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Complete elimination of methane formation in stored livestock manure using plasma technology

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Plasma-based nitrogen fixation has recently been shown to be applicable in the domain of manure management, as it has the ability to reduce ammoniacal nitrogen losses and increase the nitrogen content of organic wastes, with air and electricity as the only input. In addition, the plasma treatment confers antimicrobial properties, which we hypothesize to be transferable to methanogenic archaea and hence prevent methane formation during manure storage – a major contributor to global anthropogenic greenhouse gas emissions. In this work we compared the methane formation from cow manure to the methane formation in nitrogen enriched cow manure, kept in two outdoor storage tanks for 70–80 summer days over three consecutive years. In all instances, the methane formation was eliminated completely. To investigate the cause of inhibition, a controlled incubation experiment was conducted to show that neither the acidification nor the addition of nitrate or nitrite, alone or in combination, could explain the inhibition of methanogenesis and denitrification that occurred in plasma treated cow manure at moderate pH.

KEYWORDS
nitrogen fixation, plasma, methane emissions, sustainable livestock production, organic fertiliser

1 Introduction

The potent greenhouse gas methane (CH_4) has received recent attention for its short lifespan in the atmosphere (IEA, 2022). Compared to the 120-year half-life of carbon dioxide (CO_2), CH4 has an atmospheric lifespan of only 12 years (Lynch et al., 2020). The combination of its potency and short lifespan results in a time-dependent global warming potential (GWP) where the GWP over a 100-year period (GWP100) will be 27 times higher than that of CO_2, whereas a 20-year time-horizon (GWP20) will result in a GWP of 81 CO_2 equivalents. Consequently, measures that significantly reduce CH_4 emissions will have an impact on the rate of global warming, particularly in the short term. The Intergovernmental Panel on Climate Change (IPCC, 2018) estimate that a 40–45% reduction of CH_4 emissions can limit the global temperature rise to 1.5°C above pre-industrial level if implemented by 2030. The resultant delay in global warming may thus leave sufficient time for the complex task of decarbonizing power production, transportation, and other fossil fuel-dependent industries, and increase the probability of limiting global warming to 1.5°C throughout the 21st century.

Of global methane emissions, close to one third stems from agriculture, whereof the livestock sector is the main contributor. Methane emissions from livestock are dominated by enteric fermentation in ruminants and are estimated to account for 70% of global

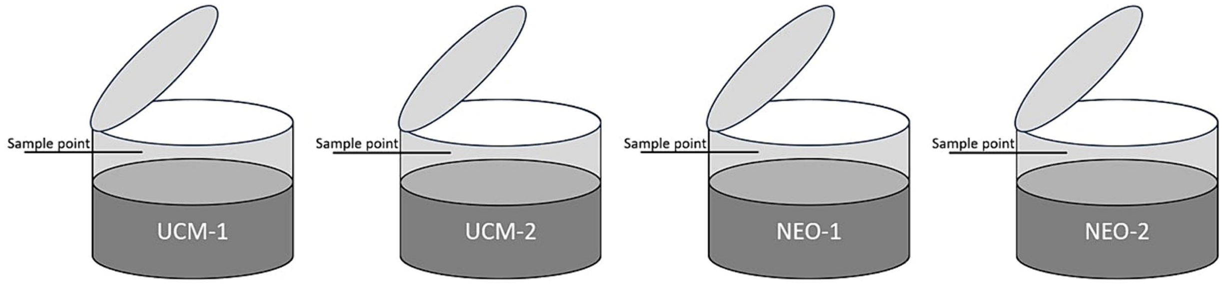

FIGURE 1
The four outdoor tanks had a retractable lid to ensure realistic exposure to the environment, except during the brief period of measurement. Samples were drawn through the sampling point with a syringe, transferred to a gas vial and the CH_4 concentration was measured on a gas chromatograph.

agricultural CH_4 emissions, followed by manure management (7%) (Kuylenstierna et al., 2021). However, there are regional differences in the distribution of sources: in livestock-intense regions, where liquid manure is stored for prolonged periods, such as California, CH_4 emissions from manure management equal those from enteric fermentation (CARB, 2020). Identifying and implementing measures that reduce CH_4 emissions from manure management is therefore central to the 1.5°C target, as these measures must counteract a growing global demand for milk and meat (OECD-FAO, 2022).

Anaerobic digestion of livestock manure (AD) has been presented as a sensible mitigatory measure, as it captures the CH_4 emitted from livestock manure and uses it to replace fossil gas. However, AD will often lead to increased methane production downstream (Balde et al., 2016; Rodhe et al., 2018) and exacerbated ammonia (NH_3) emissions (Holly et al., 2017). An alternate or complimentary approach is acidification of livestock manure, commonly with sulfuric acid (H_2SO_4), to reduce the slurry pH and hence the ammonia volatilization and methanogenic activity (Hou et al., 2017). Retaining the effect during storage and at field application does however lead to a sulphur dose that often exceeds crop needs and increases the risk of soil acidification and sulphate leaching (Lamers et al., 1998).

A novel approach to manure management, that can work alone or in conjunction with the above technologies, is plasma treatment. In this process, a strong electric field ionises air, leading to the formation of a reactive nitrogen gas (NO_x). The reactive gas is absorbed in livestock manure as nitrogen oxyanions (NO_x^-) resulting in a nitrogen enriched organic fertiliser (NEO), containing more plant available nitrogen at a lower pH, thus reducing NH_3 volatilisation (Ingels and Graves, 2015). Since crops require more nitrogen than sulfur, the nutrient balance is improved, which in turn reduces the risk of soil acidification. The treatment is energy intense but electricity-based and is therefore suitable for integration with intermittent renewable energy.

In addition to the electricity-based nitrogen production and reduced losses of NH_3, the plasma treatment has been shown to have antimicrobial effects (Hiis et al., 2023). This is important, as microbial activity in NEO would lead to denitrification and the subsequent loss of the plasma-generated nitrate (NO_3^-) and nitrite (NO_2^-). In this work we hypothesise that the inhibitory effect is transferable to methanogenic archaea. We measured the impact on CH_4 emissions during the summer months over three consecutive years, in an outdoor storage facility, to approximate a farm scenario. To elucidate the mechanism behind the inhibition of CH_4 emissions, we also conducted a controlled laboratory experiment investigating the effect of acidification (by H_2SO_4) and NO_2^- alone and in combination to imitate the effect of the plasma treatment.

2 Methodology

2.1 Farm-scenario experiment

Four outdoor monitoring tanks, each holding 2 m^3 of slurry were used to estimate the CH_4 emissions from untreated cow manure (UCM), which served as the non-plasma treated control, and nitrogen enriched organic fertiliser (NEO). All tanks were equipped with a lid that remained open, except during the 40-min measurement period when the lid was closed and gas samples were drawn at 10-min intervals to estimate CH_4 emission. Figure 1 shows a schematic of the system. Continuous measurements of the pH and temperature in all tanks were conducted the first year.

The UCM originated from Skollenborg, Norway and was filtered with a screw press before being transferred to two of the tanks. The same filtered UCM was used as input material in the plasma process, which converts UCM to NEO. Figure 2 illustrates the plasma process and Table 1 shows the difference in chemical composition between the UCM and NEO (averaged over the three years). Each year, the tanks were drained before a new batch of UCM and NEO was transferred to the tanks, with approximately the same composition as the year prior.

2.2 Laboratory experiment

In the laboratory experiment, the UCM was compared to UCM acidified with sulfuric acid (H_2SO_4) to pH 6.3 and pH 5.0 ($UCM_{pH6.3}$ and $UCM_{pH5.0}$) and to NEO at pH 6.3 and pH 5.0 ($NEO_{pH6.3}$ and $NEO_{pH5.0}$). Each UCM treatment was set up in nine gas tight serum-vials, the NEO treatment in three. Conditions in the vials were made anoxic by washing the bottles (repeated cycles of evacuation and He-filling while stirring the slurries) to accelerate methanogenesis. The vials were incubated at 30°C for 180 h in a water bath connected with a multi-column GC (Model

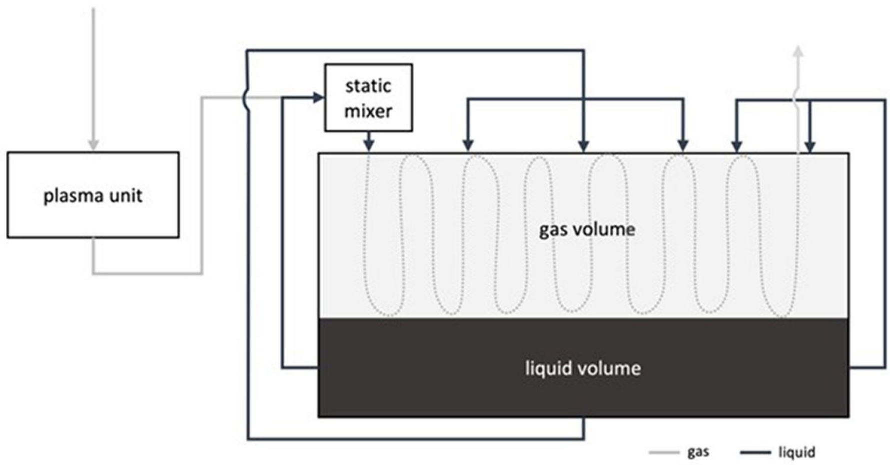

FIGURE 2
A schematic representation of the enrichment process. Air passes through the plasma generator, the resultant reactive nitrogen gas is subsequently absorbed in the organic substrate as NO_3^- and NO_2^-. The process is pH-controlled and produces material at a requested pH.

TABLE 1 The pH and nitrogen composition of the cow slurry before and after nitrogen enrichment.

Treatment		[kgN m^{-3}]		
	pH	NH_4^+	NO_3^-	NO_2^-
UCM	7.1	1.3	0	0
NEO	5.3	1.3	1.2	0.7

7890A, Agilent, Santa Clara, CA, USA) and a chemoluminescence NO analyser (Model 200 A, Advanced Pollution Instrumentation, San Diego, USA) via an autosampler and a peristaltic pump for repeated headspace analyses as described by Molstad et al. (2007). The methane concentrations in the headspace were measured six times a day.

Approximately halfway through the incubation, three vials from the UCM and $UCM_{pH6.3}$ received a potassium nitrate (KNO_3) dose resulting in a concentration of 1,000 mgNO_3^-- N/L, and another three vials received a potassium nitrate (KNO_3) and potassium nitrite (KNO_2) dose resulting in a concentration of 1,000 mgNO_3^-- N/L and 750 mgNO_2^-- N/L, which corresponds to the nitrogen oxyanion concentration in NEO. No CH_4 formation was detected in the $UCM_{pH5.0}$ vials. Therefore, no NO_3^- or NO_2^- was added to the $UCM_{pH5.0}$ treatments. The setup aimed to determine whether pH, nitrate or nitrite, or a certain combination of the three, yielded a complete inhibition of methanogenesis or whether the inhibition is a result of particular properties of the plasma treatment.

3 Results and discussion

3.1 Farm-scenario experiment

Temperature readings suggested differences in microbial activity between the two treatments. As shown in Figure 3, the temperature at a slurry depth of 30 cm displayed higher peaks in UCM than in NEO, and more pronounced diurnal fluctuations, likely caused by higher microbial activity in the UCM that affected the slurry temperature, whereas air temperature was the main driver in NEO. However, the minima are lower in the UCM – resulting in comparable average temperature over the entire storage period.

The pH during storage also indicated a clear inhibition of microbial activity in NEO (Figure 4). The pH in NEO rose slowly from pH 5.3 to pH 5.9 throughout the 80 days of storage. The slow increase can be attributed to deprotonation of carbonates, combined with the reaction of NO_2^- with organic matter. The onset of microbial denitrification would have been associated with an abrupt pH increase and elevated temperatures as described by Hiis et al. (2023), suggesting an absence of denitrification in the stored NEO.

The apparent inhibition of microbial activity had a clear effect on the CH_4 fluxes. Figure 5 shows the cumulative CH_4 emissions for all tanks in the 2021–2023 measurement season, where the methane formation is completely inhibited by the nitrogen enrichment. A minimal yet consistent CH_4 uptake is found in all NEO treatments.

The low measurement frequency in 2022 did not yield an appropriate resolution of the fluxes, especially since the deviation in emissions between the two tanks in 2021 was large, mainly as a result of crust formation on UCM-1 and no crust formation on UCM-2. CH_4 emissions were substantially higher in 2023 compared to 2022, a finding we currently have no explanation for, other than speculating that the annual temperature variations may have influenced the rate of CH_4 formation. Despite emission-variation in the UCM, the reduction resulting from the plasma-based nitrogen enrichment was reliably above 100% throughout the storage periods. The complete emission reduction distinguishes the plasma-based nitrogen enrichment from acidification alone, which reports CH_4 reductions in the range of 69 to 84% (Habtewold et al., 2018).

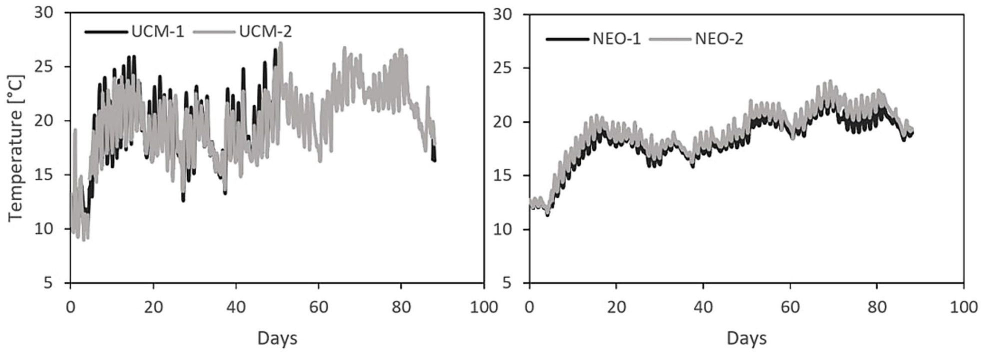

FIGURE 3
The temperature at 30 cm depth in all tanks, year 2 – ambient temperatures were used to determine the impact of temperature the following years.

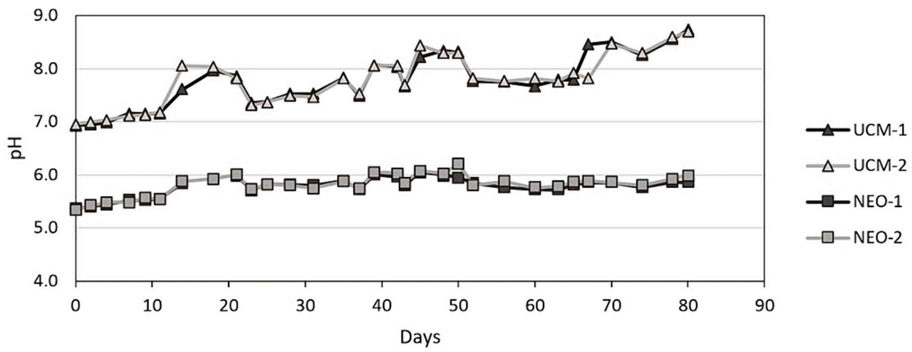

FIGURE 4
The pH development in all tanks, year 2.

3.2 Laboratory experiment

The controlled incubation experiments demonstrated a pronounced effect of acidification and NO_3^- and NO_2^- addition on CH_4 production, although their combinatory effect did not match that of the plasma treatment entirely. The unacidified UCM (pH 7.1) showed a steady methane formation, until either NO_3^- or $NO_3^- + NO_2^-$ was added (Figure 6). The addition of $NO_3^- + NO_2^-$ resulted in inhibition of methanogenesis throughout the incubation period, whereas the methane formation resumed in the NO_3^- treatment.

Both treatments where the nitrogen oxyanion concentration was artificially elevated resulted in denitrification, as was evident from N_2 formation shown in Figure 7. The pronounced drop in CH_4 concentration was unexpected; the increased formation of N_2, N_2O, NO and CO_2 and could not entirely explain the reduced concentration. A potential explanation of the effect is denitrifying anaerobic methane oxidation (DAMO), as described by Wei et al. (2022), a thermodynamically favourable denitrification pathway where CH_4 acts as electron donor and is oxidised to CO_2.

Figure 8 shows the impact of acidifying the UCM with H_2SO_4 to pH 6.3. Acidification alone resulted in a 45% reduction in CH_4 accumulation. The nitrogen oxyanions were added in identical concentrations as in the prior experiment after 86 h. Both treatments resulted in complete inhibition of methanogenesis. NEO (UCM plasma treated to pH 6.3) showed a complete inhibition of methanogenesis throughout the experiment.

Both $UCM_{pH6.3+NO3}$ and $UCM_{pH6.3+NO3+NO2}$ exhibited clear signs of denitrification. The pH at the end of the experiment was 8.1 and 8.2, which indicates that the alkalization occurred due to consumption of nitrogen oxyanions. In contrast, pH in NEO rose moderately from pH 6.3 to pH 6.5 and did not display the same N_2O peaks as the comparable UCM treatments, as shown in Figure 9.

When acidifying the UCM to pH 5.0, no methane formation occurred over the duration of the experiment. However, the amount of H_2SO_4 required to reach this pH results in a sulphur content that often exceeds agronomic needs (5.4 kg 98%H_2SO_4 m^{-3}). Targeting such low pH levels with sulfuric acidification should therefore be avoided.

The exact inhibitory mechanisms of plasma-based nitrogen enrichment cannot be determined by this set of experiments alone. However, some important observations can be made: the addition of nitrogen oxyanions suppresses CH_4 formation, likely because of a shift to NO_3^- and NO_2^- as the favoured electron acceptors. Consequently, denitrifiers consume the nitrogen oxyanions whereafter methanogenesis is likely to resume (as in UCM_{NO3}). Our results also indicate the occurrence of the DAMO

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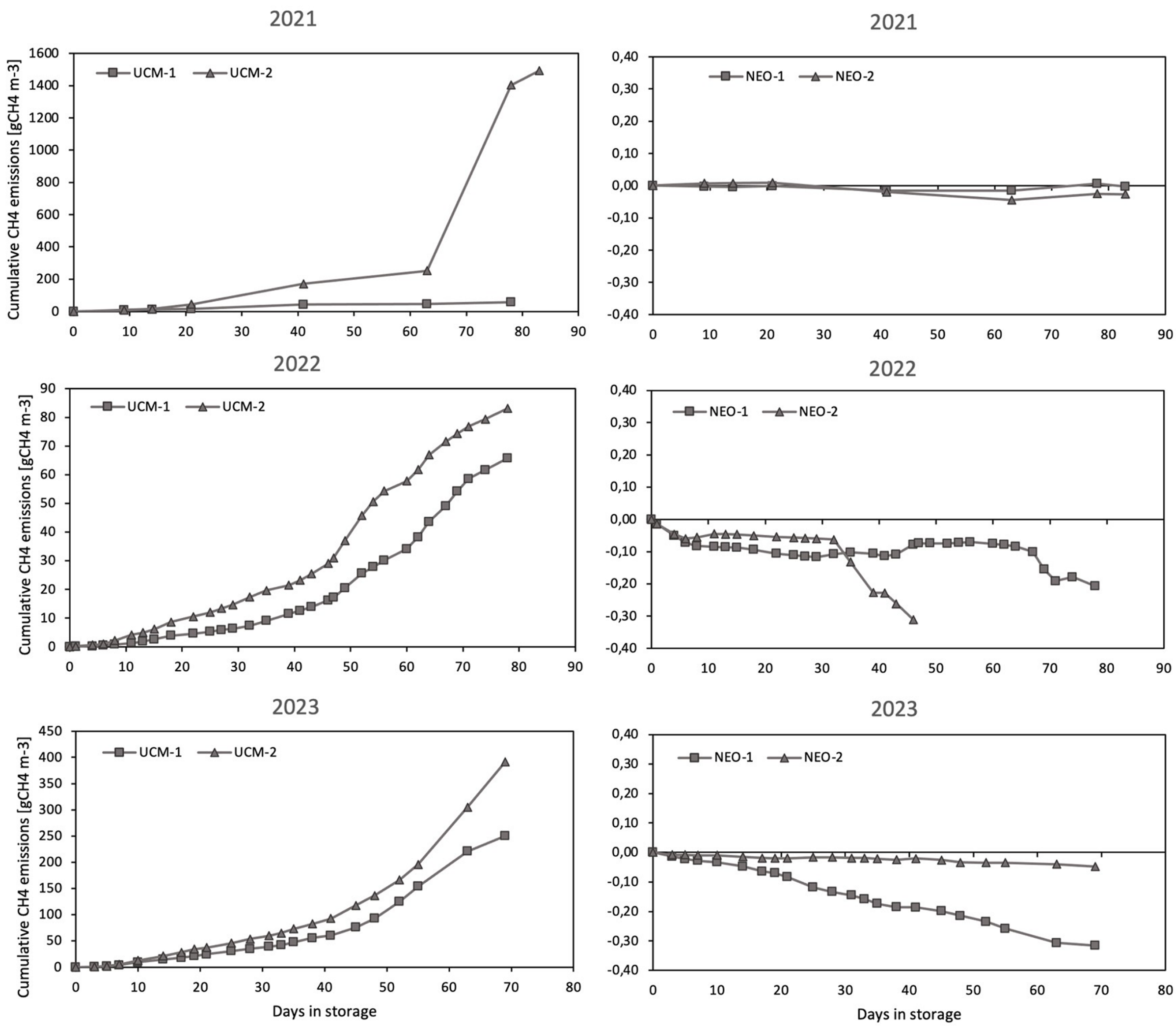

FIGURE 5
The cumulative CH_4 emissions from all tanks over the three measurement periods. The tank lid-closing mechanism malfunctioned after 48 days in NEO-2 in 2022, which is why the measurements stop.

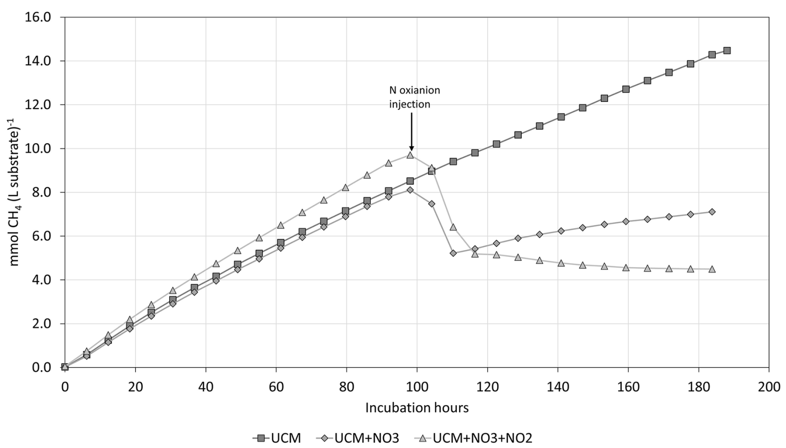

FIGURE 6
The cumulative CH_4 formation in UCM, with and without elevated N oxyanion concentrations (injected after 98 h).

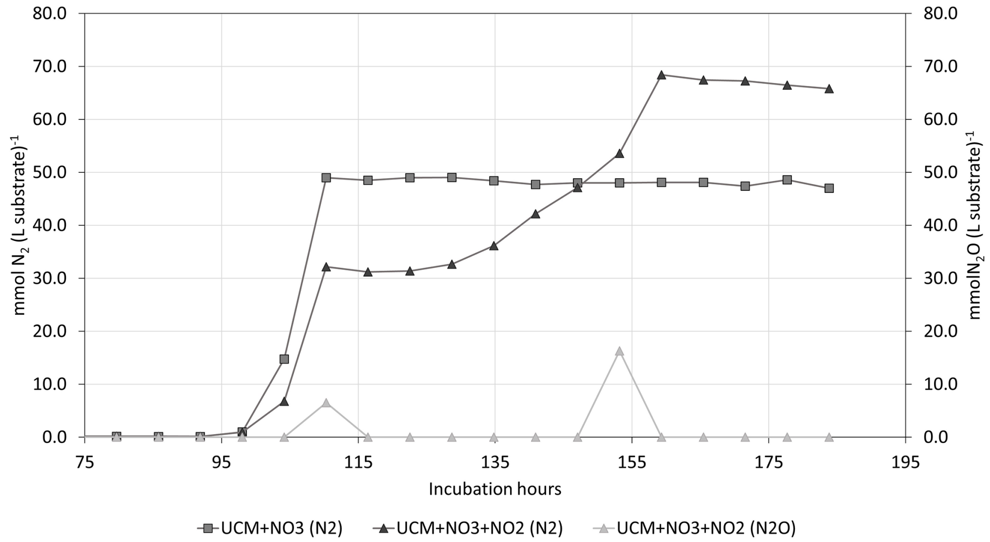

FIGURE 7
The N_2 and N_2O accumulation in the non-acidified treatments. All replicates displayed the same pattern, although shifted in time. Therefore, only one replicate of each treatment is displayed.

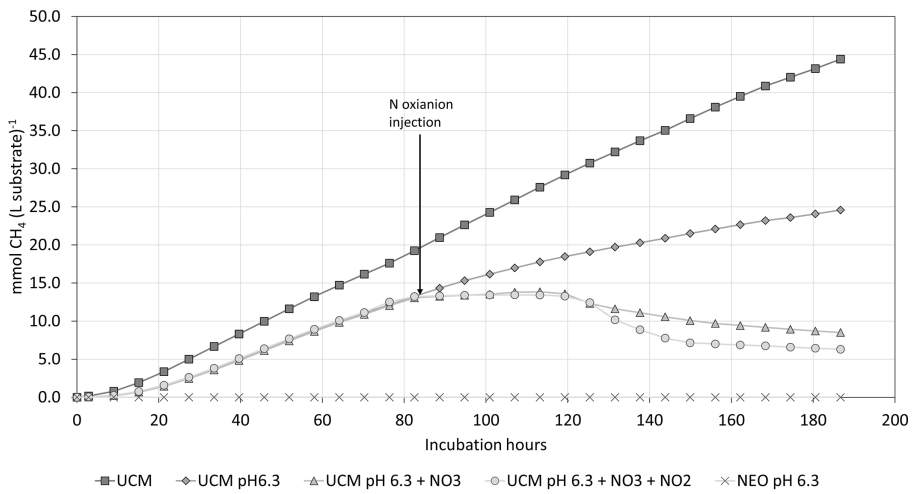

FIGURE 8
CH_4 formation in the substrate acidified with H_2SO_4 to pH 6.3 compared to a non-acidified control (UCM). Nitrogen oxyanions were added after 86 h of incubation.

process, as denitrification coincides with a reduction in the CH_4 concentration that cannot be accounted for by dilution alone. Surprisingly, the NEO, even at a moderate pH of 6.3, did not result in the denitrification that occurred in its chemical imitations, suggesting that the plasma treatment itself has antimicrobial properties beyond the acidification and addition of nitrogen oxyanions. One hypothesis worthwhile exploring is that the plasma treatment occurs under aerobic conditions when the facultative anaerobic denitrifiers are more likely to respire oxygen and therefore cannot easily counter the elevated levels of undissociated nitrite (HNO_2).

4 Conclusion

In this work we demonstrate that plasma-based nitrogen enrichment of cow manure inhibited methanogenesis completely in exposed outdoor storage tanks during the warm period of the year over three consecutive years. A laboratory experiment showed that the CH_4 inhibition could be achieved by reducing the slurry pH below 5, or by amending the cow manure with nitrate and nitrite. However, the latter strategy resulted in rampant denitrification, which renders plasma-based nitrogen enrichment the only method by which both methanogenesis and denitrification can be avoided at moderately low pH.

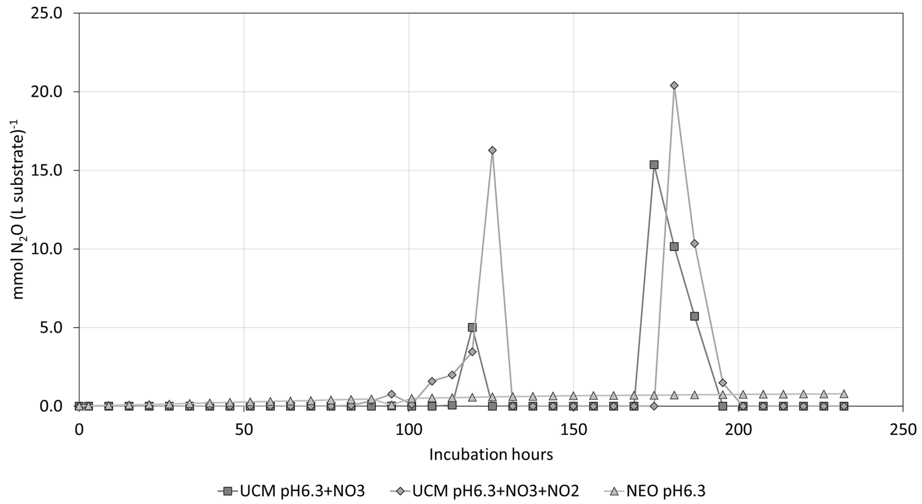

FIGURE 9
The N_2O development show abrupt peaks during microbial denitrification compared to a low but steady N_2O formation in NEO, which is likely a result of chemical interactions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MN: Writing – original draft, Writing – review & editing. PD: Writing – original draft, Writing – review & editing.

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Conflict of interest

The work was part of an industrial PhD where the MN works for a company that develops Plasma technology.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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