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PERSISTENCE OF NITRIFICATION INHIBITORS AND THEIR EFFICACY TO REDUCE NITROUS OXIDE EMISSIONS FROM CONTRASTING PASTURE SOILS AMENDED WITH URINE

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Abstract

Nitrification inhibitors (NIs) play a key role in mitigating nitrogen (N) losses from pastoral agricultural systems. The effect of NI, dicyandiamide (DCD) on reducing nitrous oxide (N_2O) emissions from urine patches on pasture soils is well-documented. However, information on the comparative effectiveness of other most used NIs [3,4-dimethylpyrazole phosphate (DMPP), 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) and A2 (a confidential NI)] is lacking. Therefore, laboratory incubation experiments were conducted to test the persistence of DMPP, nitrapyrin and A2 (using DCD as a reference NI) and assess their effectiveness to mitigate N_2O emissions from two urine-amended pasture soils (Te Kowhai and Tokomaru soils) varying in texture and organic matter. The half-life values (at 15°C) of the NIs varied with soil organic matter and clay content, and were 12-17 days for DMPP, 18-29 days for nitrapyrin and 20-33 days for DCD. All the NIs reduced N_2O emissions, but their effectiveness differed with the amount of NI and soil type. The reductions in N_2O emission were 19-46% with DMPP, 45-59% with nitrapyrin, 12-43% with A2 and 43-47% with DCD. Higher rates of both nitrapyrin and A2 achieved significantly ($p \leq 0.05$) greater reductions on the Tokomaru soil than on the Te Kowhai soil. There was no effect of NIs on the levels of soil microbial biomass carbon and nitrogen (MBC and MBN) during the study period.

Introduction

The use of nitrification inhibitors (NIs) to manage nitrogen (N) losses from agricultural systems has been practiced for decades. In livestock grazed pastures, where urine is a major source of N_2O emission, the NI, dicyandiamide (DCD) has been used extensively to mitigate N losses to the environment. However, the discovery of DCD residue in New Zealand milk product led to its withdrawal for use in New Zealand and Australia. Thus, current research with NIs is focused on targeted management of urine patches, as opposed to the blanket application of NIs across the whole paddock, to limit the risk of NI residue entering the food chain through grazing animals.

Other commonly used NIs with the potential to mitigate N_2O emissions from urine patches are 3,4-dimethylpyrazole phosphate (DMPP) and 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin). However, there is currently limited research on the use of these NIs on intensively managed pasture soils, despite the potential of both NIs to mitigate N_2O emissions from urine patches (Adhikari *et al.*, 2021b), In particular, there is a paucity of data on the half-life and

efficacy of DMPP and nitrapyrin on grazed pasture soils containing urine patches, as influenced by soil properties such as texture and organic matter. Information on their effect on non-target soil microbes is also limited. Bridging these knowledge gaps is critical to optimise their application, and hence maximise their effectiveness for managing N_2O emissions from urine patches on pasture soils.

The objectives of this study were to determine the persistence and efficacy of commonly used NIs (DMPP, nitrapyrin) and a confidential NI (termed A2) on pasture soils amended with urine, test their effect on non-target soil microbes, and assess the effect of soil properties on their persistence and efficacy. The widely researched DCD in New Zealand dairy grazed pasture soils was included as a standard.

Materials and methods

Experimental set-up

Two pasture soils varying in soil organic carbon (C) and texture – Te Kowhai (total C: 67.17 g kg^{-1}; clay content: 35%) and Tokomaru (total C: 42.33 g kg^{-1}; clay content: 24%) were used in this study. The soils were collected from 0-10 cm soil depth and sieved with a 2 mm sieve to achieve homogeneity. They were subsequently pre-incubated for 2 weeks to stabilise the soil microbial population. Nine treatments were applied to the pre-incubated soils as follows:

1. Control (water only)
2. Urine
3. Urine + DMPP-2.5 (2.5 mg DMPP kg^{-1})
4. Urine + DMPP-5.0 (5 mg DMPP kg^{-1})
5. Urine + Nitrapyrin-3.0 (3 mg nitrapyrin kg^{-1})
6. Urine + Nitrapyrin-6.0 (6 mg nitrapyrin kg^{-1})
7. Urine + A2-R1 (R1 mg A2 kg^{-1})
8. Urine + A2-R2 (R2 mg A2 kg^{-1})
9. Urine + DCD-10 (10 mg DCD kg^{-1}).

The treatments were replicated four times. For the confidential NI (A2), this study only assessed its efficacy to reduce N_2O emission from urine. Its persistence and effect on non-target organisms were not determined.

Fresh dairy cattle urine was applied at a rate of 660 mg N kg^{-1} to the appropriate treatments. The NIs were mixed in the urine before soil application, with subsequent thorough mixing of the amended soil to ensure an even distribution of the amendments.

The treatments were incubated at a constant temperature of 15°C. Soil moisture content was maintained at 70-80% field capacity by monitoring weight changes and adding deionised water to compensate for any weight loss. Soil samples were collected periodically for 56 days and analysed for NI concentration, microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and mineral-N concentration.

A simultaneous laboratory incubation experiment with the same treatments and soils was conducted to measure the effect of the NIs on N_2O emissions from urine (applied at a rate of 624 mg N kg^{-1}). Gas samples were collected periodically as follows: 2 h after treatment application, every alternate day for the first and second week, twice per week for the third week, weekly for the next four weeks, and biweekly till the emissions from the treatments reached the background levels.

Soil and gas analyses

DCD in the soil samples was extracted by shaking 10 g of soil in 20 mL of deionised water for 1 h, with subsequent centrifugation (at 5000 rpm for 5 min) and filtration (with No. 42 Whatman filter paper). The extract was analysed for DCD concentration using high-performance liquid chromatography (HPLC). DMPP and nitrapyrin extraction and analysis were carried out as described by Adhikari *et al.* (2021a). Mineral-N concentrations were determined on KCl extracts colorimetrically by continuous flow analysis (Technicon® AutoAnalyser II). Soil MBC and MBN were determined by the chloroform fumigation extraction method (Vance *et al.*, 1987). Soil C in the resulting extracts was determined by the semi-automated dichromate method (O'Dell, 1993) with modifications as described by Chibuike *et al.* (2019), while soil N was obtained by automated colorimetry after alkaline persulphate digestion of the extracts.

The N_2O concentration of the gas samples was analysed on the Shimadzu Gas Chromatograph (GC-17A) equipped with a ^{63}Ni electron capture detector as outlined in Hedley *et al.* (2006). Thereafter, N_2O flux (mg N_2O-N kg^{-1} soil day^{-1}) was calculated from the equation of Mosier and Mack (1980) as described by Saggar *et al.* (2004). The cumulative N_2O flux was then calculated by internal extrapolation from the area under the curve which relates daily fluxes to the measurement period. The N_2O emission factor (EF), which is N_2O-N emitted as a percentage of urine-N applied, was calculated as follows:

$$\text{EF (\%)} = \left(\frac{\text{Cumulative } N_20\text{-}N_{urine} - \text{Cumulative } N_20\text{-}N_{control}}{\text{Total urine-N applied}}\right) \times 100$$

Statistical analyses

Both the soil and gas data were checked for normality and equal variances (using Anderson-Darling test and Levene's test, respectively) before they were analysed using Analysis of Variance (ANOVA). Tukey comparison procedure was used to detect significant differences ($p \leq 0.05$) in treatment means. The log-transformed NI concentration data were plotted against time and fitted to a linear regression model to obtain the NI degradation rate constants and half-life values via first-order kinetics. All statistical analyses were conducted using Minitab statistical software (Minitab® 19.1.1, 64-bit).

Results and discussion

NI persistence as influenced by soil properties

The half-life values of the NIs are summarised in Table 1, with a graphical presentation of the log-transformed NI concentration plotted over time in Figure 1. DCD had the longest half-life

on the examined soils (20-33 days), followed by nitrapyrin (18-29 days) and DMPP (12-17 days). The observed DCD half-life values are within the range (10-37 days at 15°C) reported in previous laboratory incubation experiments (Bronson *et al.*, 1989; Barneze *et al.*, 2015; McGeough *et al.*, 2016). No study to date has tested the persistence of nitrapyrin at 15°C. However, Herlihy and Quirke (1975) reported nitrapyrin half-life values of 13 and 54 days at 10 and 20°C, respectively, and these compare well with the results of the current study. The DMPP half-life values reported above are lower than the values (29-53 days at 15°C) reported by Zhao *et al.* (2017). The difference may be linked to the lower soil C and clay content of the previous study (total C: 10 g kg^{-1}; clay content: 20%) compared to the present study (total C: 42-67 g kg^{-1}; clay content: 24-35%). Higher soil organic C (and clay) content has been showed to promote greater microbial degradation of NI, which result in shorter NI half-life (Singh *et al.*, 2008; McGeough *et al.*, 2016). This also explains why the half-life values of both DCD and DMPP were shorter in the Te Kowhai soil compared to the Tokomaru soil. Conversely, nitrapyrin half-life values were higher in the Te Kowhai soil compared to the Tokomaru soil. Greater persistence/half-life of nitrapyrin in soils with high organic matter has previously been observed (Briggs, 1975; McCarty and Bremner, 1990; Rakhi *et al.*, 2019). This may be linked to the sorption of nitrapyrin to soil organic matter which results in slower hydrolysis to its degradation product and/or slower volatilisation.

Greater ($p \leq 0.05$) half-life values were observed for the higher rate of nitrapyrin on both soils. It is worth noting that the results of a preliminary experiment showed that the recovery of DCD, DMPP and nitrapyrin was higher on the Tokomaru soil compared to the Te Kowhai soil as follows: 96 vs 93% for DCD, 74 vs 53% for DMPP and 78 vs 68% for nitrapyrin. The lower recovery of the NIs in the Te Kowhai soil is associated with its higher number of sorption sites.

Table 1: Summary of decay rate constants, half-life values, coefficients of determination and regression equations for DCD, DMPP and nitrapyrin

Soil	**Computed values**	**DCD**	**DMPP**		**Nitrapyrin**	
		10 mg kg^{-1}	***2.5 mg kg^{-1}***	***5.0 mg kg^{-1}***	***3.0 mg kg^{-1}***	***6.0 mg kg^{-1}***
Te Kowhai	*k* (days^{-1})	0.035 ± 0.002	0.056 ± 0.006	0.045 ± 0.007	0.035 ± 0.003	0.024 ± 0.001
	$t_{½}$ (days)	19.8 ± 1.13	12.4 ± 1.33^{a}	15.4 ± 2.40^{a}	19.8 ± 1.70^{b}	28.9 ± 1.20^{a}
	R^2	0.97	0.92	0.87	0.91	0.96
	Regression equation	y = -0.035x + 2.43	y = -0.056x + 0.20	y = -0.045x + 0.83	y = -0.035x + 0.29	y = -0.024x + 0.35
Tokomaru	*k* (days^{-1})	0.021 ± 0.001	0.048 ± 0.003	0.040 ± 0.006	0.039 ± 0.004	0.031 ± 0.002
	$t_{½}$ (days)	33.0 ± 1.57	14.4 ± 0.90^{a}	17.3 ± 2.60^{a}	17.8 ± 1.82^{b}	22.4 ± 1.44^{a}
	R^2	0.97	0.97	0.86	0.89	0.94
	Regression equation	y = -0.021x + 2.36	y = -0.048x + 0.69	y = -0.040x + 1.28	y = -0.039x + 0.30	y = -0.031x + 0.41

k: decay rate constant; $t_{½}$: half-life; R^2: coefficient of determination. Values are means ± standard error of the mean (n = 4). Half-life values followed by different letters indicate significant difference ($p \leq 0.05$) between application rates for each NI and each soil.

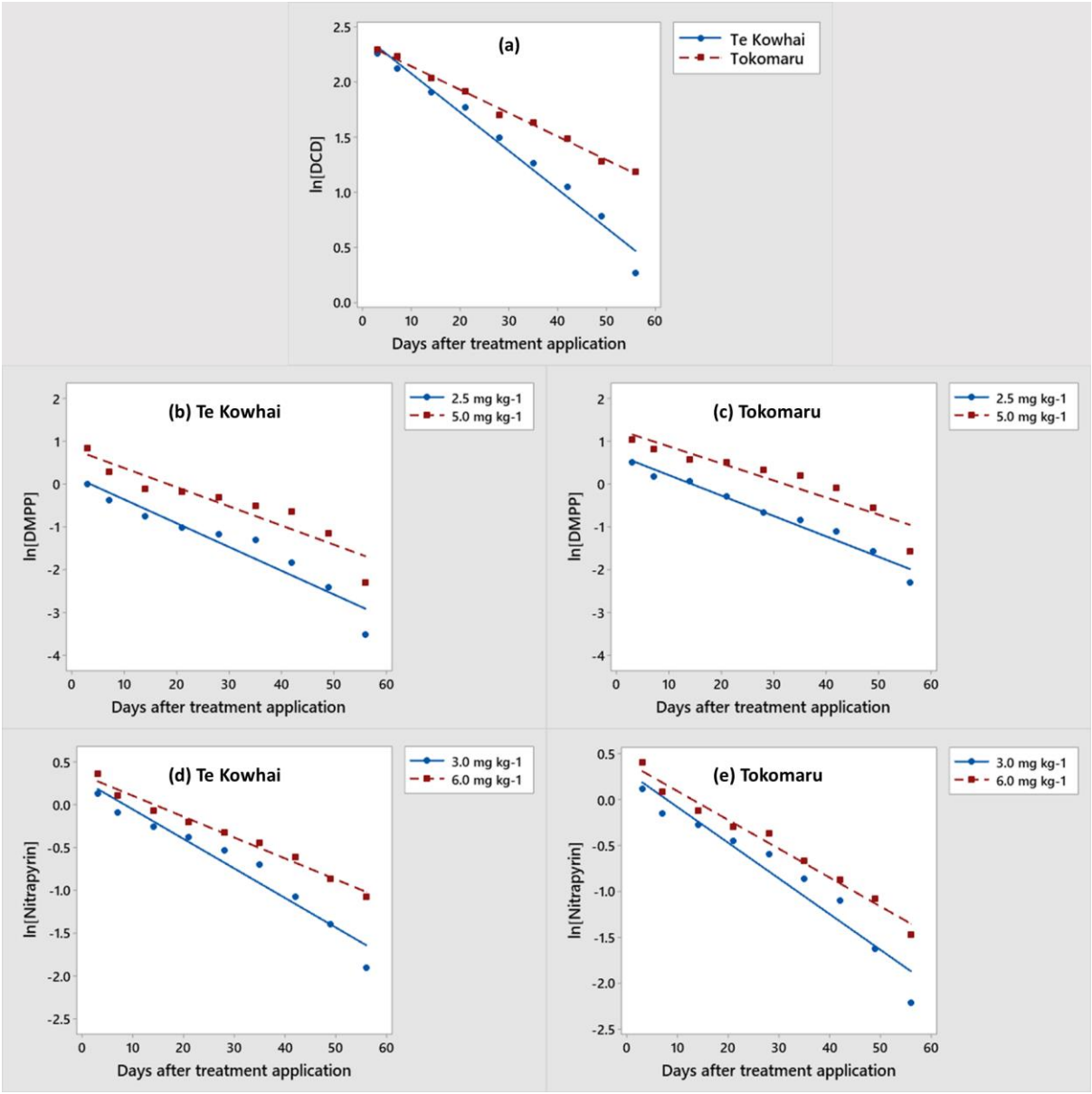

Figure 1: Natural log transformation of DCD, DMPP and nitrapyrin concentrations over time in Te Kowhai and Tokomaru soils

Efficacy of NIs to inhibit nitrification in soils amended with urine

The results of mineral-N analysis (Figure 2) show that compared to the urine-only treatment, slower nitrification occurred in the treatments amended with NI. The concentration of ammonium-N decreased with time in both soils, with a more rapid decrease observed in the urine-only treatments relative to the treatments with NI. Conversely, nitrate-N concentrations increased with time, and this was also more rapid in the urine-only treatments.

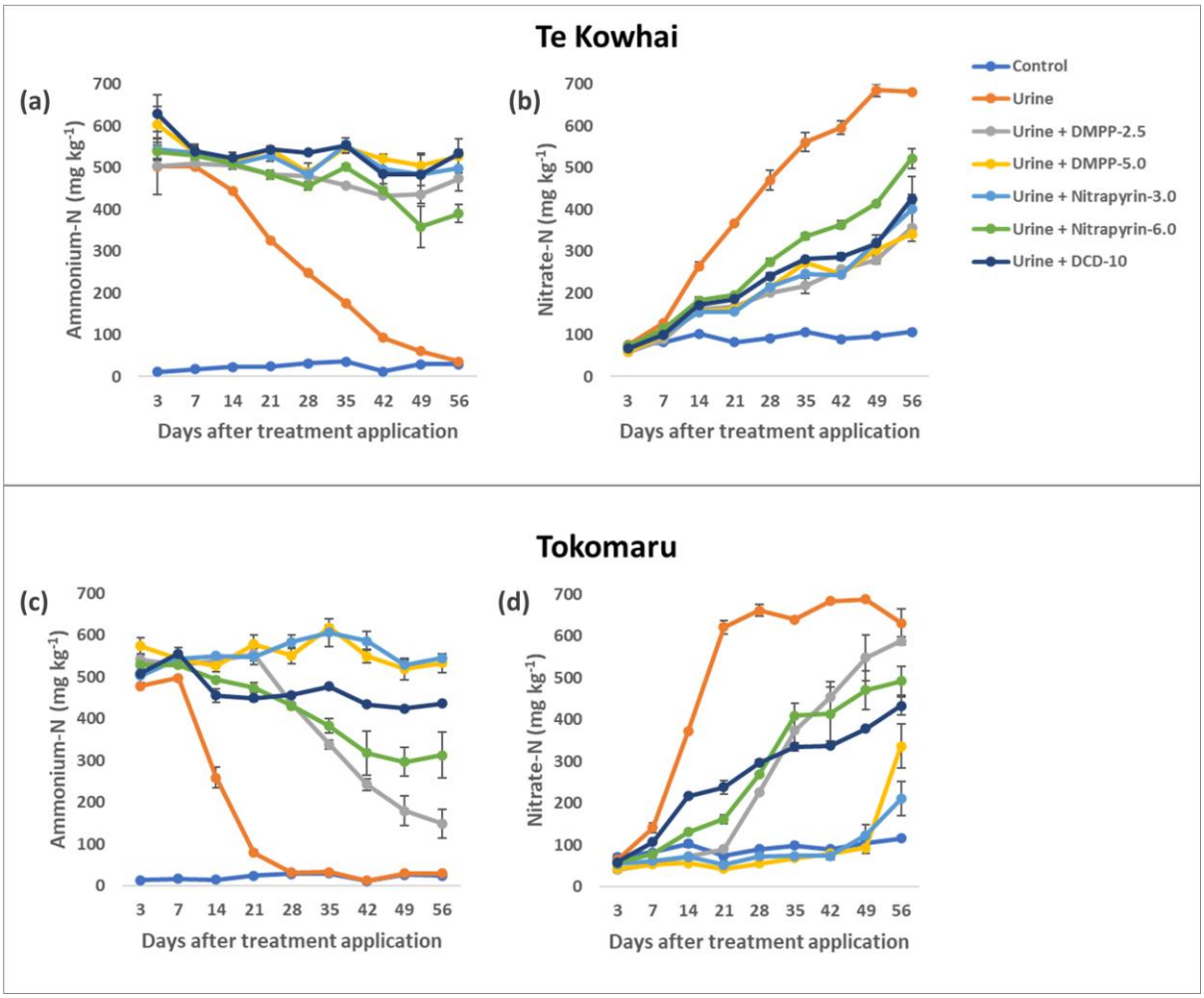

Figure 2: Changes in soil mineral-N over time in Te Kowhai and Tokomaru soils. *Each value represents the mean (n = 4), with error bars showing the standard error of the mean.*

Nitrous oxide emission factor (EF) of the urine-only treatment was 2.9-3.0% on both soils. These values are higher than the N_2O EF (0.98%) reported in New Zealand inventory (van der Weerden *et al.*, 2020), and this is attributed to lower N-uptake mechanism in the incubated soils compared to field studies that typically have higher N-uptake mechanism mainly due to the presence of growing plants. It is also possible that the incubation temperature (15°C) might have limited the reduction of N_2O to dinitrogen (N_2), as increasing temperature has been found to increase N_2/N_2O (Wang *et al.*, 2021). The N_2O EF of the urine-only treatments was also significantly ($p \leq 0.05$) higher than the N_2O EFs of the treatments amended with NI (Figure 3a). This result aligns with the results of mineral-N reported above. Both soils had similar N_2O EFs except for the higher rate of nitrapyrin (6 mg kg^{-1}) and A2 (R2) treatments, where the Tokomaru soil showed significantly ($p \leq 0.05$) lower N_2O EFs compared to the Te Kowhai soil.

Figure 3b shows the per cent reduction in N_2O emission for the treatments with NIs relative to the urine-only treatment. The reductions in N_2O emission were 19-46% with DMPP, 45-59% with nitrapyrin, 12-43% with A2 and 43-47% with DCD. The observed per cent reduction in N_2O emission by nitrapyrin is higher than the average efficacy (28 ± 5%) reported for field studies with urine (Adhikari *et al.*, 2021b). Again, this higher efficacy may be linked to the absence of plants. The varying climatic conditions in the field could also lower NI

effectiveness, given that nitrapyrin is a volatile compound (0.43-0.64 Pa at 25°C). On the other hand, the efficacy of both DMPP and DCD observed in this study compare well with the average values reported for field studies, i.e., 28 ± 38 and 44 ± 2% , respectively (Adhikari *et al.*, 2021b). Higher rates of both nitrapyrin and A2 achieved significantly ($p \leq 0.05$) greater reductions on the Tokomaru soil than on the Te Kowhai soil.

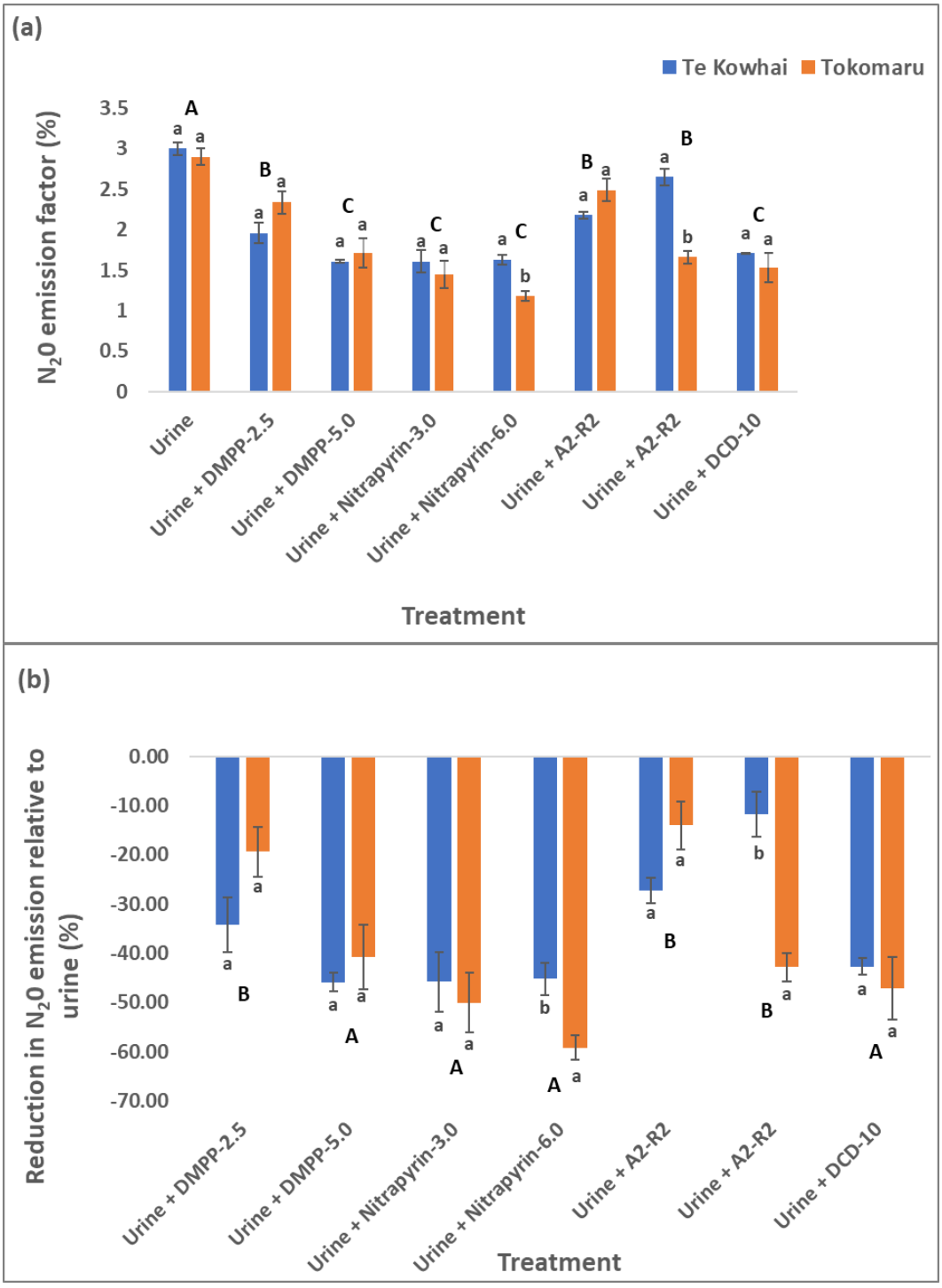

Figure 3: (a) N_2O emission factor of the treatments, and (b) Reduction in N_2O emission relative to urine in Te Kowhai and Tokomaru soils. *Each bar represents the mean (n = 4), with error bars showing the standard error of the mean. Different capital letters indicate significant difference ($p \leq 0.05$) among treatments, while different small letters indicate significant difference ($p \leq 0.05$) between soils for a particular treatment.*

Effect of NIs on non-target soil microbes

There was no effect of NI application on the MBC concentration of both soils (Figures 4a and b). The DCD treatment initially had a higher mean MBN concentration (on the Te Kowhai soil), though this was not significantly different ($p > 0.05$) from the other treatments with urine (Figure 4c). These observations suggest that the applied NIs had no unintended consequences on soil microbial biomass. As the experiment progressed, a significant decline in MBC was observed on both soils (this was also true for MBN on the Te Kowhai soil). Similar temporal changes in soil microbial biomass have been reported by previous researchers (Zaman *et al.*, 1999; Di and Cameron, 2004; Li *et al.*, 2018), and this may be linked to the life cycle of soil microbes and C availability.

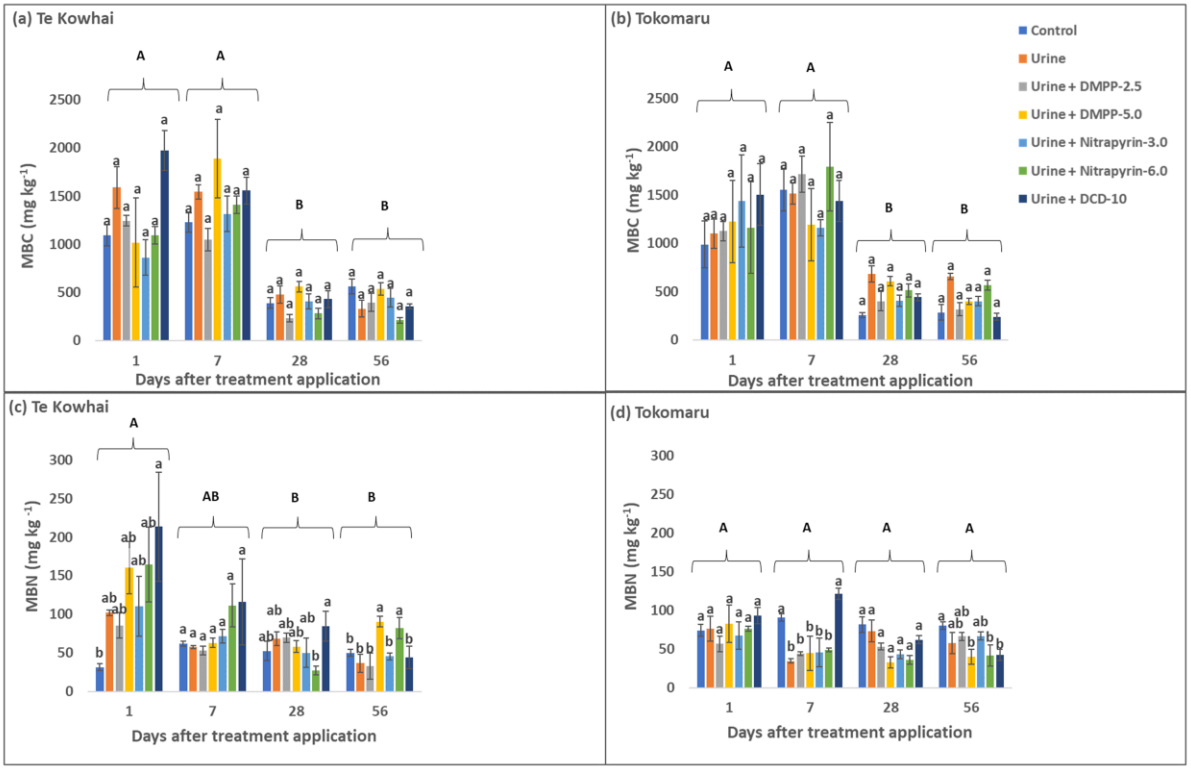

Figure 4: Changes in soil microbial biomass C and N (MBC and MBN) over time. *Each bar represents the mean (n = 4), with error bars showing the standard error of the mean. Different capital letters indicate significant difference ($p \leq 0.05$) in sampling days after treatment application, while different small letters indicate significant difference ($p \leq 0.05$) among treatments for a particular day.*

Conclusion

The half-life values for DCD, DMPP and nitrapyrin obtained in this study generally compare well with half-life values quoted in the literature. Soil organic matter and clay influenced NI persistence on the two examined soils, as shorter DCD and DMPP half-life values were observed on the Te Kowhai soil, which had greater amounts of organic matter and clay compared to the Tokomaru soil. The reverse was the case for nitrapyrin. All the NIs inhibited nitrification and hence mitigated N_2O emissions from the amended soils. These NIs also showed no adverse effect on soil microbes, within the context of this experiment.

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