RESEARCH PAPER

Soil Use and Management | British Society of Soil Science | WILEY

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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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 behaviour 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 analysed 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 CO_2-C emissions by 43%–46% and increased N_2O-N emissions by 15%–48%, whereas 1.0 g kg^{-1} $MgCl_2$ decreased CO_2-C emissions by 72% and N_2O-N emissions by 19%. KCl and $MgCl_2$ retarded the decrease of the NH_4^+-N concentration and increase of the NO_3^--N concentration. PIADIN reduced the emissions of CO_2-C by 113% and N_2O-N by 97% and maintained a high soil NH_4^+-N concentration and low NO_3^--N concentration. $MgCl_2$ addition at 1.0 g kg^{-1} was an effective treatment as the Mg both fertilized the soil and inhibited CO_2-C and N_2O-N emissions. Moreover, 1.0 g kg^{-1} $MgCl_2$ could retard soil nitrification, the decrease of NH_4^+-N concentration and the increase of NO_3^--N concentration. While PIADIN had no fertilizing value, it was a more effective nitrification inhibitor than Cl^- salts.

KEYWORDS
KCl, $MgCl_2$, N_2O emissions, nitrification, nitrification inhibitor

1 | INTRODUCTION

Nitrogen (N) is one of the most important nutrients in farming systems (Fixon & 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 sulphate (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 & 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 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).

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 & Ely, 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 & Ely, 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, Ito, et al., 2006) and reduce associated N_2O emissions and NO_3^- leaching (Subbarao, Ishikawa, et al., 2006).

The use of nitrification inhibitors (NIs) is one of the most common practices to inhibit nitrification (Abbasi et al., 2003; Di & 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; Pereira et al., 2013; Serna et al., 2000). 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 & Blennerhassett, 2010; Zaman et al., 2008, 2009). 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 & Rao, 1998; Westerman & Tucker, 1974). The inhibition of nitrification by salts results in accumulation of NH_4^+-N and increased availability of N to plants (Azam & 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 (Chen & Wong, 2004; Wong et al., 1988). 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 (Chen & Wong, 2004; Chowdhury et al., 2011; Darrah et al., 1987; Golden et al., 1981; McGuire et al., 1999; Wade, 1997; Wickramasinghe et al., 1985; Yuan et al., 2007). 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 $MgCl_2$ could decrease soil CO_2 and N_2O emissions to a level that is comparable with commercial NIs since KCl and $MgCl_2$ are often used to fertilize soil. Therefore, it is important to investigate the effect of different Cl^- salts (KCl and $MgCl_2$) under different Cl concentrations (0.5 and 1.0 g kg^{-1}) on soil NH_4^+ and NO_3^- dynamics and CO_2 and N_2O emissions compared with PIADIN.

The aim of this study was to (a) determine the effects of different Cl^- salts on soil NH_4^+ and NO_3^- dynamics and CO_2 and N_2O emissions, (b) determine the effects of different

Cl^- concentrations on soil NH_4^+ and NO_3^- dynamics and CO_2 and N_2O emissions, and (c) compare Cl^- with PIADIN to identify the optimal NI. We hypothesized that (a) different Cl^- salts have varying ability to reduce the CO_2 and N_2O emissions, (b) Cl^- concentration can also affect the CO_2 and N_2O emissions, and (c) Cl^- is useful to reduce the CO_2 and N_2O emissions compare with PIADIN. These findings may help us to decrease CO_2 and N_2O 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 analysed for the following initial soil properties following standard methods: particle size distribution (sand and silt), pH, total carbon (C) and N, NH_4^+-N, NO_3^--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); $MgCl_2$-Cl, 0.5 g kg^{-1} soil (0.5MgCl$_2$); $MgCl_2$-Cl, 1.0 g kg^{-1} soil (1.0MgCl$_2$); 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 NH_4^+-N kg^{-1} soil using ammonium sulphate salt (($NH_4)_2SO_4$) 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 a.m. The pots were left open outside of the sampling periods.

The concentrations of CO_2 and N_2O in the gas samples were measured by gas chromatography (Agilent 7890A GC; Agilent). An electron capture detector (ECD) was used to detect the N_2O, and the ECD temperature was set at 300°C with an N_2 carrier gas. A thermal conductivity detector (TCD) was used to detect the CO_2 concentration, with a TCD temperature of 250°C and a He carrier gas. The CO_2 and N_2O concentrations per minute from each pot (ppm min^{-1}) during lid closure were calculated using headspace volume and a linear relation between the CO_2 and N_2O concentration and time (Venterea et al., 2020). The flux of CO_2-C (µg hr^{-1} kg^{-1}) and N_2O-N (ng hr^{-1} kg^{-1}) was calculated with the following equations:

$$EN_2O - N = \frac{R \times 60 \times V_{gas} \times AR}{W_{soil} \times V_m} \times 2 \times 1,000, \quad (1)$$

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

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)

where ECO_2-C and EN_2O-N are the CO_2-C ($\mu g \ hr^{-1} \ kg^{-1}$) and N_2O-N (ng $hr^{-1} \ kg^{-1}$) fluxes, respectively; R represents the CO_2 or N_2O emissions from each pot (ppm min^{-1}); 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^{-1} at 15°C.

Total CO_2 and N_2O emissions during the experimental period were calculated from the daily emissions of the gases. We used the fluxes of CO_2-C ($\mu g \ hr^{-1} \ kg^{-1}$) and N_2O-N (ng $hr^{-1} \ kg^{-1}$) multiplied by the number of hours during sampling (24 hr in the first week, 48 hr in the second week and 72 hr in the last six weeks) and added all of them together to obtain the total emissions (mg kg^{-1} or $\mu g \ kg^{-1}$).

2.4 | Analysis of NH_4^+ and NO_3^- 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 NH_4^+ and NO_3^- concentrations.

Each soil sample was divided into two subsamples; one was oven-dried at 105°C for 8 hr to calculate the soil water content, while the other was used for the determination of NH_4^+ and NO_3^- concentrations. For the analysis of soil mineral N, 10 g of fresh soil were mixed with 40 ml of 0.0125 M $CaCl_2$ solution (1:4) and shaken for 1 hr. 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 analysed for NH_4^+ and NO_3^- concentrations using a continuous flow analyser (San^{++} Automated Wet Chemistry Analyzer–Continuous Flow Analyzer, Skalar).

2.5 | Statistical analysis

We used one-way and repeated measures analysis of variance (ANOVA) to test the effects of incubation time on soil CO_2-C and N_2O-N flux. Treatment means for total CO_2 and N_2O emissions were compared using one-way analysis of variance. Incubation time means for NH_4^+-N and NO_3^--N concentrations were compared using one-way analysis of variance. Differences between individual treatments and incubation time were determined using Tukey's honest significant difference (HSD) test. The statistical analyses were performed by R statistical software 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 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). The variance decomposition of N_2O emissions was computed using the 'VARPART' function of the 'vegan' library for R (Oksanen & O'Hara, 2005).

3 | RESULTS

3.1 | Fluxes of CO_2-C and N_2O-N

The flux of CO_2-C and N_2O-N first significantly increased and then significantly decreased in the control and the KCl and $MgCl_2$ treatments (Table 2 and Figure 1). The maximum fluxes occurred after 25 and 15 days of incubation for CO_2-C and N_2O-N in the control. PIADIN addition significantly maintained the CO_2-C and N_2O-N flux ($p > 0.05$) at a low level around 0 throughout the incubation period (Figure 1).

0.5 KCl, 1.0 KCl, 0.5 $MgCl_2$ and 1.0 $MgCl_2$ had smaller CO_2-C fluxes compared with the control before 40 days (Figure 1) but had greater CO_2-C fluxes than the control after 40 days
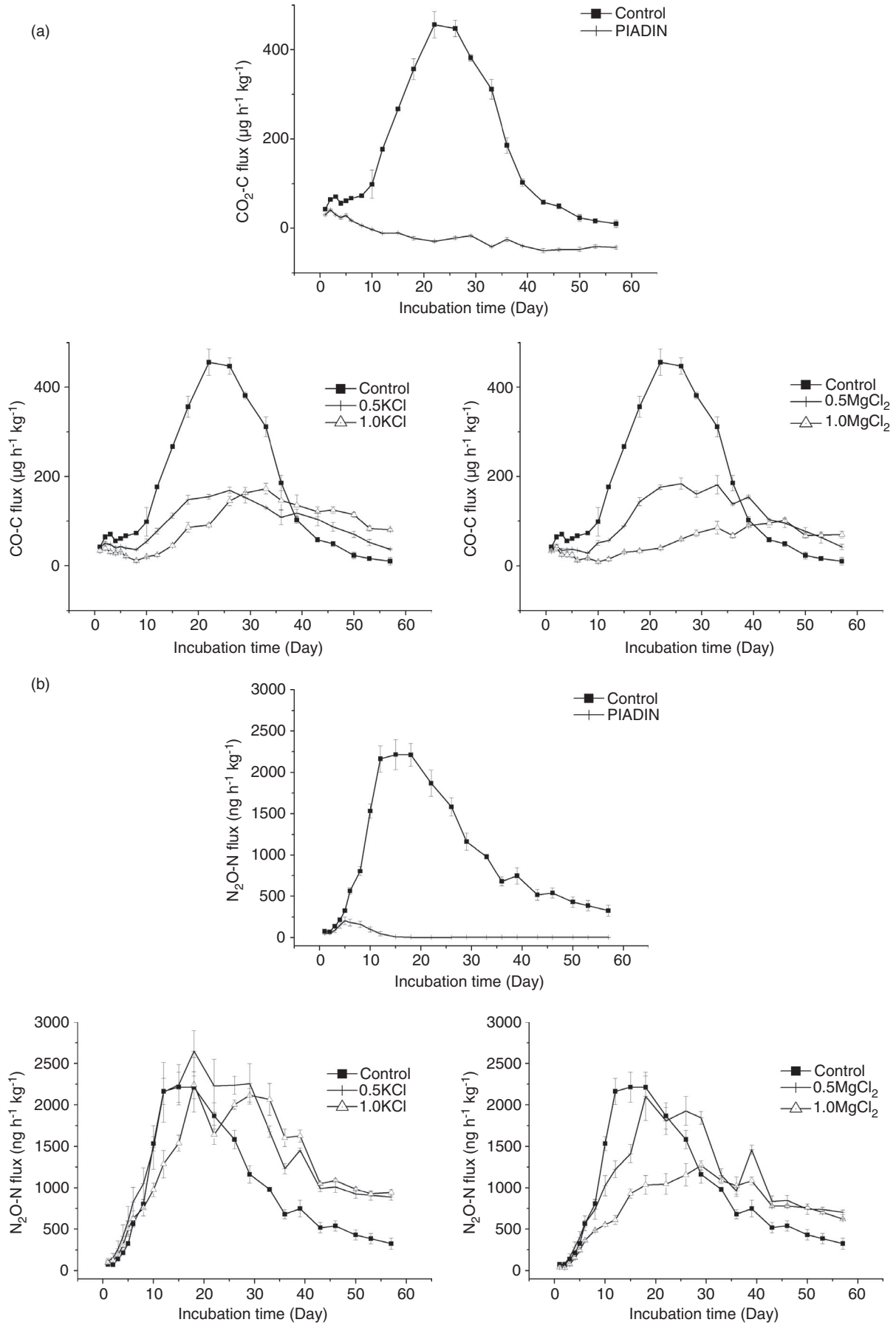
0.5 and 1.0 KCl had the same N_2O-N flux as the control before 20 days (Figure 1) and had greater N_2O-N flux than the control after 20 days. 0.5 $MgCl_2$ and 1.0 $MgCl_2$ had a smaller N_2O-N flux compared with the control before 25 days, whereas 0.5 $MgCl_2$ and 1.0 $MgCl_2$ had greater N_2O-N fluxes compared with the control after 25 days.

3.2 | Total emissions of CO_2-C and N_2O-N

The greatest CO_2-C emission (248.3 mg kg^{-1}) was from the control, while the smallest (32.8 mg kg^{-1}) was from PIADIN. PIADIN, 0.5 KCl, 1.0 KCl, 0.5 $MgCl_2$ and 1.0 $MgCl_2$ significantly decreased the CO_2-C emissions compared with the control (Table 3). PIADIN had the highest decrease in CO_2-C emissions, while 0.5 KCl, 1.0 KCl and 0.5 $MgCl_2$ had the lowest decrease. 1.0 $MgCl_2$ had an intermediate decrease compared with the control.

The greatest N_2O-N emission (1,999 $\mu g \ kg^{-1}$) was from the 0.5KCl, while the smallest (36.4 $\mu g \ kg^{-1}$) was from PIADIN. PIADIN significantly decreased N_2O-N emissions compared with the control. 0.5 and 1.0 KCl significantly increased N_2O-N emissions (Table 3) compared with the control. 0.5 $MgCl_2$ and 1.0 $MgCl_2$ showed no significant influence on N_2O-N emissions; however, 0.5 $MgCl_2$ had a tendency to

FIGURE 1 The flux of CO_2-C (a) and N_2O-N (b) as affected by KCl, $MgCl_2$ and PIADIN (0.5KCl, 0.5 g kg^{-1} KCl-Cl; 1.0KCl, 1.0 g kg^{-1} KCl-Cl; 0.5$MgCl_2$, 0.5 g kg^{-1} $MgCl_2$-Cl; 1.0$MgCl_2$, 1.0 g kg^{-1} $MgCl_2$-Cl). The data are means of four independent pot replicates, and error bars represent standard error of the mean ($n = 4$)

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(a)

(b)

increase N_2O-N emissions and 1.0 $MgCl_2$ had a tendency to decrease N_2O-N emissions compared with the control.

3.3 | Soil NH_4^+-N and NO_3^--N concentrations

In the control, NH_4^+-N concentration rapidly decreased in the first 30 days and then remained constant at a low concentration afterwards ($p < 0.05$). The NO_3^--N concentration rapidly increased in the first 30 days and then slightly decreased after 30 days ($p < 0.05$; Figure 2). Under 0.5 KCl, 1.0 KCl, 0.5 $MgCl_2$ and 1.0 $MgCl_2$, the NH_4^+-N concentration decreased and NO_3^--N concentration increased over the whole incubation period ($p < 0.05$). However, the decrease in the NH_4^+-N concentration and the increase in the NO_3^--N concentration were slower in all of the Cl^- salt treatments (0.5 KCl, 1.0 KCl, 0.5 $MgCl_2$ and 1.0 $MgCl_2$) than the control (Figure 2). The intersection points of NH_4^+-N concentration and NO_3^--N concentration occurred at 15 days under control, 20 days under 0.5 KCl and 0.5 $MgCl_2$, and 32 days under 1.0 KCl and 1.0 $MgCl_2$ (Figure 2). Under PIADIN, the NH_4^+-N concentration high over the first 40 days and then slightly decreased, and NO_3^--N concentration remained at a low concentration over the whole incubation period ($p > 0.5$). There was no intersection of NH_4^+-N and NO_3^--N concentrations under PIADIN.

3.4 | Attribution of N_2O emissions to NH_4^+-N and NO_3^--N

The variance decomposition of NH_4^+, NO_3^- and their shared fraction for the first 22 days, and from 23 to 57 days are shown in Figure 3a,b, respectively. NH_4^+, NO_3^- and their shared fraction explained 1%, 35% and 43% of the N_2O emissions in 0–22 days, respectively (Figure 3a). There was no shared fraction between NH_4^+ and NO_3^- from 23 to 57 days. NH_4^+ and NO_3^- variables explained 4% and 10% of the N_2O emissions from 23 to 57 days (Figure 3b).

4 | DISCUSSION

4.1 | The effect of different Cl^- salts

We found that both KCl and $MgCl_2$ treatments decreased soil respiration, and therefore, 0.5 KCl/$MgCl_2$ and 1.0 KCl/$MgCl_2$ had smaller CO_2-C flux compared with the control in the first 40 days of incubation (Figure 1), and the total CO_2-C emissions was decreased (Table 3). Azam and Müller (2003) also found that NaCl significantly decreased CO_2 emissions.

The combination of nitrification and denitrification played a key role in the N_2O 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 (Figure 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 (Figure 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 (Figure 2), providing clear evidence that KCl can retard nitrification.

TABLE 2 The p values of CO_2-C flux, N_2O-N flux, NH_4^+-N and NO_3^--N and changes with time for the different treatment

Treatment	CO_2-C flux	N_2O-N flux	NH_4^+-N	NO_3^--N
Control	0.000	0.001	0.000	0.000
0.5 KCl	0.006	0.015	0.000	0.000
1.0 KCl	0.013	0.011	0.000	0.000
0.5 $MgCl_2$	0.060	0.018	0.000	0.000
1.0 $MgCl_2$	0.009	0.018	0.000	0.000
PIADIN	0.010	0.059	0.005	0.785

TABLE 3 Soil CO_2-C and N_2O-N total emissions and decreased CO_2-C and N_2O-N emissions under KCl, $MgCl_2$ and PIADIN (0.5 KCl, 0.5 g kg^{-1} KCl-Cl; 1.0 KCl, 1.0 g kg^{-1} KCl-Cl; 0.5$MgCl_2$, 0.5 g kg^{-1} $MgCl_2$-Cl; 1.0$MgCl_2$, 1.0 g kg^{-1} $MgCl_2$-Cl)

NI	CO_2-C emissions		N_2O-N emissions	
	Total (mg kg^{-1})	Decrease (% of control)	Total (µg kg^{-1})	Decrease (% of control)
Control	248.3 (5.96)d	–	1,365 (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
0.5$MgCl_2$	141.7 (2.61)c	42.91	1564 (149.97)b	−14.63
1.0$MgCl_2$	69.1 (3.79)b	72.15	1,100 (69.79)b	19.40

Note: 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.
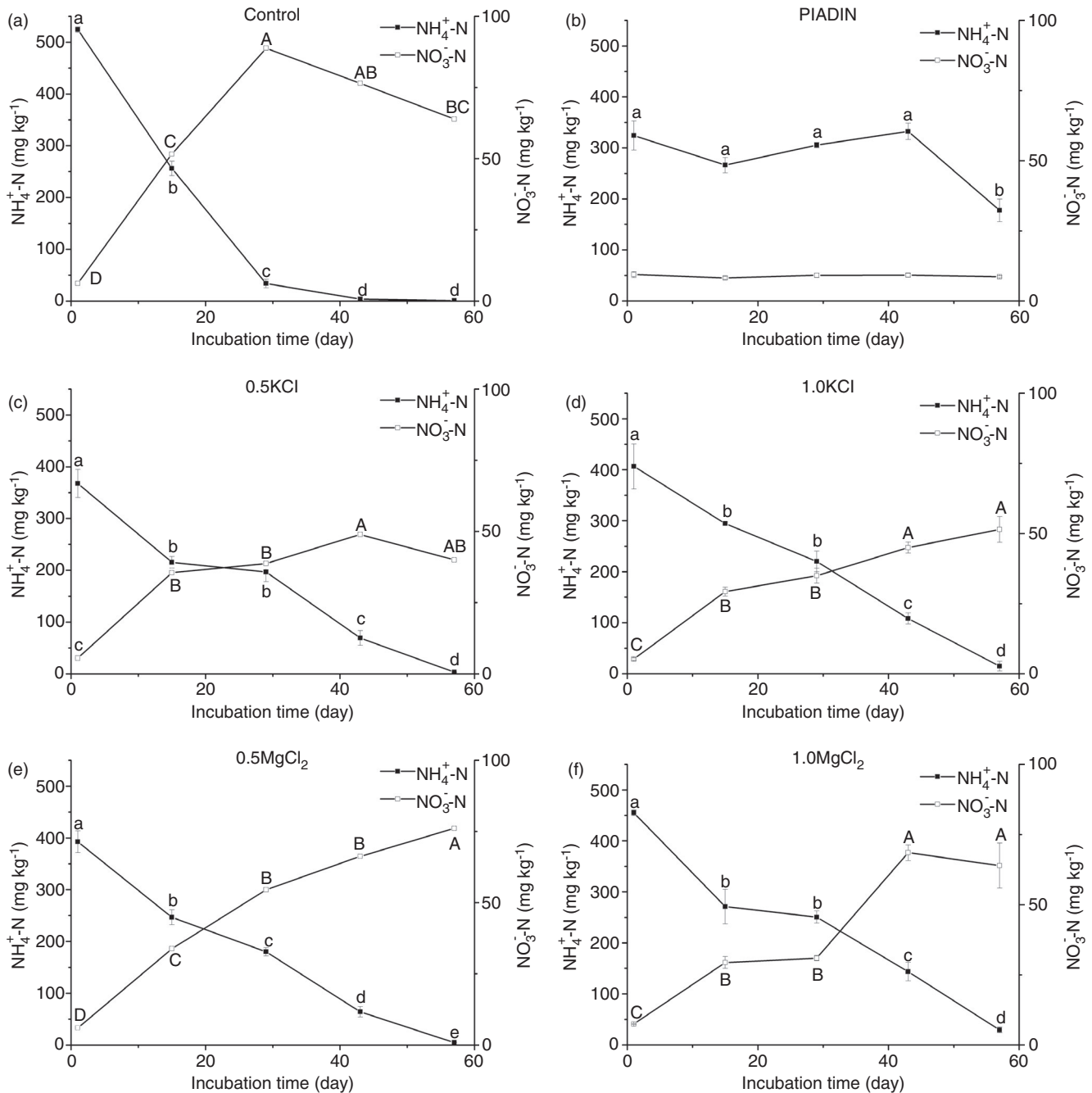
FIGURE 2　Changes of NH_4^+-N and NO_3^--N concentrations in soils without nitrification inhibitor Control (a) and with nitrification inhibitors KCl, $MgCl_2$ and PIADIN (b) (0.5KCl (c), 0.5 g kg^{-1} KCl-Cl; 1.0KCl (d), 1.0 g kg^{-1} KCl-Cl; 0.5$MgCl_2$ (e), 0.5 g kg^{-1} $MgCl_2$-Cl; 1.0$MgCl_2$ (f), 1.0 g kg^{-1} $MgCl_2$-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

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 (Darrah et al., 1987; Golden et al., 1981; McGuire et al., 1999). In addition, Cl^- and its different derivatives can act as strong oxidizers and potent biocides (Chen & Wong, 2004). The mechanism of Cl^- nitrification inhibition is associated with increased solute concentration and osmotic pressure in the soil solution (Darrah et al., 1987; Golden et al., 1981; Souri, 2010). However, in the

current study, KCl had a greater N_2O-N flux than the control after 20 days (Figure 1), and the total N_2O-N emission was greater under the two rates of the KCl treatment (Table 3). KCl treatment also resulted in the low NO_3^--N concentration at the end of the incubation (Figure 2). Denitrification is the main mechanism of N loss occurring after nitrification of NH_4^+ (Abbasi & Adams, 2000a, b) and the emissions of N_2O mainly originate from the denitrification process (Kool et al., 2011; Wrage et al., 2001). 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 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 (Figure 1). $MgCl_2$ had no significant influence on the overall N_2O-N emissions (Table 3); 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 (Jiang et al., 2009; Stauber, 1998), 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 (Figure 2). Some studies have shown that aluminium 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; Smith et al., 2001, 2004). $MgCl_2$ could also inhibit nitrification by decreasing soil pH and reducing the NH_4^+ availability, which directly inhibits the effects of Mg. Previous studies (Choi & Moore, 2008; Smith et al., 2001, 2004) 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 1.0 KCl had a smaller N_2O-N flux compared with 0.5 KCl before 20 days of incubation (Figure 1), which delayed the intersection point of NH_4^+-N concentration and NO_3^--N concentration (Figure 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 (Figure 1) and 1.0 $MgCl_2$ tended to decrease the total N_2O-N emissions (Table 3). The intersection point of NH_4^+-N concentration and NO_3^--N concentration was delayed under 1.0 $MgCl_2$ (Figure 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 0.5 $MgCl_2$ had a larger CO_2-C flux than 1.0 $MgCl_2$ before 40 days (Figure 1) and 1.0 $MgCl_2$ led to a greater decrease in CO_2-C emissions than 0.5 $MgCl_2$ (Table 3). 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
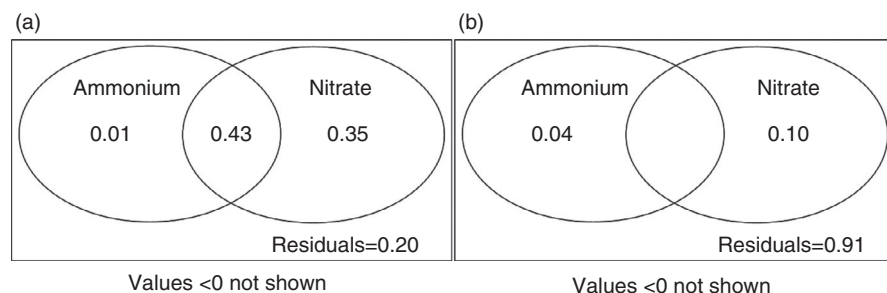
FIGURE 3 Variation partitioning of N_2O between ammonium and nitrate during 0–22 days (a) and 23–57 days (b)

increasing the $AlCl_3$ concentration did not significantly reduce gaseous N emissions. Kandeler (1993) found that chlorate at a weak concentration could inhibit the oxidation of NO_2^- to NO_3^-. 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 NO_3^--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 NH_4^+-N and NO_2^--N was inhibited in the presence of 10 mM chlorate (Xu et al., 2011). Monaghan and Barraclouch (1992) found that at greater NaCl concentrations, there was a significant accumulation of NH_4^+, suggesting an inhibition of nitrification. The difference between 0.5 and 1.0 g $MgCl_2$ kg^{-1} soil concentration in our experiment was mostly caused by the corresponding decrease in soil pH, which reduced the NH_4^+ 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 $MgCl_2$, 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 CO_2-C and N_2O-N flux at a low level during the whole incubation period (Figure 1). In addition, it significantly decreased the total CO_2-C and N_2O-N emissions (Table 3) and retained a high soil NH_4^+-N concentration and low NO_3^--N concentration (Figure 2). The amendment with NIs increases the NH_4^+ concentration in soil (Chaves et al., 2006; Pasda et al., 2001; Weiske et al., 2001; Zerulla et al., 2001). PIADIN has been shown to maintain NH_4^+-N in the mineral-N pool for a long period by blocking its oxidation and stabilizing NH_4^+ in bulk soil (Malla et al., 2005; Prasad & Power, 1995), and inhibiting nitrification and subsequently denitrification by restricting the supply of NO_3^- to the denitrifying organisms (Abbasi & Adams, 2000b; Chen et al., 1994; Freney et al., 1993; Rochester et al., 1996; Souri, 2010).

PIADIN decreased soil nitrification. PIADIN is more effective than KCl and $MgCl_2$ on inhibiting the soil nitrification and keeping soil NH_4^+-N in the mineral-N pool.

5 | CONCLUSION

Different Cl^- salts and concentrations had varying effects on soil NH_4^+ and NO_3^- dynamics and CO_2 and N_2O emissions compared with the control. Both KCl and 0.5 g kg^{-1} $MgCl_2$ decreased CO_2-C emissions and increased N_2O-N emissions compared with the control. $1.0MgCl_2$ decreased both CO_2-C and N_2O-N emissions and retarded the decrease of NH_4^+-N concentration and the increase of NO_3^--N concentration. However, Cl^- did not have such a strong NI effect as PIADIN. PIADIN decreased the CO_2-C and N_2O-N emissions and preserved the soil NH_4^+-N concentration at a high level and NO_3^--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 CO_2-C and N_2O-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.

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