.

Furthermore, these results are in the range reported by Pampuro et al. [30, 33], who found N_2O emissions of about $30\text{--}240 \text{ g N}_2\text{O-N ha}^{-1}$ at a fertilizer level of 200 kg N ha^{-1} . Although the period of measurements in their work was considerably longer, most emissions were also registered during the first week after application [30, 33].

Conclusions

Our measurements clearly show that the indigenous microflora of the pellets has a substantial potential for N_2O production and release. The degree of N_2O release from the pellets was strongly affected by pellet moisture which, under field conditions, is controlled by the moisture of the soil after pellet application. The N_2O emissions in our lab experiment were in the same order of magnitude as the N_2O emissions in a field study. Therefore, we assume that the pellets themselves were the main contributor for the field emissions. To quantify the contribution of the pellets to the total N_2O emission in the field, relatively simple experiments, like measuring trace gas fluxes with the pellets and from the same sampling area immediately after removing the pellets, would be useful. For a closer understanding of the microbial N and C transformations in the pellets, additional in-depth studies on indigenous microbial structure as well as on changes in microbial diversity would be expedient.

In order to avoid high N_2O emission after pellet application, we suggest the utilization of pellets as big as possible because the N_2O emission increased with decreasing pellet fraction size. However, the impact of pellet size on nutrient availability should also be taken into consideration.

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Compliance with Ethical Standards

Conflicts of interest The authors declare that they have no competing interests.

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