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THE EFFECT OF COPPER BIOAVAILABILITY ON NITRIFICATION RATE IN NEW ZEALAND PASTORAL SOILS

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ABSTRACT

In New Zealand livestock industry urine patches contribute significantly to NO_3^- leaching and N_2O emissions in grazed pastures. To mitigate the environmental impacts from grazed pastures, different approaches such as use of alternative forage species, and nitrification and urease inhibitors have been applied. However, to date there is no cost-effective and environmentally friendly mitigation strategy that is adopted. Therefore, this study is conducted to explore an alternative strategy that analyses the influence of bioavailable Cu^{2+} in controlling nitrification rate. A laboratory incubation study was conducted using two soils types; Allophanic soil (Waikato soil) and Pallic soil (Canterbury soil). The following treatments were applied; control (only water was added), urea (300 mg kg^{-1}) , DCD (10 mg kg^{-1}) + urea, DMPP (5 mg kg^{-1}) + urea, undisclosed product A (10 mg kg^{-1}) + urea, undisclosed product B (120 mg kg^{-1}) + urea, and undisclosed product C (10 mg kg^{-1}) + urea and soil sample analysis were done at 3 and 7 days after incubation. The results showed that, in the Waikato soil, at 7 days after incubation, the high molecular weight organic acids (HMWOAs) had significantly ($p<0.05$) higher NH_4^+ than the urea only applied. Further, in both Waikato and Canterbury soils, Cu^{2+} concentration was significantly lower ($p<0.05$) at urea only application when compared to the HMWOAs treatment at both day 3 and 7. In conclusion, management of bioavailable Cu^{2+} in soil is showing some potential glimpse in reducing nitrification rate through application of various HMWOAs.

Key words: Nitrification, Grazed pastures, ammonia monooxygenase (AMO), Cu^{2+} bioavailability and High molecular weight organic acids (HMWOAs)

Introduction

Legume-ryegrass based pastoral farming is the dominant farming system in New Zealand agriculture. To meet livestock food demand, this system mostly relies on application of synthetic fertilizers (100-400 kg N ha^{-1} yr^{-1}) and some dairy effluent to promote pasture production. However, only 5-30 % of ingested N is converted into products, and a higher percentage (70-95 %) of the ingested N is excreted in urine and dung (Oenema et al. (2005). As a result, the N excreted by livestock onto grazed pastures provides highly localised concentrations of available N in soils. On average cows excrete around 21 L urine day^{-1} over 10.2 urine patches (Saggar et al., 2004). Each urine patch has an estimated area of around 0.2-0.4 m^2 and N concentration equivalent to 200-2000 kg N ha^{-1} (Moir et al., 2011). Depending on the stocking rate urine patches are estimated to cover about 20-30% of grazed pastures (Moir et al., 2011). Therefore, urine patches are the main primary targets to combat NO_3^- leaching and N_2O emissions in NZ grazing systems. Development of different technologies and

practices which manage the effect of urine patches have attracted much attention for the sustainable NZ farming system. The present study pinpoints the management strategy of soil bioavailable Cu^{2+} in controlling nitrification rate and narrates relevant research concepts and an ongoing laboratory study.

Nitrogen losses in agriculture

The livestock industry has been shown to contribute a significant amount to environmental contamination through NO_3^- leaching and N_2O emissions in grazed pastures (Davidson, 2009). Nitrate leaching is mostly high during late autumn, winter and early spring period (April to September) due to high rainfall and low plant nitrate uptake (Di et al., 2016), contributing to contaminating water resources. Silva et al. (2000) observed that NO_3^-N concentrations in drainage water under the urine patch can reach a peak of 120 mg NO_3^-N L^{-1} and equivalent to 124 kg N ha^{-1} yr^{-1}. New Zealand agriculture is responsible for 49 % of national GHG emissions and 22% of them are contributed by N_2O emissions.

Existing mitigation strategies to reduce N losses in NZ

In the last few decades different approaches and techniques have been implemented to mitigate N losses in pastoral soils such as use of nitrification and urease inhibitors, use of alternative forages (Judson et al., 2019; Mangwe et al., 2019), and restricted grazing (De Klein et al., 2006; Harty et al., 2016). Di and Cameron (2002) reported that application of DCD at 15 kg ha^{-1} resulted to a significant decrease (42 %) of NO_3^-N leaching in urine applied at 1000 N kg ha^{-1} in spring, and 76 % in autumn which resulted to an average reduction of 89 % percent. Although inhibitors have proved effective and efficient in reducing N losses, residual toxic effect to the environment (Marsden et al., 2015) and inconsistent results (Vilarrasa-Nogué et al., 2020) are limiting their usage in NZ agriculture.

Another recently established approach is the use of alternative forages such as plantain (*Plantago lanceolate* L.), which increase nitrogen uptake, and therefore, reduce the nitrate losses. Byrnes et al. (2017) noticed that in order to realise plantain benefits in reducing N concentration in urine, more than 30 % plantain forage must be maintained in animal diet. Therefore, the use of alternative forages is costly, and still not yet provided conclusive results on the mechanism of how these forages reduce N losses

Functioning of Cu^{2+} in the nitrification process

Ammonia monooxygenase enzyme (AMO) encoded in the *amoA* gene of the ammonia oxidizing bacteria (AOB) and ammonia oxidizing archaea (AOA) is responsible for the first process of ammonia oxidation into hydroxylamine (Wood, 1990). This enzyme uses Cu^{2+} for the electron transfer during the oxidation proces (Lees, 1946). Application of organic amendments complex with soil Cu^{2+} could hinder the nitrification rate ((Bédard & Knowles, 1989)

Copper complex with organic acids

Cu^{2+} is known to complex with functional groups of dissolved organic matter clay minerals, and iron, manganese, and aluminium oxyhydroxides. These materials contain different organic functional groups such carboxyl's (-COOH), phenols (-OH), thiols (-SH), and amines ($-NH_2$). All of these groups may play a significant role in the complexation of Cu^{2+} in soil. Organic acids such as HMWOAs can enhance Cu^{2+} complexing because of their enhanced functional

groups. In our agriculture system, the more critical aspect is to choose right organic amendments with high complexation with AMO-bound Cu^{2+} to affect the functioning of the AMO enzyme to manage nitrification rate.

Study Objective

The main aim of the study is to analyse the effect of Cu^{2+} bioavailability on influencing nitrification rate in pastoral soils through application of different HMWOAs.

Laboratory incubation study

Soil preparation

An incubation study was undertaken to determine the influence of applying HMWOAs on Cu^{2+} bioavailability and related effect on nitrification rate at different rates of N fertiliser applied soil. Two soil types, contrast in their characteristics were used to conduct this experiment namely; Allophanic soil (Waikato soil) and Pallic soil (Canterbury soil). Briefly, field moist soil was sieved through 2 mm sieve and then 10 g was added into 50 ml centrifuge tubes.

Treatments

The following treatments were used in the experiment; control (only water was added), urea (300 mg kg^{-1}) , DCD (10 mg kg^{-1}) + urea, DMPP (5 mg kg^{-1}) + urea, undisclosed product A (10 mg kg^{-1}) + urea, undisclosed product B (120 mg kg^{-1}) + urea, and undisclosed product C (10 mg kg^{-1}) + urea. These organic acids material are not referred to their specific names in this paper as they are still waiting to be patented. A 0.5 ml of each treatment solution was added to soil contained centrifuge tubes and thoroughly mixed. The tubes were incubated for 7 days at 25℃.

Analysis

Soil sampling and analysis were done 3- and 7-days after incubation. In each sample, 30 ml 0.05 M $CaCl_2$ was added, extracted in an end-over-end shaker for two hours, and then centrifuged at 1100 x g for 10 min to determine the bioavailable Cu. Samples were then filtered through Whatman 42 filter papers. Using the remaining soil samples, NH_4^+ was extracted by adding 30 ml of 2 M KCl and repeated the same procedure. Both bioavailable Cu and NH_4^+ extractants were stored at < 4 ℃ before being analysed using the graphite furnace atomic absorption spectrophotometer for Cu^{2+} and Auto analyser for NH_4^+.

Results

Effect of HMWOAs on NH_4^+-N concentration after incubation period

Waikato soil did not show any initial significant difference in NH_4^+ concentration in all the treatments, however, at 7 days the HMWOAs had significantly ($p<0.05$) higher NH_4^+ than the urea only applied (Table 1). Both, Product A + urea and Product B + urea in day 7 significantly minimized ammonium loss by 32.5 % when compared to urea only application. Urea only application in the Canterbury soil produced significantly higher NH_4^+ than HMWOAs at day-3, however, reduced at day-7, except for DCD and DMPP treatments.

Table 1: Effect of HMWOAs on NH_4^+-N concentration after 3 and 7 days incubation period of the Waikato and Canterbury soil.

Treatments	Allophanic soil (Waikato soil)		Pallic soil (Canterbury soil)	
	Day 3	Day 7	Day 3	Day 7
	(mg kg^{-1})		(mg kg^{-1})	
Urea only	88.2 ±9.0[a]	58.7 ±3.2[c]	104.7 ±8.3[b]	72.8 ±3.3[b]
Urea + DCD	98.1 ±9.8[a]	104.9±6.8[a]	119.6 ±4.8[a]	112.7 ±9.5[a]
Urea + DMPP	99.1 ±11.6[a]	108.0±2.3[a]	109.4 ±2.1[b]	111.7 ±8.5[a]
Product A + Urea	105.0±11.7[a]	77.8 ±1.9[b]	90.7 ±5.6[c]	60.6 ±4.0[b]
Product B + Urea	97.9 ±5.2[a]	77.9 ±2.3[b]	92.1 ±1.5[c]	64.8 ±3.6[b]
Product C + urea	97.3 ±1.1[a]	83.5 ±13.7[b]	89.2 ±0.6[c]	59.6 ±9.2[b]
Control	0	0	0	0

Effect of HMWOAs on Cu^{2+} bioavailability after incubation

In both Waikato and Canterbury soils, Cu^{2+} concentration was significantly lower ($p<0.05$) at urea only application when compared to the HMWOAs treatment at both day 3 and 7 (Table 2). The lower bioavailable Cu^{2+} in the urea only treatment might be due to the sharp increase in pH due to urea fertilizer application which result to significant decrease in Cu^{2+}. Sommer et al. (2004) observed that during the first few days of urea application, the pH can escalate above pH 8 which normally affect the Cu^{2+} bioavailability. However, we cannot conclude with the available data.

Table 2: Effect of HMWOAs on Cu^{2+} after incubation for 3 and 7 days incubation period of the Waikato and Canterbury soil.

Treatments	Allophanic soil (Waikato soil)		Pallic soil (Canterbury soil)	
	Day 3	Day 7	Day 3	Day 7
	(µg L^{-1})		(µg L-1)	
Control	55.0 ±3.0[a]	53.0 ±3.2[a]	57.8 ±1.2[a]	51.6 ±1.9[a]
Urea only	16.5 ±1.2[e]	11.0 ±0.6[e]	26.0 ±2.3[e]	15.3 ±2.0[a]
Urea + DCD	31.9 ±1.3[c]	13.3 ±1.5[e]	49.0 ±0.8[c]	32.0 ±0.6[b]
Urea + DMPP	44.8 ±2.0[b]	31.3 ±1.5[b]	52.3 ±1.8[b]	22.6 ±1.2[c]
Product A + Urea	45.1 ±1.1[b]	26.6 ±1.0[c]	36.6 ±1.7[d]	26.1 ±1.8[c]
Product B + Urea	30.6 ±1.0[c]	22.1 ±1.0[d]	36.9 ±2.0[d]	24.7 ±1.2[c]
Product C + urea	21.3 ±3.5[d]	22.1 ±2.4[d]	38.8 ±1.0[d]	29.2 ±3.0[bc]

Effect of HMOWAs on nitrification rate after incubation

Product C in the Waikato soil reduced nitrification rate by 26.1 % when compared to urea only application. However, product A + urea and product B + urea recorded 6.8 and 5.0 mg NO_3^- kg^{-1} day^{-1} respectively which was non significantly higher than urea only treatment, which was recorded 4.6 mg NO_3^- kg^{-1} day^{-1}. In the Canterbury soil, product A + urea, product B + urea, product C + urea recorded 7.5, 6.8 and 7.3 mg NO_3^- kg^{-1} day^{-1} respectively compared to 8.0 mg NO_3^- kg^{-1} day^{-1} in the urea only (Figure 1-1). Overall, product C + urea has a potential in reducing nitrification rate in both Canterbury and Waikato soil.

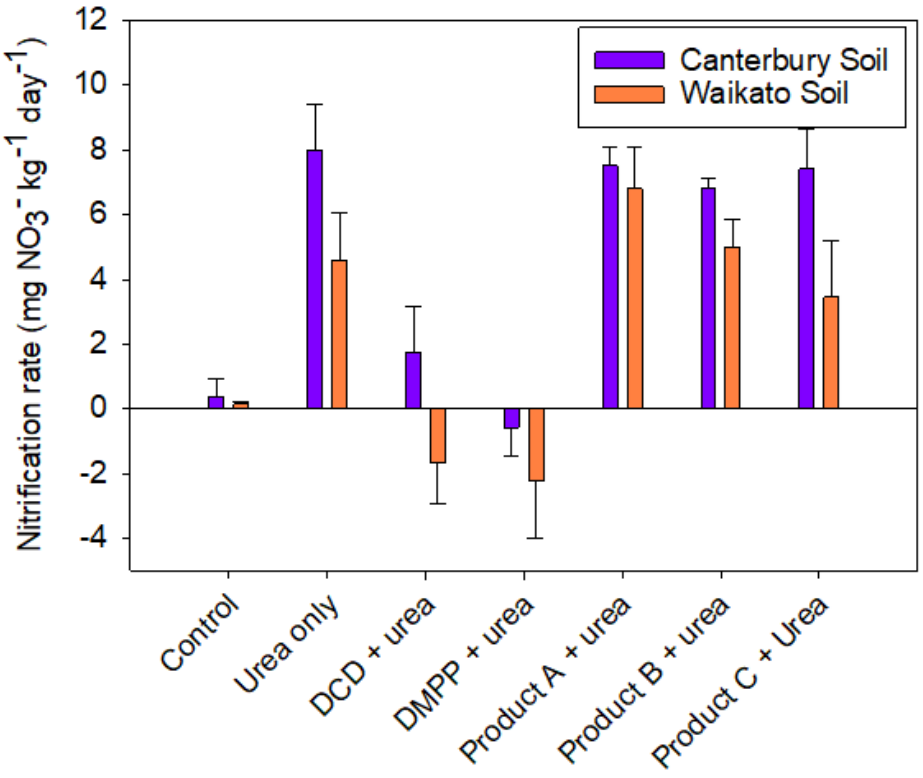

Figure 1-1 The effect of HMWOAs on nitrification rate after 7 days incubation period in Canterbury (Pallic soil) and Waikato soil (Allophanic soil)

Conclusion

From the initial results, HMWOAs can alter nitrification rate, however, their effectiveness is mostly influenced by the soil type. The on-going research activities will critically analyse the effectiveness of these HMWOAs on influencing bioavailable Cu^{2+} and nitrification rate.

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