Nitric oxide and nitrous oxide emissions from cattle-slurry and mineral fertiliser treated with nitrification inhibitor to an agricultural soil: A laboratory approach

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Abstract

The application of organic and mineral fertilisers to soil can result in increased gaseous emissions to the atmosphere such as nitric oxide (NO) and nitrous oxide (N₂O) gases. The aim of this study was to evaluate under laboratory conditions the effects on mineral N dynamics and NO and N₂O emissions of application to soil of cattle slurry derived liquid fraction (LF) obtained by screw press and mineral fertiliser (MF), both treated with or without the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). An aerobic laboratory incubation was performed over 93 days with a Dystric Cambisol amended with mechanically separated LF or mineral fertiliser ammonium sulphate only or combined with DMPP. Two additional treatments were included: soil only and soil amended with DMPP. Nitrogen immobilisation was the dominant process with MF amendment, whereas N mineralisation has been observed with LF. The application of LF reduced significantly NO emissions by 80% relative to mineral but no differences were observed with N₂O emissions. The addition of DMPP to MF induced a decrease of 18 and 29% in NO and N₂O emissions whereas DMPP combined with LF reduced (numerically but not statistically) these emissions in 20 and 10%, respectively. Results obtained in our study suggest that N (NO + N₂O) losses can be mitigated by adding DMPP to mineral fertilisers or replacing mineral fertiliser by LF.

Additional key words: carbon dioxide; DMPP; N emissions; N mineralisation; solid-liquid separation.

Abbreviations used: DMPP (3,4-dimethylpyrazole phosphate); LF (cattle-slurry derived liquid fraction); NNM (net N mineralisation); WFPS (water-filled pore space).

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Introduction

The intensive cattle production generates large amounts of slurries (liquid manure) that are commonly applied to agricultural soils to recycle nutrients to plants, mainly nitrogen (N) and phosphorus, and increase or maintain the soil organic matter contents (Webb et al., 2013). Nowadays, the surface of agricultural soil available to receive the derived slurry tends to diminish while livestock production continues to increase (duplication in 2050). The solid-liquid separation of cattle slurry has been referred as a good solution for manure management at farm scale and with potential to reduce the environmental impact of cattle-slurry (Hjorth et al., 2010). The derived solid fraction is generally exported out of the farm and the derived liquid fraction (LF) can be used to substitute mineral N application since it contains a high amount of ammonium (NH₄⁺) (Villar & Guillamares, 2010). According to Galloway et al. (2008), a duplication of the fertiliser N input is expected in agriculture by 2050 relative to the actual inputs. Fertilisa-
tion using animal slurry has the advantage to provide mineral N but also a significant part of organic N that is then slowly converted in available N for plants. Minimising use of chemical fertilisers is not just a goal for agriculture, but also for an environmentally friendly development. Another recent aspect that has to be considered is that production of phosphate fertilisers will not be possible forever because phosphorous rocks are not a renewable resource and current global reserves may be depleted in 50–100 years (Cordell et al., 2009).

Gaseous emissions [ammonia (NH$_3$), carbon dioxide (CO$_2$), nitric oxide (NO) and nitrous oxide (N$_2$O)] are generally observed following soil application of cattle-slurry or derived liquid fraction (Weiske et al., 2001; Menéndez et al., 2006; Fangueiro et al., 2008; Dorno et al., 2013; Pereira et al., 2013). The NO emissions contribute to the formation of tropospheric ozone, photochemical oxidants and acid rain. Nitrous oxide emissions are an important greenhouse gas that leads to the destruction of the ozone layer and global warming (UNEP, 2013). Furthermore, LF application can lead to significant losses of N by leaching (Sørensen & Rubæk, 2012). Indeed, a large part of NH$_4^+$ applied can be quickly nitrified in soils and then leached (Diez et al., 2010).

Previous studies showed that the application to the soil of mineral fertilisers (Weiske et al., 2001) or slurries (Hatch et al., 2005; Pereira et al., 2010; Mieselbrook et al., 2014) treated with nitrification inhibitors delays the bacterial oxidation of the NH$_4^+$ to nitrite temporarily (Zerulla et al., 2001). Ammonia-oxidizing bacteria are considered to be mainly responsible for ammonia oxidation, being significantly affected by 3,4-dimethylpyrazole phosphate (DMPP) addition, with the reduction in ammonia-oxidizing bacteria population size and activity (Gong et al., 2013). Recently, no negative impact on soil bacterial ecosystem reversion has been observed from long term application of DMPP to a Cambisol in northeast China (Dong et al., 2013). However, DMPP application to LF was rarely considered even if LF might represent a high risk of NO$_3^-$ leaching due to its high NH$_4^+$ content. Furthermore, previous studies indicate that DMPP might also affect N$_2$O emissions but did not consider the NO emissions that can reach significant values after application of mineral and organic fertilisers to soils (Menéndez et al., 2006; Vallejo et al., 2006; Pereira et al., 2010).

The aim of this study was to evaluate the effect of application to soil of cattle-slurry derived liquid fraction treated or not with DMPP on N dynamics in soils with special emphasis on NO and N$_2$O emissions. The potential substitution of mineral fertiliser by LF will also be evaluated in terms of N dynamics.

**Material and methods**

**Soil and cattle-slurry preparation**

The soil used in this study was classified as a Dystric Cambisol, with a silt loam texture (45% coarse sand, 4% fine sand, 39% silt and 12% clay), and was located at central Portugal (40° 38’ 29” N, 7° 54’ 37” W). The soil was sampled from the upper layer (0-100 mm) in an agricultural field cultivated with ryegrass. Then, soil was sieved (<2 mm), homogenized and stored (4 °C) in the three weeks before beginning the study. Soil had been wetted with deionised water one week before the start of the study to avoid the interference of pulses of NO and N$_2$O associated to first wetting of dry soil (Sánchez-Martin et al., 2008). The physico-chemical properties of the soil were: bulk density, 1.1 g/cm$^3$; pH, 6.1; water retention capacity at pH 2.0, 24.8% (w/w); total C, 15.6 g/kg dry soil; total N, 1.4 g/kg dry soil; NH$_4^+$-N, 0.6 mg/kg dry soil; and NO$_3^-$-N, 13.0 mg/kg dry soil.

The LF used in the present study was collected from a commercial dairy-cattle farm from Northwest Portugal where the diet is mainly based in maize silage. The raw slurry (10% dry matter) after being stored in a concrete storage open pit for five months, has been mechanically separated with a screw press separator equipment (FAN model S655, BAUER, Austria) generating a LF with low dry mater content and average particle size <1 mm. The collected LF was homogenized and kept refrigerated (4 °C) to preserve N. At the beginning of the experiment, LF subsamples were analysed using standard laboratory methods to assess the following parameters: dry matter, 5.7%; pH, 8.0; total C, 25.0 g/kg; total N, 3.7 g/kg; NH$_4^+$-N, 2.1 g/kg; and NO$_3^-$-N, 1.3 mg/kg (expressed on a fresh weight basis). A comprehensive description of the methods used in the analysis of the soil and slurries samples is available in Pereira et al. (2010 and 2013).

**Experiment details**

An aerobic laboratory incubation was conducted with the following treatments (with four replicates each): (i) Control, soil only; (ii) DMPP, soil amended with DMPP; (iii) Mineral, soil amended with mineral fertiliser; (iv) Mineral-DMPP, soil amended with mineral fertiliser treated with DMPP; (v) LF, soil amended with slurry derived liquid fraction; and (vi) LF-DMPP, soil amended with slurry derived liquid fraction treated with DMPP.

The mineral fertiliser used was ammonium sulphate (99.9% active component) (Panreac, Spain). The nitri-
fication inhibitor, 3,4-dimethylpyrazole phosphate (DMPP) (COMPO GmbH & Co. KG, Germany) was applied at a dosage of 1.0 kg of active ingredient per ha (in liquid form). According to Zerulla et al. (2001), an application rate of 0.5 to 1.5 kg DMPP active ingredient per ha is sufficient for effective nitrification inhibition. At the start of the study, each amended treatment received a rate of 120 mg total N/kg dry soil, based on the equivalent amount of soil occupying the 0-100 mm depth under field conditions. The amounts of total C and NH4
+ applied in LF treatment alone or with DMPP were 702 and 54 mg/kg dry soil, respectively. The soil was mixed with the respective amounts of fertiliser and nitrification inhibitor and soil moisture content was previously elevated to close to 60% water-filled pore space (WFPS) by adding deionised water. To evaluate the mineral N dynamics, half of the treated soil (80 mm depth) of each treatment was packed into PVC boxes (Ø=240 mm, h=200 mm) to achieve a bulk density of 1.1 g/cm³. To follow the gaseous fluxes in each treatment, the other half of the treated soil was used to fill Kilner jars (1.2 L) with 0.6 kg soil each (Ø=98 mm, h=80 mm), being packed at a bulk density equal to PVC boxes. The aerobic incubation was performed during 93 days at 20 °C, 60% WFPS and under controlled conditions (CONVIRON, model E15, Canada). Additional information of the incubation technique used in our study is given in Pereira et al. (2013).

Gaseous fluxes and soil mineral nitrogen content

Nitric oxide, CO2 and N2O emissions from soil in the Kilner jars were measured with modified lids fitted with two septa and a Teflon tube to allow air sampling. A detailed description of the system used to measure gas fluxes are given in Pereira et al. (2010 and 2013). The NO fluxes were measured with a chemiluminescent N oxide analyser (SIR MODEL S-5012, Spain; detection limit of 0.5 µg/m³). The CO2 and N2O fluxes were measured with a photoacoustic field gas-monitor (INNOVA 1412i-5, Denmark; detection limit of 2910 and 58.1 µg/m³ for CO2 and N2O, respectively), equipped with optical filters for CO2 (filter type UA0982) and N2O (filter type UA0985). The CO2 and N2O fluxes were measured after the measurements of NO fluxes. Gas measurements were carried out daily during the first 9 days of the experiment and then on days 13, 23, 30, 41, 58, 72 and 93.

To follow the soil mineral N dynamics, one sample from each replication (n=4) of each treatment was collected from the entire depth (80 mm) of soil in the PVC boxes on days 1, 3, 5, 9 and then once a week until the end of the experiment. Mineral N was determined after extraction with 2 mol/L KCl (1:5 w/v). Then, the supernatant was analysed for NH4
+ and NO3
− content by automated segmented-flow spectrophotometry using the Berthelot and hydrazinium reduction followed by optical filters for NH4
+ and NO3
−, respectively (Houba et al., 1988). The net N mineralisation (NNM) rates were determined by considering the mineral N in each treatment at 0, 1, 3, 5, 9, 16, 23, 30, 37, 44, 51, 58, 65, 72, 79 and 93 days. The following equation was applied to assess NNM rates expressed in mg N/kg/day dry soil: NNM(t)=Mineral N(t) – Mineral N(0). Apparent N mineralisation was calculated from NNM rates by subtracting the Control treatment to each amended treatment, and then was expressed as a percentage of total N applied and/or as a percentage of organic N applied.

Statistical analysis

The data distribution normality of the CO2, NO and N2O fluxes, and soil NH4
+ and NO3
− concentrations was verified using the Friedman statistic test (Snedecor & Cochran, 1980). Results were analysed by analysis of variance (software STATISTIX 7.0, USA) considering the measurement time as a split factor over the two experimental factors (fertiliser and inhibitor). Then, Tukey comparisons of means tests (p<0.05) were carried out for the two factors and their interaction.

Results

Soil nitrogen dynamics

The NH4
+ and NO3
− concentrations in soils from each treatment are shown in Fig. 1. The initial concentrations of NH4
+ in the Control and DMPP treatments were very low (<2 mg NH4
+ N/kg dry soil) and remained constant until the end of the experiment (Fig. 1A). As can be seen, the addition of fertilisers to soil led to an increase of the NH4
+ content close to 110 mg NH4
+ N/kg dry soil in Mineral treatments, against 75 mg NH4
+ N/kg dry soil in LF treatments. A strong decrease of the NH4
+ concentration was observed in Mineral and LF treatments. In the Mineral treatment, the NH4
+ concentration reached the Control values only after 30 days of incubation whereas such base values were reached after only 16 days in LF treatment (Fig. 1A). In Mineral-DMPP and LF-DMPP
treatments, the \( \text{NH}_4^+ \) concentration decreased more slowly and DMPP has a stronger effect on Mineral than on LF. Indeed, \( \text{NH}_4^+ \) concentration reached the Control values after 28 days in LF-DMPP and after 72 days in Mineral-DMPP (Fig. 1A).

At the beginning of the experiment, the \( \text{NO}_3^- \) concentration was about 14 mg \( \text{NO}_3^-\cdot\text{N/kg} \) in all treatments including the Control. The \( \text{NO}_3^- \) concentration increased during the 93 days of the experiment in all treatments, being significantly higher (\( p<0.05 \)) in Mineral and LF treatments (Fig. 1B). In the Mineral treatment, a strong increase of the \( \text{NO}_3^- \) concentration was observed in the first 23 days of experiment. Similar \( \text{NO}_3^- \) concentrations were observed in Mineral and Mineral-DMPP treatments at day 72. The addition of the DMPP to LF affected significantly (\( p<0.05 \)) the pattern of the \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) concentration, particularly in the first 30 days after fertiliser addition (Fig. 1B).

Figures 2A and 2B shows N mineralisation/immobilisation observed in treatments during the experiment. As can be seen, N mineralisation varied along the experiment and decreased in some periods in Mineral treatment. As for the LF treatment, a strong N immobilisation occurred in the first 9 days after LF and LF-DMPP application while N mineralisation was observed later. At the end of the experiment, N mineralisation in Mineral treatment was significantly lower (\( p<0.05 \)) than in all other treatments. The addition of DMPP to Mineral increased (\( p<0.05 \)) N mineralisation/immobilisation relative to Mineral alone, whereas the addition of DMPP to LF had a significant effect (\( p<0.05 \)) on net N mineralisation (in some sampling dates) and apparent N mineralisation relative to LF alone (Fig. 2A-B).

Over the whole incubation, 14.6% of total N applied was immobilised in the Mineral treatment, whereas 6.3% of total N applied was mineralised in LF treatment (Fig. 2B). The addition of DMPP to Mineral treatment induced mineralisation of 4.9% of total N applied. However, the mineralisation in LF-DMPP treatment was negligible (less than 2% of organic N, Fig. 2B); while 11.5% of organic N applied in LF treatment without DMPP was mineralised over the incubation.
Nitrogen emissions from cattle-slurry and mineral fertiliser with nitrification inhibitor

The mean values of daily NO fluxes in all treatments along the experiment are shown in Fig. 4A. Comparatively to Control, application of Mineral increased \((p<0.05)\) daily NO fluxes during the whole 93 days of experiment while LF amendment increased daily NO fluxes only during the first 13 days. During the whole experiment, the average daily NO fluxes were lower \((p<0.05)\) in ca. 73\% when LF was applied relative to Mineral amendment. The addition of DMPP with Mineral reduced \((p<0.05)\) the daily NO emissions between day 2 and 41 (ca. 77\% lower) and then increased \((p<0.05)\) by about 105\% until the end of the experiment. Daily NO fluxes, when DMPP was applied with LF, were reduced \((p<0.05)\) between day 2 and 13 (ca. 86\% lower) when compared to the application of LF alone (Fig. 4A). Cumulative NO emissions were higher \((p<0.05)\) in Mineral treatments relative to LF treatments (Table 1): the application of LF reduced \((p<0.05)\) by 80\% the cumulative NO emissions compared to Mineral. The addition of DMPP to Mineral significantly reduced \((p<0.05)\) cumulative NO emissions by ca. 18\% relative to Mineral alone but there were no effect \((p>0.05)\) of DMPP on LF relative to NO emissions (Table 1).

Over the first 3 days of incubation, significant \(\text{N}_2\text{O}\) emissions were observed in all treatments with about 15 and 40\% of the total \(\text{N}_2\text{O}\) losses occurring during this period from treatments Mineral and LF, respectively (Fig. 3). The average daily \(\text{CO}_2\) fluxes from LF amendment increased significantly \((p<0.05)\) in ca. 132\% during the first 9 days relative to Mineral amendment. The organic treatments (LF and LF-DMPP) significantly increased \((p<0.05)\) \(\text{CO}_2\) fluxes, and no effect \((p>0.05)\) of the inhibitor was observed. The cumulative \(\text{CO}_2\) emissions were 27\% higher \((p<0.05)\) from LF than from Mineral (Table 1). However, no significant differences \((p>0.05)\) in terms of cumulative \(\text{CO}_2\) emissions were observed between treatments receiving DMPP or not (Table 1).

**Table 1.** Cumulative gaseous emissions during the experiment

| Treatment                  | mg CO₂/kg dry soil | NO/N₂O ratio | NO-N (%) | N₂O-N (%) | N (%) | NH₄-N (%) | N (%) |
|----------------------------|--------------------|---------------|----------|-----------|-------|-----------|-------|
| Control                    | 1241 b             | 0.18 d        | 0.46 b   | 0.6 d     | 0.3 a | 3.7 a     | 4.1 a |
| DMPP                       | 1396 b             | 0.21 d        | 0.34 b   | 0.6 d     | 0.4 a | 3.0 a     | 3.2 a |
| Mineral                    | 1335 b             | 4.57 a        | 0.93 a   | 5.5 a     | 3.9 a | 0.6 a     | 0.8 b |
| Mineral-DMPP               | 1215 b             | 3.77 b        | 0.66 ab  | 4.4 a     | 0.8 a | 0.5 a     | 1.4 a |
| LF                         | 1692 c             | 0.93 c        | 0.75 ab  | 1.7 c     | 0.7 a | 0.5 a     | 1.0 a |
| LF-DMPP                    | 1800 c             | 0.74 c        | 0.68 ab  | 1.4 c     | 0.7 a | 0.5 a     | 1.0 a |
| \(p_{\text{treatment}}\) (A) | ***                | ***           | ***      | ***       | ***   | ***       | ***   |
| SE (A)                     | 69.03              | 0.090         | 0.093    | 0.129     | 0.424 | 0.069     | 0.075 |
| \(p_{\text{treatment}}\) (B) | ns                | ***           | ***      | ***       | ***   | ***       | ***   |
| SE (B)                     | 56.36              | 0.073         | 0.076    | 0.106     | 0.346 | 0.057     | 0.090 |
| A × B                      | ns                 | ***           | ***      | ***       | ***   | ***       | ***   |
| SE (A × B)                 | 97.62              | 0.127         | 0.131    | 0.183     | 0.600 | 0.098     | 0.106 |

Values from the interaction fertiliser × inhibitor are presented with different superscripts within columns, are significantly different \((p<0.05)\) by Tukey test. SE: standard error from the ANOVA for each factor (A, B and A × B). ns, ** and *** mean that the factor or interaction effects were, respectively, not significant or significant at the 0.01 and 0.001 probability level.

**Figure 3.** Average CO₂ fluxes following the application of each treatment along the experiment (vertical bars represent standard deviation of 4 replicates). LF: liquid fraction of cattle-slurry.
of LF increased ($p<0.05$) the daily $N_2O$ emissions by ca. 306% relative to Mineral amendment. When LF was added with DMPP, the daily $N_2O$ emissions were reduced ($p<0.05$) in ca. 16% relative to LF alone (Fig. 4B). As can be seen in Table 1, the cumulative $N_2O$ emissions from Mineral and LF were numerically higher relative to Control. However, there were no differences ($p>0.05$) on cumulative $N_2O$ emissions from Mineral and LF with or without DMPP.

The application of the LF or Mineral led to significant increases ($p<0.05$) on N ($NO + N_2O$) emissions relatively to the Control (Table 1). Nevertheless, the application of LF reduced ($p<0.05$) by 69% the N emissions, compared to Mineral. The addition of DMPP to Mineral significantly reduced ($p<0.05$) N emissions by ca. 20% relative to Mineral but there was no effect ($p>0.05$) of DMPP on the N emissions in LF treatment (Table 1). The NO losses from LF treatment were significantly lower ($p<0.05$) in about 84% of total N applied (or 62% of NH$_4^+$-N applied) relative to Mineral treatment, while the $N_2O$ losses were not significantly different ($p>0.05$) between these treatments (Table 1). The addition of DMPP to Mineral treatment reduced significantly ($p<0.05$) NO and $N_2O$ losses in 19 and 50% of total N applied (or NH$_4^+$-N applied), respectively, compared to Mineral alone. The NO losses from total N applied (or NH$_4^+$-N applied) represented 90% and 72% of total N losses in the Mineral (with or without DMPP) and LF treatments, respectively.

**Discussion**

A significant amount of N applied to soil via LF is in the organic form and need to be mineralised to become available to plants. This aspect may be seen has a limitation of LF application to soil for plant nutrition relative to mineral fertilisers containing only available N. However, if not immediately used by plants, the NH$_4^+$-N (available N) can be released from soil by NH$_3$ volatilisation or lost by leaching after nitrification. Furthermore, part of the mineral N can be immobilised. The use of DMPP prevents nitrification and potential losses by leaching (Díez-López et al., 2008). Therefore, when considering the possibility of mineral fertiliser replacement by LF, the combined application of LF with DMPP appears as an efficient solution. At the present study, the mineral N content in soil receiving Mineral was always higher than in LF treatment (Fig. 1). Also, DMPP was able to inhibit nitrification in Mineral treatment during 72 days, whereas it was less effective with LF (only 30 days) (Fig. 1). The minor efficiency of the nitrification inhibitor with organic fertilisers relative to mineral fertilisers is in agreement with previous studies (Vallejo et al., 2006; Pereira et al., 2010) and may be explained by some nitrification of the NH$_4^+$ derived from organic N in microsites not penetrated by nitrification inhibitor. Besides, the C content of organic fertilisers may cause denitrification to be an important process in these treatments (LF, LF-DMPP), so that the efficiency of DMPP could have been reduced. Further studies addressing the relationship between the rates of inhibitor and their efficiency are recommended since a higher DMPP rate applied to LF should have led to a more significant effect.

Results from this study showed that N immobilisation was the dominant process in the first 9 days following soil amendment of Mineral and LF (Fig. 2A), being related with the large NH$_4^+$ input in amended treatments (Pereira et al., 2010). Hence, the higher N immobilisation in LF is related with the higher soil
microbial activity comprised by the high CO₂ fluxes observed in the first 9 days (Fig. 3). Results are in agreement with previous studies (Jarvis et al., 1996) who reported that the addition of large amounts of mineral N led to microbial immobilisation and latter re-mineralisation. Consequently, the higher N immobilisation in LF had reduced the NH₄⁺ availability for nitrification and denitrification, explaining the lower N₂O emissions observed at this treatment (Fig. 4B). On the other hand, the absence of significant effect of DMPP on mineralisation has been previously observed in other studies (Chaves et al., 2006; Pereira et al., 2010) under controlled conditions.

The N mineralisation observed in LF (11.5% of organic N applied) (Fig. 2B) is in the same range of those reported in previous studies (Chadwick et al., 2000; Fangueiro et al., 2010, 2012), ranging between 1 and 20% of the applied organic N content. Reasons that could explain the occurrence of N mineralisation rather than N immobilisation in LF are the following: (i) removal of large pieces of undigested material through the mechanical separation of the raw slurry led an average particle size <1 mm in LF; (ii) presence of the finest particles in separated LF with low C:N organic ratio, NH₄⁺-N:total N ratio, lignin: N ratio relative to raw slurry (Fangueiro et al., 2010, 2012).

In our study, NO emissions from LF addition were lower (~62% of NH₄⁺-N applied) than from Mineral and N₂O emissions were similar from the two treatments (Table 1). Although NO is generated by denitrification process, nitrification is the most important source of NO, especially under aerobic conditions (Maljanen et al., 2007; Medinet et al., 2015). Regarding NO emissions, nitrification and/or denitrification are (depending on the WFPS) the main processes leading to N₂O losses, even though nitrifier denitrification can be also a relevant source of this gas, especially under certain soil conditions (Bateman & Baggs, 2005; Kool et al., 2011). In our study, the WFPS (60%) is in the threshold of nitrification/denitrification (Bateman & Baggs, 2005). Therefore, nitrification or nitrifier denitrification may be the major processes of NO and N₂O losses, but denitrification in the micropores should also be considered, especially in the case of LF treatments. In our study, the high NO/N₂O ratio (>1.0) observed in the Mineral suggests that nitrification has been the dominant process in this treatment (Table 1). Therefore, denitrification may have been a major source for N₂O in LF fertiliser, whereas nitrification may have dominated in the Mineral. Consequently, the higher importance of denitrification in LF treatments caused NO fluxes (which is originated mainly from nitrification) to be lower in this treatment (compared to Mineral), and caused the nitrification inhibitor to be less effective in mitigating N losses.

Previous studies reported that DMPP is effective in reducing emissions from mineral (Weiske et al., 2001) and organic fertilisers (Hatch et al., 2005; Merino et al., 2005; Menéndez et al., 2006). In our study, the application of DMPP with Mineral induced a decrease of 18 and 29% in NO and N₂O emissions whereas addition of DMPP in LF led to reductions (numerically but not statistically) of 20% in NO and 10% in N₂O (Table 1). Differences in terms of DMPP effectiveness for reducing NO and N₂O emissions when combined with Mineral or LF have been reported in other studies (Menéndez et al., 2006; Vallejo et al., 2006) and could be related with the initial NH₄⁺ concentrations, N release by mineralisation and mainly with soluble carbon addition in LF treatments. Our results agree with data reported by Menéndez et al. (2006) who found a decrease of 25 and 29% and in NO and N₂O emissions with the application of DMPP to untreated cattle LF to a grassland.

Globally, N (NO + N₂O) losses from Mineral were higher than losses from LF (Table 1). Despite the amount of NH₄⁺-N added by LF represent only 62% of NH₄⁺-N added by Mineral, the lower N losses from LF could be related with the slow-release characteristics on the N availability as mineral forms at short term and long term periods (Gutser et al., 2005; Webb et al., 2013). On the other hand, NH₃ losses could have also been important in the case of LF treatments, although LF was mixed with the soil (Pereira et al., 2010). Since National and International regulations establish a target field application based on the amount of total N applied instead of NH₄⁺ amount (EC, 1991) to reduce N pollution from agricultural sources, it might be of interest to replace mineral fertiliser with LF.

In dairy farms, large amounts of LF are available and could be applied to agricultural soils as N source and also as substitutes of mineral fertilisers, improving N use efficiency on a farm scale. Mechanical separation of LF would be the best practical tool for manure management at farm scale. Results obtained in our laboratory study suggest that the addition of DMPP to mineral fertiliser is an efficient mitigation technique to reduce NO and N₂O emissions from agricultural soils, and the addition of DMPP to LF also tended to mitigate both N losses. Nevertheless, our results need to be validated under field conditions. Consequently, cropping phase should be compared with subsequent fallow phase to gain insight on pre- and post-harvest conditions in the soil (Scheer et al., 2014). In addition, more research is required to assess under field conditions, the effect of long term soil applications of DMPP on alterations.
in the diversity of ammonia-oxidizing archaea/bacteria communities.

In summary, at controlled conditions, nitrification inhibitor DMPP was less efficient in preventing nitrification when added to mechanically separated LF relative to mineral fertiliser, with a soil persistency of 72 and 30 days, respectively. The application of LF reduced significantly NO emissions by 80% and led to similar N₂O emissions relative to mineral fertiliser. The addition of DMPP with Mineral induced a decrease of 18 and 29% in NO and N₂O emissions whereas DMPP combined with separated LF reduced (numerically but not statistically) these emissions in 20 and 10%, respectively. Up to 11.5% of organic N applied was potentially available in LF, which represents an extra amount. It can be concluded that N (NO + N₂O) losses can be mitigated by adding DMPP to mineral fertilisers or replacing mineral fertiliser by LF. Further studies will be required to validate our results.

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