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NITROGEN LOSSES FROM PLANTAIN: WHAT CAN WE SAY?

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Abstract

Losses of nitrogen (N) from urine patches as nitrat e (NO_3^--N) leaching, and nitrous oxide (N_2O) and ammonia (NH_3) emissions are important contributors to the degradation of the environment. Plantain *(Plantago lanceolata*) pastures can reduce N concentrations in cow urine and modify the soil N cycle by inhibiting nitrification.

Nitrogen losses from plantain pastures, and associated mechanisms, have been studied in a major field trial and a lysimeter experiment. Three pasture treatments were established in December 2016 at Massey University's Dairy 4 farm including; a standard ryegrass/white clover sward, a plantain pasture, and a pasture mix of 70% plantain and 30% red and white clover. Each treatment was replicated five times. Each treatment plot (~800 m^2) had an isolated mole-pipe drain system that allowed for the quantification of NO_3^--N leaching. The pastures were grazed by lactating cows over a 10-day period on March and April 2017, and over a 8-day period from September 2017 until June 2018 and from September 2018 until May 2019. Nitrous oxide emissions were evaluated during two seasons, spring and autumn/winter. This paper focuses on the results from the pure plantain and ryegrass /white clover treatments. In addition, a lysimeter study was also conducted to determine the effect of aucubin, a secondary metabolite produced by plantain, on N_2O emissions and NO_3^--N leaching. The treatments evaluated in the lysimeters were two forage types (ryegrass/white clover and plantain), two aucubin rates (0 and 10 mg g^{-1} DM plantain) and two urine treatments (urine from cows grazing ryegrass/white clover (583 kg N ha^{-1}) and water as a control).

In 2017 and 2018, NO_3^--N leaching was lower from plantain field plots compared to ryegrass/white clover. However, in 2019, NO_3^--N leaching losses were similar for both pastures. The N_2O losses from the field experiment are discussed in an associated poster. The lysimeter experiment shows that plantain pastures reduced N_2O emissions and that aucubin had an inhibitor effect on N_2O emissions for the first 20 days after application. However, NO_3^--N leaching was variable and the effect of aucubin was not clear.

Introduction

Urine patches are the largest source of N losses from grazed dairy pastures and can cover between 4% to 29% of the grazed pasture area per year (Haynes & Williams, 1993; Moir, Cameron, Di, & Fertsak, 2011). N concentration in a urine patch can be up to 2000 kg N ha^{-1} (Selbie et al., 2015). This large N concentration in a small area is susceptible to loss to the environment due to exceeding immediate plant requirements (Ledgard, Schils, Erikson, & Luo, 2009). Nitrogen not up taken by plants can be lost as nitrous oxide (N_2O) and nitrate leaching causing environmental issues (NO_3^--N) (Cameron, Di, & Moir, 2013).

One strategy to reduce N_2O emissions and NO_3^--N leaching is the use of alternative forages to decrease N concentration in urine and also to modify the N cycle into the soil. Plantain (*Plantago lanceolata* L.) has been identified as a forage pasture which can release bioactive compounds (Tamura & Nishibe, 2002) to the soil that could potentially reduce nitrification rates in soils (de Klein, van der Weerden, Luo, Cameron, & Di, 2019; Dietz, Machill, Hoffmann, & Schmidtke, 2013), and subsequently decrease N losses to the environment. Aucubin is one of the bioactive compounds released by plantain and aucubigenin, the aglycone of aucubin, can inhibit cytochrome P-450 and its structure indicates that it can inhibit the enzyme ammonia monooxygenase (AMO) in soil, inhibiting the first step of the nitrification process (Bartholomaeus & Ahokas, 1995; Davini, Javarone, Trogolo, Aureli, & Pasolini, 1986).

Field studies have shown that pastures including plantain reduced NO_3^--N leaching (Carlton, Cameron, Di, Edwards, & Clough, 2018; Woods, Cameron, Edwards, Di, & Clough, 2018) and N_2O emissions from urine patches (Luo et al., 2018; Simon, de Klein, Worth, Rutherford, & Dieckow, 2019). Recently, studies have evaluated the effectiveness of aucubin to reduce N_2O emissions showing that aucubin can inhibit the nitrification in a short period of time after urine application. However, it did not reduce the cumulative N_2O emissions from urine patches (Gardiner et al. 2019). Different aucubin rates have been mixed with urine previously to apply to the soil and N_2O measured. Those aucubin rates ranged from 47 to 450 kg ha^{-1} (Dietz et al. 2013; Gardiner et al. 2019). However, no study has determined the effect of aucubin application immediately before urine deposition on N_2O fluxes and NO_3^--N leaching at the same time.

The main goal of the research presented in this paper was to provide a comprehensive understanding of N losses from pure plantain pasture compared to a standard ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture.

Materials and methods

Experiment 1

The experiment was set up on Massey University's Dairy Farm 4, near Palmerston North. The area used for this trial was subdivided in fifteen plots of 800 m^2 each. There were three pasture treatments: (i) plantain, (ii) plantain-clover mix, containing plantain, red clover (*Trifolium pratense* L.) and white clover, and (iii) perennial ryegrass/white clover which were grazed by lactating dairy cows. These pastures were established in December 2016 in a complete randomised design with 5 replicates each treatment. Three additional paddocks (approximately 1 ha) of each pasture treatment were also established near the experimental plots. These paddocks were used to fully adapt the cows' diet to the pasture treatments before grazing in the experimental plot treatments.

Grazing management

Sixty lactating dairy cows were selected from the Dairy Farm herd and separated in 3 groups (n=20) to graze one of the three pastures treatments through the 2017-2018 and 2018-2019 grazing seasons. Cows grazed the pasture treatments for a period of 8 days at each grazing period. During days 1 to 6 cows' diet was adapted to the pasture treatments in an area outside the plots (adaptation period) and days 7 to 8 cows grazed the plots (experimental period). During the experimental period, the 15 plots were grazed by a group of 4 cows simultaneously for two consecutive days. The pastures were grazed on seven and eight occasions during 2017-2018 and 2018-2019 grazing seasons, respectively.

During 2017-2018, the three pastures were fertilised and received 95 kg N/ha. In the second grazing season, plantain and ryegrass/wc pastures received 80 and 30 kg N/ha, respectively.

Drainage measurements and water analysis

The drainage plots contained an isolated mole and pipe drain system. Mole channels were ploughed at 2 m intervals at 0.45m of depth. These channels were intercepted by a gravel backfilled trench above a perforated pipe drain (0.11 m diameter and installed at 0.60 m of depth). This system was established before pasture establishment. Each plot was connected through a drainage pipe into a tipping- bucket flow meter (5 L capacity per tip) which was installed at the end of the drainage pipe to monitor drainage flow rates. During a rainfall event, every second tip was automatically collected, giving a 'proportion' of the total volume. Water samples (100 mL) were collected in each drainage event and a subsample was analysed for NO_3^-—N concentration using the IC (Ion Chromatography).

Data for this experiment was analysed using the model for a complete randomise design.

Experiment 2

Forty intact soil lysimeters (200 mm diameter x 250 mm depth) were collected in March 2019 from the pre-established pure plantain and ryegrass/wc pastures in experiment 1. The lysimeters were placed on tables which were previously set up for collection of drainage water. The experimental design for this trial was a 2 x 2 factorial design with two sward types (plantain and ryegrass-wc) and two aucbin rates (0 and 10 mg g^{-1} DM).

Aucubin (Xian Plant Bio-Engineering Co., Ltd ,China) was applied at a rate of 10 mg g DM^{-1} before urine deposition. The aucubin application rates were on average between the aucubin concentration in plantain leaves from the plots used in the main field experiment (experiment 1) and the rates found by Box and Judson (2018). Aucubin powder was dissolved in water at a rate of 600 L ha^{-1}, therefore each lysimeter received 1.7 mL of aucubin solution.

Urine from cows grazing ryegrass/wc was collected at Massey University Dairy Farm 4, 24 hours before application, and stored at 4°C to avoid urea hydrolysis. A sub-sample was analysed for total N and urea concentration. Cow urine was applied at a rate of 10 L m^{-2}, and the control treatments received the same volume as water, so moisture inputs were consistent across all lysimeters.

N_2O emissions

Nitrous oxide emissions were monitored during 87 days after treatment applications. The PVC chambers used for gas sampling were sealed for 60 minutes and sampling occurred at 0, 30 and 60 minutes. In the first month of the experiment, N_2O samples were taken twice a week and afterwards, samples were taken weekly until background level was reached. On each sampling day, three air samples were also taken at each time. The N_2O fluxes were calculated using the slope of the linear increase of N_2O concentration in the chamber over time (de Klein et al., 2003) using the following equation:

$$N_2O\ flux = \frac{\delta N_2O}{\delta T} * \frac{M}{Vm} * \frac{V}{A}$$

Where δ N_2O is the increase in N_2O in the headspace over time; δT is the enclosure period (hours); M is the molar weight of N in N_2O; Vm is the molar volume of the gas at the sampling temperature (L mol^{-1}); V is the headspace volume (m^3) and A is the area covered (m^2).

NO_3^--N leaching

Leachate was collected once per week, the volume of leachate was recorded and a subsample was taken to determine NO_3^- -N concentration by Ion Chromatography (IC).

Statistical analysis

Data were analysed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, 2009). Means were compared using the least squares means test and significance was declared at $P<0.05$.

Results and discussion

Experiment 1

The cumulative NO_3^--N leaching losses during this experiment are shown in Fig. 1. During the establishment phase (2017), although the pastures were grazed only twice (March and April 2017) and overall losses from the three pastures were low, the cumulative NO_3^--N losses from ryegrass/wc (1.22 ± 0.48 kg NO_3^--N/ha) pastures were higher ($P < 0.0238$) than plantain (0.15 ± 0.03 kg NO_3^--N/ha) and plantain-clovers mix (0.19 ± 0.03 kg NO_3^--N/ha). In 2018, after grazing the pastures 7 times during the 2017/2018 grazing season, cumulative NO_3^- -N losses were reduced by 48% in plantain pastures (2.78 ± 0.37 kg NO_3^--N/ha) compared to ryegrass/wc (5.35 ± 0.83 kg NO_3^--N/ha) and by 58% in comparison to plantain-clovers mix (6.64 ± 0.42 kg NO_3^--N/ha). Similarly, a lysimeter study found that NO_3^--N leaching losses from a ryegrass/wc/plantain sward were reduced by 78% compared to ryegrass/wc sward (Carlton et al. 2018). However, in 2019, NO_3^--N leaching between plantain (4.04 ± 0.37 kg NO_3^--N/ha) and ryegrass/wc (3.05 ± 0.53 kg NO_3^--N/ha) were lower ($P < 0.0003$) than plantain-clovers mix (9.42 ± 1.23 kg NO_3^--N/ha). The lack of difference in the cumulative NO_3^--N leaching during the 2019 drainage season could be due to the high clover content in plantain pastures compared to ryegrass/wc pastures (Fig.2). At the end of the 2018-2019 grazing season, clover content in plantain pasture was higher than in ryegrass/wc pastures and therefore could fix more N_2 from the atmosphere. Plantain pastures also received more N fertilisation than ryegrass/wc (80 *vs* 30 kg N/ha) in the 2018/2019 grazing season

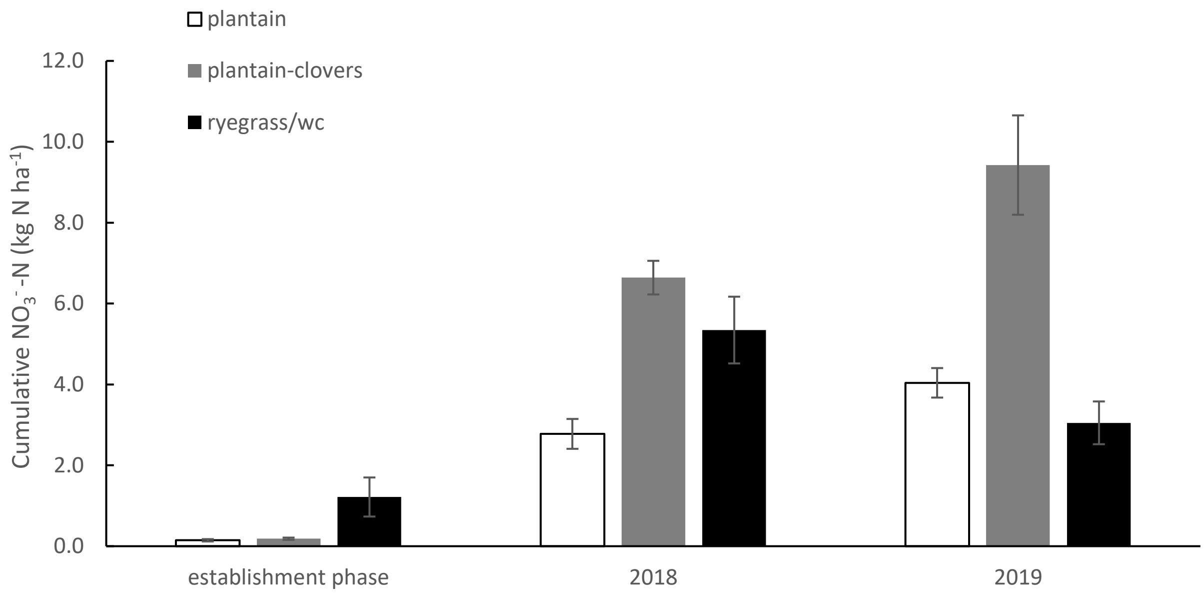


Fig. 1. Cumulative NO_3^--N (kg NO_3^--N/ha). leaching in drainage water from plantain (white bar), plantain- clovers mix (grey) and ryegrass/wc (black) pastures during the establishment phase of the experiment, 2018 and 2019 drainage seasons.

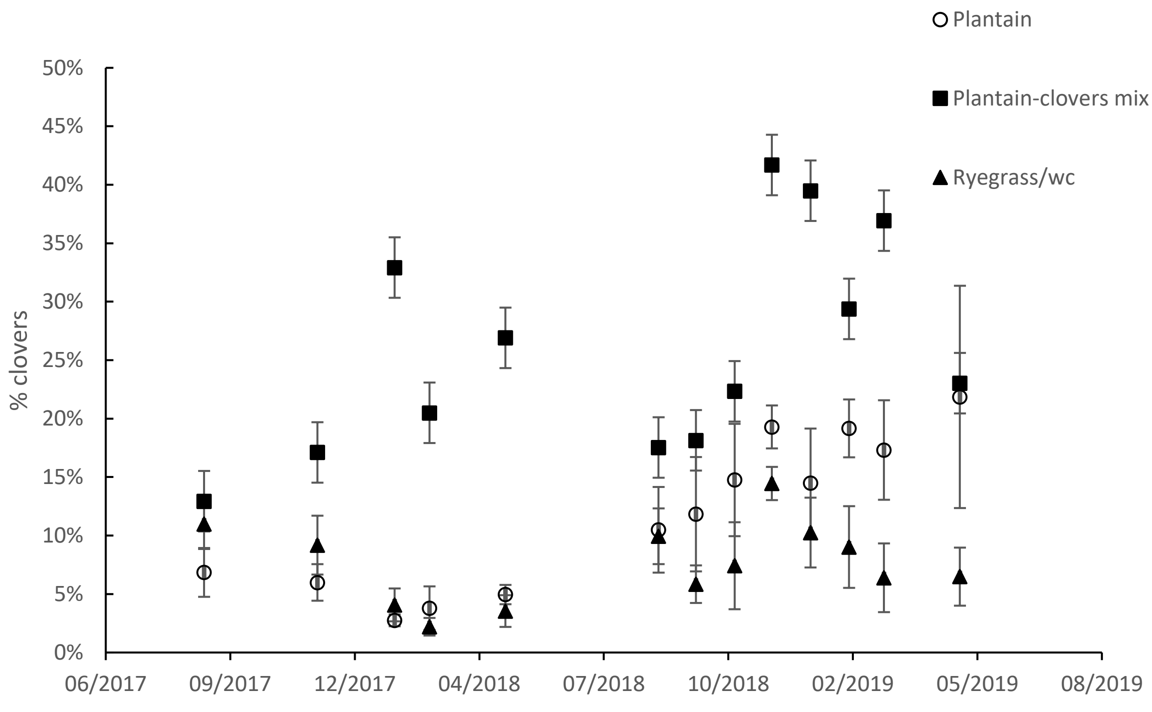
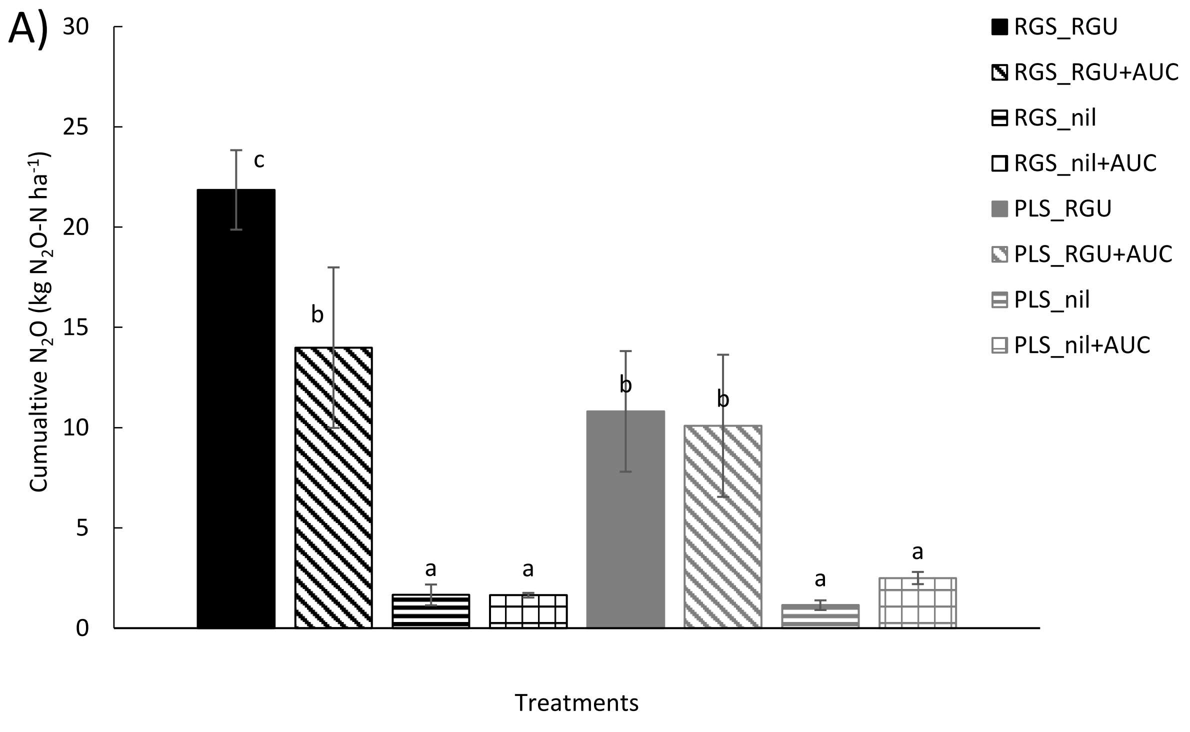


Fig. 2. Clover (white and red clovers) content (%) in plantain (white circle), plantain-clovers mix (black square) and ryegrass/wc (black triangle) pastures during the two full seasons (2018 and 2019).

Experiment 2

In experiment 2, plantain sward reduced N_2O emissions by 34% compared to ryegrass/wc sward (Fig 3A) in agreement with previous studies reporting lower N_2O emissions from plantain swards (Luo et al., 2018). Nitrous oxide emissions from urine patches were also reduced when aucubin was applied at a rate of 10 mg g^{-1} DM (243 kg/ha) in ryegrass/wc. In a laboratory experiment where aucubin was applied to the soil, at three different rates (47, 243 and 487 kg/ha) with urine (700 kg N/ ha), was found a reduction in N_2O emissions only from days 5-11 compared to urine treatment (Gardiner et al., 2019). However, the cumulative N_2O emissions over the 35 days of measurement were no different. They suggested an inhibition in the nitrification process during days 5-11 only.

In this experiment, aucubin application did not decrease NO_3^--N leaching under either the ryegrass/wc or plantain swards (Fig 3B).



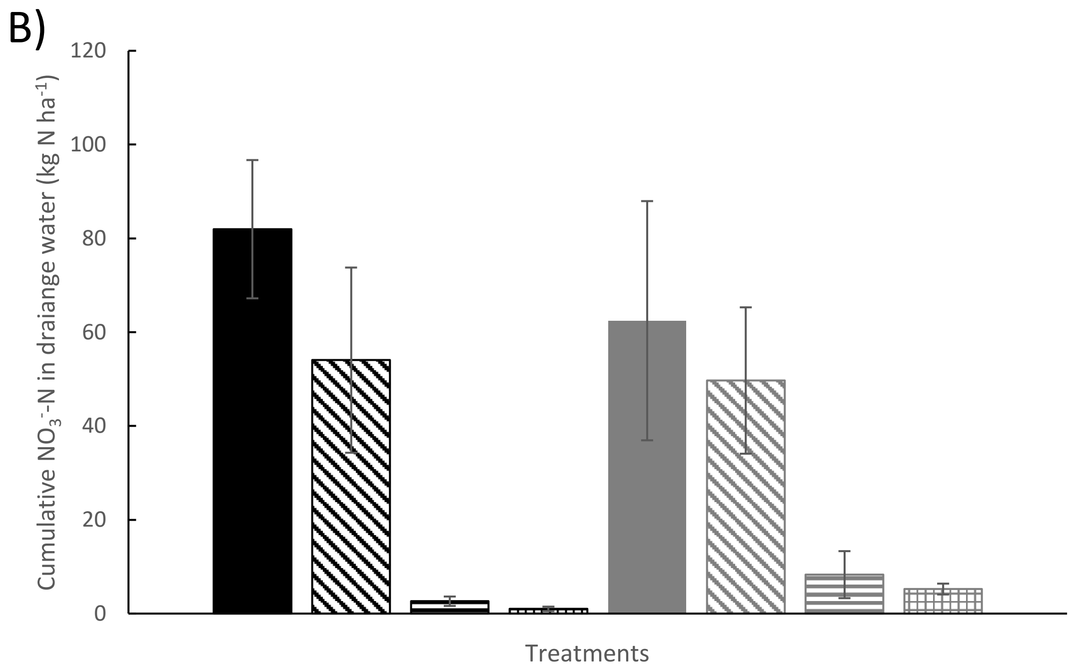


Fig. 3. Nitrous oxide (a) and Nitrate leaching (b) losses after treatments application. RGS_RGU= ryegrass/wc sward and ryegrass/wc urine; RGS_RGU+AUC= ryegrass/wc sward, ryegrass/wc urine and aucubin; RGS_nil= ryegrass/wc sward with water; RGS_nil+AUC= ryegrass/wc sward with water and aucubin; PLS_RGU= plantain sward and ryegrass/wc urine; PLS _RGU+AUC= plantain sward, ryegrass/wc urine and aucubin; PLS _nil= plantain sward with water; PLS_nil+AUC= plantain sward with water and aucubin.

Conclusions

The field experiment suggests that pure plantain reduced NO_3^--N leaching during the first season (2018) but not in the second season which was likely due to the high clover content and the decrease of plantain proportion in the animal diet.

The results from the lysimeter experiment suggest that aucubin application can reduce N_2O emissions from urine patches on ryegrass/wc swards and that plantain pastures may have potential as an effective alternative to ryegrass/wc to reduce N_2O losses.

References

Box, L. A., & Judson, H. G. (2018). The concentration of bioactive compounds in Plantago lanceolata is genotype specific. *Journal of New Zealand Grassland, 80*, 113- 118.

Cameron, K. C., Di, H. J., & Moir, J. L. (2013). Nitrogen losses from the soil/plant system: a review. *Annals of Applied Biology, 162*(2), 145-173. doi:10.1111/aab.12014

Carlton, A. J., Cameron, K. C., Di, H. J., Edwards, G. R., & Clough, T. J. (2018). Nitrate leaching losses are lower from ryegrass/white clover forages containing plantain than from ryegrass/white clover forages under different irrigation. *New Zealand Journal of Agricultural Research*, 1-23. doi:10.1080/00288233.2018.1461659 de Klein, C. A. M., van der Weerden, T. J., Luo, J., Cameron, K. C., & Di, H. J. (2019). A review of plant options for mitigating nitrous oxide emissions from pasture-based systems. *New Zealand Journal of Agricultural Research*, 1-15. doi:10.1080/00288233.2019.1614073

Dietz, M., Machill, S., Hoffmann, H. C., & Schmidtke, K. (2013). Inhibitory effects of *Plantago lanceolata* L. on soil N mineralization. *Plant and Soil, 368*(1-2), 445-458. doi:10.1007/s11104-012-1524-9

Gardiner, C. A., Clough, T. J., Cameron, K. C., Di, H. J., & Edwards, G. R. (2019). Ruminant urine patch nitrification and N_2O flux: effects of urine aucubin rate in a laboratory trial. *New Zealand Journal of Agricultural Research*, 1-8.

Haynes, R. J., & Williams, P. H. (1993). Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in agronomy, 49*, 119-199.

Ledgard, S., Schils, R., Erikson, J., & Luo, J. (2009). Environmental impacts of grazed clover/grass pastures. In: Teagasc, Oak Park, Carlow, Ireland.

Luo, J., Balvert, S. F., Wise, B., Welten, B., Ledgard, S. F., de Klein, C. A. M., . . . Judge, A. (2018). Using alternative forage species to reduce emissions of the greenhouse gas nitrous oxide from cattle urine deposited onto soil. *Science of The Total Environment, 610–611*, 1271-1280. doi:https://doi.org/10.1016/j.scitotenv.2017.08.186

Moir, J. L., Cameron, K. C., Di, H. J., & Fertsak, U. (2011). The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. *The Journal of Agricultural Science, 149*(4), 473-485.

Selbie, D. R., Buckthought, L. E., & Shepherd, M. A. (2015). The Challenge of the Urine Patch for Managing Nitrogen in Grazed Pasture Systems. *Advances in agronomy, Volume 129*, 229-292. doi:http://dx.doi.org/10.1016/bs.agron.2014.09.004