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Effects of a microbial-based soil conditioner on nitrate leaching.

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Abstract

The biological activity in soil is important to ensure plant growth and prevent leaching. However, soils that have been exposed to inorganic minerals and acidic chemicals have a depleted microbiome. Replacing this using specialised probiotics can reverse these problems. In this trial, lysimeters to collect ground water leachate were set up to measure the effect of microbial-based soil and plant products (MagnifyNZ Ltd) on nitrate leaching on a dairy farm in Canterbury (2.5 cows/ha). MagnifyNZ fermentation products establish an effective soil microbiome, creating opportunity for high output-low input agriculture/horticulture. The soils were stony shallow silt loams (400 mm deep), classified as Ruapuna and Darnley (medium and high leaching). Fertiliser was applied monthly to provide 190 kg of N/ha/yr. Two treatments were used (soil conditioner and plant biostimulant) in September 2022, January and August 2023 using Magni-Life (2 l/ha) and Magni-Grow (7 l/ha), with and without stored urine, to mimic cow N contributions (600 kg N/ha). The untreated control was Ruapuna soil using the same applications. Lysimeters generated 3-4 drainage collections monthly. Non-urine soil leaching averaged 0.77 mg NO_3-N/l for the MagnifyNZ samples compared to 3.98 mg NO_3-N/l ($P<0.0001$) for the control. There was no significant difference in drainage volumes between treatments, which was 325 mm from 901 mm rainfall plus irrigation over 12 months. Urine patch N lasted 40 d in MagnifyNZ soil *versus* 215 d for the control. Total nitrate loss was 1.18 and 15.04 mg NO_3-N/l for the MagnifyNZ treatment and control, respectively ($P<0.0001$). For the year starting September 2022, estimated total leaching was 3.1 kg N/ha for the treated soils *versus* 48.99 kg N/ha for the control, compared to 17.15 kg N/ha for the calculated dicyandiamide (DCD) response (Di and Cameron, 2007). There was a 91% reduction in total nitrate leaching from the MagnifyNZ treatment, due to a more stable microbiome which affected absorption of available N by pasture and soil microbes. Modelling the results for DCD (Di and Cameron, 2007) using 600 kg N/ha urine, DCD's would have given 20-65% reduction in leaching.

Research highlights:

- Nitrate leaching is a major problem in NZ dairy pastures, leading to toxic water courses.
- Farm soil management needs to change to reduce nitrate and improve pasture growth and quality.
- Adding MagnifyNZ boosts the soil microbiome, improving nutrient availability to plants.
- MagnifyNZ reduces leaching by allowing nitrate to be utilised by plants, even under urine patches.

Keywords: DCD, leaching, microbiome, nitrate, soil microbiome

Introduction

Globally, drinking water nitrates in both well and surface water sources significantly threaten human and ecosystem health. From surface water sources alone, during 1970-2010, global populations potentially affected by potential chronic health risks increased from 169 to 1361 million persons per year (Wang *et al* 2023). Elevated nitrates in water impact human, animal and environmental health, plus - indirectly - global warming via nitrous oxide emissions. It is essential to reduce the nitrates from both surface runoff plus drainage from intensively managed agricultural soils.

Intensive farming practices in the last 70 years have relied on acidic and inorganic fertilisers and chemicals to promote plant growth. However, this has depleted the soil of its biological activity, *i.e.*, the micro-organisms (soil microbiome) which are essential for the correct transformation and valency of nutrients for uptake by root systems. Where cattle are fed high protein (>15%), which is common in NZ, increased levels of nitrates are excreted in urine, which can further exacerbate leaching into ground water and water courses (Wilkinson and Waldron, 2017). This has had a major effect on the availability of nitrates, which may lead to increased nitrogen leaching and less availability for plant growth.

The synergy between soil and its microbiome has evolved over time to efficiently use N without excess losses through leaching and to ensure good uptake in plants. Magni-N-Enviro manufactured by MagnifyNZ Limited is a biofertiliser product which is composed of specific micro-organisms that can re-establish correct soil biology and regenerate soil. In addition it includes a biostimulant (fermentation based) which promotes the soil microbiome and allows nutrients to be better utilised by plants.

Reducing soil nitrogen (N) leaching into waterways is essential for preventing pollution and protecting both animal and human health. Excess N in water (mainly in the form of nitrates) increases eutrophication, destroys ecosystems and increases the growth of pathogenic organisms, including toxic algae. The N in soil is naturally derived from atmospheric sources and the breakdown of organic matter in soil. More recently, the use of chemical fertilisers and the increase in intensive farming systems have added to soil reserves. Nitrogen as ammonium can be held in high levels in soil as it is positively charged and is held by negative surfaces in soil. It is subsequently converted by nitrifying bacteria creating high levels of water soluble nitrates which then drain through the soil and enter water courses. As water availability and quality is an on-going concern around the world, protecting sources, especially those used for drinking and leisure activities, is crucial. In New Zealand it has become common in the last 20 years for many rivers and lakes to be 'red zoned', meaning they are contaminated with pathogenic bacteria or toxic algae that can cause illness or even death in the case of smaller animals and children.

Levels of leached nitrates into water is dependent on several factors, including soil type and structure, historical fertiliser inputs (and whether these were matched to requirements or applied *ad hoc*) and type of farming (animals, intensity, production system). For example, the grass-only dairy system promoted in New Zealand utilises high protein-low fibre rye grass/clover legume leys, which typically exceed dietary protein requirements (often by a large margin), which results in high levels of nitrogen being excreted from cows, mainly in urine (Wilkinson and Waldron, 2017). Hence, there is a need to control the excess levels of nitrates in soil to prevent pollution and protect human and animal health.

Soil and water nitrates are, of course, correlated to atmospheric N_2O. For example, Di and Cameron (2006) found that a 59% reduction in nitrates leached yielded an 82% reduction in nitrous oxide. The global warming potential of N_2O in the long-term is about 320 times that of carbon dioxide (CO_2). The amount of N_2O directly emitted from agricultural fields may account for 20-30% of the total N_2O emitted annually from the earth's surface (Mosier, 1994). In grazed grassland systems, a major source for emissions is the N from animal excreta, predominantly urine (Oenema *et al.*, 1997). For example, in New Zealand, N emissions from animal excreta account for approximately 50% of the country's total emissions (de Klein *et al.*, 2001). Total N_2O emissions make up about 20% of New Zealand's greenhouse gas emissions inventory. A significant reduction in N_2O emissions from animal excreta in grazed pastures will make an important contribution to reducing total greenhouse gas emissions in the country.

The New Zealand Government (November 2022) set drinking water standards (DWS) at 11.3 mg NO_3-N/l at all times of the year and proposed reducing the maximum N levels in fresh water (FWS) to 2.4 mg NO_3-N/l. Many small freshwater streams in New Zealand are predominantly supplied from drainage through farmland soils and so leachate from the farms have to be below 2.4 mg NO_3-N/l to meet FWS. Another pressure for future DWS levels comes from human health concerns. Historically, DWS was set to protect against infant methemoglobinemia (blue baby syndrome), as excess nitrate intake from water causes gut inflammation and infections related to the syndrome. When nitrates in water are above 11 ppm, incidents of methemoglobinemia become apparent and then escalate rapidly, more than doubling when nitrates move from 30-80 ppm (Avery, 1999).

There is an increasing body of epidemiological evidence showing an elevated risk of human colorectal cancer, even at nitrate levels below the current DWS (Schullehner *et al*., 2017; Ward *et al*, 2018; Temkin *et al*., 2019; Mathewson *et al*., 2020; Chambers *et al*., 2022; Richards *et al*., 2022; Jacobsen *et al.,* 2023). Earlier studies (McElroy *et al*., 2008) reported a 2.9-fold increased risk of proximal colonic cancer when drinking water nitrates exceeded 10 mg NO_3-N/l . This same pollution level resulted in an increased odds ratio of 1.49 for colorectal cancer occurrence in a study in a Spanish and Italian population (Espejo-Herrera *et al.,* 2016). Schullehner *et al*. (2018) showed that the hazard ratio for colorectal cancer was 1.16 in subjects exposed to high levels of nitrates in water, which significantly increased when water levels exceeded 3.87 mg NO_3-N/l. Jacobsen *et al.* (2023) showed major economic benefits for Denmark by reducing DWS to 2.13 mg NO_3-N/l. Hence, there is a benefit in reducing farming nitrate leaching to below 2.4 mg NO_3-N/l.

High nitrates and the concurrent increases in toxic algae growth have major health implications in animals. Farm animals have been known to be at high risk for a long time (Francis, 1878), whereby, when a dam was contaminated with toxic algae in Australia and used as a drinking water supply, it caused the death of sheep within 10-8 h, horses 8-24 h, dogs 4-5 h and pigs within 3-4 h. Many reports of algal toxicity in cattle have been published, starting with Fitch *et al.* (1934) in Minnesota, USA. In terms of damage to ecosystems, fish exposed to algal toxins in polluted waterways typically died within 6 h (Prescott, 1948). More recent work, under controlled conditions relating to toxic algae in contaminated water conducted in Canada, have shown that contaminated water supplies can reduce milk output from dairy cows by 1.4 litres per day, and reduce growth of calves by 9%, yearling heifers by 23% and body weight of suckler cows by 25%. In yearling cattle, contamination reduced water intake from an average of 154 litres per day down to 113 litres, and corresponding feed intake from 32 to 28 kg (Willms *et al*., 2002).

Bio-stimulants are a growth sector in agriculture and several products have been developed for commercial use, including MagnifyNZ. For example, humates and biochar are considered to be valuable farm bio-stimulants for soil and environmental health. In NZ, Espie (2023, preprint) showed adding 10-20% humate to urea by weight reduced nitrates leached by 50-61%, but only 9% was seen when this was modelled under Russian farming conditions. However, both studies used low rates of nitrogen (5-22 kg N/ha; Korsakov *et al.,* 2023) and no urine applications were included. In addition, NZ trials showed that overall microbial biomass decreased 30%, while in Russia it increased by 7%. Güereña *et al.* (2013) showed that nitrate leaching was reduced by 82% when 108 kg N/Ha fertiliser was applied to maize crops in temperate climates two years after 12 t/ha of biochar had been applied to the soil. However, no change occurred with only 54 kg N/ha. Although soil microbial mass increased three-fold there was no increase in N use efficiency or plant yield.

There has been significant amounts of research done with carbon containing compounds like biochar and humates regarding their influence on soil nitrogen availability/retention, although leaching studies that have included urine N loadings are hard to find. Humate can be more affordable than biochar, but there are large variations in such products and farmer acceptance is still relatively low in spite of these substances being available for over 50 years. Soil bacterial and fungal populations have been studied but results such as those from Espie (2023) suggested leaching reductions and crop yields are more complex than solely being related to biomass. There is little variation in NPKS fertilisers compared to biological products that, despite appearing similar in nature, produce markedly different results. Most bio-stimulants have been used to increase nutrient uptake in crops, although increasing nitrogen concentrations in the pasture still leads to increased leaching. Our literature searches found no published papers assessing the effects of living biological inoculants on reducing nitrate leaching.

The desire for high efficiency fertilisers has seen a trend in the use of nitrogen inhibitors. MagnifyNZ has been primarily used to reduce nitrogen fertiliser application rates as it includes competitive, beneficial microbes which replenishes the microbiome, allowing correct soil biological activity essential for plant growth and to prevent leaching of N. As part of a literature review Ghorbani *et al.,* (2008) stated that 'Plants growing in disease-suppressive soil resist diseases much better than in soils with low biological diversity.' Magnify aims to create disease suppressive soils and has suppressed common agricultural and crop diseases, such as those caused by *Fusarium*, *Sclerotinia* and *Phytophora* and *Gaeumannoyces* ('take-all' in wheat) spp. Leaching is a significant threat to Dairy farming in NZ and this study was conducted to see if the Magnify products would have a significant impact on leaching as a useful addition to already established benefits. Since the removal of DCD's from the market NZ agriculture has been hoping to find a replacement to help with a growing nitrate issues. Our hope was Magnify's natural products would create nitrate reductions equal or better than historic chemical nitrogen inhibitors. Hence, the following trial was conducted in New Zealand to evaluate the effect of using a biological product (Magni-N-Enviro) on controlling levels of nitrogen in soil leachate.

Methods

Grass growth and milk production was monitored from standard farm recording practises (*e.g*., plate meters and in-shed milking record systems). Trials to monitor the response of soil to MagnifyNZ and its effect on nitrate leaching were conducted using lysimeters to collect drainage samples. Farms in the Canterbury region of NZ, which is an intensively farmed and irrigated dairy area, were used in the trials. The soil type in this region is stony silt loam over a gravel base – which has a medium to high leaching risk. At the time, local environmental bodies required 90% reductions in nitrate leaching and the aim was to reduce leaching to under 3 mg nitrate nitrogen per litre of water.

Paddocks in the fertiliser treatment group were treated with Ravensdown Dairy Pasture Boost 200 kg in October and urea monthly, to make 150 kg of N/ha for the 2022/2023 milking season. An additional 70 kg of urea per hectare was applied in early August 2023 for the start of the new milking season. For the paddocks in the MagnifyNZ treatment group, the specific product Magni-N-Enviro (9 l/ha) was applied in September 2022, January 2023 and August 2023. This approach was used to give the maximum chance of reducing leaching. This product contained a mixture of soil organisms that have been identified as being important in optimising soil biology.

In accordance with accepted practice (Beale, 2021), the drainage data was the average of all four, medium size, above ground monoliths (250 mm diameter) lysimeters for both the control and MagnifyNZ treatments. Free draining shingle started at 400 mm deep and few roots went below this level. The monolithic lysimeters were made from 250 mm bore pipe, 400 mm deep. Soil was hand dug away from the perimeter and the lysimeters pushed down over the core. It was then turned sideways and lifted out of the hole. They were carefully inverted. The bottom 50 mm of soil was removed and replaced with 2 cm of sand and then 3 cm of fine stones followed with a fine mesh made from industrial shadecloth. The base plates were attached using industrial MS silicon sealant and 4 rachet ties per lysimeter further secured the base to the top.
Below ground level lysimeters are not always buried by soil and may not reflect field soil temperatures. (Cameron *et al.,* 1992, Selbie 2014). This has obvious advantages when you have many different soil types being studied but it is very costly and does not necessarily reflect the temperatures of the soil the lysimeters have come from. Due to resource constraints we adopted a keep it simple philosophy. Lysimeters were kept above ground and the outside insulated with wool and wrapped in thick builders aluminium foil to reflect heat.

The lysimeter soil temperatures were checked against field soil temperatures and found to be the same. Insulation coverings were brought over the top edge of the soil to prevent rain from hitting the edges directly to eliminate any direct flow of water down the sides of the lysimeter.

Petroleum jelly was pushed into any gaps between the soil and the walls of the lysimeters and regularly checked. Despite regular rainfall, for two months there was no drainage at all indicating there was a good seal between the soil and inner edge of the lysimeter casing. The drainage water was collected and analysed for nitrate levels using standard drinking water testing methods (Hill Laboratories, New Zealand; https://www.hill-labs.co.nz/media/l12kzk5i/technical-note-water-testing-for-drinking-water.pdf), before being replaced by a LAQUA TWIN nitrate reader (Horiba, Kyoto, Japan), which was verified as producing the same results. All lysimeters were tested with two drainage events to ensure lysimeters did not accidentally include urine patches.

In late Autumn, full urinations were collected from cows on the MagnifyNZ treated pastures and frozen. This urine contained 4.5 mg/l with an average of 1.5 l/urination, giving 6 g of N excreted per urination. In late Autumn, the average soil area covered by 1.5 l was tested and was found to be 0.36 m^2, giving 150 kg N/ha. Based on previous studies on leaching in NZ, a urine spread assumption of 0.2 m^2 (Beale *et al.,* 2021) was used for both the MagnifyNZ treated and control paddocks. This gave more accurate comparisons against other studies that have commonly used this default assumption.

Monolith lysimeters had urine applied at 200 kg N/ha and 700 kg N/ha for the MagnifyNZ and Control lysimeters, respectively. This allowed the lower urine N output created from MagnifyNZ lower N pastures to be included in the comparison. In unpublished studies, cow urine volumes were 0.8 - 1.5 l/urination with 0.2 - 6 g/l. This was in line with expected changes (Castillo *et al.,* 2000) due to lower dietary N. For the MagnifyNZ treatment, using larger hybrid lysimeters, urine was applied at 600 kg/ha, more than three-times the actual concentration measured and calculated in the field.

Additionally (8 months prior to the establishment of monolithic lysimeters) larger hybrid lysimeters (1.1 m x 0.9 m) were made using IBC totes to 600 mm in depth (Figure 1). These were established in March 2022. Half-sectioned PVC pipes (150 mm) with mesh covered by river shingle were placed in the bottom of the lysimeters to cover the drain hole for the IBC. The bottom 400 mm layer of the soil profile was added at 100 mm layers at a time with human bodyweight gently compacting each layer. The top 250 mm of soil was cut into squares and placed on top and any gaps were filled with topsoil. On 6 June 2022, a full rate of urine (6 g N at 1.5 l) was applied (600 kg N/ha equivalent). Measurements were then started in September and 200 days after this (22 March 2023), further urine was applied at 600 kg N/ha. One was established for a Ruapuna*f* (Sib 2) soil (medium Nitrate leaching susceptibility) and an additional one for a Darnley soil (high nitrate leaching susceptibility). Monolith lysimeters were used to support and cross check the data from these original lysimeter designs. To avoid confusion this report focuses only on lysimeters with the same leaching susceptibility - Ruapuna soils. Ruapuna*f* (Sib 2) are stony (5-35%) shallow, well drained silts. There was a low budget for this study, however, there was no evidence that the results were compromised by this.

For the MagnifyNZ paddocks, the urine adjustment factor was 0.024 ha for urine (0.2 m^2 x 14 urinations/day x 2.5 cows/ha x 35 d). Under Magnify treatment the 35 d period was used because the spike in nitrogen was measured to last for this length of time. This factor was multiplied by the nitrates under the urine patches to calculate the influence of the urine for field application. For the control, the default urine adjustment factor was 0.14 ha (0.2 m^2 x 14 urinations/day x 2.5 cows/ha x 209 d) with the spike in nitrogen lasting 209 d before returning to base levels. This trial assumed 14 urinations/d in line with a previous field study previously conducted on a Magnify treated farm (unpublished data).

Lysimeter readings started in January 2023 and were recorded approximately weekly to give a monthly average, along with the rainfall at the trial site. Irrigation was used to ensure drainage happened, even if it was at a time of year when little drainage occurs naturally in the Canterbury region. This was to simulate large rainfall events, as these have been shown to cause a trend of spiking nitrate levels in water. Measurements were taken within 1-3 days of drainage events.

Statistical analysis was performed using ANOVA in the GLM procedure of Unistat (v.10, Unistat, London, UK) with MagnifyNZ and urine application as factors, and $P<0.05$ was deemed significant. Due to the limitations regarding lysimeter numbers, the monthly averages were used as replicates over time, and as a direct comparison between the treatments (with or without urine or MagnifyNZ). Means were separated using Duncans method. Ideally, a negative control soil area would have been useful to include but this was not achievable, given that all farms on this soil type typically use some form of nitrogenous fertiliser.

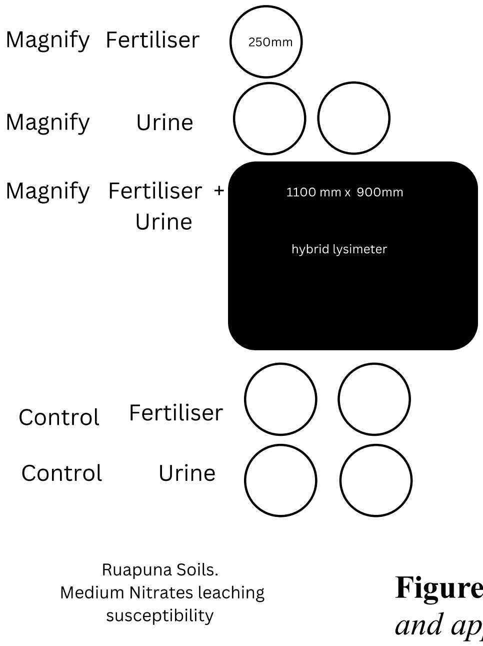


Figure 1. *Schematic of lysimeter placement and application areas for fertiliser and urine*

Results and discussion

In spite of receiving 40 kg less nitrogen, the extra grass growth generated by the MagnifyNZ products was 20-70 kg DM/day for January to April and September to October. The average growth for the control areas was 46 kg DM/day for this period. The extra growth was 300 % for the MagnifyNZ treated areas from June to September (15 kg DM/day compared to 5 kg DM/day for the control areas). The MagnifyNZ treatment showed greater consistency for growing grass with low nitrogen inputs, thus the urine nitrogen was significantly decreased from traditionally recognised standards (700-750 kg N/ha) typically used in leaching studies in NZ.

In this study the grass /clover sward tests were significantly lower than the default level of 28.75% assumed by Overseer modelling (Overseer, Wellington, New Zealand). The protein levels were 21.25% in October 2021, 17.5% in December 2021, 15.6% in March 2022 and 19.3% in September 2022. These levels still exceeded the maximum that ruminants can process, whereby the rest is excreted in the urine (Wilkinson and Waldron, 2017). Plant nitrogen levels were within the critical levels necessary for 90-99% herbage yield maximums (Langworthy *et al.,* 2023, Whitehead 1995) There was no difference in rainfall or irrigation inputs recorded between lysimeters (P=0.977).

The results for the effect of applying MagnifyNZ to soils on nitrate leaching are shown in Table 1 below.

Table 1. Average nitrates and calculated total leaching using 0.14 ha urine coverage for control and 0.024 ha for MagnifyNZ treated soils

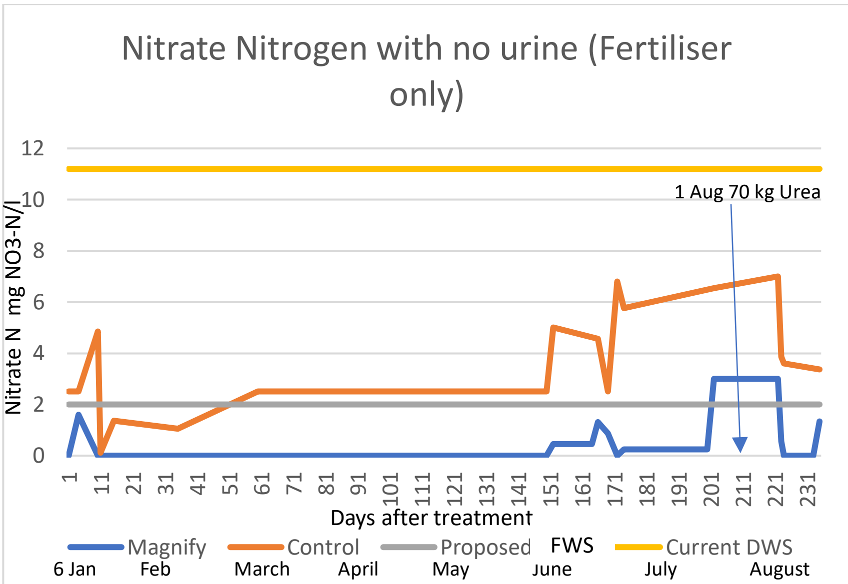
Treatment	Average nitrates mg NO3-N/l	Total drainage[1] mm	Total leaching[1] kg N/ha
Control plus urine	79.92[b]	90.51	9.82
Control no urine	3.99[a]	90.51	3.55
MagnifyNZ plus urine	17.53[a]	92.61	0.73
MagnifyNZ no urine	0.77[a]	92.16	0.077
SEM	5.45	Calculated	Calculated
P value	<0.0001		-

Means not sharing a superscript differ significantly (P<0.05). Total nitrates and leaching were calculated at the end of the trial period, and therefore were not replicated over time and statistical analysis was not performed on these parameters.

The lysimeter results showed that, from January to August 2023, for the urea fertiliser in the control group, nitrate leaching from the lysimeters (400 mm depth) was 3.99 mg NO_3-N/l compared with 0.77 mg NO_3-N/l for the MagnifyNZ group (81% less). These values were not significantly different, which was due to the large

SEM caused by the high leaching from the control plus urine treatment. After urine application, N leaching was 79.92 mg NO_3-N/l for the control soil, which was significantly higher than the 17.53 mg NO_3-N/l for the MagnifyNZ treatment ($P<0.05$), when these treatments were compared individually as part of a separate GLM analysis. The control reached a peak of 164 mg NO_3-N/l and took 209 d to return to base levels. Urine applications with MagnifyNZ reached a peak of 80 mg NO_3-N/l and took 35 d to return to base levels. Peak nitrate concentrations for 700 kg N/ha urine loading were consistent with other studies. For example, Mannings *et al.* (2012) and Selbie (2014) showed a 700 kg N/ha peak at 220 mg NO3-N/l and 163 mg NO3-N/l. Selbie (2014) also reported 300 kg N/ha peaked at 83 mg NO3-N/l. Average and total drainage for the MagnifyNZ and control lysimeters were not significantly different (82 - 98mm P=0.99). There was no significant differences in total drainage between individual lysimeters either (range 68-122 mm; P=0.6).

Figures 2-7 illustrate the effects of MagnifyNZ on leaching and nitrates over the recorded period and against accumulative drainage.



Days after 6 January	Urea fertiliser applied Kg N/ha
1	30 kg
21	30 kg
42	30 kg
107	10 kg
New season 213	35 kg

Figure 2. Fertiliser only. *Influence of MagnifyNZ soil application from each drainage event on nitrate nitrogen levels with 190 kg N/ha/year in Canterbury (January to August). X axis is days after urine application on 6 January 2023. DWS = drinking water standard. FWS = Fresh Water Standard*

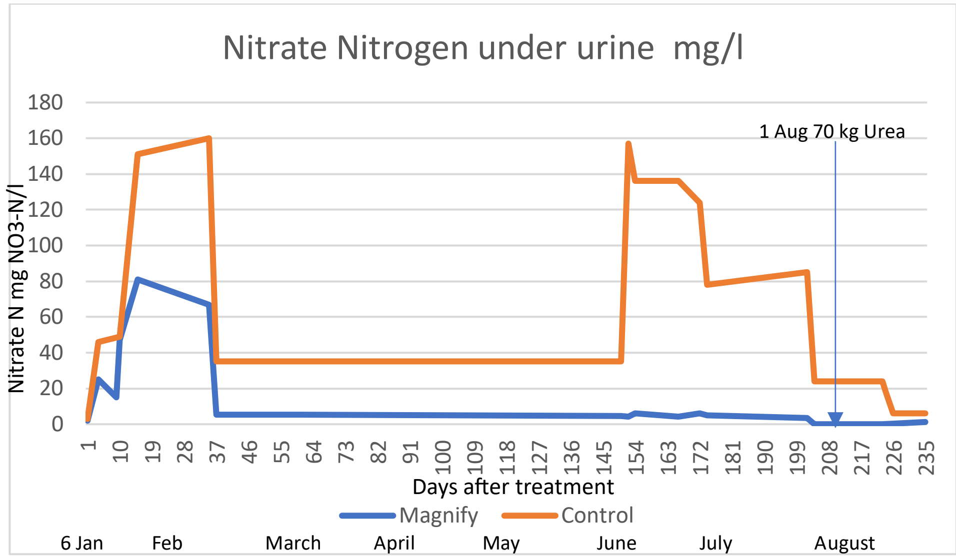


Figure 3. Under Urine. *The number of days nitrate levels from soils treated were elevated under urine patches before returning to base levels. X axis is days after urine application. The significant urine effect lasted 215 days for control and 35 days for MagnifyNZ.*

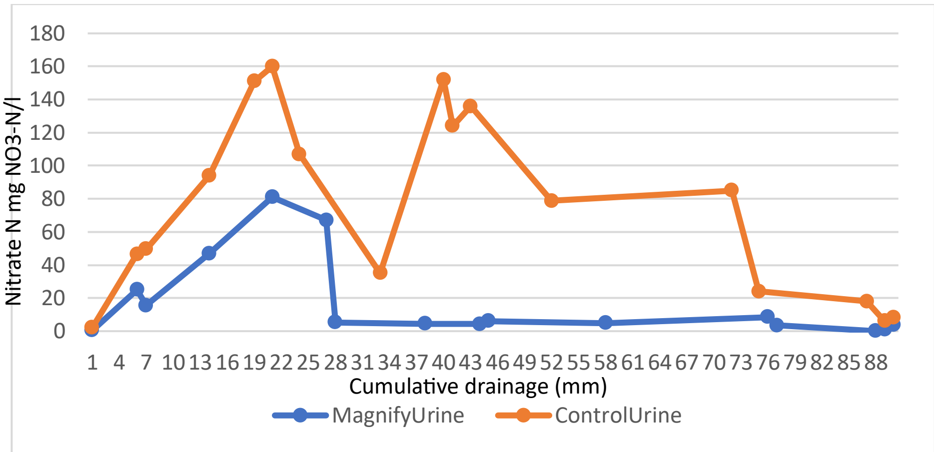
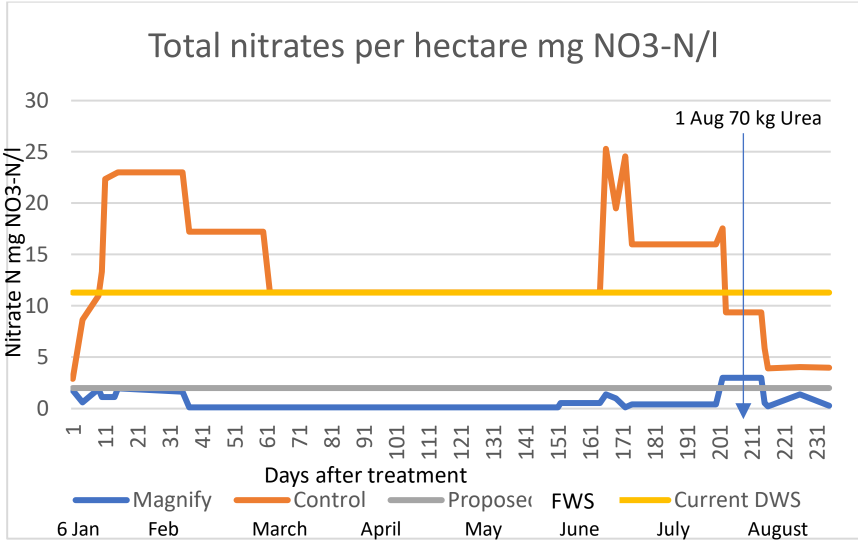
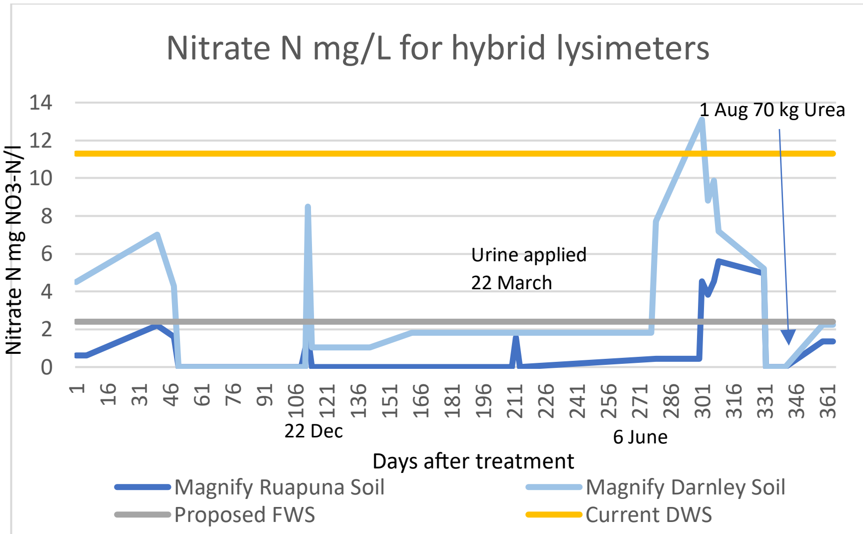


Figure 4*. Nitrate nitrogen levels (mg NO_3-N/l) under urine patches against cumulative drainage (mm).*



Days after 6 January	Urea fertiliser applied Kg N/ha
1	30 kg
21	30 kg
42	30 kg
107	10 kg
New season 213	35 kg

***Figure 5. Total nitrate nitrogen** per hectare (nitrates from urine patches + urea treatment proportioned for the respective paddock coverage area). X axis is days after urine application. Average total nitrate nitrogen were 1.18 and 15.04 mg NO3-N/l for MagnifyNZ and Control respectively.*



Date	Nitrogen fertiliser applied Kg N/ha
10 Aug	35 kg
1 Nov	30 kg
5 Dec	25 kg
6 Jan	30 kg
17 Feb	30 kg
22 March	30 kg
25 April	10 kg
New season	
8 Aug	35 kg

***Figure 6. Large Hybrid lysimeter** readings for field total nitrate nitrogen levels starting in 26 August 2022. Urine was applied at 600 kg N/ha in June2022 and late March 2023. Nitrate leaching susceptibility is medium and high for Ruapuna(sib2) and Darnley(sib7) soils respectively.*

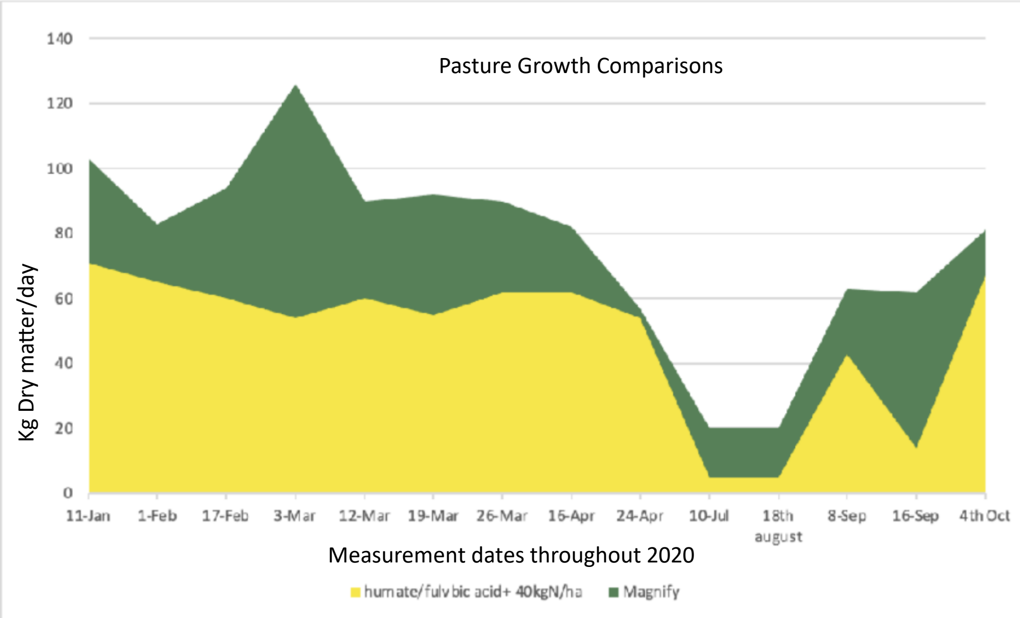


Figure 7. Pasture Growth data: *10 month field pasture growth rate data for 2020. Two applications of Magni-N-Enviro treatment replaced 2 applications of a humate/fulvic acid and liquid nitrogen (20 kg N/ha/application) product. Overall N inputs were 190 kg N/ha on the humate treated paddocks and 150 kg N/ha for MagnifyNZ treated paddocks. All paddocks were 8-10 year old ryegrass clover pastures.*

Lysimeters can be unpredictable in drainage consistency and edge-flow can be an issue. Peters and Durner (2009) showed 250 mm lysimeters had a collection efficiency of 66 and 72% for soils with 50 and 75% heterogeneity soil (standard deviation 6.7 and 8.5 with coefficient of variation 10.2 and 11.8%, respectively). Hence, the lysimeters used in the current study were operating within previously recorded ranges, although it must be borne in mind that published data for direct comparisons is limited. Edgeflow is when water flows freely between the soil monolith and the casing of the lysimeter, which increases water flow rates and can elevate nitrate concentrations (Cameron *et al.,* 1990). An edge-flow test (Cameron *et al.*, 1990) was conducted on two lysimeters and the results were consistent with no edge-flow. Interestingly, Cameron's study showed little difference in solute concentrations when edge flow was significantly affecting drainage speeds. However, it can be stated that the drainage data in this study and the nitrate concentrations for the controls were consistent with other studies on similar soils in this region, which reported similar rainfall and drainage as the MagnifyNZ large box lysimeters in the current study. Beale *et al*. (2021), in the same region, carried out trials in 2019/2020 on a farm converted from commercial forestry and reported an average of 12.6 mg NO_3-N/l and 38.9 kg total leaching.

When statistically assessed in isolation, the effects of MagnifyNZ (0.77 mg NO_3-N/l) against the control (3.99 mg NO_3-N/l) without any urine application were significantly different (81% reduction with $P<0.0001$; SEM 0.421). Statistical data was assessed, based on normal distribution responses, as no skewing was found, and hence it did not require transformation. All lysimeters were from the same soil type and depth which was confirmed at time of positioning. Although, due to cost, there were limitations within the protocol of the trial (as stated above), the responses seen in terms of reductions of nitrate made it worthwhile to conduct statistics on the dataset, given the relevant importance of such findings.

The number of days that nitrates were elevated under urine patches before returning to base levels were significantly ($P<0.0001$) different between the two treatments (control 209 d, MagnifyNZ 35 d). The average total nitrate nitrogen (applying urine adjustment factors to average urine concentrations and adding fertiliser-only nitrate concentrations) for MagnifyNZ was 1.18 mg NO_3-N/l compared to 15.04 mg NO_3-N/l ($P<0.0001$). Total nitrate loss for the MagnifyNZ non-urine group was 0.79 kg N/ha and, including urine, was 1.22 kg N/ha from 92.6 mm drainage. This was 91% less than the control group (non-urine 3.53 kg N/ha and including urine 13.74 kg N/ha from 90.51 mm drainage). The short 35 d recovery period under Magnify is not consistent with other research. Selbie (2014) found it took over 200 days for nitrates to return to base line levels regardless of the use of DCD's or urine concentrations as low as 300 kg N/ha.

Larger hybrid lysimeters were measured from 1st September for a year. For the Ruapuna soil (the same as the monolith lysimeters) average nitrates were 2 mg NO_3-N/l. A spike to 5.6 mg N/l occurred in June (winter) and lasted 40 d. This was very similar to the MagnifyNZ treated monolith lysimeters having a urea and urine combined spike reach 2.4 mg NO3-N/l and it took 35 d to return to base levels. However, in winter there was a brief subsequent spike in combined nitrates to 4 mg NO3-N/l that took 17 d to return to base levels. Both treated lysimeter types were low, considering the calculated combined spike for the control (from the monolith lysimeters) reached a peak of 25 mg NO_3-N/l through this same period. This was despite urine (600 kg N/ha) being applied to the MagnifyNZ treatment in late March *vs.* early January for the control (700 kg N/ha). Drainage was 323 mm from 901 mm rain and total leaching was 3.08 kg N/ha.

The low nitrates seen in the larger hybrid lysimeter compared to the control data from the monolith lysimeters demonstrated the effect of the MagnifyNZ treatment, and was not simply due to the lower rate of urine (200 kg N/ha) used with the MagnifyNZ monolith lysimeters. It indicated that there may be longer lasting effects of MagnifyNZ as it was applied in January and not re applied until mid-August. Presumably this was related to soil microbial changes but this would need further investigation and confirmation.

The large hybrid lysimeter aligned well with the monolith lysimeters. The overall drainage throughout the January to September period was 92 mm for monolith and 108 mm for the hybrid lysimeter. More drainage was expected to occur in the hybrid lysimeters with disturbed earth in the bottom 350 mm although it was not clear how long this would take to settle to field compaction levels. Single, large, disturbed earth lysimeters are often left for years before use to allow for field compaction to re-establish so the drainage rates and subsequent nitrate levels represent actual field levels. The nitrate average from urine and urea (after distribution assumptions) for the monolith lysimeters was 1.18 mg and 2 mg for the hybrid. However, the hybrid was exposed to urine applied at 600 kg N/ha and in late March rather than January, so this was expected to be higher. On the hybrid lysimeters there was an issue with the spread of the urine being smaller than expected due to the top soil being laid in blocks. This concentrated the urine nitrogen which increased N loading above actual field data levels. This trial aimed to apply 200 kg N/ha, but this ended up being 600 kg N/ha. The lysimeters design could be improved by making a large cutter attachment that could fit on the digger blade and cut 50% of the topsoil area at one time. It would have been more accurate if a tensiometer was used to test base level compaction (250 - 600 mm soil depth) and to replicate the base layer of the lysimeter. The goal was to trial a simple method of setting up a lysimeter that urine could be applied to during every grazing round to eliminate the assumptions that have previously been used in monolith lysimeters. Such a system would allow for more accurate and affordable field assessment of total nitrate leaching from a particular pastoral land use.

It took 65 d for total nitrates in the control areas to drop below the current drinking water standards, at 11.3 mg NO_3-N/l, before spiking again in early winter (164 d). In comparison MagnifyNZ treatments were only above the DWS for two brief periods at a maximum of 4 mg NO_3-N/l.

The trial data showed that grass and milk production were positively affected by the application of MagnifyNZ. The trial farm, running 2.5 cross-bred cows per ha, showed that applying MagnifyNZ to the soil increased Milk Solid (MS) production from 440 kg MS/cow in the 2021 lactation season to 510 kg MS/cow in 2023. Late autumn production was 1.7 kg MS/cow. As of 6th September 2023, on farm reporting from the dairy company showed 8.25% MS, 4.55% fat and 3.58% protein. Empty cow rates dropped from 24% to 11% and lame cow numbers reduced noticeably. The latter and former were likely connected not just to better nutrient balance from the pasture (higher ME, lower protein, more energy to support productive performance and immunity), but to less exposure to toxic and pathogenic organisms from the soil.

Research has suggested that urea produces an average of 10 kg extra pasture dry matter for every 1 kg of nitrogen applied. So, to get the equivalent extra growth that MagnifyNZ recorded over 10 months on this property, would take 320 kg N per hectare. The current cost of urea is NZ$795 per ton. To grow the equivalent volume of dry matter would cost NZ$565 plus multiple application costs. The current cost of Magni-N-Enviro at the time of the trial for 2 applications was $190 /ha plus application. The measured grass growth increases for this trial were consistent with other MagnifyNZ responses on dairy farms. In an unpublished field trial, Field-Dodgson (2014) measured gains of 40-49% on North Canterbury, New Zealand (sheep farms) hill country. Nineteen months after a single application and a substantial drought period, grass growth gains of 246-1142 kg DM/ha were measured.

MagnifyNZ can be applied through drones, sprayers or irrigation systems and can be mixed with effluent. The product is made using a fermentation process which is easily scalable. It is applied at 7-9 l/ha which keeps distribution costs low. There is considerable room for price movement with the scaling of the business which could provide robust pricing advantages compared to other forms of biostimulants.

No data is currently available for long term soil microbial changes and there was no budget for this in this study. MagnifyNZ has traditionally used total available soil N levels as a guideline for overall microbial mass changes. Autumn levels of 350-600 kg N/h are common after 3-5 years with two applications per year. Plant root and soil structure development is part of the medium to long term (one to three years) targets of Magni-N-Enviro. Traditionally this has been done manually using visual comparisons. On the current property there were notable increases in root strength/numbers and soil structure within 12 months compared to the other areas of the farm.

Espie and Ridgway (2020) recorded three-year average increases in pasture yield of 9.8% over and above urea by adding 10% of a locally-sourced NZ humate to urea, although this trial lacked a negative control. The price and concentrations of humates varies markedly. Field measurements on the trial farm showed that Magni-N-Enviro out performed a humate/fulvic acid plus 20 kg N/ha from liquid N (cost $150/ha/application) product by 30-300% over the 10 months measured trial period (January to October). From two applications, an extra 3200 kg of dry matter was measured from treated paddocks.

Di and Cameron (2000) calculated that most NZ dairy farms that were applying the maximum permitted amount of 190 kg N/ha through fertiliser produced the annual average. This data was generated from drainage within DWS limits, but this would be exceeded at certain times of the year. They generated this data using national averages and assumed three cows per ha and that 25% of the paddock would be covered by urine each year. In order to meet Government water regulations, this was problematic for several reasons. Firstly, previous studies on urine patch coverage (Dennis *et al.,* 2011; Moir *et al.,* 2011), which have been commonly used to calculate the total per hectare leaching, did not include dietary N inputs (*e.g.*, from high protein pasture) in their assumptions and calculations. When protein exceeds 16% of dietary intake for cattle per day, it cannot be utilised and is excreted in urine at an exponential rate (Castillo *et al.,* 2000). Levels of over 35% protein have regularly been reported from NZ pasture during lush growth (NZARN, 2018). Waldron and Wilkinson (2017) showed that, even at relatively low dietary protein in pasture (20-22%; compared to the NZ National Nutrient Management Programme; Overseer, Wellington, New Zealand), urine N levels ranged from 210-500 g/cow/day (Castillo *et al.,* 2000). This would result in paddock coverage well above the assumed 25% used in most studies as a default. In fact, the current authors have calculated that paddock urine coverage areas of 36-50% in the Canterbury region are common, which shows that many farms in this region exceed DWS, despite complying with fertiliser restrictions. Nitrate leaching from silt loam soils under grazed pastures in New Zealand typically range from 25-118 kg N/ha for dairy (three cows /ha) and on cattle farms with nitrogen inputs from 0-210 kg/ha (Cameron *et al.,* 2013). With 200 mm/yr drainage, this would equate to 12.5-28.5 mg NO_3-N/l. On free-draining stony soils, which typically have around 300 mm/yr drainage (Beale *et al.,* 2021), this would result in 8.3-18.8 mg NO_3-N/l. The current trial site had relatively low drainage volumes, as it was a dry winter. This produced low levels of total leaching (13.74 kg N/ha), however it still had levels averaging 15 mg NO3-N/l which is still above DWS. This is potentially problematic for the NZ government who currently only shows leaching on a kg N/ha basis.

The stimulus for this leaching study occurred when farmers unanimously stated at a local meeting that it was impossible to get leaching below 2.4 mg/l. The goal of this study was primarily to see if nitrate concentrations in dairy farm drainage water could get below 3 mg N/l and be 90% less than the government standard modelling has calculated for farms. Leaching needs to be based off real field data. This means including the differences in urine concentration loadings that has been recorded on different MagnifyNZ treated farms since 2017 (unpublished data). Out of 60 samples, 95% of this data showed no more than 6 g N/urination compared to historic assumptions of 26 g N/urination (750 kg N/ha assuming 0.32 m^2 urine coverage). Ideally, urine at 200 kg N/ha for untreated lysimeters would have been included, but this was outside of the scope of this trial (budget restraints), which had to rely on modelling and the hybrid lysimeters to get an estimate of how much this has influenced the results. Previous studies have shown that increasing N rates results in linear and non-linear (exponential) increases in cumulative leaching loss of NO_3^--N (Barraclough *et al.,* 1992; Ledgard, 2001; Di and Cameron, 2000), which is usually the major form of N leached under grassland (Di and Cameron, 2002). Selbie (2014) showed 26-40% reduction between 700 kg N/ha and 300 kg N/ha.

Modelling by Di and Cameron (2000) suggested that lowering urine applications from 700 to 200 kg N/ha would reduce leaching under urine patches by 68%. This would have reduced the 13.74 kg N/ha leached in the control to 6.75 kg N/ha from 90.5 mm drainage. This was still 83% greater than the leaching with MagnifyNZ using 200 kg N/ha (1.2 *vs*. 6.75 kg N/ha). Lower urine N levels are not a guarantee of less leaching. Using 200 mm diameter lysimeters on soil similar to those in this study (Templeton silt loams) and only 300 kg N/ha of urine to simulate sheep urine, leaching was calculated to be 146 kg N/ha with two peaks of 57 and 35 mg NO3-N/l with 200 mm accumulative drainage (Moir *et al.,* 2010). With Di and Cameron's modelling, incorporating both the effect of DCD and lower urine nitrogen levels, achieved the same results measured with the MagnifyNZ treatment. In addition to the modelling estimates, the hybrid lysimeters had only a slightly lower urine concentration (600 kg N/ha vs 700 kg N/ha) than the Control.

It was stated in the methodology that two hybrid lysimeters were set up, one being with a different soil type - Darnley which is very similar to Ruapuna but has a higher risk of nitrate leaching susceptibility due to differences in drainage characteristics. The hybrid Darnley lysimeter had similar (86 mm drainage compared to 90 mm) drainage to the controls from the January to September period. Following addition of 600 kg N/ha urine (on top of urea applications), nitrates spiked to a maximum of 13.1 mg NO3-N/l returning to base levels after just 54 d. Nitrate uptake from extra pasture growth was unlikely because the speed of decline was faster than pasture growth could absorb and the hybrid lysimeter did not have any extra MagnifyNZ treatment throughout the trial. The lysimeters were too small to allow accurate pasture growth measurements and there was nothing apparently special about the grass growth in the lysimeters. Interestingly, the same low spike and short recovery pattern was observed the following winter in the hybrid lysimeters, in spite of multiple urine applications, each being 800 kg N/ha (in September, October, November, December, January and June, and Magni-N-Enviro applied in August 2023 and June 2024) with no pasture growth (due to drought) for six months prior to winter.

Together the modelling and hybrid lysimeters, showed that the difference in leaching from MagnifyNZ treated soil was likely due to microbiology changes than differences in the urine N loadings. Both are necessary to control dairy farm leaching within proposed fresh water standards and to maximise nitrous oxide reductions.

The farm regulatory tool (Overseer, Wellington, New Zealand), which was originally designed as an economic model for calculating input and outputs on farm, analysed historic data and concluded a base pasture N of 3.8% (equivalent to 23.75% protein by Kjeldahl calculation) on dairy farms in New Zealand. Additional adjustments were made for the South Island (+0.23%), fertiliser additions (190 kg N fertiliser adds 0.31%) and clover content (30% clover content adds 0.26%)). Total assumed protein intake from dairy pasture, based on historic data, was 28.75%. However, the recent warmer, wetter climate conditions means that pasture protein has recently been recorded from farm testing well over 30% and sometimes as high as 40% or more, especially during springtime. Therefore, the assumptions within Overseer pose a problem, as they do not allow for mitigating strategies on farm in terms of feeding systems and soil. This is a problem in terms of practical and realistic modelling for N emissions and soil and water pollution from farming.

The commercial nitrification inhibitor DCD has proved effective at reducing N and leaching in the past. This chemical is applied at 10-30 kg/ha two or three times per year to give short term suppression of nitrifying bacteria, which reduces N leaching into water courses by typically 27-70% (Cameron *et al.,* 2007; Moir *et al.,* 2007; Moir 2010; Di and Cameron 2011; Manning *et al.,* 2012; Selbie 2014). Urine spikes after DCD application were reduced, but the length of time for nitrates under urine patches to reach base levels was still 120 and 200 d (Manning 2010; Moir 2010;

Selbie 2014) regardless of urine concentrations as low as 300 kg N/ha. However, DCD has been rejected by trading partners and consumers due to its transfer into milk, which have been linked to human health concerns.

Because of the historic success of DCD it was the most appropriate product to reference for comparing the results in this study. There were similarities, from a practical point of view, between MagnifyNZ and DCD responses, with pasture growth gains >20%, leaching reductions and reductions in nitrous oxide of over 80%. However, this related only to calculated reductions in nitrous oxide for MagnifyNZ coming from the reductions in the urine nitrogen loading (Kelliher *et al.,* 2014) and reductions in overall nitrate leaching (Di and Cameron 2006). The specific product Magni-N-Enviro had many advantages over DCD in that it can be applied without previous N fertiliser use, producing significant pasture growth and perhaps, most importantly, animal and consumer safety. Ingesting DCD is dangerous, whereas ingesting Magni-N-Enviro generally improves animal health and digestion. The use of DCD cannot reduce soil borne disease, whereas Magni-N-Enviro can help prevent common crop diseases. All MagnifyNZ-treated pasture products regenerate soil health (structure, worm life, microbial biomass, plant density, disease suppression) but DCD technical information has never mentioned soil structure improvements. In trials, DCD was usually applied immediately after urine application, presumably to maximise results, whereas the MagnifyNZ product showed similar results in the hybrid lysimeters regardless of when it was applied relative to urine application.

The specific Magni-N-Enviro product focuses on gaining momentum encouraging nature to find its best expression. Different microbes targeting different key aspects of the production food web can be included in one product; soil structure, decay cycles, nitrogen fixation, soil temperatures and plant root growth. One beneficial soil microbe giving life to another beneficial microbe and multiplying which can potentially produce effects that get stronger over time. This is a different approach to DCD or pesticide chemicals. Magni-N-Enviro had been used five times over two milking seasons on the treated paddocks prior to the current study.

Large lysimeters didn’t receive any additional MagnifyNZ product after the January application, yet the effects were holding in winter and still caused low nitrogen spikes, which indicated a longer lasting effect of Magni-N-Enviro. The large lysimeters were not treated prior to winter. Using DCD traditionally requires treatment in the two months prior to winter, creating more difficulty for spreading. Pasture responses to Magni-N-Enviro are similar in most soil types found in the South Island - peat to sandy loams - so it is highly probable the leaching responses will be consistent. Expanding trials to include different soil types and more detailed soil microbial analysis is desirable. The pasture growth gains in late autumn accounted for large increases in nitrate uptake in this study, but continued monitoring on the large lysimeters showed it was not a factor in the continuation of low nitrate levels. One important difference between MagnifyNZ products is they are non-chemical, using non-GE organisms from the natural environment. One part of the Magni-N-Enviro has proven effective on dairy effluent pond treatment, for example. Probiotics have been used in human food production for thousands of years.

Modelling estimates for DCD effectiveness do vary considerably. Modelling by Chicota *et al.* (2010) for a Horotiu soil in the lower North Island of New Zealand had January and March application of DCD producing less than 20% reductions in nitrate leaching with effectiveness greatly diminishing when urine was applied 1 month later regardless of the time of the year (over 70% less effective). In fact, when urine was applied one month following a January DCD application, the DCD had no effect on leaching at all. In this study the January treatment of Magnify has reduced leaching by 91% with no reduction in performance when urine was applied 10 weeks after treatment as observed from the hybrid lysimeters.

Published leaching studies include wide variation in how nitrates are collected. These have included one or two larger (disturbed earth) lysimeters or up to four smaller (monolith) barrel lysimeters, sometimes draining into one collection chamber (Field *et al.,* 1985; Chicota *et al., 2016;* Beale *et al.,* 2021). Monolith (Barrel) lysimeters were found to be more accurate than porous cups which are also used for leaching studies (Wang *et al.,* 2012).

The area of spread of urine (per urination) used greatly affects the overall field leaching. For example, if a urine spread of 0.36 m^2 per urination was used (which was measured in January), nitrates for the control group would have been 23 *vs.* 1.46 mg NO_3-N/l for the MagnifyNZ treatment. Total nitrogen leaching would be 3.64 kg N/ha for the MagnifyNZ treatment *vs.* 71 kg N/ha for the control (a reduction of 94%). In winter, air inversion layers are common in Canterbury, trapping smoke from fires and other air pollution. Hence, nitrates in rainfall and irrigation contribute to the levels from farming practises.

Undisturbed soil lysimeters vary in size from small (19 mm diameter) to large (250 mm – 12000 mm diameter) and it is expensive to conduct such studies - establishment costs of NZ$10,000 per 500 mm barrel lysimeter plus ongoing sample costs that could reach NZ$50,000/year are quoted by New Zealand research institutions. Size can limit the capacity to apply multiple urine applications without overlapping and restrict the urine from lateral movement, so assumptions for the influence of urine N/ha have been made and can be quite misleading. Analysing statistical data from individual lysimeters (without replication) can result in erroneous conclusions. The depth of soil can be tested but there does not appear to be technology that can test the volume of stones within a lysimeter. Although there were no differences when digging in the current lysimeters, it was still possible to have variations within the cores. For this reason the drainage data in this study was the average of all four lysimeters for both the Control and MagnifyNZ treatments. The Beale *et al.* (2021) study (which included soil scientists with large numbers of published leaching studies and lysimeter experience) was used as a guideline for best practice with similar soils. They used four lysimeters draining into one container. For the first 11 weeks of this study the hybrid lysimeters allowed for cross checking of the MagnifyNZ treated fertiliser only monolith lysimeter and no significant difference was found. The hybrid lysimeter has a larger collection surface, allowing urine to be applied at every grazing (in future studies) and adjusted for dietary nitrogen inputs, against stocking rate per grazing production at the time of grazing plus dung outputs throughout the year. This reduced any errors in total leaching measurements which would have been caused by using potentially incorrect assumptions on urine paddock coverage and inputs. The hybrid lysimeter offered a better approach, as it produced comparable results to the monolith lysimeters and was much cheaper to use. Using this equipment would allow individual farms to monitor their own leaching at low cost.

Conclusions

This study demonstrated the positive effects of treating farmland with MagnifyNZ on reducing free nitrates draining from dairy pastures, which prevent leaching into water courses. It is the first study done using MagnifyNZ products and is potentially one of the first studies using living biological soil inoculants and biostimulants together. Probiotic-type products are potentially quite different in their mechanism of action compared to chemicals. They may have longer term effects on the microbiome as the bacteria grow and develop. This could have greater effects over time and a range of other benefits in terms of cow health, crop disease control, natural nitrogen fixation and plant growth that a single chemical cannot provide. Overseer assumes monthly pasture ME levels which decreases in line with protein. MagnifyNZ-treated pastures commonly contain less than 20% protein and have ME levels higher than Overseer's assumed monthly figures. Due to these differences in pasture protein, reductions in urine nitrogen output of up to 60% can be created from lower protein pasture but this would still fall well short of the leaching reductions achieved with MagnifyNZ treatment, and may not be enough to meet proposed freshwater nitrate limits.

This study highlighted issues with other published collection models and calculations of nitrogen leached. Some forage protein levels were obtained in the current trial, but ideally analysis of pasture from all months sampled would be useful to assess the relationship between soil activity, reduced leaching and amount of protein being expressed in pasture and consumed and excreted by dairy cows. This could be introduced into an appropriate model to give farmers a more realistic response in terms of leaching reduction under specific farming and regional conditions. Applying dirt and grass around the outside of the lysimeters would likely give more accurate field drainage values by reducing any excess evaporation from the exposed above ground lysimeters, regardless of any insulation methods.

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Ethical statement

No ethics were required for this study, as no animals or practises outside common farming management were used.

Conflicts of interest

The authors report no conflicts of interest. S. Hobson is a director of MagnifyNZ.

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