
Institute for Plant Nutrition and Soil Science

Kiel University

**Mitigation of carbon dioxide and nitrous oxide emissions from soil: Elucidating
the impacts of nitrification inhibitors, environmental factors, soil characteristics
and biogas residues acidification**

Dissertation

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Chapter 1: General Introduction

1.1 The cycle of nitrogen in soil

Nitrogen (N) is one of the most important nutrients in farming systems (Fixon and West, 2002) for plant growth in agro-ecosystems (Gilsanz et al., 2016), but the effective of applied fertilizer N by crops rarely exceeds 40% (Chen et al., 2008). Nitrogen cycling through the various forms in soil determines the amount of nitrogen available for plants to uptake (Fig. 1). And chemical N fertilizers are the dominant N input in agricultural systems worldwide (Abbasi et al., 2011).

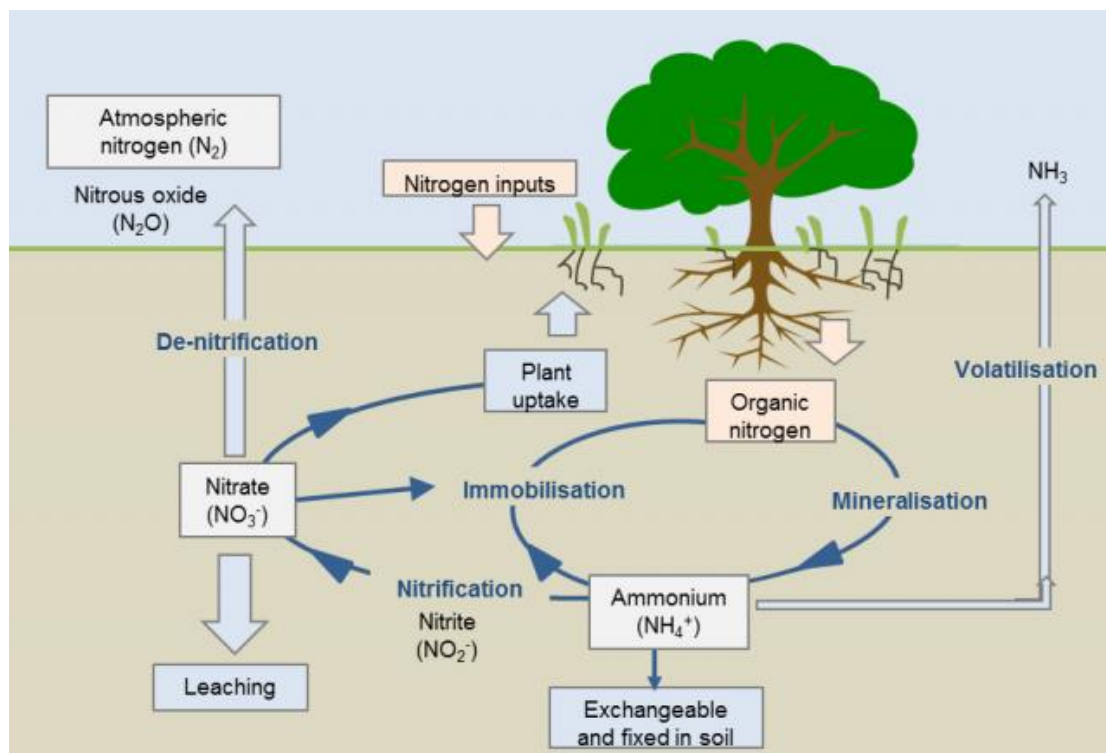


Fig. 1 Nitrogen cycle in soil (Source: <https://www.agric.wa.gov.au/soil-carbon/immobilisation-soil-nitrogen-heavy-stubble-loads>)

The most common chemical N fertilizers applied to soil are in the form of ammonium (NH_4^+) or NH_4^+ -producing compounds such as urea or ammonium sulfate (FAO, 1992). Immobilization of NH_4^+ normally occurs in the earlier stages of incubation and immobilized NH_4^+ is later released as mineral N (Abbasi et al., 2001).

These NH_4^+ fertilizers are oxidized to nitrate (NO_3^-), which is easily leached within a few days of fertilizer application to the field (Abbasi and Adams, 2000; Azam et al., 2002). This causes major losses of N from agricultural activities and represents a major threat to the environment. Half of the total land area of European Union is used for agricultural production, and nutrient losses from agriculture soil is causing a big problem since the impact on water, soil and atmosphere (Carneiro et al., 2010).

1.2 The nitrous oxide emissions from soil

Agricultural land represents 70% of nitrous oxide (N_2O) emissions (Janzen et al., 1998), of which 90% come from soils (Freney, 1997). IPCC (2007) calculated that global agricultural soils produce 2.8 (1.7–4.8) Tg N_2O -N year^{-1} . N_2O is a major greenhouse gas with a long-term global warming potential 300 times greater than that of carbon dioxide (CO_2) (Carneiro et al., 2010). It is also destructive to the ozone layer (IPCC, 2007). N_2O as a by-product of nitrification and denitrification process perform by different microbes (Bremner, 1997; Fig. 2).

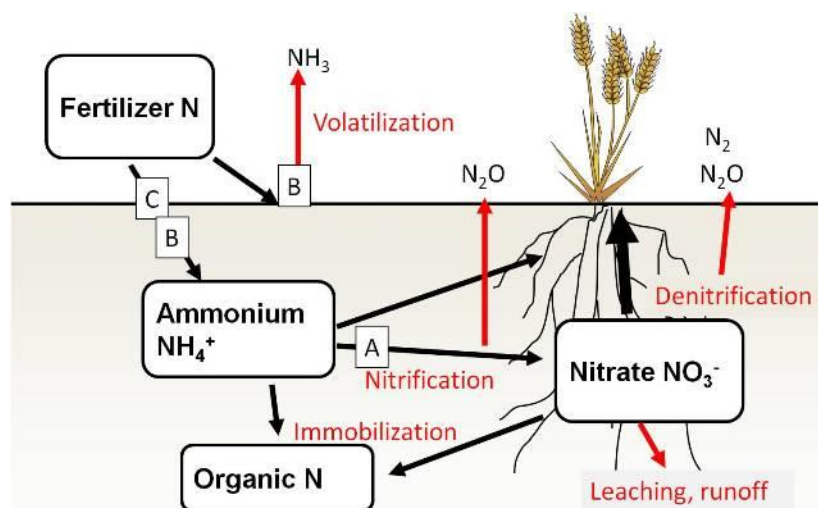


Fig. 2 Overview of soil N-cycle processes showing nitrification and denitrification (A, Nitrification inhibitor; B, Urease inhibitor; C, Controlled release; Source:

<https://www.gov.mb.ca/agriculture/crops/soil-fertility/enhanced-efficiency-additives->

for-nitrogen.html).

Nitrification is the biological oxidation of NH_3 or NH_4^+ to oxidized N in the form of nitrite (NO_2^-) and further to nitrate (NO_3^-) (Butterbach-Bahl et al., 2013; Fig. 2). Denitrification is a microbially facilitated process in which NO_2^- or NO_3^- is reduced and ultimately produces molecular nitrogen (N_2) or an oxide of N through a series of intermediate gaseous nitrogen oxide products (Butterbach-Bahl et al., 2013; Fig. 2).

Plants could take up more available N after nitrification is reduced in soil by nitrification inhibitor or urease inhibitor (Fig. 2), thereby improving the usage efficiency of N fertilizer and reducing NO_3^- -leaching (Subbarao et al., 2006b).

1.3 The inhibition of nitrification inhibitors in soil

Use of nitrification inhibitors (NIs) is one of the strategies to improve effectiveness of nitrogenous fertilizers in agriculture and reduce or minimize environmental threats caused by gaseous emissions and ground water pollution because of nitrification and denitrification and nitrate leaching (Abbasi et al., 2011). NIs could retard biological oxidation of NH_4^+ -N to NO_3^- -N and subsequently reduce N losses and N_2O emissions due to nitrification (McTaggart et al., 1997). NIs inhibit the NH_4^+ -N mono-oxygenase enzyme which is responsible for converting soil NH_4^+ -N to hydroxyl amine which is further oxidized to NO_2^- -N nitrite and then to N_2O (Malla et al., 2005). Use of NIs increases NH_4^+ -N concentration in soil (Pasda et al., 2001; Chaves et al., 2006). NIs reduce subsequent denitrification and N leaching by suppressing autotrophic nitrification, and reduce N_2O produced through this pathway (Lan et al., 2013; McTaggart et al., 1997). Some results (Menéndez et al., 2009; Zaman et al., 2009; Zaman and Blennerhassett, 2010) have shown positive effects on N dynamics and a reduction in trace gas emissions after the application of NIs from soil. Most NIs retard

microbial oxidation of NH_4^+ by depressing the activities of nitrifiers in soil (Wolf et al., 2014). NH_4^+ -N concentrations decreased and NO_3^- -N concentrations increased with incubation time. NIs application slowed down the variations of both NH_4^+ -N and NO_3^- -N (Ting et al., 2018). NIs reduced nitrification and N_2O production by inhibiting the growth of Ammonia-oxidizing (AOB) rather than Ammonia-oxidizing archaea (AOA) (Ting et al., 2018).

DCD has been studied for long times (Edmeades, 2004), and its effect in reducing both NO_3^- leaching and N_2O emission has been reported (Di and Cameron, 2012; Lan et al., 2013) by change the general microbial community composition (Patra et al., 2006). DCD decreased microbial populations and activity (Carneiro et al., 2010). DCD is able to inhibit nitrification up to 50 days under favorable conditions (Chaves et al., 2006) and 20–30 days (Vallejo et al., 2005) and at least 6 months, if the temperature did not exceed 15 °C (Guiraud and Marol, 1992).

1.4 The influence of soil conditions on nitrous oxide emissions and the efficacy of nitrification inhibitors

1.4.1 The influence of soil moisture and temperature on nitrous oxide emissions and the efficacy of nitrification inhibitors

There is a positive effect of temperature on soil nitrification activity (Ting et al., 2018) since temperature positively affects the ammonia oxidizers activity (Gubry-Rangin et al., 2017). Soil bacterial ammonia oxidizer communities may change along with the changes in temperature (Avrahami et al., 2011; Gubry-Rangin et al., 2017) and the substrate affinity or capacity to access substrates elicited by varying microbial activities were affected by temperature (Wallenstein and Hall, 2012). According to

Gubry-Rangin et al. (2017), temperature influences AOA and AOB community compositions in most soils.

Soil moisture content can affect N_2O emissions from soil and the contribution of nitrification and denitrification to N_2O emissions (Di et al., 2014; Fig. 3). Nitrifying microbes convert ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-), and can produce N_2O by nitrification under soil aerobic conditions (Wrage et al., 2001). Denitrifying microbes reduce NO_3^- to NO_2^- , nitric oxide (NO), N_2O , and N_2 by denitrification under anaerobic conditions (Mosier et al., 1998).

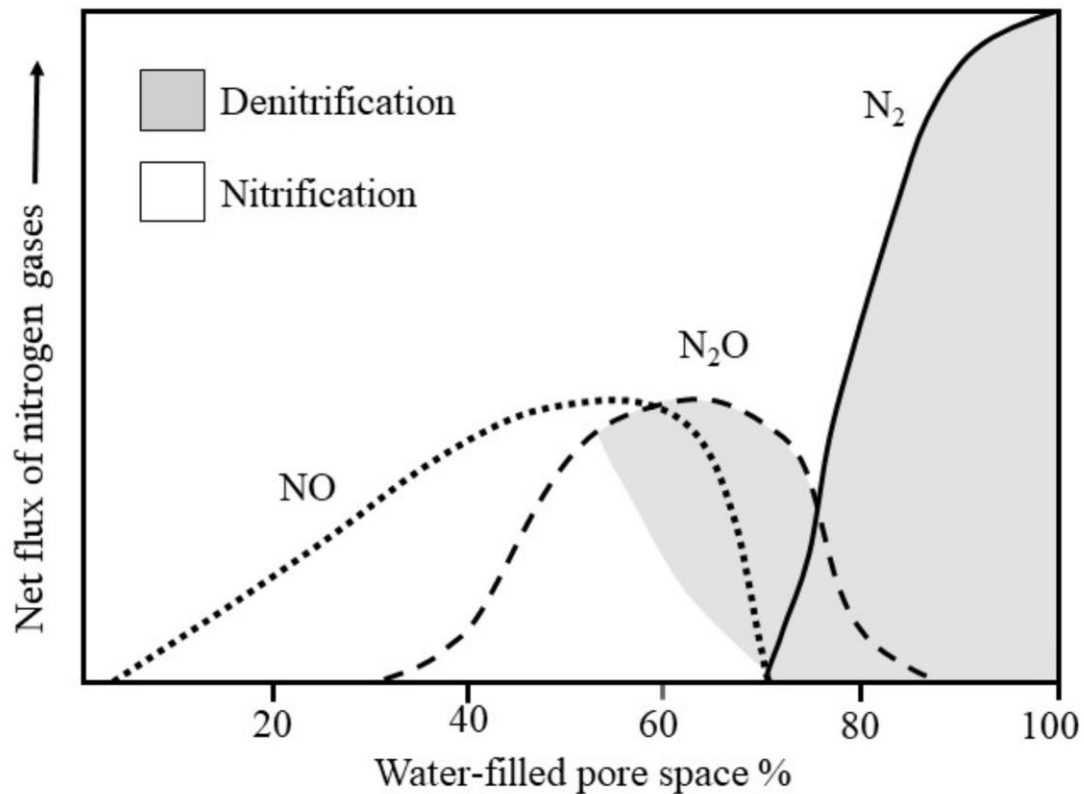


Fig.3 Relative contributions of nitrification and denitrification processes to N_2O production as a function of water-filled pore space (Congreves et al., 2019).

The inhibitory effects of NIs were temperature-dependent (Ting et al., 2018). DCD were efficient inhibitors (Puttanna et al., 1999). All NIs could reduce N_2O production rate but to different extents (Ting et al., 2018). AOB significantly decreased after NIs

application but to different extents (Shi et al., 2016; Lan et al., 2018).

1.4.2 The influence of soil chloride on nitrous oxide emissions

Apart from synthetic NIs, many chemicals can inhibit nitrification under soil and laboratory conditions (Souri, 2010). Salinity-induced retardation of nitrification has been commonly observed (Sethi et al., 1993). The inhibition of nitrification by salts results in accumulation of $\text{NH}_4^+\text{-N}$ and increased availability of N to plants (Azam and Ifzal, 2006).

Recent findings of Megda et al. (2014) demonstrated that Cl^- could inhibit soil nitrification and act as a potential biocide. Several studies under laboratory and field conditions showed that Cl^- , even at low concentrations, have the potential to inhibit soil nitrification (Yuan et al., 2007; Souri, 2010; Chowdhury et al., 2011).

All soils normally have some amount of Cl^- , which nitrifying bacteria are adapted to (Souri, 2010). This could be a critical level above which the activity of these bacteria may be affected severely. Therefore, in various soils, nitrifiers may respond differently to Cl^- concentrations (Souri, 2010). Chloride even in weak concentrations can inactivate ammonia-oxidizing bacteria in several hours, and with a stronger concentration it inactivates all ammonium-oxidizing bacteria just in results 30 min (McGuire et al., 1999). Kandeler (1993) said that chlorate could inhibit nitrite oxidation to nitrate at a very weak concentration. High Cl^- contents in the soil also lead to a reduction of microbial nitrification (Golden et al., 1981). The lowest specific activities of ammonia and nitrite oxidizers were measured at the highest NaCl concentration (Moussa et al., 2006). Results proposed by Hynes and Knowles (1983) proved that the oxidations of both $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ were inhibited in the presence of 10 mM chloride (Xu et al., 2011).

1.4.3 The influence of soil biogas residues and acidified biogas residues on nitrous oxide emissions and the efficacy of nitrification inhibitors

Fermentation processes are a method of organic waste utilization, widely known all over the world. Substrates for methane fermentation include biological waste from agriculture, food industry or urban greenery maintenance, and also sludge from sewage treatment with anaerobic co-fermentation (Quakernack et al., 2012). Meanwhile nearly one third of the total maize growing area in Germany is used for biogas production and maize silage is the most important substrate of biogas production (Wolf et al., 2014). As a result of the process, functioning of agricultural biogas plants is connected with producing large amounts of biogas residues (BR) matter (Koszel and Lorencowicz, 2015; Comparetti et al., 2013). Biogas was produced from renewable resources (organic manures, plant materials, food waste, etc.) and, with reduced dependency on the fossil fuels, expected to have no or even positive effect on the atmospheric greenhouse gas balance (Gopalakrishnan et al., 2009; Herrmann, 2013). Mostly BR are applied to soil as organic fertilizers (Köster et al., 2014). The application of BR as N-fertilizer is an essential component of cropping systems for biogas production and is considered to have a high fertilization value (Möller and Stinner, 2009). BR used as a fertilizer improves soil fertility, plants quality and their immunity to biotic and abiotic agents (Kouřimská et al., 2012). But the use of BR could increase the CO₂ and N₂O emissions and NO₃⁻ leaching when we apply it to soil (Don et al., 2012; Hennig and Gawor, 2012).

A highly effective way to reduce CO₂ and N₂O emissions and NO₃⁻ leaching from BR is to acidification before application and use of nitrification inhibitors (NIs). Acidified biogas residues (ABR) could minimize N₂O emissions, which should be reduced due to the higher NH₄⁺ concentration. NIs could minimize N₂O emissions,

which NIs reduce subsequent denitrification and N leaching by suppressing autotrophic nitrification, and reduce N₂O produced through this pathway (Lan et al., 2013). Mineral N is stabilized in the rather immobile form of NH₄⁺ instead of being transformed to nitrate (NO₃⁻) a highly mobile form which provides a much greater potential for leaching from the rooting zone or gaseous emissions in the form of N₂O (Subbarao et al., 2006a). The use of nitrification inhibitors (NIs) has been proposed to reduce N₂O emissions which contain or form NH₄⁺-N (VanderZaag et al., 2011) and thereby increase the nitrogen use efficiency of fertilizers (Subbarao et al., 2006a).

1.4.4 The influence of soil type on nitrous oxide emissions and the efficacy of nitrification inhibitors

The N₂O emissions is different under different soil type. Abbasi et al. (2011) found that the process of denitrification and production of N₂O be smaller in arable soil deficient in organic matter compared to grassland soil due to less availability of organic C.

Soil characteristics also significantly affect NIs capacity to mitigate N₂O emissions from soil. Butterbach-Bahl et al. (2013) clearly summarized that the production of N₂O in soil was a complex set of processes, and these processes is influenced by different factors such as physico-chemical characteristics of the soil. The effectiveness of NIs to decrease N₂O emissions depends on soil texture (Bronson et al., 1989; Barth et al., 2001; 2008). Same with Volpi et al. (2017) that effectiveness of NIs was strongly influenced by soil type. Kpombrekou-A and Killorn (1996) thought that the effectiveness of NIs was strongly influenced by soil type. DMPP and DCD diminished their efficacy with the addition of soil organic matter (Fisk et al., 2015) and decreased with higher clay content (Marsden et al., 2016). There are numerous field

studies assessing the effect of NIs on N₂O emissions at different locations and under different soil condition and most of them have concluded that the addition of an NIs reduces N₂O emissions (Meijide et al., 2007; Di and Cameron, 2012). Many field studies have tested the effectiveness of NIs to reduce N₂O emissions but results varied considerably because the studies were carried out under different soil conditions (Gilsanz et al., 2016).

NIs delay the nitrification process across all soil textures (sand, loam, and clay) (Barth et al., 2019) and are efficient as NIs in light soils versus heavy soils (Pasda et al., 2001; Guiraud et al., 1992). But Akiyama et al. (2010) found that the effectiveness of NIs was relatively consistent across the various types (Gilsanz et al., 2016). NIs will retain the N in the soil as NH₄⁺ form, thus minimizing the soil NO₃⁻ concentration and thereby reduce the potential for N losses by N₂O emissions or denitrification or NO₃⁻ leaching. Due to the nitrification delay, more fertilizer derived-N can be taken up by the plants, adsorbed or immobilized by the organic or mineral content (Pasda et al., 2001). This could happen in field to improve crop yield (Abalos et al., 2014). It is suggested that NIs binds to the active sites of the copper-containing AMO metalloenzyme required by AOB, and therefore can be used as a tool to reduce NO₃⁻ production and subsequent leaching (Dennis et al., 2012), and N₂O emissions (Di and Cameron, 2003).

1.5 Objectives

The use of nitrification inhibitors of dicyandiamide (DCD), 3, 4-dimethylpyrazole phosphate (DMPP), nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTECH) and active ingredients: 3.00%–3.25% 1, 2, 4-triazole and 1.50%–1.65% 3-methylpyrazole (PIADIN) could decrease the CO₂ and N₂O emissions and

maintain NH_4^+ -N concentrations at a high level and NO_3^- -N concentrations at a low level. But the efficacy of four nitrification inhibitors for the mitigation of nitrous oxide emissions is vary under different soil and environment conditions. The objective of this thesis were to find out the efficacy of four nitrification inhibitors for the mitigation of nitrous oxide emissions under different soil and environment conditions.

1.5.1 The influence of soil moisture and temperature on nitrous oxide emissions and the efficacy of nitrification inhibitors

The aim of this chapter was to (1) determine the effects of soil temperature (15 to 25°C) on soil NH_4^+ and NO_3^- dynamics and CO_2 and N_2O emissions, (2) determine the effects of soil moisture (60% to 80% water-holding capacity (WHC)) on soil NH_4^+ and NO_3^- dynamics and CO_2 and N_2O emissions, and (3) investigate the NIs function on soil CO_2 and N_2O emissions under different combinations of temperature (15 to 25°C) and soil moisture (60% to 80%).

1.5.2 The influence of soil chloride on nitrous oxide emissions

The aim of this chapter was to (1) determine the effects of different Cl^- salts on soil NH_4^+ and NO_3^- dynamics and CO_2 and N_2O emissions, (2) determine the effects of different Cl^- concentrations on soil NH_4^+ and NO_3^- dynamics and CO_2 and N_2O emissions, and (3) compare Cl^- with PIADIN to identify the optimal NI.

1.5.3 The influence of soil biogas residues and acidified biogas residues on nitrous oxide emissions and the efficacy of nitrification inhibitors

The aim of this chapter was to (1) determine the effects of biogas residues on soil NH_4^+ and NO_3^- dynamics as well as CO_2 and N_2O emissions under controlled

environmental conditions (15 °C and 80% WHC); (2) find out the influence of acidified biogas residues on soil NH_4^+ and NO_3^- dynamics as well as CO_2 and N_2O emissions under controlled environmental conditions (15 °C and 80% WHC); (3) investigate the NIs function on soil CO_2 and N_2O emissions after apply biogas residues and acidified biogas residues.

1.5.4 The influence of soil type on nitrous oxide emissions and the efficacy of nitrification inhibitors

The objective of this chapter was to evaluate the variation in soil CO_2 and N_2O emissions, and NH_4^+ -N and NO_3^- -N concentrations following application of DCD, DMPP, PIADIN and ENTEC in soils with three different texture in an incubation pot experiment as a response to: i) the effect of different soil texture on soil N_2O emissions; ii) the behavior of different NIs under different soil texture on soil N_2O emissions.

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Chapter 2: The effect of soil temperature and moisture

Efficacy of four nitrification inhibitors for the mitigation of nitrous oxide emissions
under different soil temperature and moisture

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The highlights of our study are:

- Increased temperature decreased while increased moisture increased N₂O emission
- Efficacies of all NIs improved with increase in soil moisture
- Increasing temperature had differential negative effects on the efficacies of NIs
- DMPP and PIADIN had higher inhibitory effects on N₂O emission at all temperature and moisture combinations

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Running title: Soil temperature and moisture affect efficacy of nitrification inhibitors

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Key words: N₂O emissions; Nitrification; Nitrification inhibitors; Soil moisture; Soil
temperature.

Abstract

Background: Nitrification inhibitors may be used to inhibit nitrous oxide (N₂O) emissions from agricultural soils, but their efficacy could be strongly affected by soil temperature and moisture. **Aims:** This study investigated how different nitrification inhibitors behave in a sandy soil under variable temperature and moisture levels, which currently remains unclear.

Methods: Efficacies of four nitrification inhibitors (dicyandiamide (DCD), 3, 4-dimethylpyrazole phosphate (DMPP), nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) and active ingredients: 3.00%–3.25% 1, 2, 4-triazole and 1.50%–1.65% 3-methylpyrazole (PIADIN)) were investigated in an incubation experiment under two soil temperatures (15 and 25°C) and two moisture levels (60% and 80% water-holding capacity (WHC)) for 60 days in a sandy soil. The soil received 0.5 g NH₄⁺-N kg⁻¹ soil and the inhibitors were applied at 5% of applied N. Total N₂O emissions were calculated based on gas samples collected throughout the incubation period.

Results: Increasing soil temperature from 15 to 25°C decreased N₂O emissions by 73%–191% while increasing soil moisture from 60% to 80% WHC caused a 109%–251% increase in the emissions. ENTEC followed by DCD were the least effective inhibitors to reduce N₂O emissions under all temperature and moisture combinations. Depending upon temperature and soil moisture, DMPP and PIADIN inhibited 85%–100% of N₂O emissions. At 15°C, both DMPP and PIADIN almost completely inhibited N₂O emissions. At 25°C, the efficacy of PIADIN decreased to 88% while that of DMPP ranged from 86% to 98%. Increased soil moisture content improved the efficacy of all the nitrification inhibitors.

Conclusions: Overall, DMPP and PIADIN were more effective than DCD and ENTEC

in a sandy soil under a constant soil moisture and temperature. DMPP is the most effective inhibitor, completely inhibiting N₂O emissions at 15–25°C and up to 80% WHC in this soil and is therefore recommended for use in agricultural land.

Key words: N₂O emissions; Nitrification; Nitrification inhibitors; Soil moisture; Soil temperature.

1 Introduction

Nitrous oxide (N₂O) is a major greenhouse gas emitted to the atmosphere from soils, which has a 300 times stronger global warming potential than that of carbon dioxide (CO₂) (*Carneiro et al.*, 2010). In addition, N₂O also indirectly contributes to stratospheric ozone depletion. Worldwide emissions of N₂O increased by 9% from 1998 to 2010, with agricultural soils contributing more than 70% of the total global emissions (*Carneiro et al.*, 2010). Nitrification of ammonium (NH₄⁺) by microbes plays a key role in N₂O production (*Ting et al.*, 2018). The nitrate (NO₃⁻) resulting from nitrification later undergoes denitrification and N₂O is produced as an intermediate product. Under low-oxygen environments with a sufficient amount of NO₃⁻ and metabolizable organic carbon (C), considerable quantities of N₂O can be released to the environment. Ammonia-oxidizing archaea (AOA) and bacteria (AOB) are involved in the nitrification of NH₄⁺ to NO₃⁻ in soil (*Francis et al.*, 2005).

Nitrification inhibitors (NIs) can be used to reduce emissions of N₂O from soils and thereby improve N fertilizer use efficiency (*Carneiro et al.*, 2010; *Ruser and Schulz*, 2015; *Nair et al.*, 2020). These NIs decrease the nitrification rate (*Barth et al.*, 2019) by blocking NH₃ oxidization, which is the initial and rate-limiting step of autotrophic nitrification (*Di and Cameron*, 2012). The use of NIs has been shown to effectively reduce N₂O emissions and NO₃⁻ leaching following application of mineral fertilizers to

soil (*Meijide et al.*, 2007). *Akiyama et al.* (2010) found a 31%–44% reduction in N₂O emissions following the application of different NIs to grasslands and arable fields alongside different N fertilizers. Based upon these results, it could be concluded that use of NIs has considerable mitigation potential for reducing N₂O emissions.

It has been shown that the effect of NIs in reducing the nitrification rate can be strongly affected by environmental factors such as temperature and soil moisture (*Ting et al.*, 2018). However, there are contradictory reports regarding the effect of these factors on N₂O emissions and efficiency of NIs. *Puttanna et al.* (1999) reported that the efficacy of NIs decreased from 62% to 6% with an increase in temperature from 10 to 30°C during a 30-day incubation study. Conversely, *Menéndez et al.* (2012) found that an increase in temperature from 10 to 20°C increased N₂O emission reduction efficiency of 3, 4-dimethylpyrazole phosphate (DMPP) from 17% to 42%.

The effect of NIs is also influenced by the soil properties and soil parameters (*Barth et al.*, 2001). *Barth et al.* (2008) found that DMPP was most effective in a sandy loam soil, particularly under conditions of higher soil moisture. Therefore, in this research, we only investigated a very sandy soil. However, we will also conduct research on the influence of soil type on the effect of NIs in the future.

Soil moisture has been reported to increase N₂O emissions by influencing NH₃ oxidizing and denitrifying microbial communities, with the functional genes increasing with high soil moisture (*Di et al.*, 2014). The effects of different NIs on N₂O production under different moisture conditions remain unclear (*Ting et al.*, 2018). In four-year multi-location trials in Germany, it was found that emission of N₂O was stimulated under higher soil moisture conditions, and the PIADIN (active ingredients: 3.00%–3.25% 1, 2, 4-triazole and 1.50%–1.65% 3-methylpyrazole) was ineffective to control the emission under these circumstances (*Marcus Rohwer*, pers. communication). In

contrast, *Puttanna* et al. (1999) reported that benzotriazole was equally effective under soil moisture conditions ranging from 40% to 80% of the water-holding capacity (WHC) of the soil. Although several studies have considered the individual effects of different temperatures and moisture levels on the efficacy of NIs, to the best of our knowledge, the interactive effect of the temperature, moisture and four NIs has not been studied yet.

In this study, therefore, we conducted an incubation experiment to elucidate the effects of different combinations of temperature and soil moisture levels on the efficacy of four NIs to reduce N₂O emissions and affect the dynamics of NH₄⁺ and NO₃⁻ in an N-fertilized sandy soil. We hypothesized that (1) high temperature will lead to a reduction in the efficacy of NIs; (2) high soil moisture will also lead to a reduction in the efficacy of NIs; and (3) DMPP is the most effective inhibitor under all the temperature and moisture combinations in our sandy soil. The findings of this research could help us to improve the use of soil fertilizer and reduce emission of N₂O from agricultural sandy soils.

2 Materials and methods

2.1 Collection, preparation and characterization of soil

The upper 0–20 cm layer of a sandy soil was collected from Grevenkrug, Schleswig-Holstein, Germany (54°11'09.1"N, 10°00'36.6"E). Visible plant residues and stones were carefully removed. The soil was passed through a 2-mm sieve and homogenously mixed. A representative subsample of the experimental soil was analyzed for particle size distribution (sand and silt), pH, total C and N contents, NH₄⁺-N and NO₃⁻-N concentrations, and bulk density following standard methods (Table 1).

((Table 1))

2.2 Incubation experiment

Four NIs viz. dicyandiamide (DCD, $C_2H_4N_4$), 3, 4-dimethylpyrazole phosphate (DMPP, $C_5H_{11}N_2O_4P$), ENTEC (nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer: 7.5 % NO_3^- -N, 18.5 % NH_4^+ -N and 13.0 % sulphur, 0.15% DMPP) and PIADIN (active ingredients: 3.00%–3.25% 1, 2, 4-triazole and 1.50%–1.65% 3-methylpyrazole) were tested under all possible combinations of two soil temperatures (15 and 25°C) and two soil moisture levels (60% and 80% of WHC). For comparison, a control without NI addition was also included under each combination of moisture and temperature. Thus, the following four temperature and soil moisture combinations were tested for four NIs and a control (without NIs):

- (1) 15°C with 60% WHC: Control; DCD, DMPP, ENTEC and PIADIN
- (2) 15°C with 80% WHC: Control; DCD, DMPP, ENTEC and PIADIN
- (3) 25°C with 60% WHC: Control; DCD, DMPP, ENTEC and PIADIN
- (4) 25°C with 80% WHC: Control; DCD, DMPP, ENTEC and PIADIN

Each treatment combination had four replicates, giving 80 experimental units (pots) in total.

The soil was packed into cylindrical pots (15 cm diameter and 33 cm length, the bottom of each pot was completely sealed) to achieve a bulk density of 1.4 g cm^{-3} with 20 cm depth. All pots were fertilized with $0.5 \text{ g NH}_4^+\text{-N kg}^{-1}$ soil using ammonium sulfate ($(NH_4)_2SO_4$) salt. The NIs were applied at 5% of applied $NH_4^+\text{-N}$ (i.e. 25 mg kg^{-1} soil). The application rate of nitrification inhibitors that we used in our study is the same as that used by most of the farmers. The required quantities of both the N fertilizer and NIs were dissolved in 100 ml deionized water. To achieve an even distribution in the soil, N fertilizer plus NIs solution was applied through a small perforated tube,

which was inserted in the center of the soil pot. This allowed the solution to uniformly diffuse in the soil and reach throughout the pot. Following this, deionized water was added into the tube in order to maintain the soil moisture levels to 60% or 80% of the WHC for the respective moisture treatments. The control treatment under each combination of temperature and moisture was applied with fertilizer and deionized water only. Deionized water was added each day to maintain the soil WHC. Following treatment application, pots were incubated for a period of 57 days inside an incubation chamber (controlled temperature) under the specified conditions.

2.3 Collection and measurement of emitted N₂O

The N₂O samples were collected once a day during the first 7 days, once after two days during the second 7 days and once after three days during the last days of the incubation period. Air-tight lids were placed on top of the pots. Each lid had one rubber membrane, which was the contact between the sample collecting syringe and the incubated environment. The pots were closed first and then gas samples were collected every 20 min for an hour (0, 20, 40, and 60 min). A 10-ml syringe, placed in pre-evacuated 2-ml headspace glass vials, was used to collect the gas samples using a hypodermic needle. The glass vials had a chloro-butyl rubber septum. Gas samplings were carried out between 09:00 and 11:00 am. Except for the times when samples were taken, the pots were left open. The concentration of N₂O in the gas samples was measured by gas chromatography (Agilent 7890A GC, Agilent, CA, USA). Total N₂O emission during the experimental period was calculated from the daily emitted amount of gas. The rate of N₂O emission from each pot was calculated from the total amount of N₂O emitted and the duration of lid closure, using a linear relation between the N₂O concentration and time.

2.4 Analysis of soil NH_4^+ and NO_3^-

To determine the dynamics of NH_4^+ -N and NO_3^- -N in the soil, 50 g soil samples were collected carefully from 0–20 cm depth on Days 1, 15, 29, 43 and 57 of incubation without disturbing the soil. Each soil sample was divided into two subsamples; the first was oven-dried at 105°C for 8 h to calculate the water content while the other was used for determination of NH_4^+ -N and NO_3^- -N. For this purpose, 10 g of each fresh soil sample was mixed with 40 mL of 0.0125 M CaCl_2 solution (1:4) and shaken for 1 h. The suspensions so obtained were centrifuged for 10 min and the extracts were then filtered through Whatman filter paper No. 40. The NH_4^+ and NO_3^- concentrations in the filtered extracts were determined using a continuous flow autoanalyzer (San++ Automated Wet Chemistry Analyzer - Continuous Flow Analyzer (CFA), Skalar, The Netherlands). The autoanalyzer determines the concentrations of NH_4^+ -N and total mineral nitrogen (NH_4^+ -N and NO_3^- -N) in the soil extract separately, and NO_3^- -N is obtained by subtracting NH_4^+ from the total mineral N.

2.5 Statistical analysis

Data were verified for normal distribution and the treatment means for total N_2O emission were compared using three-way analysis of variance. The NH_4^+ -N and NO_3^- -N concentrations in the soil were compared using one-way analysis of variance. Differences between individual treatments were determined using Tukey's honest significant difference test. The statistical analyses were performed by R statistical software (R Core Team 2013; Oakland, CA, USA) at a confidence level of 95% ($P \leq 0.05$).

3 Results

3.1 Rate of N₂O emission

The rate of N₂O emission was affected by temperature, moisture and applied NIs (Fig. 1). Without NIs application, the N₂O emissions were significantly higher during the first 20 days of the incubation period for all combinations of temperature and moisture levels. Irrespective of soil moisture content, the peak rate of N₂O emission in the control soil occurred during Day 22 of incubation at 15°C and earlier in beginning of Day 15 at 25°C, and thereafter it declined progressively. In NI-treated soils, a range of different N₂O emission rate trends were recorded. For example, in soils that received DMPP and PIADIN, the rate of N₂O emission throughout the incubation period was negligible at 15°C and both WHCs, whereas the rate of emission was slightly higher at 25°C than at 15°C, especially for PIADIN-treated soil at 80% WHC. Overall, DMPP and PIADIN maintained the lowest N₂O emission rate throughout the incubation period. ENTEC behaved similar to the control, whereby a peak emission rate occurred during Day 15 or 22 of incubation and thereafter emissions declined. This peak was reached faster at 25°C than at 15°C. In general, except for DCD and ENTEC at 25°C and 60% WHC, the NIs decreased the N₂O emission rate.

((Figure 1))

3.2 Total N₂O emissions

Without NI application, total emission of N₂O during a period of 60 days at 15°C was 191% and 73% higher than at 25°C under 60% and 80% WHC, respectively (Table 2). In contrast, compared with 60% WHC, total emission of N₂O at 80% WHC was 109% and 251% higher at 15 and 25°C, respectively. The effect of the NIs was dependent on soil moisture as well as incubation temperature. At 15°C under 60% WHC, all the NIs

decreased N₂O emission, with DMPP and PIADIN almost completely inhibiting N₂O emission, whereas DCD and ENTEC reduced it by 56% and 25%, respectively. At 15°C under 80% WHC, the NIs exhibited almost the same response, in which both DMPP and PIADIN decreased N₂O emission by 98%, whereas DCD and ENTEC decreased it by 85% and 43%, respectively. At 25°C and 60% WHC, DCD and ENTEC increased N₂O emission by 32% and 102%, respectively, whereas DMPP and PIADIN decreased it by 85% and 87%, respectively. At 25°C and 80% WHC, the NIs decreased N₂O emission, the decreased N₂O emission results show that DMPP (98%) and PIADIN (88%) were more effective than DCD (35%) and ENTEC (21%) in a sandy soil at 25°C and 80%.

((Table 2))

3.3 Soil NH₄⁺-N and NO₃⁻-N concentrations

In the control treatment, soil NH₄⁺-N concentration decreased over time, whereas that of NO₃⁻-N increased (Fig. 2, 3, 4, 5). However, the typical crossover of the trend lines for the two N forms occurred earlier at 25°C than at 15°C. Within the tested combinations of temperatures and moisture contents, the range of NH₄⁺-N at 57 incubation days was 0.1–2 mg kg⁻¹ soil, whereas that of NO₃⁻-N was 9.6–86 mg kg⁻¹ soil in the control treatment. The concentration of NO₃⁻-N on Day 57 of the control treatment was less at 80% WHC than at 60% WHC. Compared with the control, higher NH₄⁺-N concentrations were recorded in treatments with NIs application (Fig. 2, 3, 4, 5). In soils treated with DMPP and PIADIN, NH₄⁺-N remained fairly stable throughout the course of the experiment at low temperature (15°C). In contrast, at 25°C, the decline in NH₄⁺-N concentration was recorded but it was very slow compared with the control

treatment. The NO_3^- -N concentration was not significantly affected by NI addition. However, DCD- and ENTEC-treated soils showed a comparatively fast decline in NH_4^+ -N concentration and emission of NO_3^- -N at the fast increase rate (Fig. 2, 3, 4, 5). All added NIs were least effective in sustaining NH_4^+ -N in soil at 25°C and 80% WHC.

((Figure 2))

((Figure 3))

((Figure 4))

((Figure 5))

4 Discussion

This study investigated the efficacy of four NIs (DMPP, DCD, ENTEC and PIADIN) to reduce N_2O emissions from an N-fertilized soil incubated under different combinations of temperature and soil moisture levels in a sandy soil. Our result showed that total N_2O emission decreased with the increase in temperature, whereas it increased with the increase in soil moisture level (Table 2) in the control treatment. However, the peak N_2O emission rate was achieved earlier at 25°C than at 15°C, which suggests that the nitrification rate was higher at higher temperature (Fig. 1). Accordingly, compared with that at 15°C, NH_4^+ -N concentration declined and NO_3^- -N concentrations increased more rapidly in the control treatment at 25°C (Fig. 3, 5). The temperature-induced increase in nitrification rate, as found in this study, is in accordance with earlier findings reporting a promoting effect of temperature on nitrification in soil (Ting et al., 2018). Sahrawat (2008) reported that the nitrification rate was generally higher with an

optimum temperature ranging from 25 to 35°C. This may be attributed to a positive effect of temperature on the activity of ammonia oxidizers (*Gubry-Rangin et al., 2017*). Despite the higher nitrification rate at the higher temperature (25°C), total N₂O emissions were higher at the lower temperature (15°C) (Table 2). This may be explained by the higher shift of denitrified NO₃⁻ toward formation of nitrogen gas (N₂) at the higher temperature.

N₂O emission was higher at 80% WHC than at 60% WHC at both the incubation temperatures under the control treatment (Table 2). Accordingly, NO₃⁻-N concentration at the end of the experiment under 80% soil WHC was significantly lower than those under 60% soil WHC (Fig. 3 and 5) in the control. Similar results were found by *Senbayram et al. (2009)*, who reported that N₂O emissions were higher at 85% than at 65% WHC. Denitrification dominates N₂O emissions from soils with a high moisture content (*Diba et al., 2011*) and subsequently N₂O emissions usually increase under wet environments as the soils become more anaerobic (*Dobbie and Smith, 2001*). Denitrifying enzymes are produced under anaerobic conditions and their activities are inhibited when the oxygen concentration in the soil is high (*van Spanning et al., 2007*). *Zhu et al. (2013)* also found that growth of AOB communities (*Di et al., 2014*) responsible for producing denitrifying enzymes was promoted with increasing soil moisture content or decreasing oxygen concentration.

At lower temperature (15°C), all the NIs reduced N₂O emissions, DMPP and PIADIN were able to almost completely inhibit N₂O emissions at both moisture levels (Table 2). At lower temperature (15°C), the efficacy of DCD and ENTEC increased by 29% and 19%, respectively, with an increase in soil moisture from 60% to 80% WHC. Similarly, at 25°C, the efficacy of all the NIs increased with an increase in moisture content. DMPP completely inhibited N₂O emissions with the same efficacy at

25°C and 15°C at 80% WHC (Table 2). The efficacy of DCD, ENTEC and PIADIN was lower at 25°C compared with that at 15°C (Table 2). The present study showed that the efficacy of all the NIs was negatively affected by increasing temperature from 15 to 25°C, but the magnitude of the effect differed among the NIs. DCD and ENTEC completely lost their nitrification inhibition ability at 25°C and 60% WHC, and also showed a higher decline in activity at 80% WHC. *Menéndez et al. (2012)* and *Di et al. (2014)* also found a decline in N₂O emission inhibition efficacy of DMPP and DCD with increasing soil temperature. The variation in the effect of temperature on the efficacy of NIs may be attributed to different sensitivity of NIs to temperature-dependent degradation and decomposition by microorganisms. At higher temperatures, previous studies have reported hastened degradation of NIs in soil (*Di et al., 2014; Menéndez et al., 2012; Puttanna et al., 1999*) and accelerated microbiological degradation, with the maximum values reached at 25-33°C.

This is the novelty of the present to report that DMPP followed by PIADIN had the higher inhibition efficiency, whereby they sustained or maintained higher NH₄⁺-N concentrations and kept NO₃⁻-N concentrations at a low level at both studied temperatures and moisture levels (Fig. 2-5). These results also imply that DMPP followed by PIADIN were resistant to temperature dependent degradation and microbial decomposition. *Barth et al. (2019)* found that NIs have a better performance in reducing NO₃⁻-N formation in sandy soils compared with that of the loam and clay textured soils, which was supported by our results. *Ting et al. (2018)* reported that DMPP generally reduced N₂O emission more than DCD and 3MP + TZ at 15 and 35°C, whereas DCD performed better than DMPP and 3-methylpyrazol and 1H-1,2,4-triazol (3MP + TZ) at 25°C. When temperature does not exceed 15°C, DCD has been reported to inhibit nitrification to a variable extent and for period ranging from 20 days to at least

6 months (*Chaves et al.*, 2006; *Vallejo et al.*, 2005; *Guiraud and Marol*, 1992). Above this temperature, its efficacy has been shown to decrease substantially. Similar to the results obtained in our study, *Weiske et al.* (2001) observed a larger inhibition in N_2O emissions under field conditions after DMPP application than after DCD application. It could thus be concluded that irrespective of soil moisture and temperature, DMPP is the most effective NI for use in controlling N_2O emissions from agricultural sandy soils. Similarly, *Barth et al.* (2008) found that DMPP inhibited the oxidation of NH_4^+ in two soils, but this effect was more pronounced in a sandy loam than in a loamy soil. Most NIs retard microbial oxidation of NH_4^+ by depressing the activities of nitrifiers in soil (*Wolf et al.*, 2014) through deactivating the ammonia monooxygenase enzyme (*Di et al.*, 2010). Hence, NI application slows down the conversion of NH_4^+ to NO_3^- (*Ting et al.*, 2018) and prevents against loss via leaching or emission as N_2O (*Kirschke et al.*, 2019). In our research, we did not investigate the AOA and AOB, which play an important role during the soil nitrification and denitrification. Therefore, future studies should focus on the microbial changes with incubation time under different temperature and moisture levels with application of the four NIs.

5 Conclusion

The findings of this study reveal that N_2O emission is decreased with the increase in temperature, whereas they increased with the increase in soil moisture level without nitrification inhibitors. DMPP and PIADIN were more effective than DCD and ENTEC for nitrification inhibition in a sandy soil at both 15 and 25°C as well as 60% and 80% WHC. The efficacies of all the NIs improved with the increase in soil moisture to 80% WHC, whereas increasing temperature had various negative effects in our sandy soil. The NIs could be used to maintain the soil fertilizer by maintaining $\text{NH}_4^+\text{-N}$

concentration at a high level and NO_3^- -N concentration at a low level at both 15 and 25°C as well as 60% and 80% WHC. Atmospheric pollution and underground water pollution could thus be reduced by decreasing the N_2O emissions and NO_3^- -N leaching, respectively.

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Figures and Tables**Table 1:** Basic characteristics of the soil used in this experiment.

Silt	Sand	pH	Total carbon	Total N	NH ₄ ⁺ -N	NO ₃ ⁻ -N	Bulk density
(%)	(%)	(CaCl ₂)	(g kg ⁻¹)	(g kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)	(g cm ⁻³)
5	95	6.5	11.6	0.8	2.16	4.80	1.40

Table 2: Total emission of N₂O from soils treated with nitrification inhibitors DCD, DMPP, ENTEC and PIADIN, and incubated under different combinations of two soil moisture levels and temperatures for 57 days.

Moisture level	Treatment	N ₂ O-N emission (mg kg ⁻¹ soil 60 day ⁻¹)	
		15°C	25°C
60% WHC	Control	1.31 ± 0.08f	0.45 ± 0.03d
	DCD	0.59 ± 0.07 (−55.5)d	0.60 ± 0.11 (+31.8)d
	DMPP	0.00 ± 0.00 (−99.7)a	0.07 ± 0.02 (−85.2)b
	ENTEC	0.99 ± 0.08 (−24.5)e	0.92 ± 0.05 (+101.9)e
	PIADIN	0.01 ± 0.00 (−99.4)a	0.06 ± 0.02 (−86.9)b
80% WHC	Control	2.74 ± 0.05g	1.58 ± 0.02f
	DCD	0.42 ± 0.03 (−84.6)d	1.02 ± 0.02 (−35.4)e
	DMPP	0.05 ± 0.00 (−98.3)b	0.03 ± 0.00 (−98.2)a
	ENTEC	1.56 ± 0.11 (−43.1)f	1.26 ± 0.04 (−20.5)f
	PIADIN	0.06 ± 0.00 (−97.6)b	0.19 ± 0.01 (−88.2)c

Values (±SE) are means of four independent pot replicates. The values in parenthesis represent the percentage increase (+) or decrease (−) in N₂O emissions with nitrification inhibitors over their respective controls. WHC stands for water-holding capacity. Means indicated by the same lowercase letter are not significantly different at the $P \leq 0.05$ level according to the Tukey honest significant difference test.

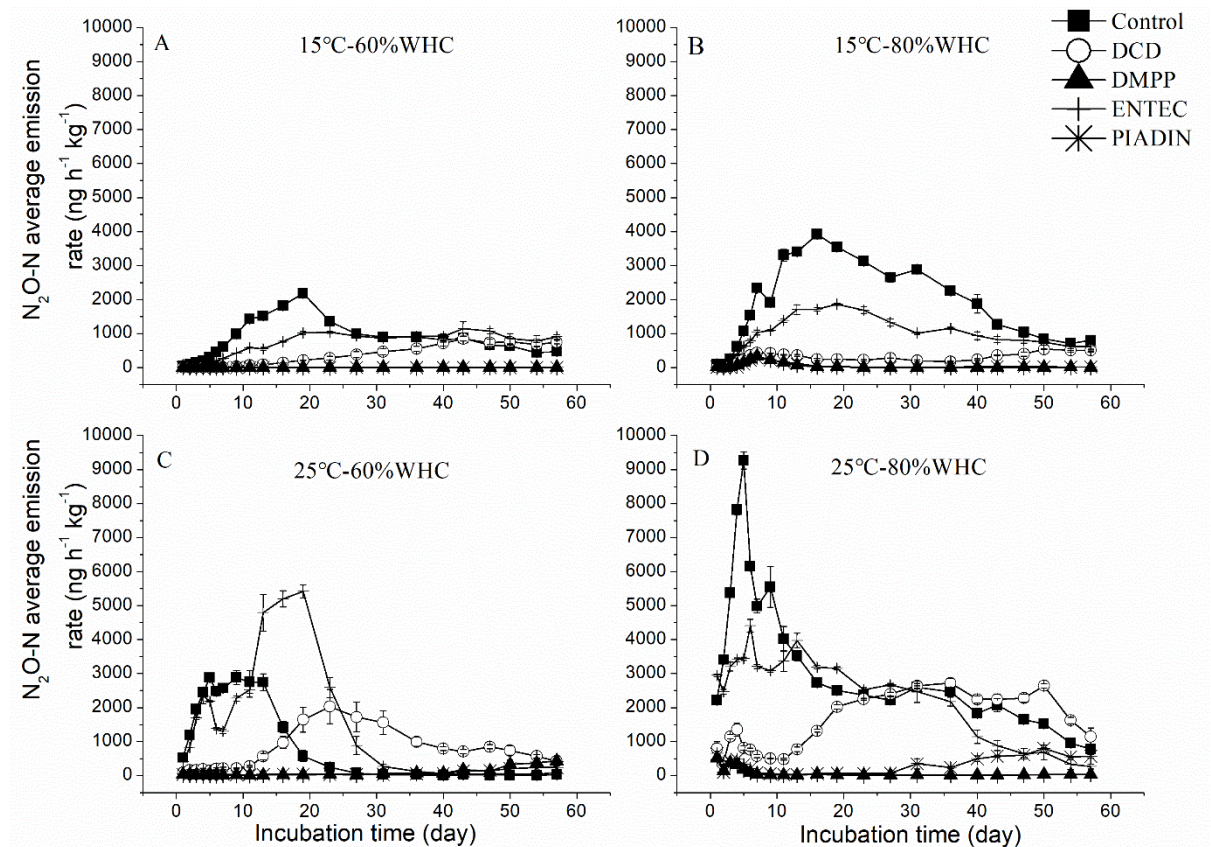


Figure 1: Rate of N_2O emissions as affected by DCD, DMPP, ENTEC and PIADIN under all possible combinations of two moisture levels (60% and 80% water-holding capacity (WHC)) and two incubation temperatures (15 and 25°C): A) 15°C and 60% WHC, B) 15°C and 80% WHC, C) 25°C and 60% WHC and D) 25°C and 80% WHC. The data values are means of four independent pot replicates and error bars represent the standard error of the mean ($n = 4$).

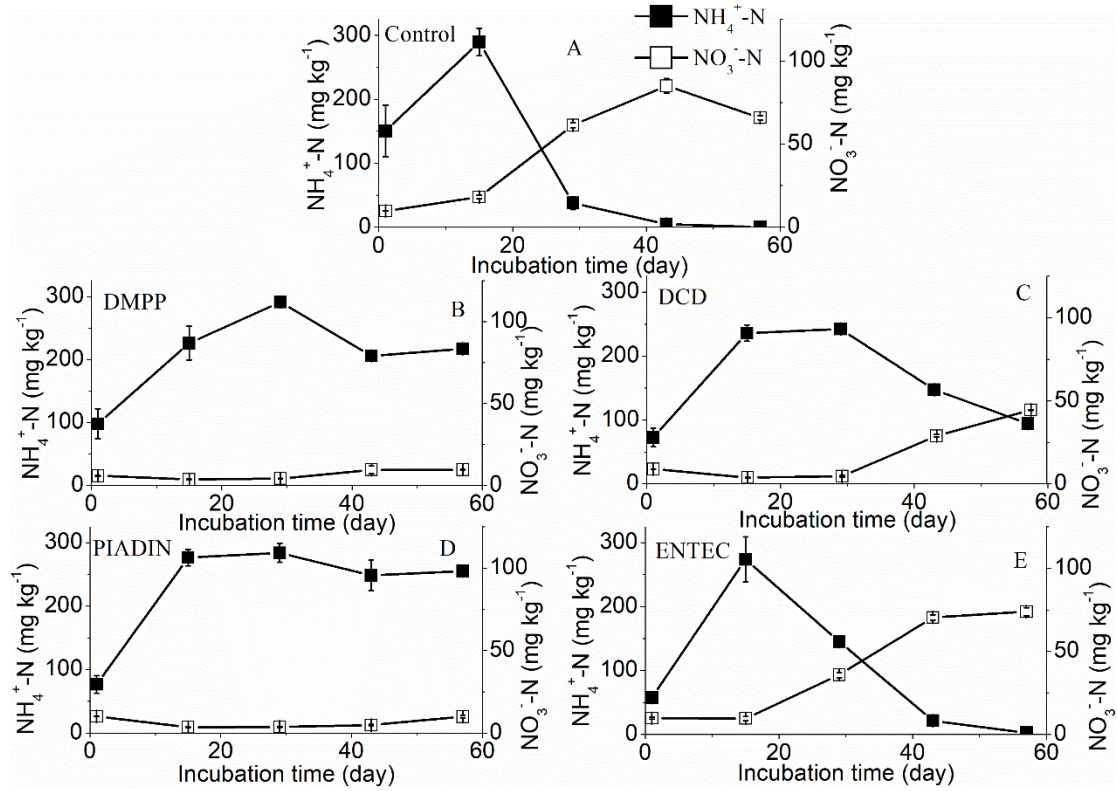


Figure 2: Changes in the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in soils treated with Control (A) and nitrification inhibitors DMPP (B), DCD (C), PIADIN (D) and ENTEC (E), and incubated at 15°C and 60% water-holding capacity for 57 days. The data values are the mean of four independent pot replicates and the error bars represent the standard error.

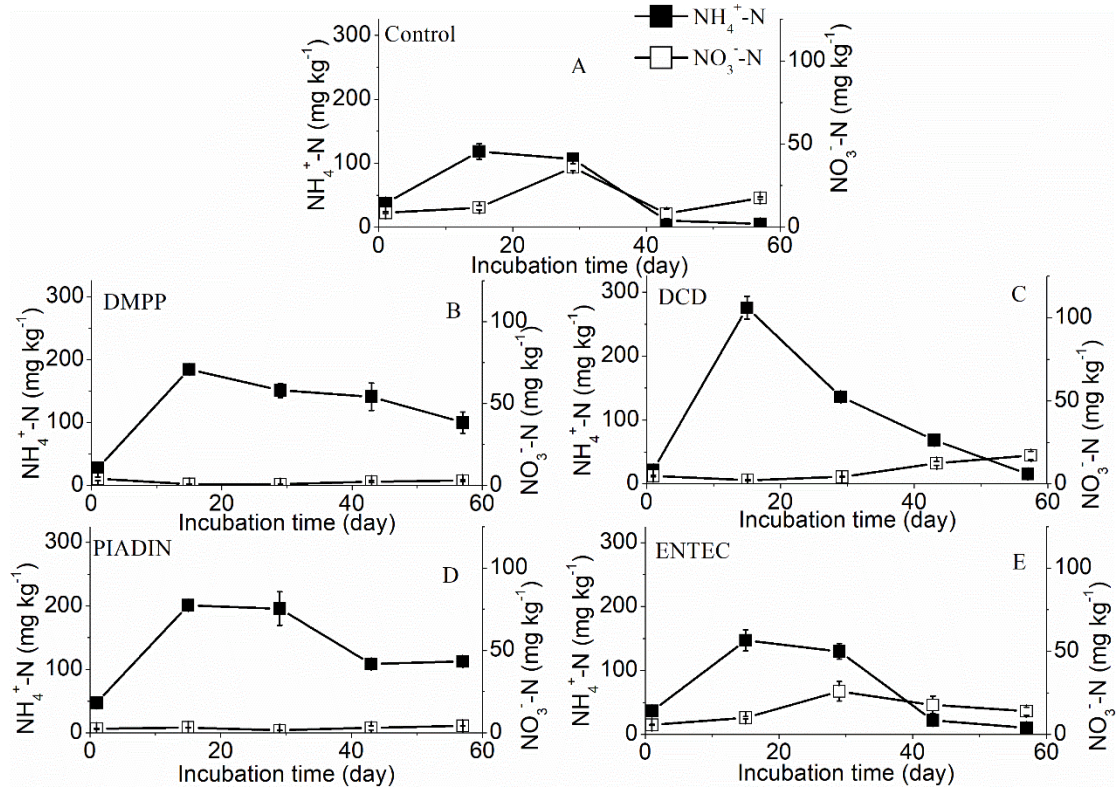


Figure 3: Changes in the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in soils treated with Control (A) and nitrification inhibitors DMPP (B), DCD (C), PIADIN (D) and ENTEC (E), and incubated at 15°C and 80% water-holding capacity for 57 days. The data values are the mean of four independent pot replicates and the error bars represent the standard error.

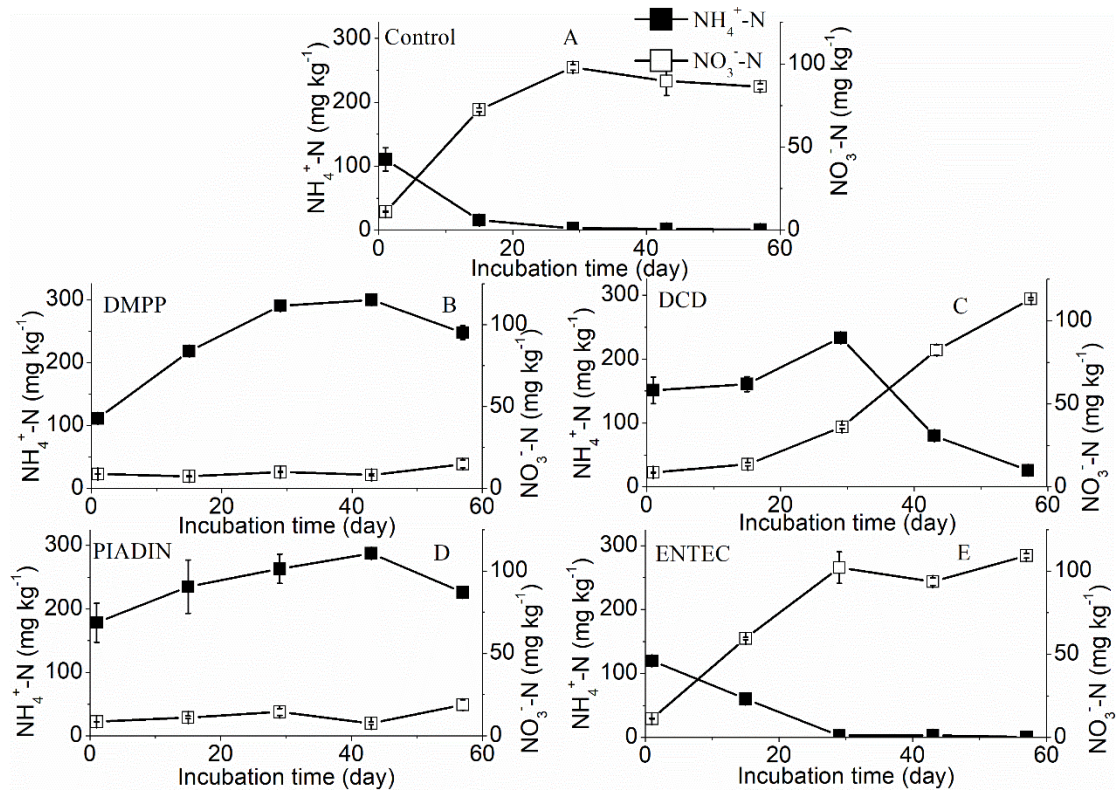


Figure 4: Changes in the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in soils treated with Control (A) and nitrification inhibitors DMPP (B), DCD (C), PIADIN (D) and ENTEC (E), and incubated at 25°C and 60% water-holding capacity for 57 days. The data values are the mean of four independent pot replicates and the error bars represent the standard error.

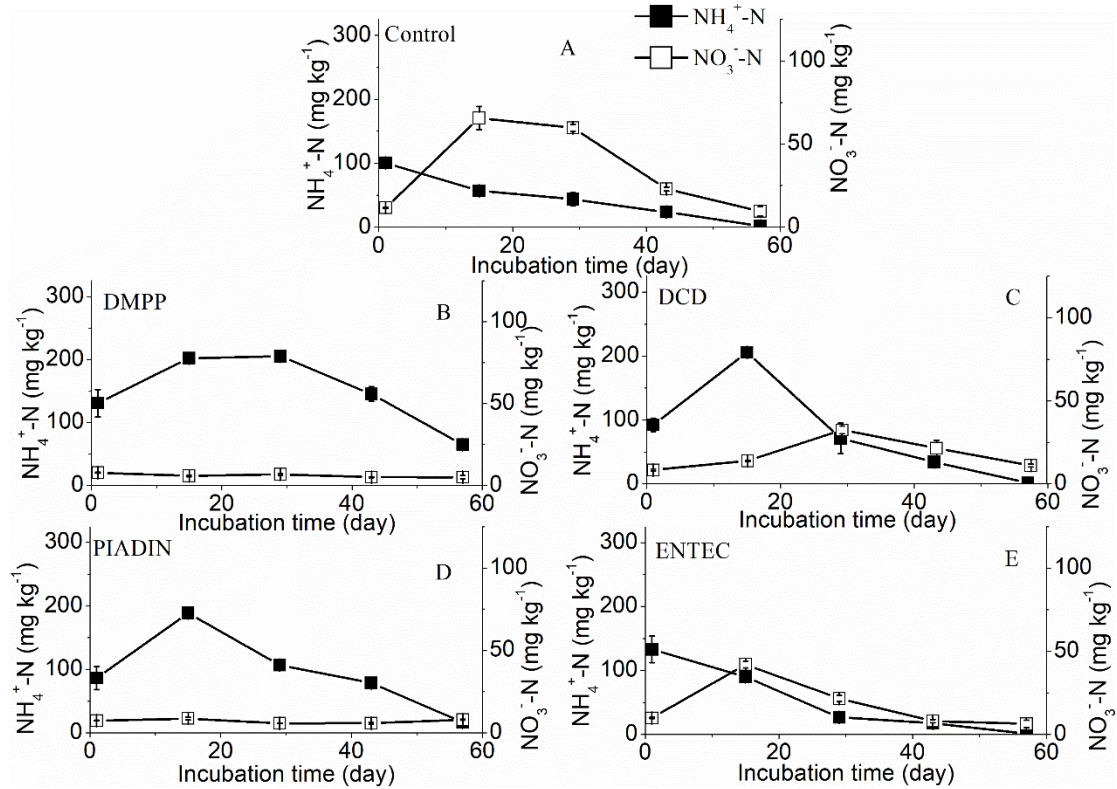


Figure 5: Changes in the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in soils treated with Control (A) and nitrification inhibitors DMPP (B), DCD (C), PIADIN (D) and ENTEC (E), and incubated at 25°C and 80% water-holding capacity for 57 days. The data values are the mean of four independent pot replicates and the error bars represent the standard error.

Chapter 3: The effect of chloride

Impact of different chloride salts and their concentrations on nitrification and trace gas emissions from a sandy soil under a controlled environment

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The highlights of our study are:

- KCl decreases CO₂ emission and increases N₂O emission.
- MgCl₂ decreases CO₂ emission without influencing N₂O.
- KCl and MgCl₂ retard the decrease of NH₄⁺ and the increase of NO₃⁻.
- The mitigation potential of potassium and magnesium salts is lower compared to PIADIN.

Impact of different chloride salts and their concentrations on nitrification and trace gas emissions from a sandy soil under a controlled environment

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Running Title: Impact of Cl⁻ on nitrification and gas emissions

Abstract

Potassium chloride (KCl) and Magnesium chloride (MgCl_2) can be used to reduce carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions, but their efficacy can be strongly affected by their Cl^- concentration. This study aimed to compare the behavior of different Cl^- salts and concentrations with that of a typical commercial nitrification inhibitor (3, 4-dimethylpyrazole phosphate, PIADIN). KCl, MgCl_2 and PIADIN were investigated under a laboratory incubation experiment for two months. KCl and MgCl_2 were applied at 0.5 and 1.0 g kg^{-1} , while PIADIN was applied at 25 mg kg^{-1} soil. CO_2 and N_2O concentrations were analyzed during the incubation period. The NH_4^+ and NO_3^- dynamics in soil were also measured. The results showed 0.5 and 1.0 g kg^{-1} KCl and 0.5 g kg^{-1} MgCl_2 decreased $\text{CO}_2\text{-C}$ emissions by 43%–46% and increased $\text{N}_2\text{O-N}$ emissions by 15%–48%, whereas 1.0 g kg^{-1} MgCl_2 decreased $\text{CO}_2\text{-C}$ emissions by 72% and $\text{N}_2\text{O-N}$ emissions by 19%. KCl and MgCl_2 retarded the decrease of the $\text{NH}_4^+\text{-N}$ concentration and increase of the $\text{NO}_3^-\text{-N}$ concentration. PIADIN reduced the emissions of $\text{CO}_2\text{-C}$ by 113% and $\text{N}_2\text{O-N}$ by 97%, and maintained a high soil $\text{NH}_4^+\text{-N}$ concentration and low $\text{NO}_3^-\text{-N}$ concentration. MgCl_2 addition at 1.0 g kg^{-1} was an effective treatment as the Mg both fertilized the soil and inhibited $\text{CO}_2\text{-C}$ and $\text{N}_2\text{O-N}$ emissions. Moreover, 1.0 g kg^{-1} MgCl_2 could retard soil nitrification, the decrease of $\text{NH}_4^+\text{-N}$ concentration and the increase of $\text{NO}_3^-\text{-N}$ concentration. While PIADIN had no fertilizing value, it was a more effective nitrification inhibitor than Cl^- salts.

Keywords:

Nitrification inhibitor, KCl, MgCl_2 , nitrification, N_2O emissions

1. Introduction

Nitrogen (N) is one of the most important nutrients in farming systems (Fixon and West, 2002) and chemical N fertilizers are the dominant N input in agricultural systems worldwide (Abbasi et al., 2011). The most common chemical N fertilizers applied to soil are in the form of ammonium (NH_4^+) or NH_4^+ -producing compounds such as urea or ammonium sulfate (FAO, 1992). Immobilization of NH_4^+ normally occurs in the earlier stages of incubation and immobilized NH_4^+ is later released as mineral N (Abbasi et al., 2001). These NH_4^+ fertilizers are oxidized to nitrate (NO_3^-), which is easily leached within a few days of fertilizer application to the field (Abbasi and Adams, 2000a; Azam et al., 2002). This causes major losses of N from agricultural activities and represents a major threat to the environment. Agricultural land represents 90% of ammonia (NH_3) emissions (Boyer et al., 2002) and 70% of nitrous oxide (N_2O) emissions (Janzen et al., 1998), of which 90% come from soils (Freney, 1997). IPCC (2007) calculated that global agricultural soils produce 2.8 (1.7–4.8) Tg $\text{N}_2\text{O-N}$ year⁻¹. N_2O is a major greenhouse gas with a long-term global warming potential 300 times greater than that of carbon dioxide (CO_2) (Carneiro et al., 2010). It is also destructive to the ozone layer (IPCC, 2007).

Nitrification is the biological oxidation of NH_3 or NH_4^+ to oxidized N in the form of nitrite (NO_2^-) and further to nitrate (NO_3^-) (Butterbach-Bahl et al., 2013). Denitrification is a microbially facilitated process in which NO_2^- or NO_3^- is reduced and ultimately produces molecular nitrogen (N_2) or an oxide of N through a series of intermediate gaseous nitrogen oxide products (Butterbach-Bahl et al., 2013).

Nitrification, which involves oxidation of NH_4^+ from soil N fertilizers, is an important source of atmospheric pollution (Souri, 2010). Ammonia-oxidizing bacteria play a vital role in global N cycling by oxidizing NH_3 or NH_4^+ to NO_2^- in a two-step

process; the NH_3 monooxygenase enzyme oxidizes NH_3 or NH_4^+ to hydroxylamine, which is then oxidized to NO_2^- by the hydroxylamine oxidoreductase enzyme (Radniecki et al., 2008). Ammonia-oxidizing bacteria are generally considered to be the most sensitive microbes to environmental factors and chemical compounds in the nitrification process, being readily inhibited by these factors (Radniecki et al., 2008). Restricting nitrification (stabilizing ammonium) for a limited time in soil systems may help to improve the use efficiency of N fertilizer and enable plants to take up more available N (Subbarao et al., 2006b) and reduce associated N_2O emissions and NO_3^- leaching (Subbarao et al., 2006a).

The use of nitrification inhibitors (NIs) is one of the most common practices to inhibit nitrification (Abbasi et al., 2003; Di and Cameron, 2005; Zaman et al., 2009). Soil amendment with NIs is a strategy to improve the effectiveness of nitrogenous fertilizers and reduce or minimize the environmental threats caused by gaseous emissions from nitrification and denitrification and groundwater pollution from nitrate leaching (Abbasi et al., 2011). NIs are intended to retard biological oxidation of NH_4^+ -N to NO_3^- -N and subsequently reduce N losses and N_2O emissions through nitrification (McTaggart et al., 1997; Serna et al., 2000; Pereira et al., 2013). There are many commercially available NIs such as dicyandiamide (DCD), 3, 4-dimethylpyrazole phosphate (DMPP), and nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) and active ingredients: 3.00%–3.25% 1, 2, 4-triazole and 1.50%–1.65% 3-methylpyrazole (PIADIN). NIs have been widely used in agriculture to improve fertilizer efficiency (Hu et al., 2015) and minimize nitrification and denitrification and reduce losses of NO_3^- via leaching by maintaining the applied fertilizer in the soil as NH_4^+ -N (McTaggart et al., 1997). Several studies have shown positive effects of NI application on N dynamics, with a reduction in trace gas emissions

from the soil after their application (Menéndez et al., 2009; Zaman et al., 2008, 2009; Zaman and Blennerhassett, 2010). Many chemicals and salts in the soil also influence the microorganisms involved in the process of nitrification (Souri, 2010). Observations have indicated that under saline conditions, nitrification may be retarded or even completely inhibited (Laura, 1977; Pathak and Rao, 1998; Westerman and Tucker, 1974). The inhibition of nitrification by salts results in accumulation of $\text{NH}_4^+\text{-N}$ and increased availability of N to plants (Azam and Ifzal, 2006). Chlorine (Cl) is a widespread salt component. Rudolph et al. (1995) found that CO_2 emission rates were reduced by Cl. Azam and Müller (2003) found that NaCl significantly decreased CO_2 emissions and MgCl_2 is involved in the synthesis of nesquehonite through reaction with CO_2 (Ferrini et al., 2009), which decreases CO_2 emissions.

Cl^- ions have a strong oxidant action and are a potent biocide with the capacity to greatly reduce the microbial population (Wong et al., 1988; Chen and Wong, 2004). In addition to commercial NIs, Cl^- can significantly inhibit microbial nitrification in soil (Souri, 2010) and act as a potential biocide (Megda et al., 2014). For example, Cl^- has been found to reduce the nitrification rate under laboratory and field conditions with a concentration of 7–50 mM (Golden et al., 1981; Wickramasinghe et al., 1985; Darrah et al., 1987; Wade, 1997; McGuire et al., 1999; Chen and Wong, 2004; Yuan et al., 2007; Chowdhury et al., 2011). Abbasi et al. (2011) showed that addition of the NI CaCl_2 resulted in a decrease in the concentration of NH_4^+ of 30%.

Soil pH, organic matter, NH_4^+ content and soil moisture can all influence the establishment of ammonium-oxidizing bacteria, leading to differences in the inhibitory effects of chemicals such as PIADIN or Cl^- (Souri, 2010). This inhibition was positively correlated with applied Cl^- concentrations in soil (Souri, 2010). The Cl^- inhibitory effect was a function of the applied concentration, for which greater

concentrations were more effective (Souri, 2010). It would be useful if amendment with KCl and MgCl₂ could decrease soil CO₂ and N₂O emissions to a level that is comparable with commercial NIs since KCl and MgCl₂ are often used to fertilize soil. Therefore, it is important to investigate the effect of different Cl⁻ salts (KCl and MgCl₂) under different Cl concentrations (0.5 g kg⁻¹ and 1.0 g kg⁻¹) on soil NH₄⁺ and NO₃⁻ dynamics and CO₂ and N₂O emissions compared with PIADIN.

The aim of this study was to (1) determine the effects of different Cl⁻ salts on soil NH₄⁺ and NO₃⁻ dynamics and CO₂ and N₂O emissions, (2) determine the effects of different Cl⁻ concentrations on soil NH₄⁺ and NO₃⁻ dynamics and CO₂ and N₂O emissions, and (3) compare Cl⁻ with PIADIN to identify the optimal NI. We hypothesized that (1) different Cl⁻ salts have varying ability to reduce the CO₂ and N₂O emissions, (2) Cl⁻ concentration can also affect the CO₂ and N₂O emissions, and (3) Cl⁻ is useful to reduce the CO₂ and N₂O emissions compare with PIADIN. These findings may help us to decrease CO₂ and N₂O pollution by applying salts as both soil fertilizer and an NI, without the need for additional chemicals.

2. Materials and methods

2.1 Collection, preparation and characterization of soil

A sandy soil from 0–20 cm soil depth was collected from Grevenkrug (54°11'09.1"N 10°00'36.6"E), Schleswig-Holstein, Germany. The sample site is a natural grassland. Visible plant residues and stones were carefully removed from the soil sample, which was then passed through a 2-mm sieve and homogenized. Four replicate subsamples of the experimental soil were analyzed for the following initial soil properties following standard methods: particle size distribution (sand and silt), pH,

total carbon (C) and N, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and bulk density (Table 1).

2.2 Incubation experiment

The experiment consisted of three NIs: KCl, MgCl_2 and PIADIN. Both of the Cl^- salts were applied at two rates, 0.5 and 1.0 g kg^{-1} soil, while PIADIN was applied at 25 mg kg^{-1} soil (Cl^- is a fertilizer for the soil and PIADIN is a typical commercial nitrification inhibitor). The PIADIN concentration that we used is the same as that used by some farmers. The soil was incubated under controlled conditions at a constant temperature of 15°C, soil moisture of 80% soil water-holding capacity and an air relative humidity of 50%. Deionized water was added daily to maintain the soil water-holding capacity. This constitutes the following six treatments: PIADIN, 25 mg kg^{-1} soil (PIADIN); KCl-Cl, 0.5 g kg^{-1} soil (0.5KCl); KCl-Cl, 1.0 g kg^{-1} soil (1.0KCl); $\text{MgCl}_2\text{-Cl}$, 0.5 g kg^{-1} soil (0.5 MgCl_2); $\text{MgCl}_2\text{-Cl}$, 1.0 g kg^{-1} soil (1.0 MgCl_2); and an untreated control. Each treatment had four replicates.

Cylindrical pots (15 cm diameter and 33 cm length) were filled with 5.48 kg soil consolidated to achieve a bulk density of 1.4 g cm^{-3} with 20 cm depth. All pots were fertilized with 0.5g $\text{NH}_4^+\text{-N kg}^{-1}$ soil using ammonium sulfate salt ($(\text{NH}_4)_2\text{SO}_4$) dissolved in 100 ml of deionized water. All NIs (KCl, MgCl_2 and PIADIN) were also dissolved in 100 ml of deionized water. The control soil was treated with 100 ml deionized water only. Following treatment application, pots were incubated for a period of eight weeks at the specified conditions in an incubation chamber.

2.3 Collection and measurement of emitted CO_2 and N_2O

The CO_2 and N_2O emissions were calculated by measuring the concentration of these gases in the headspace of closed pots at different times. The CO_2 and N_2O samples

were collected once a day during the first week, once per two days during the second week and one sampling per three days during the last six weeks. The pots were first closed by placing an airtight cover and gases were mixed and then samples were collected from the headspace every 20 min for one hour (i.e. at 0, 20, 40, and 60 min). A 10-ml syringe and hypodermic needle was used to collect the gas samples and inject them into pre-evacuated 2-ml glass headspace vials fitted with a chloro-butyl rubber septum (Chromacol). Gas sampling was carried out between 09:00 and 11:00 am. The pots were left open outside of the sampling periods.

The concentrations of CO₂ and N₂O in the gas samples were measured by gas chromatography (Agilent 7890A GC, Agilent, CA, United States). An electron capture detector (ECD) was used to detect the N₂O, and the ECD temperature was set at 300°C with an N₂ carrier gas. A thermal conductivity detector (TCD) was used to detect the CO₂ concentration, with a TCD temperature of 250°C and a He carrier gas. The CO₂ and N₂O concentrations per minute from each pot (ppm min⁻¹) during lid closure were calculated using headspace volume and a linear relation between the CO₂ and N₂O concentration and time (Venterea et al., 2020). The flux of CO₂-C (μg h⁻¹ kg⁻¹) and N₂O-N (ng h⁻¹ kg⁻¹) was calculated with the following equations:

$$EN_2O-N = \frac{R \times 60 \times V_{gas} \times AR}{W_{soil} \times V_m} \times 2 \times 1000 \quad (1)$$

$$ECO_2-C = \frac{R \times 60 \times V_{gas} \times AR}{W_{soil} \times V_m} \quad (2)$$

where ECO₂-C and EN₂O-N are the CO₂-C (μg h⁻¹ kg⁻¹) and N₂O-N (ng h⁻¹ kg⁻¹) fluxes, respectively; *R* represents the CO₂ or N₂O emissions from each pot (ppm/min); *V*_{gas} is the gas volume in the pot (L); *W*_{soil} is the weight of dry soil in pot (Kg); *AR* is the relative atomic mass, which is 12 for C and 14 for N; and *V*_m is the molar volume of gas, which is 23.7 L/mol at 15°C.

Total CO₂ and N₂O emissions during the experimental period were calculated from

the daily emissions of the gases. We used the fluxes of CO₂-C ($\mu\text{g h}^{-1} \text{kg}^{-1}$) and N₂O-N ($\text{ng h}^{-1} \text{kg}^{-1}$) multiplied by the number of hours during sampling (24 h in the first week, 48 h in the second week and 72 h in the last six weeks) and added all of them together to obtain the total emissions (mg kg^{-1} or $\mu\text{g kg}^{-1}$).

2.4 Analysis of NH₄⁺ and NO₃⁻ in soil

Soil samples to 20 cm depth were collected with a 2-cm-diameter soil sampler at random locations in each pot on Days 1, 15, 29, 43 and 57 of the incubation for the measurement of NH₄⁺ and NO₃⁻ concentrations.

Each soil sample was divided into two subsamples; one was oven-dried at 105°C for 8 h to calculate the soil water content, while the other was used for the determination of NH₄⁺ and NO₃⁻ concentrations. For the analysis of soil mineral N, 10 g of fresh soil were mixed with 40 mL of 0.0125 M CaCl₂ solution (1:4) and shaken for 1 h. After centrifugation for 10 min at 500g, the extracts were filtered through a No. 40 Whatman filter paper and stored at 4°C. The extracts were analyzed for NH₄⁺ and NO₃⁻ concentrations using a continuous flow analyzer (San⁺⁺ Automated Wet Chemistry Analyzer–Continuous Flow Analyzer, Skalar, The Netherlands).

2.5 Statistical analysis

We used one way and Repeated Measures Analysis of Variance (ANOVA) to test the effects of incubation time on soil CO₂-C and N₂O-N flux. Treatment means for total CO₂ and N₂O emissions were compared using one-way analysis of variance. Incubation time means for NH₄⁺-N and NO₃⁻-N concentrations were compared using one-way analysis of variance. Differences between individual treatments and incubation time were determined using a Tukey's honest significant difference (HSD) test. The

statistical analyses were performed by R statistical software (Oakland, CA, USA) at a confidence level of 95% ($P \leq 0.05$).

Multivariate analyses indicate which element is most important. We observed clear differences in the changes after 22 days in Figure 1. Therefore, we conducted multivariate analyses between 0–22 days and 23–57 days to quantify that difference and identify which element was the key controlling factor in each case. Multivariate analyses were performed using the R statistical language (RStudio, Version 1.3.1056, 250 Northern Ave, Boston, MA 02210). The variance decomposition of N₂O emissions was computed using the “VARPART” function of the “vegan” library for R (Oksanen and O’Hara, 2005).

3. Results

3.1 Fluxes of CO₂-C and N₂O-N

The flux of CO₂-C and N₂O-N first significantly increased and then significantly decreased in the control and the KCl and MgCl₂ treatments (Table 3 and Fig. 1). The maximum fluxes occurred after 25 days and 15 days of incubation for CO₂-C and N₂O-N in the control. PIADIN addition significantly maintained the CO₂-C and N₂O-N flux ($P > 0.05$) at a low level around 0 throughout the incubation period (Fig. 1).

0.5KCl, 1.0KCl, 0.5MgCl₂ and 1.0MgCl₂ had smaller CO₂-C fluxes compared with the control before 40 days (Fig. 1) but had greater CO₂-C fluxes than the control after 40 days.

0.5KCl and 1.0KCl had the same N₂O-N flux as the control before 20 days (Fig. 1) and had greater N₂O-N flux than the control after 20 days. 0.5MgCl₂ and 1.0MgCl₂ had a smaller N₂O-N flux compared with the control before 25 days, whereas 0.5MgCl₂

and 1.0MgCl₂ had greater N₂O-N fluxes compared with the control after 25 days.

3.2 Total emissions of CO₂-C and N₂O-N

The greatest CO₂-C emission (248.3 mg kg⁻¹) was from the control, while the smallest (32.8 mg kg⁻¹) was from PIADIN. PIADIN, 0.5KCl, 1.0KCl, 0.5MgCl₂ and 1.0MgCl₂ significantly decreased the CO₂-C emissions compared with the control (Table 2). PIADIN had the highest decrease in CO₂-C emissions, while 0.5KCl, 1.0KCl and 0.5MgCl₂ had the lowest decrease. 1.0MgCl₂ had an intermediate decrease compared with the control.

The greatest N₂O-N emission (1999 µg kg⁻¹) was from the 0.5KCl, while the smallest (36.4 µg kg⁻¹) was from PIADIN. PIADIN significantly decreased N₂O-N emissions compared with the control. 0.5KCl and 1.0KCl significantly increased N₂O-N emissions (Table 2) compared with the control. 0.5MgCl₂ and 1.0MgCl₂ showed no significant influence on N₂O-N emissions; however, 0.5MgCl₂ had a tendency to increase N₂O-N emissions and 1.0MgCl₂ had a tendency to decrease N₂O-N emissions compared with the control.

3.3 Soil NH₄⁺-N and NO₃⁻-N concentrations

In the control, NH₄⁺-N concentration rapidly decreased in the first 30 days and then remained constant at a low concentration afterwards (P<0.05). The NO₃⁻-N concentration rapidly increased in the first 30 days and then slightly decreased after 30 days (P<0.05; Fig. 2). Under 0.5KCl, 1.0KCl, 0.5MgCl₂ and 1.0MgCl₂, the NH₄⁺-N concentration decreased and NO₃⁻-N concentration increased over the whole incubation period (P<0.05). However, the decrease in the NH₄⁺-N concentration and the increase in the NO₃⁻-N concentration was slower in all of the Cl⁻ salt treatments (0.5KCl,

1.0KCl, 0.5MgCl₂ and 1.0MgCl₂) than the control (Fig. 2). The intersection points of NH₄⁺-N concentration and NO₃⁻-N concentration occurred at 15 days under control, 20 days under 0.5KCl and 0.5MgCl₂, and 32 days under 1.0KCl and 1.0MgCl₂ (Fig. 2). Under PIADIN, the NH₄⁺-N concentration high over the first 40 days and then slightly decreased, and NO₃⁻-N concentration remained at a low concentration over the whole incubation period (P>0.5). There was no intersection of NH₄⁺-N and NO₃⁻-N concentrations under PIADIN.

3.4 Attribution of N₂O emissions to NH₄⁺-N and NO₃⁻-N

The variance decomposition of NH₄⁺, NO₃⁻ and their shared fraction for the first 22 days, and from 23 to 57 days are shown in Fig. 3a and 3b, respectively. NH₄⁺, NO₃⁻ and their shared fraction explained 1%, 35% and 43% of the N₂O emissions in 0–22 days, respectively (Fig. 3a). There was no shared fraction between NH₄⁺ and NO₃⁻ from 23 to 57 days. NH₄⁺ and NO₃⁻ variables explained 4% and 10% of the N₂O emissions from 23 to 57 days (Fig. 3b).

4. Discussion

4.1 The effect of different Cl⁻ salts

We found that both KCl and MgCl₂ treatments decreased soil respiration, and therefore 0.5KCl/MgCl₂ and 1.0KCl/MgCl₂ had smaller CO₂-C flux compared with the control in the first 40 days of incubation (Fig. 1), and the total CO₂-C emissions was decreased (Table 2). Azam and Müller (2003) also found that NaCl significantly decreased CO₂ emissions.

The combination of nitrification and denitrification played a key role in the N₂O

emissions, as indicated by the shared fraction among the NH_4^+ and NO_3^- explaining most of the N_2O emissions between 0 and 22 days (Fig. 3a). Nitrification was dominant in the soil at the beginning of incubation (before 22 days) since the level of NH_4^+ -N in the soil was relatively high. Denitrification was shown to play a key role in the N_2O emissions between 23–57 days as NO_3^- variables explained most of the N_2O emissions in this period (Fig. 3b).

Different Cl^- salts have varying functions on soil nitrification and denitrification. The KCl treatments had a similar N_2O -N flux to the control at the beginning of the incubation, and KCl also slowed down the decrease of NH_4^+ -N concentration and the increase of NO_3^- -N concentration (Fig. 2), providing clear evidence that KCl can retard nitrification. Similarly, Belser and Mays (1980) demonstrated that KClO_3 had no effect on the activity of ammonia-oxidizing bacteria. Pereira et al. (2013) also found that AlCl_3 treatment had no effect on nitrification, but significantly reduced cumulative NH_3 emissions. In contrast, Agrawal et al. (1985), Souri (2010) and Megda et al. (2014) found that KCl can reduce the nitrification rate in the soil. It has long been recognized that Cl^- ions can suppress nitrification (Golden et al., 1981; Darrah et al., 1987; McGuire et al., 1999). In addition, Cl^- and its different derivatives can act as strong oxidizers and potent biocides (Chen and Wong, 2004). The mechanism of Cl^- nitrification inhibition is associated with increased solute concentration and osmotic pressure in the soil solution (Golden et al. 1981; Darrah et al., 1987; Souri, 2010). However, in the current study, KCl had a greater N_2O -N flux than the control after 20 days (Fig. 1), and the total N_2O -N emission was greater under the two rates of the KCl treatment (Table 2). KCl treatment also resulted in the low NO_3^- -N concentration at the end of the incubation (Fig. 2). Denitrification is the main mechanism of N loss occurring after nitrification of NH_4^+ (Abbasi and Adams, 2000a, b) and the emissions

of N_2O mainly originate from the denitrification process (Wrage et al., 2001; Kool et al., 2011). Xu et al. (2011) found that the chlorate selectively inhibits the activity of nitrite-oxidizing bacteria but not ammonia-oxidizing bacteria, causing nitrites to become the dominant product. Therefore, KCl -induced denitrification led to a greater N_2O -N flux and reduced soil NO_3^- -N concentration at the end of the incubation in our research. Lees and Simpson (1957) and Xu et al. (2011) showed that KClO_3 completely blocked the oxidation of NO_2^- . Azam and Müller (2003) found soil salinity inhibits denitrification mainly by restricting the oxidative processes and therefore the development of anaerobic microsites.

At both addition rates, MgCl_2 had a smaller N_2O -N flux than the control in the first 25 days; however, it had a larger N_2O -N flux than the control after 25 days (Fig. 1). MgCl_2 had no significant influence on the overall N_2O -N emissions (Table 2), thus, MgCl_2 could decrease nitrification, and increase denitrification. This supports the findings of Sloan and Anderson (1998) that MgCl_2 reduced the nitrification rate under field conditions. Chlorate is toxic to fungi, algae, invertebrates, fish and higher plants (Stauber, 1998; Jiang et al., 2009), but it is easily biodegraded by nitrate reductase in an organic-rich environment (Xu et al., 2011). The decrease in nitrification by MgCl_2 also caused the slowdown in the decrease of NH_4^+ -N concentration and the increase of NO_3^- -N concentration (Fig. 2). Some studies have shown that aluminum addition inhibits nitrification by lowering the soil pH values, which reduces the NH_4^+ availability for nitrification in the short term and directly inhibits the effects of Mg (Smith et al., 2001; Smith et al., 2004; Pereira et al., 2013). MgCl_2 could also inhibit nitrification by decreasing soil pH and reducing the NH_4^+ availability, which directly inhibits the effects of Mg. Previous studies (Smith et al., 2001, 2004; Choi and Moore, 2008) have also shown that the application of AlCl_3 to animal manure can reduce NH_3 emissions by

reducing the manure pH. The results showed that KCl can retard nitrification and increase denitrification, and MgCl_2 could decrease nitrification and increased denitrification. MgCl_2 is more successful compared with KCl for decreasing N_2O -N emissions.

4.2 The effect of Cl^- concentration

The point of intersection of NH_4^+ -N and NO_3^- -N concentrations is affected by the decrease in the rate of NH_4^+ -N and the increase in the rate of NO_3^- -N, and can provide relative information (identify which treatment leads to rapid nitrification) among the treatments when comparing different salts or NIs. However, the intersection cannot be used as an absolute since the position of the intersection is scale dependent. Nonetheless, an early intersection point indicates a fast decrease of NH_4^+ -N and increase of NO_3^- -N, and can reveal that the nitrification occurs rapidly since the same scale was used for all the treatments in our research.

Our findings indicated that different Cl^- concentrations play different roles on soil nitrification and denitrification. We found that 1.0KCl had a smaller N_2O -N flux compared with 0.5KCl before 20 days of incubation (Fig. 1), which delayed the intersection point of NH_4^+ -N concentration and NO_3^- -N concentration (Fig. 2). Megda et al. (2014) found that increased Cl content in the soil inhibited soil nitrification, and we found that an increased KCl application rate retarded soil nitrification. 0.5 MgCl_2 had a larger N_2O -N flux than 1.0 MgCl_2 before 30 days of incubation (Fig. 1) and 1.0 MgCl_2 tended to decrease the total N_2O -N emissions (Table 2). The intersection point of NH_4^+ -N concentration and NO_3^- -N concentration was delayed under 1.0 MgCl_2 (Fig. 2), indicating that 1.0 MgCl_2 had a strong inhibition effect on nitrification. The decrease in soil respiration was greater in 1.0 MgCl_2 than in 0.5 MgCl_2 , and thus the

0.5MgCl₂ had a larger CO₂-C flux than 1.0MgCl₂ before 40 days (Fig. 1) and 1.0MgCl₂ led to a greater decrease in CO₂-C emissions than 0.5MgCl₂ (Table 2). All soils contain some level of Cl, to which nitrifying bacteria have adapted (Souri, 2010); however, there is likely to be a critical level above which the activity of these bacteria may be severely affected. Therefore, nitrifiers may respond differently to the different Cl⁻ concentrations in different soils (Souri, 2010). Pereira et al. (2013) found that increasing the AlCl₃ concentration did not significantly reduce gaseous N emissions. Kandeler (1993) found that chlorate at a weak concentration could inhibit the oxidation of NO₂⁻ to NO₃⁻. Chlorite, even in weak concentrations, can inactivate ammonia-oxidizing bacteria within several hours, and at a stronger concentration it inactivates all ammonium-oxidizing bacteria in just 30 min (McGuire et al., 1999). However, increasing the amount of Cl in soil decreased the NO₃⁻-N concentration (Megda et al., 2014; Souri, 2010). High Cl⁻ contents in the soil also lead to a reduction of microbial nitrification (Golden et al., 1981). Moussa et al. (2006) observed the lowest specific activities of ammonia and nitrite oxidizers at the highest NaCl concentration. Hynes and Knowles (1983) showed that the oxidation of both NH₄⁺-N and NO₂⁻-N was inhibited in the presence of 10 mM chlorate (Xu et al., 2011). Monaghan and Barraclough (1992) found that at greater NaCl concentrations, there was a significant accumulation of NH₄⁺, suggesting an inhibition of nitrification. The difference between 0.5 and 1.0 g MgCl₂ kg⁻¹ soil concentration in our experiment was mostly caused by the corresponding decrease in soil pH, which reduced the NH₄⁺ availability and directly inhibited the effects of Mg.

DCD, DMPP and PIADIN are useful NIs (Agrawal et al., 1985; Malla et al., 2005). Our results indicate that KCl and MgCl₂, which are inexpensive and readily available, can be used to fertilize the soil, decrease C and N losses and increase retention of the

added N, achieving the same function as other expensive NIs.

4.3 The effect of PIADIN

We found that PIADIN addition decreased soil respiration and nitrification. PIADIN maintained the CO₂-C and N₂O-N flux at a low level during the whole incubation period (Fig. 1). In addition, it significantly decreased the total CO₂-C and N₂O-N emissions (Table 2) and retained a high soil NH₄⁺-N concentration and low NO₃⁻-N concentration (Fig. 2). The amendment with NIs increases the NH₄⁺ concentration in soil (Pasda et al., 2001; Weiske et al., 2001; Zerulla et al., 2001; Chaves et al., 2006). PIADIN has been shown to maintain NH₄⁺-N in the mineral-N pool for a long period by blocking its oxidation and stabilizing NH₄⁺ in bulk soil (Prasad and Power, 1995; Malla et al., 2005), and inhibiting nitrification and subsequently denitrification by restricting the supply of NO₃⁻ to the denitrifying organisms (Freney et al., 1993; Chen et al., 1994; Rochester et al., 1996; Abbasi and Adams, 2000b; Souri, 2010).

PIADIN decreased soil nitrification. PIADIN is more effective than KCl and MgCl₂ on inhibiting the soil nitrification and keeping soil NH₄⁺-N in the mineral-N pool.

5. Conclusion

Different Cl⁻ salts and concentrations had varying effects on soil NH₄⁺ and NO₃⁻ dynamics and CO₂ and N₂O emissions compared with the control. Both KCl and 0.5 g kg⁻¹ MgCl₂ decreased CO₂-C emissions and increased N₂O-N emissions compared with the control. 1.0MgCl₂ decreased both CO₂-C and N₂O-N emissions and retarded the decrease of NH₄⁺-N concentration and the increase of NO₃⁻-N concentration. However,

Cl^- did not have such a strong NI effect as PIADIN. PIADIN decreased the $\text{CO}_2\text{-C}$ and $\text{N}_2\text{O-N}$ emissions and preserved the soil $\text{NH}_4^+\text{-N}$ concentration at a high level and $\text{NO}_3^-\text{-N}$ concentration at a low level. Our hypotheses were supported by the results of the study.

Based on our findings, addition of MgCl_2 at 1.0 g kg^{-1} soil is effective in retaining fertilizer N and decreasing greenhouse gas emissions, but it is not as effective as PIADIN. Both K and Mg salts are less expensive than PIADIN and most other NIs and also have the benefit of fertilizing the soil. KCl and MgCl_2 could help plants uptake N better in field conditions which may reduce greenhouse gas emissions and close greenhouse gas reduction performance gaps with PIADIN. In summary, MgCl_2 amendment could be useful to reduce $\text{CO}_2\text{-C}$ and $\text{N}_2\text{O-N}$ emissions; however, more research is needed to evaluate these findings under field conditions with different soil types and determine the optimum rates and type of nitrification inhibitors. We realized that the concentration of KCl and MgCl_2 were high. But we will try to use the lower concentration of KCl and MgCl_2 in the future to find out the effect.

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Table 1 Soil characteristics (mean with standard error in parentheses, $n = 4$).

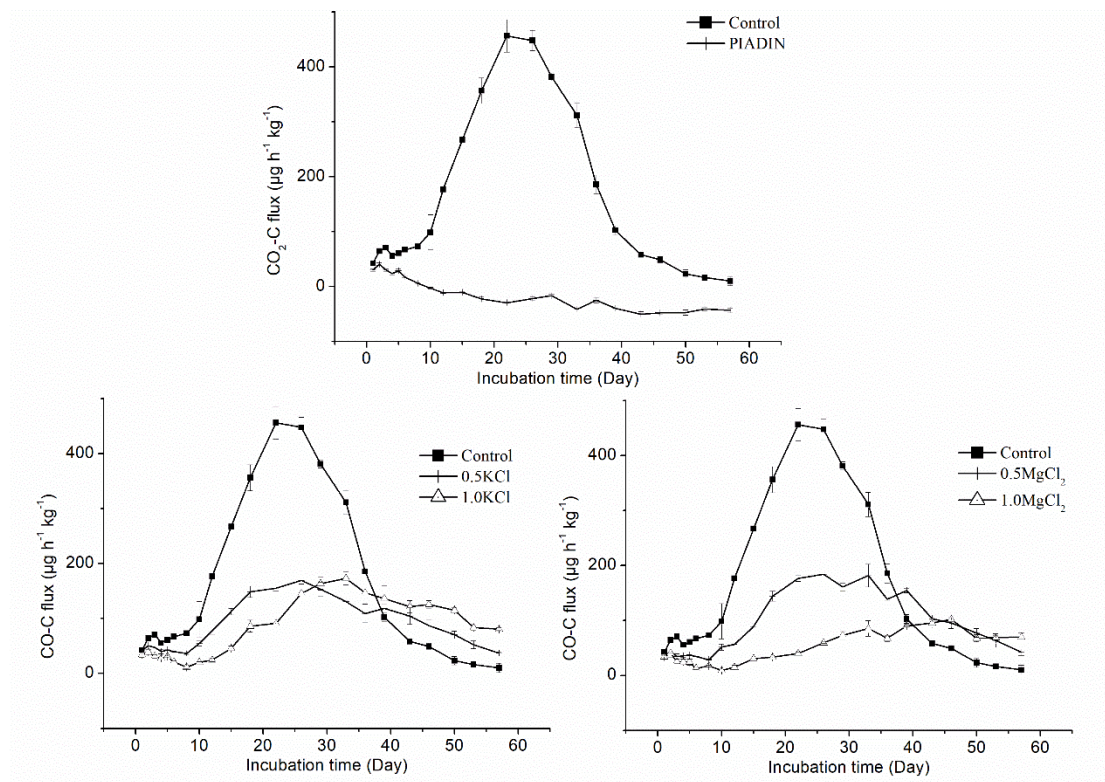
Silt (%)	Sand (%)	Total carbon (g kg ⁻¹)	Total nitrogen (g kg ⁻¹)	pH	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	Bulk density (g cm ⁻³)
5 (2)	95 (6)	11.6 (2.5)	0.8 (0.3)	6.5 (2.4)	2.16 (0.13)	4.80 (0.36)	1.40 (0.13)

Table 2 Soil CO₂-C and N₂O-N total emissions and decreased CO₂-C and N₂O-N emissions under KCl, MgCl₂ and PIADIN (0.5KCl, 0.5 g kg⁻¹ KCl-Cl; 1.0KCl, 1.0 g kg⁻¹ KCl-Cl; 0.5MgCl₂, 0.5 g kg⁻¹ MgCl₂-Cl; 1.0MgCl₂, 1.0 g kg⁻¹ MgCl₂-Cl). Values are means with standard error in parentheses (*n* = 4). Total emissions in the same column indicated by the same lowercase letter are not significantly different at the *P* < 0.05 level.

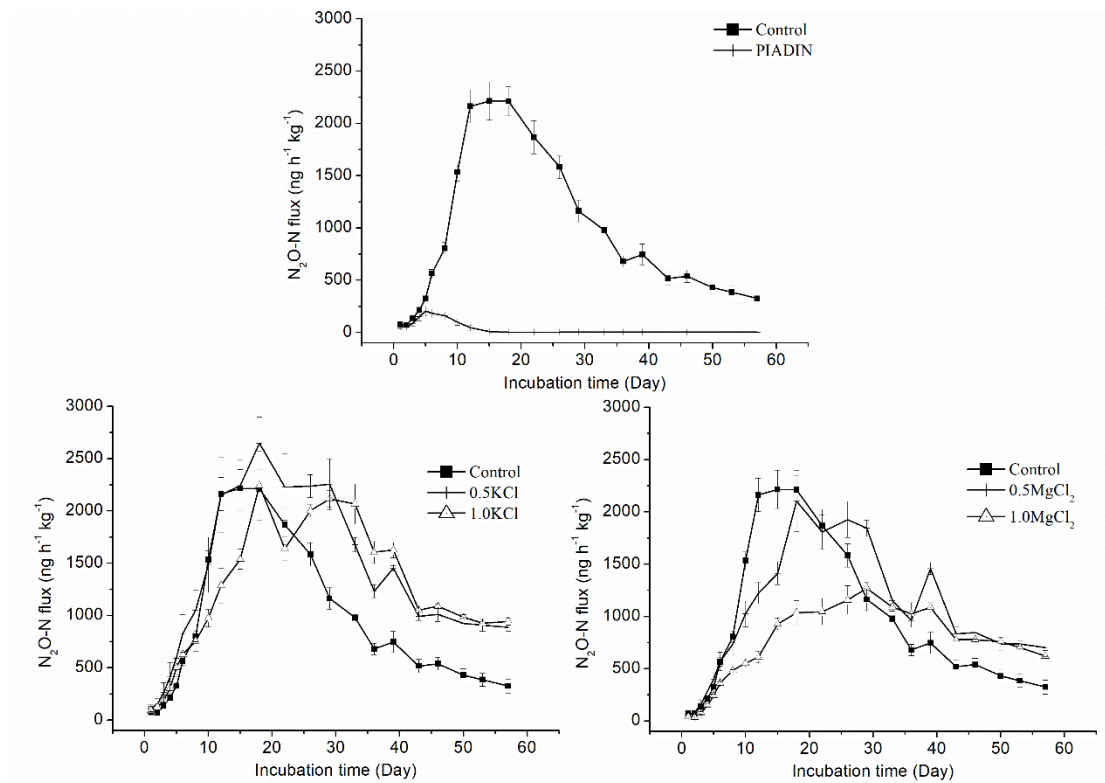
NI	CO ₂ -C emissions		N ₂ O-N emissions	
	Total (mg kg ⁻¹)	Decrease (% of control)	Total (μg kg ⁻¹)	Decrease (% of control)
Control	248.3 (5.96)d	–	1365 (97.63)b	–
PIADIN	–32.83 (0.93)a	113.23	36.43 (8.16)a	97.33
0.5KCl	134.8 (4.39)c	45.70	1999 (118.21)c	–46.49
1.0KCl	134.5 (6.36)c	45.82	1842 (92.99)c	–34.98
0.5MgCl ₂	141.7 (2.61)c	42.91	1564 (149.97)b	–14.63
1.0MgCl ₂	69.1 (3.79)b	72.15	1100 (69.79)b	19.40

Table 3 The P values of CO₂-C flux, N₂O-N flux, NH₄⁺-N and NO₃⁻-N and changes with time for the different treatment.

Treatment	CO ₂ -C flux	N ₂ O-N flux	NH ₄ ⁺ -N	NO ₃ ⁻ -N
Control	0.000	0.001	0.000	0.000
0.5KCl	0.006	0.015	0.000	0.000
1.0KCl	0.013	0.011	0.000	0.000
0.5MgCl ₂	0.060	0.018	0.000	0.000
1.0MgCl ₂	0.009	0.018	0.000	0.000
PIADIN	0.010	0.059	0.005	0.785



a



b

Figure 1 The flux of CO₂-C (a) and N₂O-N (b) as affected by KCl, MgCl₂ and PIADIN

(0.5KCl, 0.5 g kg⁻¹ KCl-Cl; 1.0KCl, 1.0 g kg⁻¹ KCl-Cl; 0.5MgCl₂, 0.5 g kg⁻¹ MgCl₂-Cl; 1.0MgCl₂, 1.0 g kg⁻¹ MgCl₂-Cl). The data are means of four independent pot replicates and error bars represent standard error of the mean ($n = 4$).

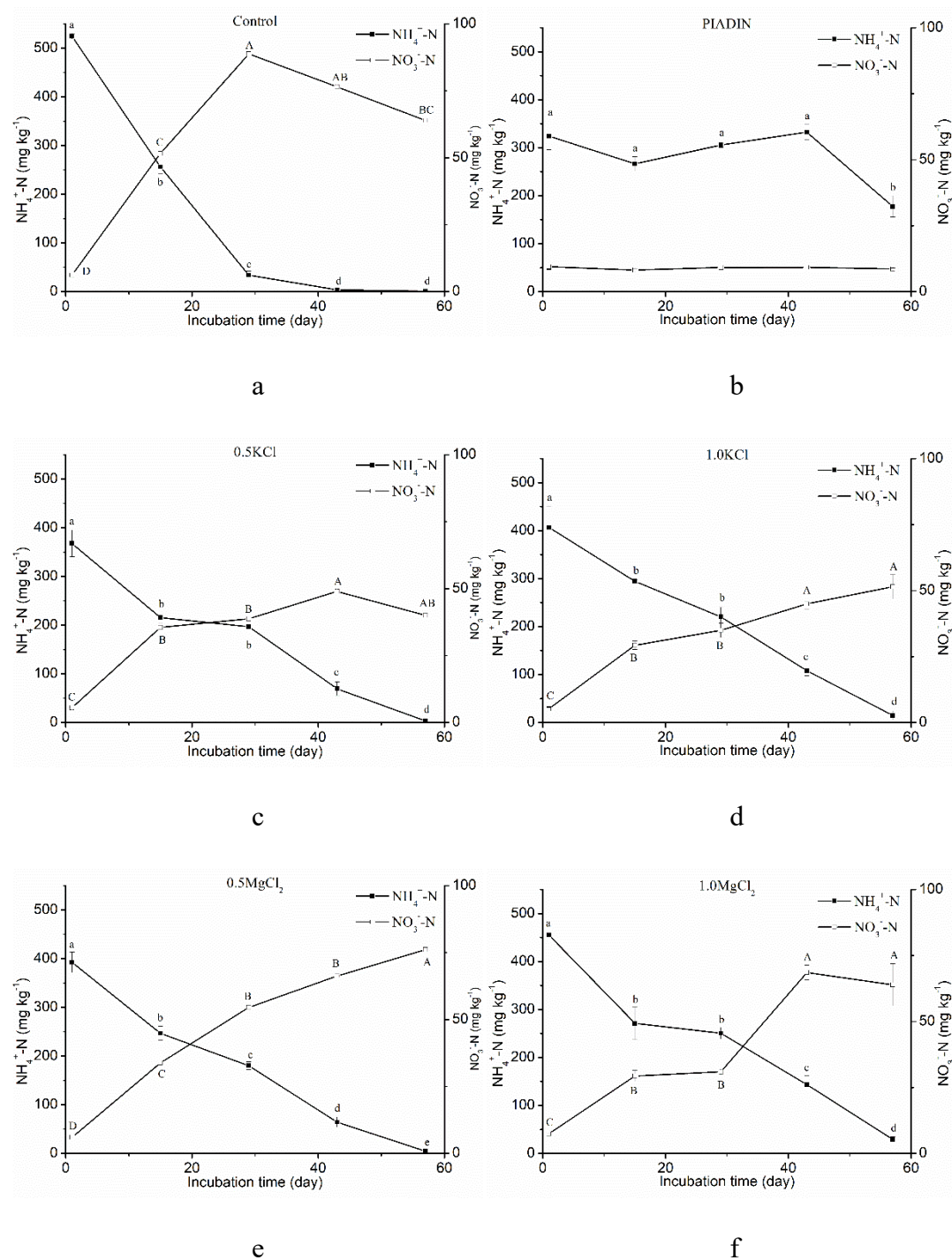


Figure 2 Changes of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in soils without nitrification inhibitor Control (a) and with nitrification inhibitors KCl, MgCl_2 and PIADIN (b) (0.5KCl (c), 0.5 g kg^{-1} KCl-Cl; 1.0KCl (d), 1.0 g kg^{-1} KCl-Cl; 0.5MgCl_2 (e), 0.5 g kg^{-1} $\text{MgCl}_2\text{-Cl}$; 1.0MgCl_2 (f), 1.0 g kg^{-1} $\text{MgCl}_2\text{-Cl}$). The data are means of four independent pot replicates and error bars represent the standard error of the mean ($n = 4$). Incubation

time indicated by the same upper case letter are not significantly different at $p < 0.05$ on the basis of one-way ANOVA for NO_3^- -N concentrations; Incubation time indicated by the same lower case letter are not significantly different at $P < 0.05$ on the basis of one-way ANOVA for NH_4^+ -N concentrations.

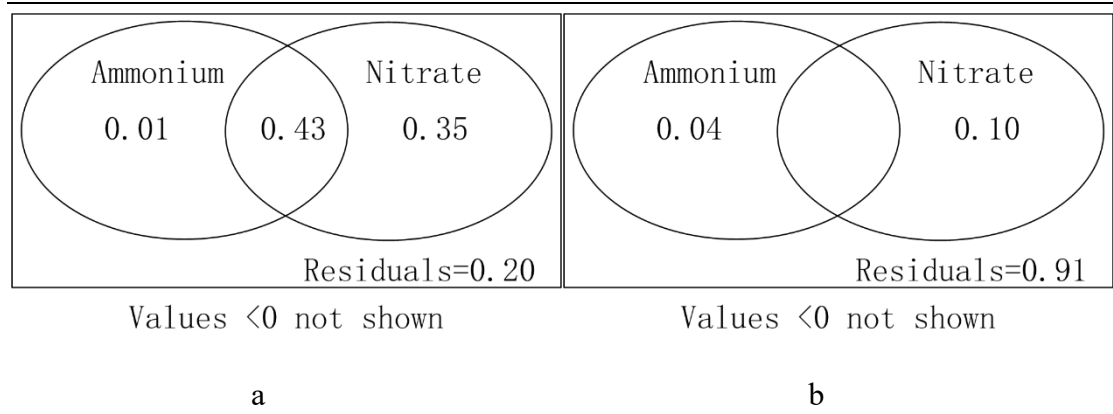


Figure 3 Variation partitioning of N₂O between ammonium and nitrate during 0–22 days (a) and 23–57 days (b).

Chapter 4: The effect of biogas residues and acidified biogas residues

Acidification of Biogas Residues and Application of Nitrification Inhibitors Efficiently Mitigate CO₂ and N₂O Emissions from Biogas Residues Amended Soils

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Water, Air, & Soil Pollution (under review)

Highlights

- Biogas residues application to soil resulted in increased CO₂ and N₂O emissions
- Acidified biogas residues decreased pH, CO₂ and N₂O emissions than non-acidified
- DMPP decreased CO₂ emissions more than PIADIN in acidified biogas residues amended
- DMPP and PIADIN were equally effective in reducing N₂O emission under both residues

Acidification of Biogas Residues and Application of Nitrification Inhibitors Efficiently

Mitigate CO₂ and N₂O Emissions from Biogas Residues Amended Soils

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Abstract

Owing to their high carbon and nitrate contents, biogas residues may lead to higher carbon dioxide (CO₂) and nitrous oxide (N₂O) emissions from soils. Acidification of biogas slurry and application of nitrification inhibitors (NIs) could mitigate the emission of these gases. An incubation experiment was therefore carried out to investigate the effect of NIs, DMPP (3, 4-dimethylpyrazole phosphate) and PIADIN (active ingredients: 3.00-3.25% 1,2,4-triazole and 1.50-1.65% 3-methylpyrazole), on CO₂ and N₂O emissions from soils fertilized with biogas residues and acidified biogas residues. Biogas residues produced higher ammonium-nitrogen (NH₄⁺-N) and nitrate-nitrogen (NO₃⁻-N) concentrations in soils which resulted in higher emissions of CO₂-C and N₂O-N than that from acidified biogas residues. Both DMPP and PIADIN significantly decreased the emissions of CO₂-C (8.1-55.8%) and N₂O-N (87-98%) and

maintained lower $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations when compared to control (without nitrification inhibitors). However, the DMPP had a higher reduction capability for $\text{CO}_2\text{-C}$ emissions than PIADIN in acidified biogas residue applied soil. In conclusion, the acidification of biogas residues and application of NIs has effect in reducing gaseous emission from biogas residue fertilized soils and thus could improve the fertilizer-effectiveness of the residues.

Key words: Acidified biogas residues; Nitrification inhibitors; N_2O and CO_2 emissions; Nutrient cycling

1. Introduction

Carbon dioxide (CO_2) and nitrous oxide (N_2O) are the primary greenhouse gases (GHGs) present in the Earth's atmosphere (IPCC, 2010). N_2O is a long-lived GHG and a major stratospheric ozone depleting substance (Thompson et al., 2019). Atmospheric CO_2 concentration, with an increase of about 120 ppm over the past 250 years, has risen to a current global average of approximately 409 ppm, and future rapid increase is expected, with values likely to reach 550 ppm by mid-century and 1000 ppm by the end of this century (IPCC, 2014). Similarly, N_2O concentration in the atmosphere has risen steadily since the mid-twentieth century (IPCC, 2013), from approximately 290 ppb in 1940 to 330 ppb in 2017 (Park et al., 2012; WMO Greenhouse Gas Bulletin, 2018).

Agricultural practices, particularly the use of nitrogenous fertilizers, substantially contribute to enhancing N_2O emission and thus increasing the concentration of reactive nitrogen (N, N_2O) in the atmospheric environment (Bouwman et al., 2013). In soils, N_2O is produced as a by-product of nitrification and denitrification processes which are carried out by different types of microbes (Bremner, 1997). Nitrification is the biological oxidation of NH_4^+ to nitrite (NO_2^-) and further to nitrate (NO_3^-). Denitrification is a microbially facilitated process in which NO_3^- is reduced, through a series of intermediate gaseous N oxide products, to molecular

nitrogen (N_2) (Köster et al., 2013). To meet the ambitious climate change adaptations, CO_2 and N_2O emissions should be minimized (IPPC, 2018).

Various techniques are used to reduce the CO_2 and N_2O emissions while meeting the growing demand for food and other agricultural products (Thompson et al. 2019). One of the approaches is to produce biogas through fermentation of renewable sources, including organic manures, plant materials, food waste, etc. The use of biogas as an energy source could reduce dependency on fossil fuels and is expected to have no or even positive effect on the atmospheric greenhouse gas balance (Herrmann, 2013). Biogas production plays an important role in the European bio-energy supply and has increased rapidly in Germany in recent years. According to the Agency of Renewable Energies, there are 837 biogas production factories in the northern part of Germany. It is evident that the recycling of the biogas residue (BR) from such a large number of biogas production factories installed in the state of Schleswig-Holstein became a problem. Mostly BR is applied to soil as organic fertilizers (Köster et al., 2014; Koszel and Lorencowicz, 2015) and is considered an essential component of cropping systems due to its high fertilization value (Möller and Stinner, 2009). The application of BR as a fertilizer improved soil fertility, plants quality and their immunity to biotic and abiotic stress agents (Kouřimská et al. 2012).

In addition to improving soil quality, the BR application to soil, due to their high carbon, ammonium and nitrate content, may increase the CO_2 and N_2O emissions and NO_3^- leaching from the soils (Hennig and Gawor, 2012). N_2O emissions and NO_3^- leaching from BR may be minimized by their acidification before soil application and by using nitrification inhibitors (NIs). The stabilization of mineral N in immobile NH_4^+ form, by preventing its transformation to highly mobile NO_3^- form, in the acidified biogas residues (ABR) would reduce NO_3^- leaching of from the rooting zone and gaseous emissions of N_2O from the soils. While application of NIs can further reduce NO_3^- leaching from BR applied soils by suppressing autotrophic nitrification and subsequent denitrification, which will ultimately also reduce N_2O emission. In mineral

fertilizer applied soil, the use of NIs has shown reduced N₂O emissions (VanderZaag et al., 2011) and thereby increased N use efficiency of the applied fertilizers (Subbarao et al., 2006).

We hypothesized that a) Application of ABR to soil will decrease soil pH and show lower CO₂ and N₂O emissions than the application of BR, b) NIs application to ABR-amended soil will further lower the gaseous emission from the soil. Keeping this in view, an incubation pot experiment was conducted to investigate CO₂ and N₂O emissions as well as NH₄⁺ and NO₃⁻ dynamics in the soils amended with BR and ABR and applied with DMPP and PIADIN nitrification inhibitors.

2. Material and Methods

2.1 Collection and preparation of soil and biogas residues

The biogas residues (BR) were collected from a large commercial biogas company in Germany in May 2019. Its digesters were used to feed with the following materials: 18 t corn silage, 55 t dry chicken faeces, 4 t whole crop silage (rye) and 7 m³ swine manure. For the incubation experiment, BR was sampled directly from the biogas residue storage pool. Acidified biogas residues (ABR) were prepared by lowering the pH of BR from 7.9 to 5.5 with the addition of H₂SO₄ (15 ml H₂SO₄ per 1 kg biogas residues).

Soil was collected from upper 20 cm layer of a natural grassland in Grevenkrug, Schleswig-Holstein (54°11'09.1" N and 10°00'36.6" E). The soil was sandy in texture, with 5% silt and 95% sand. The water-holding capacity (WHC) of the soil was 24.34% and bulk density was 1.4 g cm⁻³. The soil was sieved through a 2 mm sieve to remove visible plant residues, roots and stones. Some salient characteristics of the soil and fresh BR are given in Table 1.

2.2 Treatments and experimental design

Three treatments of biogas residues (unamended, BR and ABR) were tested against three treatments of nitrification inhibitors (control, DMPP (3, 4-dimethylpyrazole phosphate) and

PIADIN (active ingredients: 3.00-3.25% 1,2,4-triazole and 1.50-1.65% 3-methylpyrazole)), yielding 9 treatment combinations in total. Soil was filled in cylindrical pots (15 cm diameter and 33 cm length) up to 20 cm height while adjusting the bulk density to 1.4 g cm^{-3} . Both BR and ABR were applied to the respective pots at the rate of 27.8 g kg^{-1} soil (equivalent to $0.1 \text{ g NH}_4^+\text{-N kg}^{-1}$ soil), whereas unamended did not receive any amendment. The DMPP and PIADIN were applied at the rate of 5 mg kg^{-1} soil (5% of the applied $\text{NH}_4^+\text{-N}$), whereas no NI was added to the control pots. The pots were incubated for 57 days in a climatic chamber at a constant temperature (15°C), soil moisture (80% WHC) and air humidity (50%). Deionized water was added daily to maintain the desired soil moisture level. The experiment followed a two-factorial completely randomized design with four replicates.

2.3 Collection and measurement of N_2O and CO_2

The N_2O and CO_2 samples were collected daily during 1st week, once after two days during the 2nd week, and once after three days during rest of the incubation period. Before collecting gas samples, the pots were tightly closed with air-tight lids having one rubber membrane, which served as a contact between the sample collecting syringe and the incubated environment. The samples were collected every 0, 20, 40 and 60 min. A 10-ml syringe with a hypodermic needle was placed in pre-evacuated 2-ml headspace of Chromacol glass vials to collect the gas samples. The glass vials had a chloro-butyl rubber septum. Each gas sampling was carried out between 09:00 - 11:00 am. Except for the times when samples were being taken, the pots were left open. The concentrations of CO_2 and N_2O in the gas samples were measured by gas chromatography (Agilent 7890A GC, Agilent, CA, United States). For each gas measurement, the gas chromatograph was calibrated with respective certified gas standards. The rate of CO_2 and N_2O emissions from each pot (ppm/min) during lid closure was calculated using headspace volume and a linear relation between the CO_2 and N_2O concentration and time (Venterea et al., 2020). The emission rates of $\text{CO}_2\text{-C}$ ($\mu\text{g h}^{-1} \text{ kg}^{-1}$) and of $\text{N}_2\text{O-N}$ ($\text{ng h}^{-1} \text{ kg}^{-1}$) was calculated with the

following equations:

$$EN_2O-N = \frac{R \times 60 \times V_{gas} \times AR}{W_{soil} \times V_m} \times 2 \times 1000 \quad (1)$$

$$ECO_2-C = \frac{R \times 60 \times V_{gas} \times AR}{W_{soil} \times V_m} \quad (2)$$

Where, ECO_2 and EN_2O are emission rates of CO_2 -C ($\mu g\ h^{-1}\ kg^{-1}$) and N_2O -N ($ng\ h^{-1}\ kg^{-1}$), respectively; R is the rate of CO_2 and N_2O emissions from each pot (ppm/min); V_{gas} is the gas volume in pot (L); W_{soil} is the weight of dry soil in pot (kg); AR is the relative atomic mass of C and N, i.e. 12 and 14, respectively ; V_m is the molar volume of gas which is 23.7 L/mol at 15 °C.

Total N_2O and CO_2 emissions during the incubation period were calculated by adding the total daily N_2O and CO_2 emissions. For this purpose, the emission rates of CO_2 -C ($\mu g\ h^{-1}\ kg^{-1}$) and N_2O -N ($ng\ h^{-1}\ kg^{-1}$) was multiplied with the hours i.e. 24 h for 1st week, 48 h for 2nd week, and 72 h for last six weeks. The submission of all these gave the total emissions of CO_2 ($mg\ kg^{-1}$) and N_2O ($\mu g\ kg^{-1}$).

2.4 Soil sampling and measurement of NH_4^+ , NO_3^- and pH

For the measurement of NH_4^+ -N, NO_3^- -N and pH, soil samples (each 50 g approx.) were collected up to 20 cm depth using a specialized soil agar on day 1, 15, 29, 43 and 57 of incubation. Each soil sample was divided into two subsamples; the first was oven-dried at 105°C for 8 h to calculate the water content, while the other was used for determination of NH_4^+ -N, NO_3^- -N and pH. For soil mineral N analysis, 10 g fresh soil was extracted with 40 mL of 0.0125 M $CaCl_2$ solution (1:4) for 1 h on a reciprocating shaker. The suspensions so obtained were centrifuged for 10 min, filtered through Whatman filter paper No. 42 and stored at 4 °C. The concentrations of NH_4^+ and total mineral N were measured by a continuous flow autoanalyzer (San⁺⁺ Automated Wet Chemistry Analyzer - Continuous Flow Analyzer (CFA), Skalar, The Netherlands). The concentration of NO_3^- -N was obtained by subtracting NH_4^+ -N

from the total mineral N. For pH measurement, 10 g air-dried and sieved soil was mixed with 25 mL of 0.0125 M CaCl₂ solution (1:2.5) and shaken for 1 h. After centrifugation of the suspensions, the pH of the upper clear liquid was measured using a pH meter.

2.5 Statistical analysis

Data were verified for normal distribution, treatment means for total N₂O-N and CO₂-C emissions over the incubation period were compared using a two-way analysis of variance. The significance of differences between individual treatment means was determined using Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$. Tukey's HSD test was performed by R statistical software (Oakland, CA, USA).

3. Result

3.1. Rate of CO₂-C and N₂O-N emissions

In unamended soil, the rates of CO₂-C and N₂O-N emissions were substantially lower than the biogas residues amended soils and there was no consistent pattern of increase or decrease with passage of time during incubation period (Fig. 1 and 2). However, rates of both CO₂-C and N₂O-N emissions were substantially higher in BR- and ABR-amended soils than unamended soil, with BR application showing more increase than ABR application. In BR and ABR amended soils, rates of emissions reached maximum by the end of first or mid of 2nd week, and thereafter decreased gradually, reaching the minimum value at the end of incubation period. Application of DMPP and PIADIN to BR and ABR amended soils decreased the emissions rates of both the gases.

3.2 Total CO₂-C and N₂O-N emissions

Irrespective of whether NIs were applied or not, total CO₂-C emission was very low from unamended soil than that from BR- and ABR-amended soils. The total CO₂-C emission was the

highest from BR-amended soil ($169 \text{ mg kg}^{-1} \text{ soil}$), followed by ABR-amended soil ($81 \text{ mg kg}^{-1} \text{ soil}$) and the lowest from unamended soil ($37 \text{ mg kg}^{-1} \text{ soil}$) (Table 2). Compared to unamended soil, the application of BR and ABR increased total $\text{CO}_2\text{-C}$ emission by 3.60- and 1.21-fold, respectively. Total $\text{N}_2\text{O-N}$ emission was also significantly higher from BR- and ABR-amended soils than that from unamended soil (Table 2). The mean total $\text{N}_2\text{O-N}$ emission was the highest from BR-amended soil ($3678 \text{ } \mu\text{g kg}^{-1}$), followed by ABR-amended soil ($1464 \text{ } \mu\text{g kg}^{-1}$) and the lowest from unamended soil ($5 \text{ } \mu\text{g kg}^{-1}$). Compared to unamended soil, the application of BR and ABR increased the total $\text{N}_2\text{O-N}$ emission by 73- and 29-folds, respectively.

ABR-amended soil showed half $\text{CO}_2\text{-C}$ emission of what did the BR-amended soil (Table 2). The NIs (DMPP and PIADIN) significantly lowered total $\text{CO}_2\text{-C}$ emission compared to control, but their relative effectiveness depended upon the residues types (Table 2). In BR-amended soil, DMPP and PIADIN were equally effective in reducing $\text{CO}_2\text{-C}$ emission, with reduction amounted to be 55% of that from control. However, in ABR-amended soil, DMPP was more effective in reducing $\text{CO}_2\text{-C}$ emission, showing 38% decrease over control, than PIADIN which showed only 8% decrease. Overall, $\text{CO}_2\text{-C}$ emission reducing capacity of both the NIs was higher in BR-amended soil than that in ABR-amended soil.

ABR-amended soil showed 60% lower total $\text{N}_2\text{O-N}$ emission than BR-amended soil (Table 2). The application of NIs further much lowered the total $\text{N}_2\text{O-N}$ emission compared to control, with DMPP having higher reduction efficiency than PIADIN under both BR- and ABR-amended soils. The mean reduction in total $\text{N}_2\text{O-N}$ emission in BR- and ABR-amended soil by DMPP and PIDIN was 96 and 90%, respectively.

3.3 Soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations

The application of both BR and ABR substantially increased $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations compare to the control (Fig. 3). With the passage of time, ABR application showed slow gradual decline in NH_4^+ concentration and rise in NO_3^- concentration as compared

to BR-amended soil. Similarly, the residue amended soils applied with NIs showed a gradual decline in NH_4^+ -N concentration and rise in NO_3^- -N concentration whereas these changes were quite sharp in control soils. Moreover, DMPP more strongly retarded the fall in NH_4^+ -N concentration and rise in NO_3^- -N concentration than PIADIN in both BR- and ABR-amended soils. The peak NO_3^- -N concentrations were lower in NIs treated soils as compared to control and DMPP produced the lowest NO_3^- -N concentration.

3.4 Soil pH

Application of BR did not affect the soil pH throughout the course of experiment (Fig. 4). However, ABR application lowered the soil pH as compared to unamended soil and the mean decrease during the incubation period was 0.34 units. The application of DMPP and PIADIN had no influence on the soil pH.

4. Discussion

4.1. Effect of biogas residues

Application of biogas residues to agricultural soils as an organic fertilizer has become a common practice and is further growing with the increase in number of biogas plants in some European countries (Wolf et al., 2014; Köster et al., 2015). The application of BR no doubt improves soil organic matter and N availability to crops, but on other side it can also provoke CO_2 and N_2O emissions from soils (Köster et al., 2011, 2015; Senbayram et al., 2014). Thus, high application rates of BR to cultivated lands could be linked to environmental problems such as high levels of CO_2 and N_2O in the atmosphere, and NO_3^- leaching to groundwaters (Qu et al., 2014). This study revealed that BR application to soil significantly increased CO_2 and N_2O emissions (Fig. 1 and 2; Table 2). A sharp decline in NH_4^+ -N concentration and rise in NO_3^- -N concentration was recorded in BR-amended soil (Fig. 3). Concurrent presence of NH_4^+ , NO_3^- and high level of labile C in the BR could have hastened the nitrification and denitrification

processes in the soil (Jaeger et al., 2013), and thus increased CO₂ emissions (Köster et al., 2015) and N₂O emissions (Senbayram et al., 2009) compared with unamended soil. The availability of high level of NO₃⁻ as a result of nitrification together with labile C serves as a driving force for N₂O emissions (Senbayram et al., 2009). Groffman and Crawford (2003) reported that CO₂ efflux and denitrification activity can be positively correlated, and hence increased denitrification activity also increases CO₂ efflux. It is generally accepted that application of organic fertilizers stimulates soil microbial biomass, basal respiration (Ros et al., 2003) and enzyme activities (Chu et al., 2007). Soil microbial processes also produce gases such as N₂O (IPCC, 2013), and residual organic carbon substrates favor soil microbial denitrification (Robertson and Groffman, 2007). In conclusion, BR application to soil significantly increases CO₂-C and N₂O-N emissions from soils.

4.2 Acidification of BR and the gaseous emission

Soil N dynamics in BR-amended soils could depend on the BR composition and stability (Albuquerque et al., 2012). Hence, through changing the soil characteristics, BR may affect the nitrification process, and hence the amounts of NO₃⁻ lost by denitrification or leaching (Peter et al., 2004). Among the soil characteristics, pH is a master variable that affect microbial transformations in soils. Nitrification is strongly influenced by pH (Mackens et al., 2021), with maximum rates occurring at pH 7.5 (Eric et al., 2002). Oxidation of NH₄⁺ is completely inhibited at pH 5 and increases with increase in pH (Wang et al., 2018). Our result showed that application of ABR lowered soil pH than unamended soil, but this pH effect was not observed for BR-amended soil (Fig. 4). Accordingly, ABR application resulted in half CO₂-C and 60% less N₂O-N emissions from soil compared to BR application (Table 2). This is well-explained by a slow gradual decline in NH₄⁺ concentration and rise in NO₃⁻ in ABR-amended soil as compared to BR-amended soil (Fig. 3). Thus, it could be inferred that ABR application retarded nitrification process through pH-driven shift in the microbial community structure and/or

microbial activities (Ottosen et al., 2009). Fangueiro et al. (2013) also reported that lowering the pH led to decreased CO₂ emission from soil, and effect is due to the low microbial activities in the acidified soil (Fangueiro et al., 2015). In conclusion, ABR application to soil significantly decreases CO₂-C and N₂O-N emissions from soils compared to BR.

4.3 Nitrification inhibitors and the gaseous emissions

Application of NIs is recognized as one of the mitigation strategies that have been proven to be highly effective in reducing N fertilizer losses, increasing N use efficiency and crop yields under different cropping systems (Moir et al., 2012; Zhang et al., 2015). Therefore, we tested the compatibility of using the most common NIs DMPP and PIADIN with BR and ABR for reducing CO₂ and N₂O emission from soils. Our result showed that NIs reduced CO₂ and N₂O emissions in BR- and ABR-amended soil (Fig. 1 and 2; Table 2). For reducing CO₂ emission, the efficiency of NIs was relatively less in ABR-amended soil than BR-amended soil and DMPP was more effective than PIADIN only in ABR-amended soil (Table 2). However, NIs were equally effective in reducing N₂O emission in BR- and ABR-amended soil and DMPP was relatively more effective than PIADIN under both condition (Table 2). The application of PIADIN has been reported to reduce N₂O emissions by 37 - 62 % during the weeks following biogas residue application to soil (Wolf et al., 2014). In fact, DMPP and PIADIN maintained NH₄⁺-N concentration at a higher level which showed that NIs retarded nitrification process and kept NO₃⁻-N concentration at a low level (Fig. 3). Most NIs retard microbial oxidation of NH₄⁺ by depressing the activities of nitrifiers in soil (Wolf et al., 2014). Thus, NIs could have inhibited nitrification through suppressing the activity of ammonia-oxidizing bacteria or relevant enzymes, effectively delaying the oxidization process that transforms NH₄⁺ into NO₃⁻. We found long-lasting higher NH₄⁺-N concentration and lower CO₂-C and N₂O-N emission from DMPP treated soil as compared to PIADIN treated soil (Fig. 3; Table 2). This implies that DMPP was more effective than PIADIN in retarding the nitrification process and mitigating the

gaseous emission from both BR- and ABR-amended soils under given experimental conditions. Chen et al. (2010) and Fangueiro et al. (2009) also reported that DMPP maintained a high soil NH_4^+ content and a low soil NO_3^- content for a longer time than the other NIs and resulted in the highest reduction in N_2O emissions.

5. Conclusion

In conclusion, application of both BR and ABR improved soil NH_4^+ content, but ABR-amended soil showed much lower $\text{CO}_2\text{-C}$ and $\text{N}_2\text{O-N}$ emissions than BR-amended soil. DMPP and PIADIN were equally effective in reducing $\text{CO}_2\text{-C}$ emission from BR-amended soil but DMPP was more effective than PIADIN in ABR-amended soil. However, DMPP showed higher efficiency than PIADIN for reducing N_2O emission from both BR- and ABR-amended soils. The BR acidification and NIs are effect in reducing gaseous emission from soil.

Credit author statement

Yafei Guo, Methodology, Software, Writing – original draft, Writing – review & editing. Anjum, Methodology, Writing – review & editing. Ahmad Khan, Methodology, Writing – review & editing. Asif Naeem, Writing – review & editing. Karl H. Mühling, Conceptualization, Writing – review & editing, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Characteristics of experimental soil and fresh biogas residues.

Characteristic	Soil	Biogas residues
Water content (%)	-	90.3
Total carbon (C, g kg ⁻¹)	11.6	31.4
Total nitrogen (N, g kg ⁻¹)	0.8	5.6
Organic N (g kg ⁻¹)	0.7	2
NH ₄ ⁺ -N (mg kg ⁻¹)	2.16	3600
NO ₃ ⁻ -N (mg kg ⁻¹)	4.8	0
pH*	6.5	7.9

* In soil:CaCl₂ (1:4) extract

Table 2. Total emissions of CO₂-C and N₂O-N in unamended, biogas residues (BR)- and acidified biogas residues (ABR)-amended soils applied with control, DMPP and PIADIN nitrification inhibitors.

Treatment	Nitrification inhibitors	CO ₂ -C emission		N ₂ O-N emission	
		Total	Decrease (%)	Total	Decrease (%)
		(mg kg ⁻¹)	over control	(µg kg ⁻¹)	over control
Unamended	Control	37±1 e	-	5.04±0.35 e	-
	DMPP	30±5 f	17.8	8.48±0.53 e	- 68.3
	PIADIN	26±0 f	29.5	5.31±0.24 e	- 5.37
BR	Control	169±8 a	-	3678±64 a	-
	DMPP	76±7 c	55.0	84±4 d	97.7
	PIADIN	75±3 c	55.8	233±28 c	93.7
ABR	Control	81±3 b	-	1464±72 b	-
	DMPP	50±5 d	38.1	87±6 d	94.1
	PIADIN	75±4 c	8.1	187±15 c	87.2

Values (mean ± SE, n = 4) in a column followed by different letters are significantly different at P = 0.05.

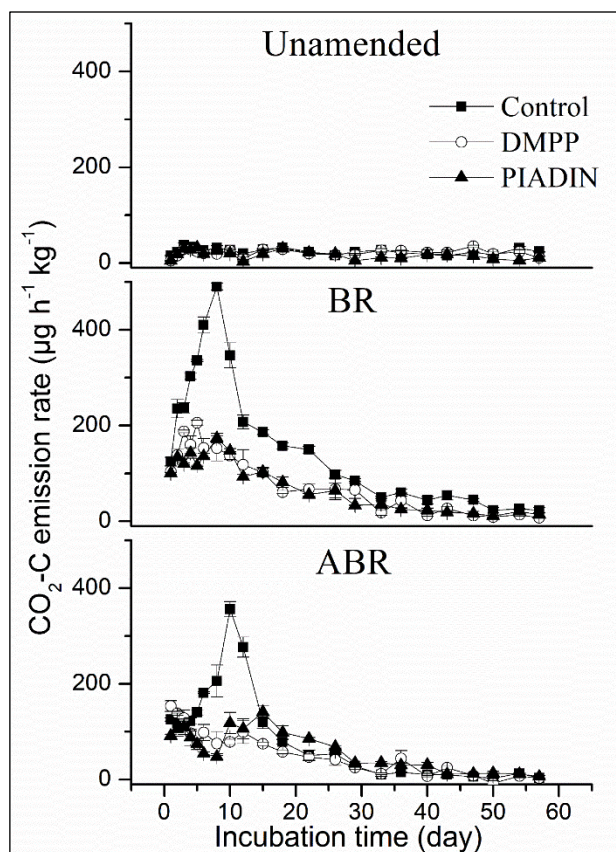


Fig. 1. Rate of $\text{CO}_2\text{-C}$ emissions from unamended, biogas residues (BR)- and acidified biogas residues (ABR)-amended soils applied with control, DMPP and PIADIN nitrification inhibitors. The vertical bars indicate the standard error of the means (n=4).

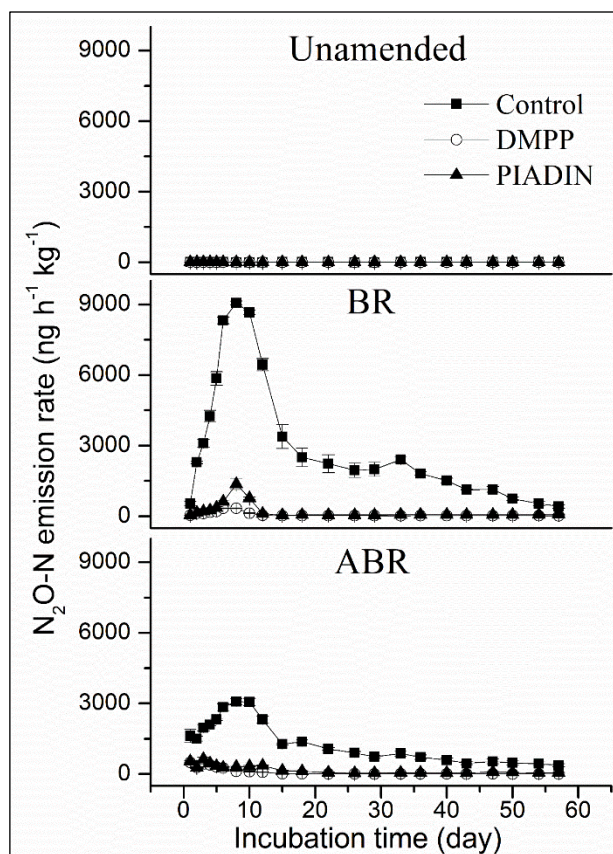


Fig. 2. Rate of N_2O -N emissions from unamended, biogas residues (BR)- and acidified biogas residues (ABR)-amended soils applied with control, DMPP and PIADIN nitrification inhibitors. The vertical bars indicate the standard errors of the means (n=4).

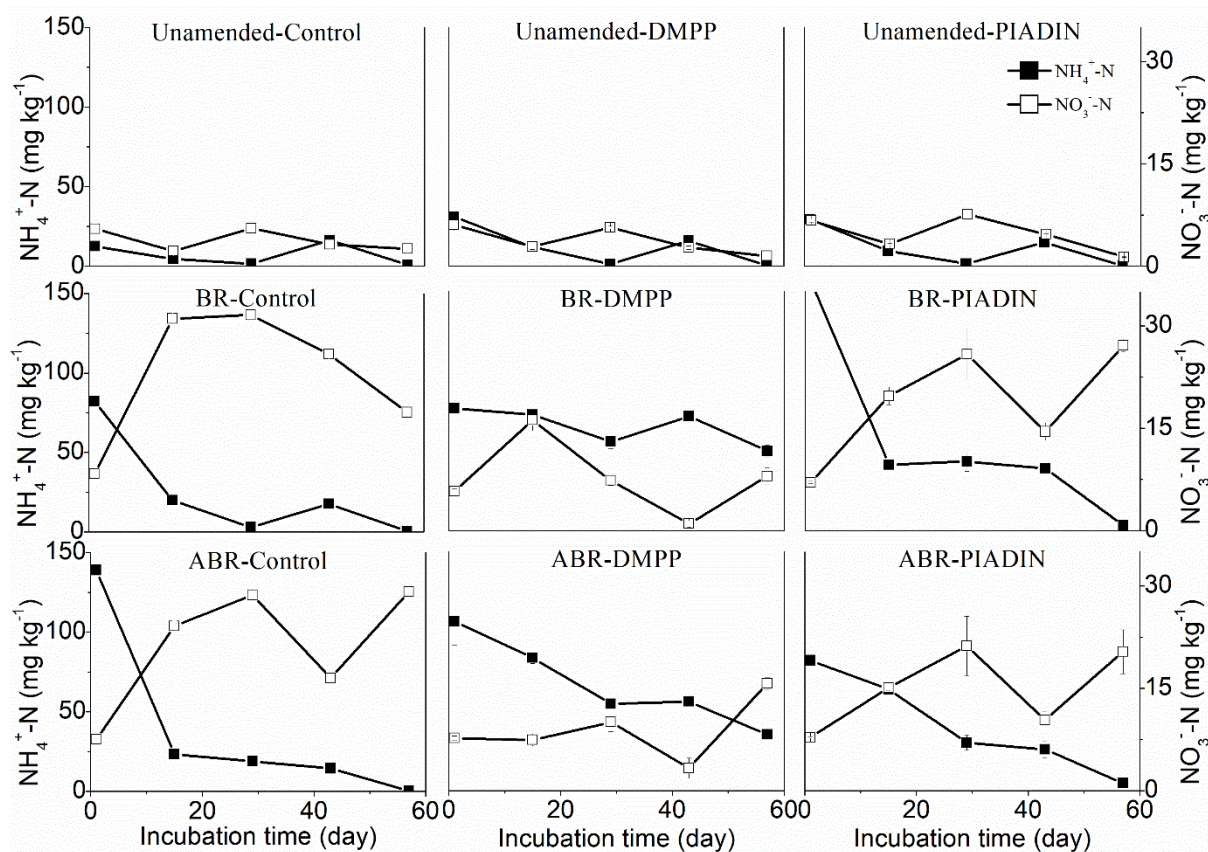


Fig. 3. Time dependent change in $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in unamended, biogas residue (ABR)- and acidified biogas residue (ABR)-amended soils applied with control, DMPP and PIADIN nitrification inhibitors under controlled environment. The vertical bars indicate the standard errors of the means (n=4).

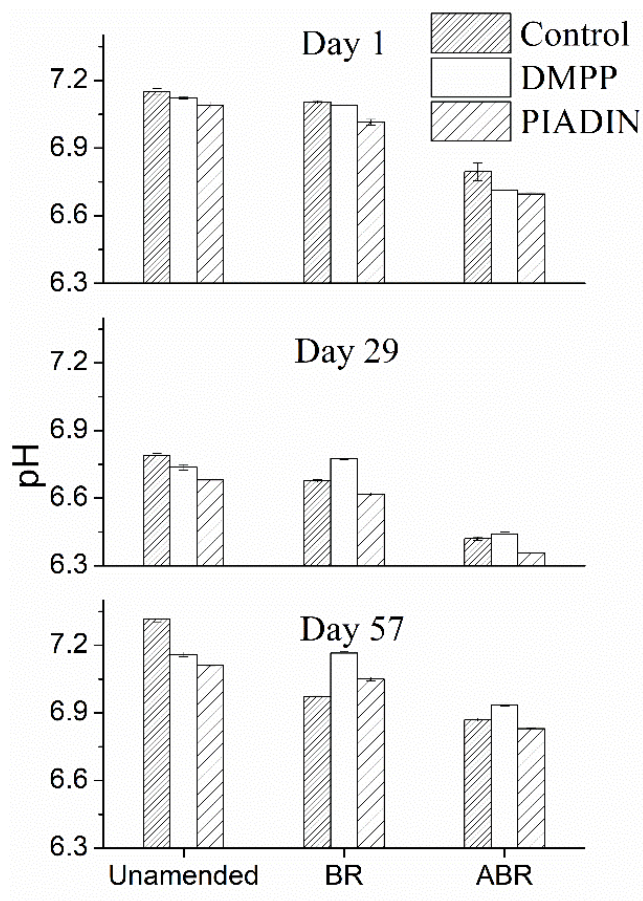


Fig. 4. Changes in pH of unamended, biogas residues (BR)- and acidified biogas residues (ABR)- amended soils applied with Control, DMPP and PIADIN nitrification inhibitors. The vertical bars indicate the standard errors of the means (n=4).

Chapter 5: The effect of soil type

Comparative effectiveness of four nitrification inhibitors for mitigating carbon dioxide and nitrous oxide emissions from three different textured soils

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Highlights

- CO₂ and N₂O emissions were higher from clayey soil than loamy and sandy soils
- DCD was the most effective NIs in decreasing emissions from clayey and loamy soils
- DMPP and PIADIN decreased CO₂ and N₂O emissions more effectively from sandy soil
- ENTEC proved ineffective in inhibiting N₂O emission from the studied soils

Comparative effectiveness of four nitrification inhibitors for mitigating carbon dioxide and nitrous oxide emissions from three different textured soils

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Abstract

Nitrification inhibitors (NIs) could be used to reduce both NO₃⁻-N leaching and N₂O-N emissions, and improve the efficiency of N fertilizer. However, the comparative efficacies of NIs could be strongly affected by soil type. Therefore, efficacies of four nitrification inhibitors namely dicyandiamide (DCD), 3, 4-dimethylpyrazole phosphate (DMPP), nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) and PIADIN (active ingredients: 3.00%–3.25% 1, 2, 4-triazole and 1.50%–1.65% 3-methylpyrazole) were investigated in three different textured N fertilized (0.5 g NH₄⁺-N kg⁻¹ soil) soils of Schleswig-Holstein viz. Marsch (clayey), Östliches Hügelland (loamy) and Geest (sandy) under constant temperature (15 °C) and soil moisture (80% water-holding capacity) condition for 8 weeks. Total CO₂-C and N₂O-

N emissions during the incubation period were significantly higher from Marsch soil than Östliches Hügelland and Geest soils. In Marsch soil, DMPP showed the highest inhibitory effect on CO₂-C emission (50%), followed by PIADIN (32%) and the lowest by ENTEC (16%). In Östliches Hügelland soil, DCD and PIADIN showed the highest and equal inhibitory effect on CO₂-C emission (73%), followed by DMPP (64%) and the lowest by ENTEC (36%). In Marsch and Östliches Hügelland soils, DCD showed the stronger inhibitory effect on N₂O-N emission (86 and 47%, respectively) than DMPP (56 and 30%, respectively) and PIADIN (54 and 16%, respectively). In Geest soil, DMPP was more effective in reducing N₂O-N emission (88%), than PIADIN (70%) and DCD (33%). Thus, it could be concluded that DCD is a better NI for clay and loamy soils while DMPP and PIADIN are better for sandy soil to inhibit soil nitrification and the gaseous emissions.

Key words: Nitrification inhibitors, Soil type, CO₂ and N₂O emissions, Soil nitrogen dynamic

1. Introduction

Nitrogen is an important element for plant growth in agro-ecosystems (Gilsanz et al., 2016), but the effectiveness of applied fertilizer N by crops rarely exceeds 40% (Chen et al., 2008). Chemical N fertilizer constitutes approximately 75% of the total EU input of reactive N (van Grinsven et al., 2014) and between 40 and 70% of the fertilizer N applied is lost to the atmosphere or the hydrosphere (Sutton et al., 2011). The majority of applied N is lost from agriculture through ammonia (NH₃) volatilization, gaseous emissions of nitrous oxide (N₂O) and di-nitrogen (N₂) and nitrate (NO₃⁻) leaching (Liu et al., 2017). Soil NO₃⁻-N leaching and N₂O emission are

processes responsible for both N losses from agricultural soils as well as environmental pollution (Harrison and Webb, 2011; McGeough et al., 2016). Beyond its powerful greenhouse effect, N₂O also is a major ozone depleting substance involved in the destruction of the protective ozone layer in the stratosphere (Ravishankara et al., 2009).

The concentration of N₂O, which about 300 times more reactive gas than CO₂, have risen from a pre-industrial value of 270 ppb to 319 ppb in 2005 (IPCC, 2007), primarily due to agricultural practices and increase in use of industrial fertilizers (Hénault et al., 2012; Gilsanz et al., 2016). It has been estimated that agricultural soils produce 2.8 (1.7-4.8) Tg N₂O-N year⁻¹ and contribute approximately 65% of the atmospheric N₂O loading (IPCC, 2007; Ravishankara et al., 2009). In Europe, N₂O emissions from agricultural soils contribute about 70% of the total annual N₂O emissions (European Environment Agency, 2015). Application of N to soils as chemical or organic fertilizers stimulates nitrous oxide (N₂O) emissions, mainly through the processes of denitrification and nitrification (Hu et al., 2015; Zhang et al., 2015). Nitrification is an aerobic process in which ammonium (NH₄⁺) is first oxidized to nitrite (NO₂⁻) and then nitrate (NO₃⁻) (Gilsanz et al., 2016), and it plays a key role in the soil N cycle (Khalil et al., 2008). During the oxidation of NH₄⁺ to NO₂⁻, N₂O can be produced as an intermediate and liberated into the atmosphere (Gilsanz et al., 2016). Denitrification is an anaerobic microbial process in which organic carbon is used as an energy source and NO₃⁻ is reduced to gaseous N compounds, including N₂ and N₂O (Volpi et al., 2017).

Nitrification inhibitors (NIs) are used to improve efficiency of N fertilizers through decreasing both NO₃⁻ leaching and gaseous N emissions (Volpi et al., 2017; Boeckx et al., 2005). NIs can decelerate the rate of soil nitrification by deactivating the enzyme ammonia monooxygenase (AMO) responsible for catalyzing ammonia oxidation, the

first and rate-limiting step of nitrification, which is encoded by produced by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) (Zhang et al., 2012). As NO_3^- is the initial required substrate for denitrification, the use of NIs decreases N_2O emissions from both processes; nitrification and denitrification (Gilsanz et al., 2016).

The production of N_2O , and in turn CO_2 emission, in soil and its inhibition by NIs are complex processes, which could be influenced by different factors such as physicochemical characteristics of the soil. Among these, texture is a major soil characteristic that govern various soil properties and hence relative effectiveness of NIs may be different in different textured soils. Soil texture could affect the effectiveness of NIs through affecting their stability/persistence and absorption in soils. Some NIs such as DMPP (3, 4-dimethylpyrazole phosphate) has shown to affect N_2O emission through decreasing soil pH (Barth et al., 2019) which could also vary with soil texture. Some studies have tested the effectiveness of NIs to reduce N_2O emissions but results varied considerably because the studies were carried out under different soil conditions (Gilsanz et al., 2016). Thus, it is difficult to draw any specific conclusions about the N_2O mitigation potential of NIs in different textured soils.

This study investigates the effectiveness of four NIs viz. dicyandiamide (DCD), DMPP, nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer (ENTEC) and PIADIN (active ingredients: 3.00%–3.25% 1, 2, 4-triazole and 1.50%–1.65% 3-methylpyrazole) under three vastly different textured soils (clayey, loamy and sandy). We hypothesized that i) clay contents have the positive effect on soil N_2O emissions; and ii) DMPP would have a better performance than DCD, PIADIN and ENTEC in reducing soil CO_2 and N_2O emissions and the conversion of $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$ under a range of soils with different textures. The objective of this study was to evaluate the variation in soil CO_2 and N_2O emissions, and $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$

concentrations following the application of above mentioned NIs in clayey (Marsch), loamy (Östliches Hügelland) and sandy (Geest) soils.

2. Materials and methods

2.1 Collection, preparation and characterization of soil

Three soils varying in soil texture were collected from the following three geological regions of Schleswig-Holstein, Germany: 1) the sandy outwash region (Geest, the outwash region is dominated by Brunic Arenosols or Cambisols, Podzols and Gleysols, as well as Histosols, 2) the Weichselian glacial region in the east (Östliches Hügelland, the Weichselian glacial deposits contain very fertile Luvisols, Cambisols, Anthrosols derived from colluvic material, Gleysols and Rheic Histosols), and 3) the marshland with alluvial deposits in the west (Marsch, the marshland includes different types of Fluvic Gleysols and Histosols) (Horn et al., 2020). The selected soils represent the major soil types or climatic zones of the state of Schleswig-Holstein.

Soils from upper 20 cm soil horizon were collected. Visible plant residues and stones were removed by passing through a 2 mm sieve. A representative subsample of each soil was analyzed for salient characteristic viz. particle size distribution (clay, sand and silt), pH, total carbon (C) and N, $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ following standard methods (Table 1).

2.2 Incubation experiment

Four NIs including dicyandiamide (DCD), 3, 4-dimethylpyrazole phosphate (DMPP), ENTEC (nitrogenous mineral fertilizers containing the DMPP ammonium stabilizer) and PIADIN (active ingredients: 3.00%–3.25% 1, 2, 4-triazole and 1.50%–1.65% 3-methylpyrazole) were tested with three different soil type (Marsch, Östliches

Hügelland and Geest). For comparison, a control without the addition of NI was also included for each soil type. The soils were packed into cylindrical pots (15 cm diameter and 33 cm length, sealed at the bottom) to achieve a bulk density of 1.4 g cm^{-3} with 20 cm depth. All the pots were fertilized with $0.5 \text{ g NH}_4^+\text{-N kg}^{-1}$ soil using ammonium sulfate $((\text{NH}_4)_2\text{SO}_4)$ salt in solution form. The NIs were applied at 5% of applied $\text{NH}_4^+\text{-N}$ (i.e. 25 mg kg^{-1} soil in solution form). The control treatment was applied with N fertilizer and deionized water only. Deionized water was added each day to maintain the moisture content equivalent to the water-holding capacity of the soils. Following treatment application, pots were incubated for a period of 57 days in a climatic chamber adjusted to a consistent temperature (15°C), soil moisture (80% soil water-holding capacity) and air humidity (50%).

2.3 Collection and Measurement of Emitted CO_2 and N_2O

The rate of CO_2 and N_2O emissions was calculated by measuring concentration of these gases from each pot at different times. The CO_2 and N_2O samples were collected once a day during the first week, once after two days during the second week and once after three days during rest of the incubation period. For sampling, the pots were closed first and then gas samples were collected at 0, 20, 40, and 60 min. A 10-ml syringe having a hypodermic needle was used to collect the gas samples. The gas samples were stored in pre-evacuated Chromacol glass vials having chloro-butyl rubber lids that prevent the leakage of gas samples. Each gas sampling was carried out between 09:00 and 11:00 am. Except for the times when gas samples were collected, the pots were left open.

The concentrations of CO_2 and N_2O in the gas samples were measured by gas chromatograph (Agilent 7890A GC, Agilent, CA, United States). The rate of CO_2 and

N₂O emissions from each pot (ppm/min) during lid closure was calculated using headspace volume of the pot and a linear relation between CO₂ and N₂O concentrations and time (Venterea et al., 2020). The flux of CO₂-C (μg h⁻¹ kg⁻¹) and N₂O-N (ng h⁻¹ kg⁻¹) was calculated with the following equation:

$$EN_2O-N = \frac{R \times 60 \times V_{gas} \times AR}{W_{soil} \times V_m} \times 2 \times 1000 \quad (1)$$

$$ECO_2-C = \frac{R \times 60 \times V_{gas} \times AR}{W_{soil} \times V_m} \quad (2)$$

Where ECO₂/N₂O is the flux of CO₂-C (μg h⁻¹ kg⁻¹) and N₂O-N (ng h⁻¹ kg⁻¹), R is the rate of CO₂ and N₂O emissions from each pot (ppm/min), V_{gas} is the gas volume in pot (L), W_{soil} is the weight of dry soil in pot (kg), AR is the relative atomic mass of C and N i.e. 12 and 14, respectively, and V_m is the molar volume of gas which is 23.7 L/mol at 15 °C. Total CO₂ and N₂O emissions during the experimental period were calculated from the daily emissions of the gases.

2.4 Analysis of NH₄⁺ and NO₃⁻ in soil

Soil samples (0-20 cm depth) were collected from the pots on day 1, 15, 29, 43 and 57 of incubation for the measurement of NH₄⁺ and NO₃⁻ concentration. Each soil sample was divided into two subsamples; one was oven-dried at 105 °C for 8 h to calculate water content while the other was used for determination of NH₄⁺ and NO₃⁻. For the analysis of soil mineral N, 10 g of fresh soil were mixed with 40 mL of 0.0125 M CaCl₂ solution (1:4) and shaken for 1 h. After centrifugation for 10 min, the extracts were filtered through Whatman filter paper No. 40 and stored at 4 °C. The extracts were analyzed for NH₄⁺ and NO₃⁻ concentrations using a continuous flow analyzer (San⁺⁺ Automated Wet Chemistry Analyzer - Continuous Flow Analyzer (CFA), Skalar, The Netherlands).

2.5 Statistical analysis

Soil CO₂-C and N₂O-N total emissions and NH₄⁺-N and NO₃⁻-N concentrations across different incubation times were compared by one-way Analysis of Variance (ANOVA). Treatment means for total CO₂ and N₂O emissions were compared using two-way ANOVA. The significance of differences between individual means and incubation times were determined using a Tukey's honest significant difference (HSD) test. The statistical analyses were performed by R statistical software (Oakland, CA, USA) at a confidence level of 95% ($P \leq 0.05$).

3. Result

3.1 Fluxes of CO₂-C and N₂O-N

Marsch had the highest CO₂-C flux, followed by Geest and the lowest in Östliches Hügelland (Fig. 1). The flux of CO₂-C progressively decreased with time in all the NIs treatments under Marsch and Geest soils (Fig. 1). In Marsch, DMPP had the lowest CO₂-C flux during the incubation period (Fig. 1). CO₂-C flux did not differ among the NIs treatments during the incubation period in Geest soil (Fig. 1). DCD, DMPP and PIADIN had the lowest CO₂-C flux at 7 days of incubation in Östliches Hügelland soil (Fig. 1).

Marsch had the highest N₂O-N flux, followed by Östliches Hügelland and the lowest in Geest soil (Fig. 2). Marsch and Östliches Hügelland soils applied with DCD and ENTEC had the lowest and the highest N₂O-N flux, respectively than the other NIs during the incubation period (Fig. 2). In Geest, ENTEC showed the highest N₂O-N flux during the incubation period while DMPP and PIADIN showed the lowest N₂O-N flux (Fig. 2).

3.2 Total emissions of CO₂-C and N₂O-N

Total CO₂-C emission during the incubation period was significantly higher from Marsch soil (274 mg kg⁻¹) than Östliches Hügelland soil (54.6 mg kg⁻¹) and Geest soil (60.3 mg kg⁻¹) (Table 2). In Marsch soil, DMPP showed the highest inhibitory effect on CO₂ emission (50%), followed by PIADIN (32%) and the lowest by ENTEC (16%). DCD did not influence CO₂-C emission from Marsch soil (Table 2). In Östliches Hügelland soil, DCD and PIADIN showed the highest and equal inhibitory effect on CO₂ emission (73%), followed by DMPP (64%) and the lowest by ENTEC (36%). The effect of the NIs on CO₂ emission from Geest soils was nonsignificant.

Total N₂O-N emission was also the highest from Marsch soil (8051 µg kg⁻¹), followed by Östliches Hügelland soil (3516 µg kg⁻¹) and the lowest from Geest soil (1313 µg kg⁻¹) (Table 3). In Marsch and Östliches Hügelland soils, DCD showed the stronger inhibitory effect on N₂O-N emission (86 and 47%, respectively) than DMPP (56 and 30%, respectively) and PIADIN (54 and 16%, respectively). In Geest soil, DMPP was more effective in reducing N₂O-N emission (88%), than PIADIN (70%) and DCD (33%).

3.3 Soil NH₄⁺-N and NO₃⁻-N concentrations

In Marsch soil, DCD, DMPP and PIADIN slowed down the nitrification process and maintained higher NH₄⁺-N concentration and lower NO₃⁻-N concentration as compared to control (Fig. 3). ENTEC did not affect NH₄⁺-N and NO₃⁻-N concentrations which showed the same trend as that of control Marsch soil (Fig. 3). NH₄⁺-N concentration decreased and NO₃⁻-N concentration increased progressively during the incubation period in control of Östliches Hügelland soil whereas DCD, DMPP and

PIADIN maintained NH_4^+ -N concentration at high level and kept NO_3^- -N at lower level as compared to control (Fig. 3). Similarly, DCD, DMPP, ENTEC and PIADIN slowed down the decrease of NH_4^+ -N and the increase of NO_3^- -N concentrations as compared to control in Geest soil (Fig. 3). DMPP and PIADIN showed lower NO_3^- -N concentration as compared to DCD and ENTEC in Geest soil (Fig. 3).

4. Discussion

4.1 Effect of soil type

Our result showed that CO_2 -C emissions from Marsch soil was higher than Östliches Hügelland and Geest soils (Fig. 1, Table 2). This may be explained by the fact that Marsch soil had more organic matter and clay content than the other soils, and CO_2 emission is positively linked to soil organic carbon (SOC) content (Volpi et al., 2017). Soil organic carbon content is linearly correlated to soil respiration (Lou and Zhou, 2006). Thomsen et al. (2003) found that the emissions of CO_2 from the sandiest soil was low than the heavier textured soils due to a low content of potentially mineralizable native SOC. Moreover, CO_2 -C emissions from native SOC increased with increasing clay content but this relationship was ascribed to the different mineralogy of the soils (Parfitt et al., 2001).

Marsch soil showed the highest N_2O -N emission, followed by Östliches Hügelland soil and the lowest by Geest soil (Table 3). Organic C and clay content of the three soils also followed the same decreasing order (Table 1). Microbial community structure of a soil plays an important role in defining the rate of nitrification and denitrification in soil (Butterbach-Bahl et al., 2013) and a soil with higher microbial activity could result in higher N_2O emissions (Schaufler et al., 2010). Nitrogen mineralization and N_2O emissions are influenced by soil organic matter (Stehfest and Bouwman, 2006) and

microbial population (Hart et al. 1994). Since nitrification is primarily autotrophic process, with heterotrophic nitrification accounting for only 20% (Liu et al., 2015), C-substrate availability and N₂O emissions from denitrification and nitrification are always positively related to each other (Li et al., 2005, Wan et al., 2009). Abbasi et al. (2011) found that the process of denitrification and production of N₂O were smaller in arable soil deficient in organic carbon compared to grassland soil having plenty of organic carbon. Cébron et al. (2003) reported presence of organic carbon promoted the population of nitrifying bacteria in a clay-textured soil. Thus, higher organic carbon content in Marsch soil could be one possible reason to higher N₂O-emission from this soil.

Nitrification is also directly influenced by soil texture (Stehfest and Bouwman, 2006). A fine-textured soil can retain more water and create frequent and longer anaerobic conditions than a coarse- and medium-textured soil and thus may result in faster denitrification and N₂O emission (Köster et al., 2013). Overall, the cumulative N₂O-N emission from a clay soil was significantly higher than those from the loamy soil (Köster et al., 2013) and therefor N₂O emissions increase with increasing clay content.

4.2 Effect of NIs

Except for CO₂-C emission from Geest soil and N₂O-N emission from ENTEC treated soil, NIs significantly decreased CO₂-C and N₂O-N emissions in all other cases but magnitude of inhibitory effect did not show any specific consistent trend with the soil texture (Table 2 and 3). Different results have been reported by the previous researchers regarding the relative efficacy of NIs in varying textured soils. Barth et al. (2019) found that NIs are more efficient in light soils than heavy soils whereas Akiyama

et al. (2010) found that the effectiveness of NIs was relatively consistent across the various soil types. According to Fisk et al. (2015), efficacy DMPP and DCD diminished with the addition of soil organic matter, and Marsden et al. (2016) reported decreased efficacy with higher clay content. Volpi et al. (2017) found that NIs were effective only in the soil with the highest nitrification activity and the lowest clay content.

In our study, we found that the comparative effectiveness of the NIs in inhibiting CO₂-C and N₂O-N emission depended on soil type (Table 2 and 3). DCD had the highest inhibitory effect on soil nitrification in clay soil of Marsch, followed by loam soil of Östliches Hügelland and lowest in sandy soil of Geest (Table 3). Ernfors et al. (2014) reported that the efficacy of DCD was soil specific whereas Wakelin et al. (2014) found that the efficacy of DCD was not related to the soil type. The lowest efficacy of DCD in sandy soil as found in our study may be explained by the lowest decomposition of this NIs in sandy soil as compared to heavy textured soils (Gilsanz et al., 2016). Moreover, McGeough et al. (2016) found that DCD may not be an effective NI under heterotrophic nitrification which is proportionally dominant form of nitrification in sandy soil as compared to a heavy textured soil.

DMPP was reported to be highly specific and more effective in inhibiting nitrification as compared to DCD (Gilsanz et al., 2016). We found that the inhibitory effect of DMPP on N₂O emission was more pronounced in the sandy soil of Geest than in the loamy and clay soils (Table 3). In short-term incubation experiments, Barth et al. (2001) found that decreasing sand content reduced the efficacy of DMPP to retard NH₄⁺ oxidation. They found that adsorption capacity of DMPP is positively correlated with clay content, and that a lower effectiveness of DMPP may be due to the adsorption of the NI on silt and clay particles. In their later study, the same group of researchers reported that DMPP had more pronounced inhibition of N₂O emission in the sandy than

the loam soil (Barth et al., 2008). The same explanation as given above for the effect of DMPP stands true for PIADIN which showed the same trend of effectiveness in different textured soils as that of DMPP. DMPP has relatively low mobility (Barth et al. 2001, 2008; Di et al. 2007, 2010), mineralized slowly and thus have long lasting inhibitory effect on nitrification than DCD (Weiske et al. 2001; Irigoyen et al. 2003).

Soil texture may affect organic carbon turnover by adsorption of organic carbon onto surfaces of clay or organic complexes (Oades, 1988). Thus, the effect of different NIs on organic carbon decomposition could be also affected by soil texture. Our result showed that DCD could not influence the soil organic carbon decomposition in Marsch soil and thus CO₂-C emission from DCD treated soil was the same as that from control soil (Table 3). DMPP has the best inhibitory effect on the organic carbon decomposition in Marsch soil. The NIs of DCD, DMPP and PIADIN effectively inhibited the decomposition of organic carbon in Östliches Hügelland soil and resulted in the lowest CO₂-C emissions (Table 3). ENTEC has the smallest inhibitory effect on the soil organic carbon decomposition in Östliches Hügelland soil.

5. Conclusion

CO₂-C and N₂O-N emissions are higher from N fertilized clayey soils than the lighter textured loamy and sandy soils. DCD was proved the most effective NIs in decreasing CO₂-C and N₂O-N emissions and inhibiting nitrification in the clayey and loamy soils. On the other hand, DMPP and PIADIN could decrease CO₂-C and N₂O-N emissions more effectively in the sandy soil. ENTEC remained least effective in inhibiting CO₂-C emission whereas did not inhibited N₂O-N emission from the studies soils. The future research should focus on the microbial dynamics in the different texture soil amended with various nitrification inhibitors.

It is concluded that clayey soil has more gaseous emissions and NIs perform different in dependence of soil texture to inhibit soil nitrification and gaseous emissions. DCD is a better NI for clay and loamy soils while DMPP and PIADIN are better for sandy soil to inhibit soil nitrification and gaseous emissions. ENTEC is ineffective in all soil texture.

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Table 1. Salient characteristics of the experimental soils.

Characteristic	Marsch	Östliches Hügelland	Geest
Silt (%)	10.6±3.9	22.3±9.1	19.2±7.8
Sand (%)	44.3±5.4	62.3±8.7	75.0±6.6
Clay (%)	45.1±3.8	15.4±4.6	5.8±2.3
Texture	clayey	loamy	sandy
Total C (g kg ⁻¹)	20.8±0.34	12.0±0.50	13.2±0.19
Total N (g kg ⁻¹)	1.37±0.01	1.13±0.05	1.11±0.02
NH ₄ ⁺ -N (mg kg ⁻¹)	0.86±0.02	1.39±0.06	3.56±0.06
NO ₃ ⁻ -N (mg kg ⁻¹)	10.9±0.32	13.47±0.59	0.08±0.02

Values (mean±SE, n = 4)

Table 2. Total CO₂-C emission during the incubation period from Marsch, Östliches Hügelland and Geest soils applied with control, DCD, DMPP, ENTEC and PIADIN under controlled conditions.

NI	Marsch		Östliches Hügelland		Geest	
	Emission (mg kg ⁻¹)	Decrease (%)	Emission (mg kg ⁻¹)	Decrease (%)	Emission (mg kg ⁻¹)	Decrease (%)
Control	274±1.3a	-	54.6±2.3e	-	60.3±0.4e	-
DCD	261±11.5a	5	14.8±1.7g	73	64.4±1.2e	-6.8
DMPP	136±3.8d	50	19.8±0.3g	64	59.6±4.4e	1.2
ENTEC	231±11.3b	16	35.1±0.5f	36	65.8±1.4e	-9.1
PIADIN	187±8.4c	32	14.5±1.0g	74	65.0±3.3e	-7.8

The values (mean±SE) are means of four independent pot replicates. The values indicated with the same lower-case letter(s) are not significantly different at P = 0.05.

Table 3. Total N₂O-N emissions during the incubation period from Marsch, Östliches Hügelland and Geest soils applied with control, DCD, DMPP, ENTEC and PIADIN under controlled conditions.

NI	Marsch		Östliches Hügelland		Geest	
	Emission (mg kg ⁻¹)	Decrease (%)	Emission (mg kg ⁻¹)	Decrease (%)	Emission (mg kg ⁻¹)	Decrease (%)
Control	8051±279b	-	3516±24d		1313±19i	-
DCD	1157±222i	86	1861±102g	47	879±34j	33
DMPP	3533±81d	56	2467±74f	30	157±23k	88
ENTEC	9849±280a	-22	4169±42c	-18	1569±17h	-19
PIADIN	3708±408dc	54	2960±105e	16	387±83k	70

The values (mean±SE) are means of four independent pot replicates. The values indicated with the same lower-case letter(s) are not significantly different at P = 0.05.

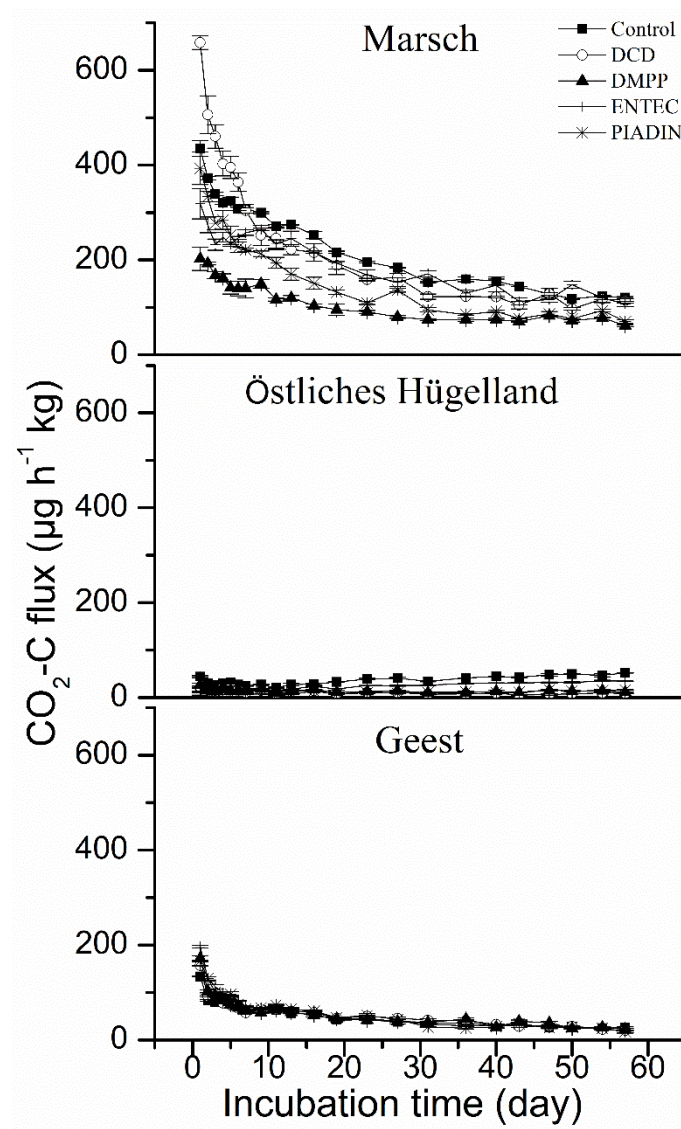


Fig. 1. The flux of CO₂-C emission as affected by control, DCD, DMPP, ENTEC and PIADIN in Marsch, Östliches Hügelland and Geest soils. The data points are means of four independent pot replicates and error bars represent standard errors of the means (n=4).

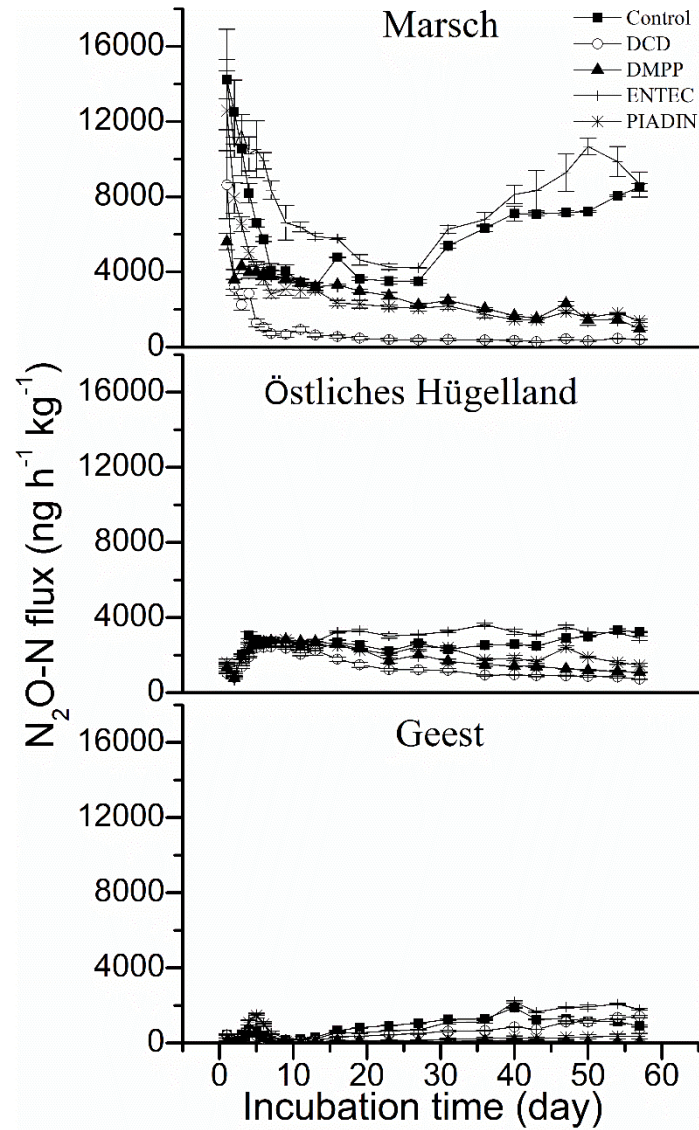


Fig. 2. The flux of $\text{N}_2\text{O-N}$ emission as affected by control, DCD, DMPP, ENTEC and PIADIN in Marsch, Östliches Hügelland and Geest soils. The data points are means of four independent pot replicates and error bars represent standard errors of the means (n=4).

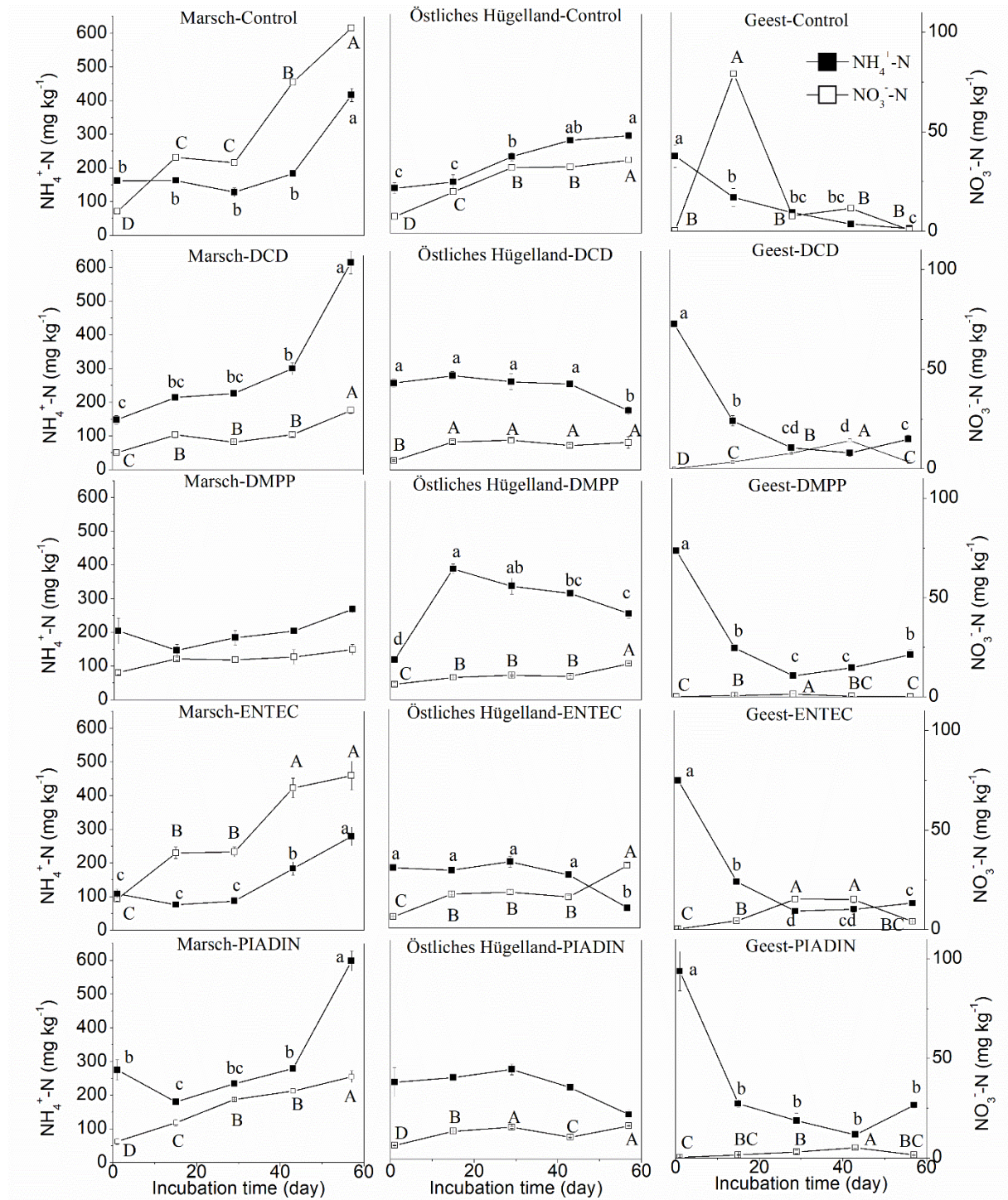


Fig. 3. Changes in $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations in Marsch, Östliches Hügelland and Geest soil applied with control, DCD, DMPP, ENTEC and PIADIN. The data values are means of four independent pot replicates and error bars represent standard errors of the means ($n=4$). Incubation time indicated by the same lower-case (NH_4^+) or upper-case (NO_3^-) letter(s) do not differ significantly at $p \leq 0.05$.

Chapter 6: General Discussion**6.1 Effects of soil temperature and moisture on nitrous oxide emissions and the efficacy of nitrification inhibitors**

The nitrification rate of control increased with the increased of temperature in our experiment. Sahrawat (2008) reported that nitrification generally with an optimum temperature ranging from 25 to 35 °C. Previous had shown that variation in soil temperatures may result in changes in soil microbial populations for denitrification (Braker et al., 2010) or differential expression of the enzymes responsible for different $\text{N}_2\text{O}/\text{N}_2$ emissions during denitrification (Saleh-Lakha et al., 2009). Phillips et al. (2015) found that the denitrified N shifts toward N_2O when temperature increases, different with our result that denitrified N shifts toward N_2 when temperature increases in our research. So N_2O -N total production of control was decreased with the increased of temperature (Chapter 2: Table 2).

The denitrification rate of control increased with the increased of moisture. NH_4^+ -N concentrations in the end of experiment in 80% WHC is significantly greater than that in 60% WHC and the NO_3^- -N concentrations in the end of experiment in 80% WHC is significantly smaller than that in 60% WHC (Chapter 2: Fig. 2, 3, 4 and 5). The denitrifying enzymes are produced in anaerobic conditions (van Spanning et al., 2007). The activities of the denitrification enzymes are inhibited when the concentration of O_2 is high (60% WHC) (van Spanning et al., 2007). Denitrification dominated the emission from soil with a high soil moisture (Bateman and Baggs, 2005; Diba et al., 2011). N_2O emissions usually increase in wetter soils as the soils become more anaerobic (Smith et al., 1998; Dobbie et al., 1999; Dobbie and Smith, 2001). However, other studies (Maag and Vinther, 1996; Zhu et al., 2013) found that N_2O emissions from nitrification also

increased with increasing soil moisture content or decreasing oxygen concentration by growth of AOB communities (Di et al., 2014). N₂O-N total production of control in 80% WHC is significantly greater than that in 60% WHC. N₂O-N total production of control was increased with the increased of soil moisture (Chapter 2: Table 2).

In the present investigation the inhibitory effects of DCD, DMPP, ENTEC and PIADIN decreased with the increased of temperature, so the decreased N₂O-N emissions in 25 °C is significantly smaller than that in 15 °C (Chapter 2: Table 2). This is confirmed by Lan et al. (2018) who observed that the inhibitory effects of NIs decreasing with increasing temperature from 15 to 35 °C. DCD and DMPP are the two most commonly used NIs (Ting et al., 2018). Evidence has showed that declining inhibitory efficacy with increasing soil temperature of DMPP (Menendez et al., 2012) and DCD (Di et al., 2014). Increase soil temperatures can lower the NIs efficiency owing to the faster molecular degradation (Ting et al., 2018). On the one hand, high temperatures hastened NI degradation in soil (Di et al., 2014; Menendez et al., 2012; Puttanna et al., 1999).

The inhibitory effects of DCD and ENTEC increased with higher moisture, so the decreased N₂O-N emissions in 80% WHC is significantly greater than that in 60% WHC. The inhibitory effects of DMPP and PIADIN were same in 80% WHC and 60% WHC (Chapter 2: Table 2). DCD was highly effective in inhibiting the growth of AOB communities, and reducing N₂O emissions at anaerobic condition (Di et al., 2014). The nitrification inhibitor DCD reduced N₂O emission by 60-80% (Di and Cameron, 2002, 2006; Smith et al., 2008) and 44%-65% (de Klein et al., 2011) and 11%-96% (Kumar et al., 2000), which could be attributed to the inhibits ammonia oxidizer growth and activity by deactivating the ammonia monooxygenase enzyme (Di et al., 2009, 2010). DCD works as a nitrification inhibitor by blocking the active site of the ammonia

monooxygenase enzyme (Edmeades, 2004). It has been shown that N₂O emissions can be decreased by 37 and 62 % (Wolf et al., 2014) and 30 to 45 % (Parkin and Hatfield, 2010) by the use of PIADIN with stabilization of ammonium and lower rates of nitrification and denitrification (Subbarao et al., 2006). DMPP was demonstrated as one of the most efficient NIs (Menendez et al., 2012; Benckiser et al., 2013).

The inhibitory effects of DCD is smaller than DMPP and PIADIN at all temperature (15 °C and 25 °C) and moisture (60% and 80%) in the present study under controlled environment (Chapter 2: Table 2). Weiske et al. (2001) also observed a more significant inhibition of DMPP in N₂O emissions than DCD, but Di and Cameron (2012) cannot find any difference between DCD and DMPP in terms of inhibition of N₂O emission. The inhibitory effects of DCD is greater than ENTEC at all temperature (15 °C and 25 °C) and moisture (60% and 80%).

DMPP and PIADIN had the best inhibit efficiency, and DMPP and PIADIN could maintain the NH₄⁺-N concentrations at a higher level and maintain the NO₃⁻-N concentrations at the lower level in all temperature (15 °C and 25 °C) and moisture (60% and 80%). N₂O-N emissions were low throughout the trial period of the DMPP and PIADIN at all temperature (15 °C and 25 °C) and all moisture (60% and 80%) in the present study.

6.2 Effects of soil chloride on nitrous oxide emissions

It was shown that different Cl⁻ salts and concentrations had varying effects on soil NH₄⁺ and NO₃⁻ dynamics and CO₂ and N₂O emissions compared with the control (Chapter 3: Table 2). The Cl⁻ ions can suppress nitrification, and this effect has long been recognized (Golden et al., 1981; Darrah et al., 1987; McGuire et al., 1999). Cl⁻ and its different derivatives can act as strong oxidizers and potent biocides (Chen and

Wong, 2004). Pereira et al. (2013) found that AlCl_3 treatment had no effect on nitrification, but significantly reduced cumulative NH_3 emissions. In contrast, Azam and Müller (2003) demonstrated that NaCl significantly decreased N_2O and CO_2 emissions. Different Cl^- salts have varying functions on soil nitrification and denitrification. In the present study, 0.5 and 1.0 g kg^{-1} KCl-Cl and 0.5 g kg^{-1} $\text{MgCl}_2\text{-Cl}$ decreased $\text{CO}_2\text{-C}$ emissions and increased $\text{N}_2\text{O-N}$ emissions compared with the control. 1.0 g kg^{-1} $\text{MgCl}_2\text{-Cl}$ decreased both $\text{CO}_2\text{-C}$ and $\text{N}_2\text{O-N}$ emissions and retarded the decrease of $\text{NH}_4^+\text{-N}$ concentration and the increase of $\text{NO}_3^-\text{-N}$ concentration (Chapter 3: Table 2; Fig. 1 and 2). The decrease in nitrification by 1.0 g kg^{-1} $\text{MgCl}_2\text{-Cl}$ caused the slowdown in the decrease of $\text{NH}_4^+\text{-N}$ concentration and the increase of $\text{NO}_3^-\text{-N}$ concentration (Chapter 3: Fig. 2). Some studies have shown that aluminum addition inhibits nitrification by lowering the soil pH values, which reduces the NH_4^+ availability for nitrification in the short term and directly inhibits the effects of Mg (Pereira et al., 2013). Undergoing some (chemical or biological) changes in soil, Cl^- might produce other reactive compounds similar to chlorate or chlorite that are toxic for nitrifying bacteria (Souri, 2010). Cl^- could inhibit nitrification by increasing solute concentration and osmotic pressure in soil solution (Souri, 2010) through increase the osmotic pressure of the soil (Darrah et al., 1987; Golden et al., 1981). Soil salinity could restrict the oxidative processes and development of anaerobic microsites, denitrification will mainly be inhibited in this situation (Azam and Müller, 2003). The chlorate selectively inhibited the activity of nitrite-oxidizing bacteria but not ammonia-oxidizing bacteria, so nitrites became the dominant product (Xu et al., 2011). Chlorate is toxic to the fungi, algae, invertebrates, fish, and higher plants (Stauber, 1998; Jiang et al., 2009) but it is easily biodegraded by nitrate reductase in an organic-rich environment (Xu et al., 2011). However, Cl^- did not have such a strong NI effect as PIADIN. PIADIN decreased the

CO₂-C and N₂O-N emissions and preserved the soil NH₄⁺-N concentration at a high level and NO₃⁻-N concentration at a low level in the present investigation (Chapter 3: Table 2; Fig. 1 and 2).

6.3 Effects of soil biogas residues and acidified biogas residues on nitrous oxide emissions and the efficacy of nitrification inhibitors

In the present study biogas residues and acidified biogas residues significantly increased the emissions of CO₂-C and N₂O-N compare without fertilizer under control conditions, but the ABR has a smaller effect compare with BR on increased CO₂-C and N₂O-N emissions (Chapter 4: Table 2; Fig. 1 and 2). Fangueiro et al. (2015) calculate that H₂CO₃ would increase from 9% to 91% after pH decrease from 7.5 to 5.5, based only on this calculation, lowering the pH from 7.5 to 5.5 would lead to an increase the emissions of CO₂ (10 times greater). But Fangueiro et al. (2013) observed lower CO₂ emissions from acidified than from non-acidified over the whole storage period, same with our result. Such decreased CO₂-C and N₂O-N emissions may be due to the lower microbial activity in the soil, with a negative impact on nutrients cycling (Fangueiro et al., 2015).

Fangueiro et al. (2015) also found that an obvious way to minimize ammonia emissions from slurry is to decrease slurry pH by addition of acids or other substances, which minimize the concentration of NH₃ relative to NH₄⁺, namely by lowering the slurry pH. The microbial community must also be influenced or, at least, changed in its activity levels by acidification (Ottosen et al., 2009). BR and ABR increased the NH₄⁺-N concentrations and NO₃⁻-N concentrations compare without fertilizer under the control (Chapter 4: Fig. 2). ABR significantly decreased the soil pH (Chapter 4: Fig. 3). However, only a limited number of studies (Ottosen et al., 2009; Zhang et al., 2011)

can be found in relation to the impact of acidification on the composition and activity of the microbial community. Acidification of organic slurry has been used commonly since 2010 in countries such as Denmark (Fangueiro et al., 2015). DMPP and PIADIN could significantly decreased the emissions of CO₂-C and N₂O-N compare with control under BR and ABR. DMPP and PIADIN could retarded the decrease of NH₄⁺-N concentrations and the increase of NO₃⁻-N concentrations compare with control under BR and ABR. The effect of DMPP is much better compare with PIADIN.

6.4 Effect of soil type on nitrous oxide emissions and the efficacy of nitrification inhibitors

Comparing typical soil types of North Germany demonstrated that CO₂-C emissions of a Marsch soil is higher compared with soils originated from the regions of Geest and Östliches Hügelland (Chapter 5: Table 2; Fig. 1). Nitrification also was influenced by soil texture (Stehfest and Bouwman, 2006). Marsch soil has the highest N₂O-N emissions, Geest soil has the lowest N₂O-N emissions, and Östliches Hügelland has the intermediate N₂O-N emissions (Chapter 5: Table 3; Fig. 2). The most reason is that Marsch soil has more clay content, organic matter and a higher water content. The CO₂ emissions were positively linked to SOC content in soil (Volpi et al., 2017). Nitrogen mineralization, N₂O emissions and nitrification appear to be influenced by soil organic matter (Stehfest and Bouwman, 2006) and microbial establishment (Hart et al., 1994). There are a positive relationship between C-substrate availability and N₂O emissions of denitrification and nitrification (Tiedje, 1988; Robertson and Klemmedtsson, 1996; Li et al., 2005) and CO₂ emissions (Lou and Zhou, 2006). Emissions are mostly higher for fine-textured soil than for coarse- and medium-textured soils, because of the more frequent occurrence of anaerobic conditions associated with higher water contents

(Parton et al., 1996; FAO, 2001).

DCD, DMPP and PIADIN could inhibit the soil nitrification, so the N_2O -N emissions was lower compared with control conditions under Marsch, Geest and Östliches Hügelland. However, it was shown that soil texture had big effect on the inhibitory effect of Nis (Chapter 5: Table 3; Fig. 2). The present study showed that DCD has the best effect on inhibit the soil nitrification under clay soil of Marsch and loam soil of Östliches Hügelland. DCD has the smallest effect on inhibit the soil nitrification under sandy soil of Geest (Chapter 5: Table 3; Fig. 2). The organic matter content of the soil affects the efficacy of DCD as a nitrification inhibitor (McGeough et al., 2016). Singh et al. (2008) found that as soil organic matter content increased the decomposition rates of DCD and the efficacy of DCD in reducing N_2O emissions decreased, which could be attributed to both DCD adsorption on organic matter surfaces and the microbial activities of the soils. McGeough et al. (2016) found that DCD may not be as effective under heterotrophic nitrification dominates. DMPP strongly inhibited nitrification in the sandy soil (Barth et al., 2008). This could be confirmed in the present study, DMPP has the best effect on inhibit the soil nitrification under sandy soil of Geest. It is very likely that the adsorption of DMPP to inorganic soil constituents was a crucial factor under these conditions (Barth et al., 2008). Nitrification was less inhibited in the soil with the higher clay and silt content, where DMPP may have been adsorbed (Barth et al., 2008). The same authors reported that adsorption capacity is positively correlated with clay content, and that a slowdown of the effectiveness of DMPP may be due to the adsorption of the NI on clay particles and soil components. PIADIN and DMPP has the smallest effect on inhibit the soil nitrification under Marsch. ENTEC could promote the soil nitrification. This might be caused by the fertilizer component which was included in ENTEC.

6.5 General Conclusions

The nitrification inhibitors are useful to increase fertilizer use efficiency and reduce the emissions of greenhouse gas of CO₂-C and N₂O-N by maintaining NH₄⁺-N concentration at a high level and NO₃⁻-N concentration at a low level at both temperature and soil moisture. The efficacies of all the nitrification inhibitors are higher at low temperature and high water-holding capacity in our sandy soil. DMPP and PIADIN are more effective than DCD and ENTEC for mitigation the greenhouse gas of CO₂-C and N₂O-N in a sandy soil.

MgCl₂ amendment and acidified the biogas residues could be useful to reduce the emissions of greenhouse gas of CO₂-C and N₂O-N and fertilizer the soil. DCD is a good choice to use for mitigation the greenhouse gas when the soil is more clay. DMPP and PIADIN are good choice to use for mitigation the greenhouse gas when the soil is more sandy.

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Chapter 7: Summary

The mineral N fertilizer (NH_4^+) and the organic N fertilizer (biogas residues) applied to fertilize soils may lead to higher CO_2 and N_2O emissions. Nitrification inhibitors (NIs) and chloride are used to curb this effect. However, their effectiveness is strongly influenced by environmental factors, soil and fertilizer properties. This study investigated how different NIs and chlorides behave under different environmental factors, soil and fertilizer properties.

In an incubation experiment the efficacies of four NIs (DCD, DMPP, ENTEC and PIADIN) and two chlorides (KCl and MgCl_2) under different environmental factors (two soil temperatures (15 and 25 °C), two moisture levels (60% and 80% water holding capacity)), three types of soils (clay, loam and sand) and two types of fertilizer (biogas residues and acidified biogas residues) were tested for 8 weeks. The soil received 0.5 g $\text{NH}_4^+\text{-N kg}^{-1}$ soil and the inhibitors were applied at 5% of the applied N. KCl and MgCl_2 were applied at 0.5 and 1.0 g kg^{-1} , respectively.

The results of this study show that DMPP and PIADIN were more effective than DCD and ENTEC in inhibiting nitrification in sandy soils at 15 and 25 °C and at 60% and 80% water holding capacity. The efficacy of all nitrification inhibitors improved with increasing soil moisture, while increasing temperatures had various negative effects on the sandy soil used. 1.0 g kg^{-1} MgCl_2 reduced both CO_2 and N_2O emissions. The acidified biogas residues had less influence on the increased CO_2 and N_2O emissions compared to the unacidified biogas residues. DMPP and PIADIN were able to significantly reduce the emissions of CO_2 and N_2O compared to the control treatment when applied as biogas residues and acidified biogas residues. DCD reduced CO_2 and N_2O emissions in clay and loam soil. DMPP and PIADIN reduced N_2O emissions in

sandy soil. ENTEC increased N₂O emissions in clay, loam and sandy soils.

The NIs, MgCl₂ amendment and acidification of biogas residues are useful to increase fertilizer use efficiency and to mitigate CO₂ and N₂O emissions. If the soil contains more clay, DCD is more efficient. If the soil contains more sand, strong inhibition occurs with DMPP. It was demonstrated in the present study that efficacy of nitrification inhibitors strongly depends on soil texture.

Chapter 8: Zusammenfassung

Der mineralische $\text{NH}_4^+\text{-N}$ haltige Dünger und der organische N-Dünger Biogastrückstand, die bei der Düngung von Böden angewendet werden, können zu höheren CO_2 - und N_2O -Emissionen und einer höheren NO_3^- -Auswaschung führen. Nitrifikationsinhibitoren (NIs) und Chlorid können verwendet werden, um diesen Effekt einzudämmen. Ihre Wirksamkeit wird jedoch stark von den Umweltfaktoren, den Boden- und Düngemiteleigenschaften beeinflusst. Diese Studie untersuchte, wie sich unterschiedliche NIs und Chloride unter verschiedenen Umweltfaktoren, Boden- und Düngemiteleigenschaften verhalten.

In einem Inkubationsexperiment wurden die Wirksamkeiten von vier NIs (DCD, DMPP, ENTEC und PIADIN) und zwei chloridhaltigen Salzen (KCl und MgCl_2) unter verschiedenen Umweltfaktoren (zwei Bodentemperaturen (15 und 25 °C), zwei Feuchtigkeitsniveaus (60% und 80% Wasserhaltekapazität)), drei Arten von Böden (Ton, Lehm und Sand) und zwei Düngern (Biogastrückstände und angesäuerte Biogastrückstände) jeweils acht Wochen lang getestet. Der Boden erhielt $0,5 \text{ g NH}_4^+\text{-N kg}^{-1}$ Boden und die Inhibitoren wurden mit 5% des aufgebrauchten N eingesetzt. KCl und MgCl_2 wurden mit $0,5$ und $1,0 \text{ g kg}^{-1}$ angewendet.

Die Ergebnisse dieser Studie zeigen, dass DMPP und PIADIN bei der Hemmung der Nitrifikation in sandigen Böden bei 15 und 25 °C sowie bei einer Wasserhaltekapazität von 60% und 80% wirksamer als DCD und ENTEC waren. Die Wirksamkeit aller Nitrifikationsinhibitoren verbesserte sich mit zunehmender Bodenfeuchtigkeit, während steigende Temperaturen verschiedene negative Auswirkungen auf den verwendeten sandigen Boden hatten. $1,0 \text{ g kg}^{-1} \text{ MgCl}_2$ verringerten sowohl die CO_2 - als auch die N_2O -Emissionen. Die Ansäuerung von Biogastrückständen reduzierte die

CO₂- und N₂O-Emissionen. DMPP und PIADIN konnten die Emissionen von CO₂ und N₂O im Vergleich zur Kontrollbehandlung unter Biogastrückständen und angesäuerten Biogastrückständen signifikant senken. DCD verringerte die CO₂- und N₂O-Emissionen im Ton- und Lehmboden. DMPP und PIADIN verringerten die N₂O-Emissionen im sandigen Boden. ENTEC erhöhte die N₂O-Emissionen in Ton-, Lehm- und Sandböden.

Die NIs, MgCl₂- und die Ansäuerung von Biogastrückständen sind nützlich, um die Düngemittelnutzungseffizienz zu erhöhen und die CO₂- und N₂O-Emissionen zu reduzieren. Enthält der Boden mehr Ton, ist DCD besser geeignet. Bei sandigen Böden tritt eine starke Hemmung mit DMPP ein. Es konnte gezeigt werden, dass die Wirkung von Nitrifikationshemmern von der Bodenart abhängig ist.

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Publications

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