Assessment of current understanding of the effects of plant species on nitrous oxide emissions

Cecile de Klein[1], Tony van der Weerden[1], Jiafa Luo[1], Keith Cameron[2], Hong Di[2]

[1]AgResearch [2]Lincoln University

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Contents

1. Executive Summary

This report summarises the results from experiments conducted in objective 6.3 of the New Zealand Agricultural Greenhouse gas Research Centre (NZAGRC) programme that ran from 2014 to 2017. The aim of the objective was to investigate the effects of different plant and forage species on nitrous oxide (N_2O) emissions from grazed pastures. The report also assesses these results in the context of the international literature, and provides hypotheses of the potential mechanisms underlying the key findings.

The research experiments produced nine key findings:

1. **Soil with plants had lower N_2O emissions compared to bare soil.**

A field trial measuring N_2O emissions for 6 weeks following application of fresh cow urine to bare soil and to monocultures of 17 different plant species suggested that the presence of plants reduced N_2O emissions from soils, compared with bare soil. This could be due to a reduction in soil inorganic N as a result of plant N uptake, and/or modifications in the biological or abiotic environment. The study provided no conclusive evidence about the potential mechanism of this plant effect.

2. **Different plant species resulted in different N_2O emissions from urine applied to soil.**

The same field experiment showed that N_2O emissions over a 6-week period following urine application varied with plant species (ranging from 0.7 kg N_2O-N ha^{-1} for Italian ryegrass Grassland Moata to 3.2 kg N_2O-N ha^{-1} for upland brome Grassland Gala). Plants with high N uptake had low emissions, but the majority of N_2O emissions occurred in the first two weeks following urine application when soil mineral N was in excess of plant N demand. The authors therefore concluded that high N uptake *per se* was not the driver for low emissions, but that other traits that are related to high N uptake are the primary driver. There is evidence in the international literature that the effect of plant species on soil nitrification could be such a trait.

3. **For some plant species, different cultivars of the same species had different N_2O emissions from urine applied to soil.**

Italian ryegrass cultivar Grasslands Moata had lower N_2O emissions from urine over a 6-week measurement period than Italian ryegrass cultivar Tama (0.7 vs. 2 kg N_2O-N ha^{-1}). We found no other published studies comparing N_2O emissions from different cultivars of the same species. It is unclear what the underlying mechanism of the observed difference is.

4. **Lower N loading in individual urine patches resulted in lower N_2O emissions and associated emission factors.**

Some of the NZAGRC studies showed that the N_2O emission factor (EF) from urine applied at 500 kg N ha^{-1} was 20-40% lower than the EF from urine applied at 700 kg N ha^{-1}. This effect was not observed in another study where N_2O emissions were very low due to dry conditions. The review of the literature also showed conflicting results on the effect of N loading rate on N_2O emission factors. Some studies have found no effect, whilst others have found higher emission factors with increasing N loading rates. However, on balance, the NZ and international evidence suggests that the N_2O emission factor for urine is lower with lower urine N loading rates.

5. **'Diverse' pastures had lower N_2O emissions from urine applied at 500 kg N ha^{-1} compared with standard pastures receiving urine at 700 kg N ha^{-1}.**

'Diverse' pastures[1] receiving urine at a typical 'diverse pasture' N loading rate (500 kg N ha^{-1}) had 46% lower N_2O emissions than standard ryegrass/white clover pastures receiving urine at a 'standard' N loading rate (700 kg N ha^{-1}). There was no difference in N_2O emissions from diverse vs standard pasture when the N loading rate was the same. This suggests that if 'diverse' pastures reduce the urinary N loading rate in individual urine patches then total N_2O emissions will be reduced, even if the total urinary N output is the same as for standard ryegrass/white clover pastures. If, in addition, the total urinary N output of animals on 'diverse' pastures is lower than for standard pastures, then the N_2O emissions will be further reduced.

6. **In field trials with plant monocultures, plantain had significantly lower N_2O emissions following urine application, compared with ryegrass monocultures that received the same urine application.**

N_2O emissions from urine applied to plantain monocultures were on average 28% lower than from urine applied to ryegrass monocultures. As the same rate and the same type of urine was used in these monoculture trials, the observed differences were plant-induced rather than urine-induced. The literature suggested that plantain does not appear to reduce animal N intake compared to ryegrass, but there was some evidence that the observed reduction in urine N concentration from animals on plantain may be due to a diuretic effect. There also was some evidence in the international literature that plantain can produce biological nitrification inhibitors.

7. **'Winter-active' plant species had no effect on N_2O emissions, but reduced N leaching and thus indirect N_2O emissions.**

Field experiments where urine was deposited onto 'winter-active' Italian ryegrass either in autumn or spring showed no reduction in N_2O emissions compared with urine deposited on standard ryegrass pasture. Literature showed that Italian ryegrass can reduce N leaching, due to higher winter plant growth rates that allows plants to take up more N during autumn and winter when the risk of leaching is greatest. The increased plant N uptake was not due to root architecture.

8. **N_2O emissions and emission factors from urine applied to land planted with fodder beet were lower than from urine applied to land planted in kale.**

N_2O emissions from urine collected from cattle grazing fodder beet and applied to a fodder beet crop were 39% lower than urine collected from cattle grazing kale and applied to a kale crop. The rate of urine-N applied was very similar (c. 300 kg N ha^{-1}). The observed differences in N_2O emissions could be due to a plant effect, a urine composition effect, or

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[1] Diverse pasture consisted of a mixture of plantain, chicory, ryegrass and white clover.

a combined effect. There is no evidence in the literature that describes a potential mechanism for lower emissions from fodder beet.

9. **The plant metabolites *glucosinolate* and *aucubin* reduced N_2O emissions in laboratory studies but these results were not always observed under field conditions.**

The laboratory and field studies on the effect of plant metabolites glucosinolate (GLS) and aucubin on N_2O emissions were inconclusive. The literature review indicated that both GLS and aucubin exhibit biological nitrification inhibition (BNI) properties. However, there are no published studies on the effect of these compounds on N_2O emissions. The reasons for the inconclusive results from the New Zealand field studies are unclear.

Summary

The results to-date have highlighted the potential of plants to reduce N_2O emissions, with plantain and fodder beet showing significantly lower emissions compared to ryegrass and kale, respectively. Plant species that reduce the N concentration of urine may also reduce N_2O emissions – even if the total amount of urinary remains the same – as the evidence is pointing towards a reduction in the N_2O emission factor with lower urine N loading rate. None of the New Zealand studies has provided conclusive evidence of any mechanisms by which these plants reduce N_2O emissions.

Based on the results to-date and the evidence from internationally published studies, the key hypothesis for the mechanism by which forage species such as plantain and fodder beet decrease N_2O emissions is that:

- Plantain and fodder beet have root exudates that inhibit nitrification and/or increase initial nitrogen immobilisation by increasing supplies of available C near roots.

Other hypotheses that could be tested are that plantain and fodder beet reduce soil moisture content and/or increase soil pH, resulting in reduced N_2O emissions. However, the review of the international literature suggested that there is limited evidence to support these.

2. Background

In pasture-based systems, animals consume more nitrogen (N) than they need for growth and production, and subsequently a large proportion of the surplus N is excreted as urine (Selbie et al. 2015). These animal urine patches are the key source of nitrous oxide (N_2O) emissions from grazed systems (de Klein and Ledgard 2005). The amount of urinary-N excreted is largely determined by dietary N content and plant chemical components that may increase the partitioning of N into dung. Plants can also affect N_2O emissions through their impact on N_2O-producing soil processes and the N_2O emission factor for urine.

Forage plant options for reducing N_2O emissions from predominantly grazed systems can thus be split into two broad categories:

- Plants that manipulate urine N excretion (i.e. reduce total urinary output)
- Plants that modify soil N processes (i.e. reduce the proportion of the urinary N that is emitted as N_2O).

A significant amount of work has been conducted in the past few years to assess the impacts of a range of plant species on urinary N output and N_2O emissions. The results to-date are not always conclusive and at times confounded by multiple differences between treatments, which makes interpretation difficult. This report summarises the results to-date from the N_2O plant programme of the New Zealand Agricultural Greenhouse gas Research Centre (NZAGRC), assesses the findings in the context of the international literature, and provides hypotheses of the potential mechanisms underlying the key findings.

3. Summary of New Zealand findings from the NZAGRC programme

The aim of objective 6.3 of the NZAGRC N_2O programme that ran from 2014-2017 was to assess the role of different plant species on N_2O emissions from grazed pastures. A series of laboratory and field trials were conducted to measure the effects of plants on N_2O emissions from urine patches, and to identify possible reasons for the observed results. Many of these studies were conducted in association with existing research programmes such as the Forages for Reduced Nitrate Leaching (FRNL) and Pastoral 21 (P21) research programmes. Aligning the NZAGRC programme with existing trials was very valuable and also very efficient in terms of resources. However, it also meant that the N_2O experiments had unavoidable dependencies on these other programmes, which, at times, compromised the ability to establish the most appropriate experimental designs and control to specifically meet the objectives of the NZAGRC N_2O programme. Nevertheless, the studies have resulted in some significant findings on the impacts of plants on N_2O emissions (Balvert et al. 2017; Bowatte et al. 2018; Di et al. 2016; Gardiner et al. 2016, 2017; Luo et al. 2018; Smith et al. unpublished).

Summary assessment

Overall, the results from NZAGRC studies have highlighted the potential of plants to reduce N_2O emissions, with the plantain and fodder beet showing significantly lower emissions compared to ryegrass and kale, respectively. Plant species that reduce the N concentration of urine are also likely to reduce N_2O emissions, even if the total amount of urine N is not reduced, as the N_2O emission factor for urine was lower at a lower urine N loading rate. None of the New Zealand studies has provided conclusive evidence of any mechanisms by which these plants reduce N_2O emissions.

More details of the key findings and relevant results are provided below.

Key findings

Key research findings 1 to 3

1. Soil with plants had lower N_2O emissions compared to bare soil.
2. Different plant species resulted in different N_2O emissions from urine applied to soil.
3. For some plant species, different cultivars of the same species had different N_2O emissions from urine applied to soil.

These findings are from a plot study conducted in Manawatu to measure N_2O emissions for 6 weeks following application of fresh cow urine (equivalent to 530 kg N ha^{-1}; collected from animals on standard ryegrass/white clover pasture) to bare soil and to monocultures of 17 different plant species and a conventional ryegrass/white clover pasture mix (Bowatte et al. 2018).

The results of the study showed that bare soil emissions averaged 5.0 kg N_2O-N ha^{-1} while the average cumulative N_2O emissions from plots with plants ranged from 0.7–3.2 kg N_2O-N ha^{-1}. The lowest emissions were from Italian ryegrass Grasslands Moata and the highest from upland brome Grasslands Gala. Cumulative emissions from the Italian Ryegrass cultivar Tama averaged c. 2 kg N_2O-N ha^{-1}. Perennial ryegrass cultivars and plantain (cultivar Tonic) had generally low emissions (~1 kg N_2O-N ha^{-1}), while the white clover cultivars had high emissions (~3 kg N_2O-N ha^{-1}). Emissions from the plantain and ryegrass monocultures were not significantly different from average emissions from a ryegrass/white clover mix. It should be noted that the measurement period was only six weeks and thus did not include the full envelope of urine-induced emissions.

There was a negative relationship between plant biomass and N uptake and the amount of N_2O produced. However, 49-78% of emissions occurred in the first two weeks after urine application when soil N was in excess of plant demand, and it therefore seems unlikely that N_2O emissions were controlled by plant N uptake per se. But there were significant differences in the soil mineral N pool among species 7 days after urine was applied and the researchers also found a positive relationship between soil nitrification potential (SNP) measured at the end of the experiment and N_2O emissions, for all species except for plantain and forage rape. These plant species did not fit this relationship as their plots both had a high SNP, yet low N_2O emissions. The researchers suggest that this may be related to another factor that influences rates of immobilization, mineralization and or N leaching of the applied urine-N. For example, if plants have biological nitrification inhibition (BNI) properties, N will remain in the less mobile ammonium form for longer. This will reduce N_2O emissions and enable more opportunity for plants to utilise soil derived urine-N.

The researchers also observed a weak relationship ($R^2 = 0.20$) between the bulk soil pH and total N_2O emissions, although the relationship was not influenced by whether the plant species was a legume or not. They noted that the soil pH ranged from 5.2 to 5.8, which is considered to be near-optimal for nitrifier activity for the soil type used in their study. The bare soil treatment had the lowest soil pH (4.6) and also the lowest soil moisture content, however these were measured 6 weeks after the urine was applied and therefore may not reflect the conditions during the first 2 weeks when emissions were greatest. Soil moisture content or proportion of bare ground under different plant species could not explain differences in N_2O emissions. It is possible that ammonia losses from the bare soil were negligible given the low pH, thereby increasing the amount of soil mineral N available for N_2O producing processes.

In conclusion, plants could affect N_2O emissions in different ways: i) through influencing the characteristics of the soil microbial community under different species, conferring different capacities to respond to large pulses of N such as those resulting from urine deposition; ii) more specifically through inducing BNI activity in the soil, which enables more opportunity for plant uptake and reduces the risk of N_2O emissions; and/or iii) plant and root morphology differences resulted in differences in soil microclimate characteristics that affect N conversion and loss processes.

Key research findings 4 to 6

4. Lower N loading in individual urine patches resulted in lower N_2O emissions and associated emission factors.
5. ‘Diverse’ pastures (including perennial ryegrass, white clover, plantain and chicory) had lower N_2O emissions from urine applied at 500kg N ha^{-1} compared with standard pasture receiving urine at 700kg N ha^{-1}. There were no differences in N_2O emissions between diverse and standard pastures at the same urine-N loading rate.

6. In a field trial with plant monocultures, plantain had significantly lower N_2O emissions following urine application, compared with ryegrass (and lucerne) monocultures that received the same urine application.

A number of lysimeter and plot studies were conducted in Canterbury and Waikato, comparing the effect of different plant species in mixed swards and in monocultures on N_2O emissions (Di et al. 2016; Luo et al. 2018; Luo unpublished results). Table 1 provides a summary of the results.

The studies with mixed swards (Table 1, trials 1 to 3) indicated that in Canterbury differences in N_2O emissions and associated emission factors were largely due to changes in the rate of urine-N applied, rather than due to plant effects on N_2O emissions. The N_2O emission factors were significantly higher at the higher N loading rates compared with lower loading rates. At the same rate of urine N, N_2O emissions were not significantly different when applied to diverse pastures or Italian ryegrass/plantain mixed pastures compared with ryegrass/white clover pasture (trials 1 and 3). The diverse pasture field trial in Waikato (trial 2) also did not show any significant differences in N_2O emissions between plant species. However, urine N application rate did not have an effect on N_2O emissions in this trial either. This was probably due to the low emissions observed during the trial as a result of relatively dry weather conditions.

The monoculture trials clearly showed a plant effect on N_2O emissions (Table 1, trials 4 to 8). In both Canterbury and Waikato, these trials used a standard rate of urine N from cows grazing ryegrass/white clover pasture, and any differences in N_2O emissions were therefore solely due to differences in plant species. Lucerne had significantly lower N_2O emissions compared with either a perennial ryegrass/white clover mix or a ryegrass monoculture in two trials (trials 4 and 5), but not in the other monoculture trials (trials 6-8). Plantain had significantly lower emissions than ryegrass in three trials (trials 5, 7 and 8). In trial 6, emissions from plantain were higher, but not significantly different from the ryegrass monoculture. However, averaged over all trials, plantain was the only plant species that had significantly lower N_2O emissions from urine than ryegrass.

Table 1: Summary results of the effects of mixed swards and monocultures on N_2O emissions from urine.

Trial #	Trial details	Plant species and urine N rate (kg ha^{-1})	Total N_2O (kg N_2O-N ha^{-1})	Emission factor (% of N applied)
Mixed swards				
1	Standard fresh cow urine applied at 500 and 700 kg N ha^{-1} Canterbury; lysimeters Period: May-Nov	Diverse pasture[1] 500 Diverse pasture 700 Ryegrass/WC 500 Ryegrass/WC 700	4.2 a 7.2 b 3.5 a 7.8 b	0.84 a 1.03 b 0.69 a 1.12 b
2	Standard fresh cow urine applied at 500 and 700 kg N ha^{-1} Waikato; field plots (Troughton farm) Period: May-Nov	Diverse pasture[1] 500 Diverse pasture 700 Ryegrass/WC 500 Ryegrass/WC 700	0.86 0.98 1.04 1.34	0.16 0.13 0.18 0.18
3	Plant species-specific fresh cow urine applied at 507 and 672, and standard fresh cow urine at 700 kg N ha^{-1} Canterbury; lysimeters Period: March-Aug	Italian+plantain 507 Italian+plantain 700 Ryegrass/WC 672 Ryegrass/WC 700	15 a 33 c 20 ab 25 bc	2.91 a 4.74 c 3.10 ab 3.95 bc
Monocultures				
4	Standard fresh cow urine applied at 700 kg N ha^{-1} Canterbury; lysimeters Period: May-Nov	Lucerne Perennial ryegrass/WC	7 a 11 b	0.97 a 1.50 b
5	Standard fresh cow urine (622 kg N ha^{-1}) applied to monoculture field plots Waikato; field plots Period: Jun-Sep	Plantain Lucerne White clover Perennial ryegrass	0.79 a 1.41 b 2.93 c 3.03 c	0.12 a 0.20 b 0.32 c 0.47 c
6	Standard fresh cow urine (622 kg N ha^{-1}) applied in February Waikato; lysimeters Period: Feb-Nov	Plantain Lucerne Perennial ryegrass	3.34 ab 4.64 b 2.33 a	0.51 ab 0.70 b 0.34 a
7	Standard fresh cow urine (622 kg N ha^{-1}) applied in March Waikato; lysimeters Period: Mar-Nov	Plantain Lucerne Perennial ryegrass	1.17 a 1.82 b 1.92 b	0.17 a 0.27 b 0.29 b
8	Standard fresh cow urine (622 kg N ha^{-1}) applied in May Waikato; lysimeters Period: May-Nov	Plantain Lucerne Perennial ryegrass	0.91 a 1.23 b 1.39 b	0.14 a 0.19 b 0.23 b

[1] Diverse pasture consisted of a mixture of plantain, chicory, ryegrass and white clover.

Key research finding 7

7. 'Winter-active' species had no effect on N_2O emissions, but reduced N leaching and thus indirect N_2O emissions.

Two trials were conducted to examine the effect of the 'winter-active' species Italian ryegrass on N_2O emissions from urine (Table 2; Di et al. 2016 and Smith et al. unpublished).

Table 2: Summary results of studies comparing the effect of Italian ryegrass on N_2O emissions from urine.

Trial #	Trial details	Plant species	Total N_2O (kg N_2O-N ha^{-1})	Emission factor (% of N applied)
I	Standard fresh cow urine applied at 700 kg N ha^{-1} Canterbury; lysimeters Period: May-Nov	Italian ryegrass Perennial ryegrass/WC	9 11	1.35 1.50
II	Standard fresh cow urine applied at 470 kg N ha^{-1} Southland; grazed field plots Period: Sep-Dec	Italian ryegrass/WC Perennial ryegrass/WC	1.39 1.55	0.30 0.30

Italian ryegrass has been shown to reduce nitrate leaching losses as this 'winter activity' enabled the plants to take up more N during the high leaching risk period in autumn and winter. However, there was no significant difference in direct N_2O emissions from either autumn or spring applied urine patches.

Key research finding 8

8. N_2O emissions and emission factors from urine applied to land planted in fodder beet were lower than from urine applied to land planted in kale.

In association with the P21 Canterbury study, N_2O measurements were conducted from urine applied to a kale crop or a fodder beet crop (Table 3; Di et al. 2016).

Table 3: Summary results of an N_2O study with urine applied to a kale or a fodder beet crop.

Trial details	Plant species	Total N_2O (kg N_2O-N ha^{-1})	Emission factor (% of N applied)
Species-specific cow urine applied at 300 kg N ha^{-1} Canterbury; field plots Period: July-Nov	Kale Fodder beet	6.3 a 3.9 b	1.1 a 0.85 b

In this trial, ***plant species-specific*** urine was used, which meant that the kale crop received urine from animals grazing kale, while the fodder beet crop received urine from cows grazing fodder beet. The N concentration of the two urine types was very similar and the loading rate for each species was 300 kg N ha^{-1}. Total N_2O emissions from fodder beet were significantly lower than from the kale crop, but as both the plant species and the urine-type differed it is unclear if this was a plant effect or a urine composition effect, or a combined effect.

Key research finding 9

9. Plant metabolites *glucosinolate* and *aucubin* reduced N_2O emissions in laboratory studies but these results were not always observed under field conditions.

A series of laboratory and field studies were conducted to investigate the effect of secondary plant metabolites on N_2O emissions from urine (Balvert et al. 2017; Gardiner et al. 2017). This work focused on the plant metabolites glucosinolate (GLS and its derivatives) found in brassicas, and aucubin, a metabolite in plantain (Table 4).

The laboratory studies (studies I and III) suggested that GLS derivatives and plantain leaf extract or aucubin could reduce N_2O emissions, and soil mineral N analyses indicated that this could be due to nitrification inhibition. However, where inhibition occurred, the results suggested that the effect was generally short-lived.

The field study using 3 GLS hydrolysis products (study II) did not reveal any significant difference in N_2O emissions from urine affected soil. The field trial using aucubin or plantain leaf extract (study IV) suggested a reduction in the mean N_2O EF of up to 70% but this was not statistically significant. In addition, soil mineral N analyses did not indicate nitrification inhibition.

Table 4: Summary results of the effects of secondary metabolites on N_2O emissions from urine.

Trial #	Trial details	Metabolite	Total N_2O (µg N g^{-1} soil)	Emission factor (% of N applied)
I	Urea (600 µg/g soil) + GLS derivatives applied to homogenised soil Waikato; lab incubation	Urea only	1.31	0.19
		Penylethyl ITC @ 30 µg/g soil	1.08	0.15
		60	0.74 *	0.09 *
		4 pent-1-yl ITC 30	1.04	0.14
		60	1.17	0.16
		2 propenyl nitrile 30	1.13	0.16
		60	1.13	0.15
		2 propenyl ITC 30	1.33	0.19
		60	1.25	0.17
		4 pentene nitrile 30	1.24	0.17
		60	1.78 *	0.26
		All combined 30	1.24	0.17
		All combined 60	1.60	0.22
II	Artificial cow urine (600 kg N ha^{-1}) + GLS derivatives applied to homogenised soil Waikato; field plots	Urine only	0.19	0.029
		DCD @ 10 kg ha^{-1}	0.15	0.023
		Penylethyl ITC @ 60 kg ha^{-1}	0.15	0.022
		4 pent-1-yl ITC 60	0.18	0.027
		2 propenyl nitrile 60	0.10	0.015
		Penylethyl ITC (30) + 2 propenyl nitrile (30)	0.11	0.016
		Penylethyl ITC (60) + 2 propenyl nitrile (60)	0.22	0.033
III[a]	Fresh cow urine or Urea + metabolite applied to sieved soil Canterbury; lab incubation	Urine only	ng[b]	0.030
		Urine + Plantain leaf extract		0.020
		Urine + Aucubin		0.027
		Urea + Plantain leaf extract		0.018
		Urea + Aucubin		0.020
IV	Fresh cow urine or Urea + metabolite applied to sieved soil Canterbury; field plot	Urine only		2.8
		Urine + Plantain leaf extract		1.4
		Urine + Aucubin		0.8

* Significantly different from 'urea only' treatment; all other results were non-significant in trials I, II and IV.

[a] Statistical analysis was not possible in study III as the emission factors were calculated from the average values from all replicates; [b]ng, not given.

4. Summary of international literature

4.1 Plant effects on total urinary N output

The effect of plant-presence on N_2O emissions varies with plant species, and can be due to a reduction in total urinary output, or due to a reduction in the N_2O emission factor from the urine. This section summarises existing literature on the effects of plants on reducing total urinary output, while the effects on the N_2O emission factor are summarised in section 4.2.

There are two key mechanisms by which plants can reduce total urinary N output from grazing animals: (i) by lowering the total N intake to reduce the total amount of excreta N and/or (ii) by partitioning more of the excreta N into dung, rather than urine.

4.1.1 Lower N intake

Several studies have shown a strong positive relationship between N intake and N excretion (e.g., Castillo et al. 2000; Kebreab et al. 2001; Mulligan et al. 2004; Huhtanen et al. 2008). As a result, reducing the amount of N consumed in the diet is an effective way of reducing N_2O emissions, provided dietary N, crude protein (CP) levels and energy content (e.g. sugars and starch (SS)) remain sufficient to meet the animal's metabolic requirements. For example, Dalley et al. (2017) suggested that feeds most likely to result in less N excretion have <12% CP and >50% SS. However, lactating cows require dietary CP contents of 16-20% (Dalley et al. 2017; de Klein and Eckard 2008).

The use of low N supplements such as maize, grain, kale, fodder beet, swedes or low N citrus pulp can reduce the N content in the diet and thus reduce total and urinary N excreted in ruminants (e.g. Burke et al. 2008; Edwards et al. 2014; Huhtanen et al. 2008; Kebreab et al. 2001; Luo et al. 2008; Misselbrook et al. 2005; Mulligan et al. 2004; Nielsen et al. 2003; Steinshamn et al. 2006; van der Weerden and Styles 2012). However, reducing the N content in the diet in pasture-based systems is often challenging. High producing pasture species generally have high N contents and reducing the amount of dietary N by using low-N supplements is often costly and/or impractical.

Although many of the recent New Zealand studies on urinary N excretion from animals grazing plantain (*Plantago lanceolate L*.), or mixed pastures that included plantain, showed a reduction in the N concentration of the urine as well as in total amount of N excreted (e.g. Box et al. 2016; Cheng et al 2017a, b; Totty et al. 2013), most of these studies found no differences in the N concentration of the DM, nor in total N intake by the animals, compared with standard ryegrass pastures (e.g. Box et al. 2016; Cheng et al. 2017a, b). The observed reduction in N excretion from animals on plantain or diverse pastures can therefore not be explained by a reduction in N intake. However, a recent animal metabolism stall study by O'Connell et al. (2016) found that that sheep on a plantain diet had consistently higher urine volumes than sheep on a ryegrass diet. As feed intake and feed water content were similar between the two diets, these authors suggested that plantain could cause diuresis, possibly by reducing reabsorption of water in the kidneys. This diuretic effect of plantain could explain the findings of the studies mentioned above where animals on plantain had lower urine N concentrations despite their N intake being the same as animals on ryegrass pastures.

4.1.2 Increased N partitioning into dung

Partitioning more of the excreted N into dung rather than urine will reduce N_2O emissions as the emission factor for dung is 4 times lower than that for urine (0.25 vs 1% of the excreted N emitted as N_2O, respectively) (Luo et al. 2009). Increased partitioning into dung can be achieved through reducing the N content of the diet, as the proportion of total excretal N that is excreted as urine is positively related to the N content of the diet (Luo and Kelliher 2010):

$$\text{\% of excretal N excreted as urine} = 10.5\ (\pm\ 1.1) \times \text{\% N in diet} + 34.4\ (\pm\ 3.4)$$

Feeding the animals condensed tannin-rich diets can also increase N partitioning into dung (Carulla et al. 2005). Condensed tannins (CT) complex with proteins in the rumen and protect them from microbial digestion, resulting in either more efficient digestion in the lower intestine or the tannin-protein complex being excreted in the dung (de Klein and Eckard 2008). Sheep that were fed a CT extract from *Acacia mearnsii* (Black Wattle) increased their partitioning of N from urine to faeces, reducing urinary N by 9.3% as a proportion of total N excreted (Carulla et al. 2005). Adding a CT extract from Black Wattle to the diet of lactating dairy cattle reduced urinary N excretion by 45-59% relative to a non-tannin control diet (Grainger et al. unpublished). Furthermore, Misselbrook et al. (2005) showed that dairy cows on a 3.5% CT diet excreted 25% less urine N, 60% more dung N and 8% more N overall, compared with cows on a 1% CT diet. In all these studies, inclusion of CT either as a dietary supplement or in forages fed to ruminants reduced N excretion in the urine, increased N excreted in faeces and improved N retention in the animal.

Condensed tannins have also been found in plantain (Ramírez-Restrepo and Barry 2005), which could partly explain the reduced urinary N excretion that was observed from cows grazing diverse pastures that included plantain (Box et al. 2016; Cheng et al. 2017a, b; Totty et al. 2013).

4.2 Plant effects on the N_2O emission factor

Plants can also reduce N_2O emissions from urine by affecting the percentage of excreted urine N that is lost as N_2O, i.e. the N_2O emission factor for urine. The main mechanisms by which plants can affect the emission factor are:

- A reduction in the N concentration in the urine and an associated reduction in the N loading rate in individual urine patches
- Exudates from plant roots that impact on soil N processes (e.g. through BNI or changes in pH or available C)
- Compounds excreted in animal urine that impact on soil N processes
- The effect of plant shoot and root morphology on soil N processes through changes in the soil microclimate

4.2.1 N concentration in the urine

The N concentration in urine and the associated N loading rate of urine patches has been shown to be positively related to the N_2O emission factor (e.g. de Klein et al. 2014). de Klein et al. (2014) showed an effect of urine N loading on the N_2O emission factor for simulated cow urine patches on free draining soils with relatively low emissions (emission factors between 0.03 and 0.3%). However, in a poorly draining soil with higher N_2O emissions (emission factors between 0.5 and 0.9%), de Klein et al. (2014) found that the N_2O emission factor was independent of the N application rate. These authors suggested that the urine N concentration only affects the N_2O emission factor when emissions are low. Marsden et al. (2016) did not observe any urine N loading effects on the N_2O emission factor for simulated sheep urine patches with relatively low N_2O emission factors (0.1-0.2%).

In studies using N fertiliser rather than urine N, a lack of a significant effect of the N application rate on the N_2O emission factor has been attributed to factors other than N availability determining the N_2O emissions (e.g. dry conditions; Peng et al. 2011; and/or low available soil carbon; Rochette et al. 2010; competition for N between vegetation and soil microbes; Kim et al. 2013). However, it should be noted that these studies used inorganic fertiliser N rather than urine N and were conducted at N application rates of up to 400 kg N ha^{-1} only. Although some of these studies included grazing animals, they did not directly measure the effect of urinary N applications on N_2O emissions.

4.2.2 Plant root exudate effects on soil N processes

There is extensive evidence to show that plant tissues contain a wide variety of chemical compounds that are not involved in primary metabolism. These compounds, termed ‘secondary metabolites’, vary according to plant family and species and play a variety of roles in protecting plants from a range of stresses (e.g. predation and competition, Bennett and Wallsgrove 1994; Erickson et al. 2000) including through suppression or inhibition of soil N transformation processes. Immobilisation of inorganic N species or inhibition of soil N processes, such as urea hydrolysis and nitrification, have the potential to reduce N_2O emissions from animal excreta deposited to land (Cameron et al. 2014; de Klein et al. 2011; Di et al. 2010; Li et al. 2014).

Plant roots can secrete a wide range of compounds and transfer about 5-40% of total photosynthetically-fixed carbon into the rhizosphere through root exudates (e.g. Walker et al. 2003; Badri and Vivanco 2009). Root exudates include carbohydrates, amino acids, organic acids, sugars, secondary metabolites, polysaccharides and proteins and enzymes. There are several mechanisms through which plant roots can affect soil N cycling (Paterson 2003; Dijkstra et al. 2013; Cheng et al. 2014).

4.2.2.1 Biological nitrification inhibition

The suppression of nitrification has been observed to occur naturally in some ecosystems where certain plant species release organic molecules from their roots that suppress the function and growth of nitrifying bacteria, a phenomenon termed biological nitrification inhibition (BNI) (Subbarao et al. 2007b, 2015). Subbarao et al. (2007a) used a developed assay involving recombinant luminescent *Nitrosomonas europaea* to detect and quantify biological nitrification inhibitors in root exudates released from plants. Among pasture grasses, *Brachiaria humidicola* and *Brachiaria decumbens*, which are adapted to a low N environment, showed the highest BNI capacity in root systems. Field studies suggest a 90% decline in soil ammonium oxidation rates and N_2O emissions within three years of establishment of *B*. *humidicola* pasture (reviewed by Subbarao et al. 2015). Subbarao et al. (2015) suggest that these reduced emissions were due to extremely small nitrifier populations present in the established pastures. Based on monitoring of N_2O emissions over a 3-year period from fields planted with tropical grasses with a wide range of BNI-capacity, a negative relationship was observed between the BNI-capacity of a species and N_2O emissions (Subbarao et al. 2013). A recent study conducted at CIAT in Colombia has shown that N_2O emissions from urine patches deposited on plots of tropical forage grass with high BNI capacity (*Brachiaria humidicola cv. Tully*) were three times lower than from urine patches in plots of tropical forage grass with low BNI capacity (*Brachiaria hybrid cv. Mulato*) (Byrnes et al. 2017).

It has been suggested that BNI has evolved as part of some plants' adaptation mechanisms to conserve and use N efficiently in systems that are naturally limited in mineral N (Al-Ansari and Abdulkareem 2014; Subbarao et al. 2007b), but it appears to be stimulated by high NH_4^+ concentration in the soil (Subbarao et al. 2012; 2015). Plants that use nitrate as their N source did not release BNIs from roots, whereas BNIs were released from plants that use ammonium as their N source (Zakir et al. 2008). BNI release from roots is a localised phenomenon confined to the part of the root system exposed to ammonium ions and is not extended to the remaining parts of the root system (Zhu et al. 2012). It is also suggested that soil pH and texture may influence the release of BNIs from roots, but more work is required to understand this possible mechanism (Subbarao et al. 2015).

Several BNIs have been isolated from root exudates and plant tissues and BNIs that belong to many different chemical classes have been identified (Gopalakrishnan et al. 2007; Subbarao et al. 2013). A biological nitrification inhibitor, named "brachialactone", in the root exudates of the tropical grass *B. humidicola* was identified (Subbarao et al. 2008). Brachialactone is a cyclic diterpene with a unique 5-8-5-membered wing system and a ɤ-lactone ring. It contributed to 60-90% of the inhibitory activity released from the roots of *B. humidicola*. Brachialactone appears to block both ammonia mono-oxygenase (AMO) and hydroxylamine oxidoreductase enzymatic pathways involved in ammonia oxidation in *Nitrosomonas* (Subbarao et al. 2008). This is in contrast to most synthetic nitrification inhibitors such as DCD and nitrapyrin that suppress *Nitrosomonas* function mostly by blocking the AMO pathway (McCarty 1999). The biosynthesis pathway for brachialactone is still unknown, but release of this inhibitor is a regulated plant function, triggered and sustained by the availability of ammonium ions in the root environment which results in the inhibitor being released precisely where the majority of the soil nitrifier population resides (Subbarao et al. 2015). Research also found that BNI compounds isolated from root exudates of *B. humidicola* remained effective when added to the soil and incubated for up to 55 days at 20°C (Gopalakrishnan et al. 2009). It is expected that the longevity of BNI effectiveness would depend on individual BNI compounds and their concentrations in the soil. As well, significant genetic variability exists for BNI capacity in *B. humidicola*, suggesting that there is a strong potential to breed *Brachiaria* for enhanced BNI capacity by selection and recombination (Subbarao et al. 2013).

Although early observations of nitrification inhibition were mostly made on tropical grassland systems, BNI function does not seem to be confined to plants either from humid-or sub-humid tropics as certain temperate forest ecosystems also suppress nitrification (Smolander et al. 2012). It is likely that some temperate grasses also have BNI capacity in their root systems. In fact, differences in N_2O emissions between plants can be very large as evidenced from the recent NZAGRC-funded research trial (section 3). Furthermore, a recent FRNL-funded study (A. Carlton, unpublished) discovered that soil ammonia oxidiser bacteria (AOB) population abundance was lower under plantain soil compared to ryegrass/white clover soil. This resulted in slower nitrification rates and smaller nitrate leaching losses under the plantain soil. These results suggest that plantain could produce BNIs, which can be released to the soil through exudation from the roots and reduce nitrification rates and N_2O emissions. Limited available literature suggests that plantain can produce many secondary metabolites, most of which are potential BNIs (Dietz et al. 2013). These metabolites include the iridoid glycosides aucubin and catalpol (reviewed by Gardiner et al. 2016). Soil incubation experiments with plantain leaf materials and extract confirmed they significantly inhibited N mineralisation and nitrification (Dietz et al. 2013). Dietz et al. (2013) also found that the addition of aucubin at 0.6 mg g^{-1} soil can decrease soil nitrate and increase ammonium concentration/levels in the soil.

It is also known that glucosinolates (GLSs) are an important group of bioactive phytochemicals and these compounds are naturally abundant in brassica crops (e.g. Agneta et al. 2013; Kushad et al. 1999). GLSs or their hydrolysis products (GLS-HPs) (e.g. Isothiocyanates, thiocyanates and nitriles), have been shown to inhibit N processes in soil (Bending and Lincoln 2000; Reardon et al. 2013).

The understanding of BNI function in plants is new and still developing. Efforts will be required to test relationships between specific plant traits and BNIs in root exudates in various soil types and environmental conditions. Further work is also needed in order to determine whether the major temperate forage species have the capacity to produce BNIs with an aim to introduce this capacity into New Zealand grazed pastures.

4.2.2.2 N immobilisation

In urine patches, the N reaching the soil is often far in excess of the capacity of plants to take it up (Selbie et al. 2015). A number of studies have demonstrated that this excess N does not simply enter the mineral N pool and become immediately available for nitrification and denitrification (Bol et al. 2004; Carran et al. 1982; Davidson et al. 1991; Whitehead and Bristow 1990; Williams and Haynes 1994). There are two key mechanisms by which urine N can by immobilised: microbial immobilisation or N fixation on clay particles.

For example, Qian et al. (1997) found that labile C additions from rhizodeposition stimulated microbial N immobilisation and retained N in a maize-cropped soil. Additionally, Fisk et al. (2015) observed that C from root exudate additions temporarily decreased the risk of N loss in a semi-arid soil as a result of increased microbial N immobilisation. Carbon from root exudates may thus reduce N_2O emissions derived from urine deposition due to immobilisation of urine-N.

Davidson et al. (1991) calculated that almost 50% of applied NH_4^+ was likely to have been fixed on clay particles within minutes of being applied. Although this is largely a physical process and very soil dependent (i.e. according to clay mineralogy), plants can influence NH_4^+ fixation through the balance of soil cations (Nieder et al. 2011).

4.2.3 Secondary metabolites in urine

A recent review suggested that forage plants are also able to affect N_2O emissions through changing animal urine composition after the plants have been consumed (Gardiner et al. 2016). Some plant compounds (e.g. secondary metabolites) can be excreted in urine and dung and can subsequently affect the N cycle and N_2O emissions. An advantage of having plant compounds excreted in urine and dung is that they are delivered directly with the main N source for N_2O production.

Luo et al. (2015) reported reduced N_2O emissions from urine patches derived from animals fed a brassica rape (*Brassica napus subsp. oleifera* L.) compared to urine from animals fed on perennial pasture. The N concentration of urine, and the associated N loading rate, from brassica-fed animals was lower than that of ryegrass-fed animals and therefore lower N_2O emissions would be expected. However, the authors also reported a reduced N_2O emission factor, which is the function of total N_2O emitted and N loading rate. One possible explanation for this reduction in emission factor is that brassica-derived compounds, such as glucosinolate (GLS) and GLS hydrolysis compounds, may be delivered to the soil in the urine (e.g. Munday et al. 2008; Ye et al. 2002) and nitrification may subsequently be inhibited in the urine patch. However, Hoogendoorn et al. (2016) did not find reduced N_2O emissions from the urine of animals consuming brassicas. The reasons for these different findings are unclear but may be related to soil types as the former field study was carried out on a free-draining allophanic soil (Luo et al. 2015) and the latter on a poorly draining fluvial soil (Hoogendoorn et al. 2016). It is unclear how long it takes for changes in urine chemical composition to occur after a change in diet (David Pacheco pers. comm.). This timing matters in relation to experimental protocols and from a practical standpoint, as the urine returned in grazing systems may reflect a previous diet.

There is an array of potential GLS and GLS hydrolysis compounds in animal urine that can be utilised as BNIs for reducing N_2O production in agricultural systems. In New Zealand systems, forage crops that are known to have BNI potential include: leafy turnips, bulb turnips, swedes, kale, forage rape and plantain (de Ruiter et al. 2009). However, there is little research that has examined the potential for urine from animals consuming these feeds to reduce nitrification and N_2O emissions in practice.

An advantage of targeting compounds in urine for reducing N_2O emissions is that the active compounds is delivered with the main N source for N_2O production. Similarly, Ledgard et al. (2008) and Welten et al. (2013) showed that urine patches could be individually targeted with a synthetic nitrification inhibitor (in this instance dicyandiamide) by orally administering the inhibitor to grazing animals that was subsequently excreted in the urine. It is possible that this delivery mechanism could also be used for administering plant-derived compounds/inhibitors to urine patches.

4.2.4 Plant and root morphology effects

Plants are strong modifiers of nitrogen cycling processes in soils and can therefore be expected to influence N_2O emissions (e.g. Haichar et al. 2014). In New Zealand's grazed pastoral systems, where urine is the largest source of N_2O emissions (Ministry for the Environment 2016), the size and shape of the plant canopy may affect the volume and wetted area of urine reaching the soil surface. Likewise, the root system plays an important role in a plant's ability to access water and nutrients within the soil environment. There are two general root system structures: a primary (tap) root with lateral roots is typically found in dicotyledonous species (e.g. clover, lucerne) while monocotyledonous species (e.g. ryegrass) have an extensive adventitious root system (White and Hodgson 2000). The root system architecture will vary by plant species, with some plants being able to explore the soil volume more effectively than others. Changes in the root system architecture will occur under conditions with high nitrate and phosphate levels (Badri and Vivanco 2009). Urine patches are prime examples of nutrient-rich environments; it could therefore be expected that root activity may increase in the vicinity of these patches, particularly in low-fertility soils.

The release of exudates from roots helps plants to mineralise soil nutrients and assists with plant–microbe interactions (Pierret et al. 2007). Therefore, modified root growth and branching in regions of nutrient-rich patches may coincide with increased root exudation that could affect the nutrient dynamics and microbial community (Badri and Vivanco 2009). The effect of root exudates on soil processes that influence N_2O production has been covered above (section 4.2.2). Below we examine the role of plants and root morphology on factors that influence N_2O emissions.

4.2.4.1 Effects on abiotic environment (soil moisture, pH)

The presence or absence of plants appears to influence N_2O emissions from soils, with greater emissions occurring from soils with no plant cover (Maljanen et al. 2004). These researchers found that N_2O emissions from organic soils sown in grass and barley were only 12-36% of the annual N_2O emissions measured from bare organic soil in Finland. Bare soils may emit more N_2O than soils supporting living plants for two reasons. Firstly, in soils supporting living plants, a proportion of N inputs (e.g. from mineralisation or fertiliser application) will be utilised by plants, thereby reducing the available N for N_2O-producing processes (Maljanen et al. 2004). Secondly, bare soils may be warmer than soils shaded by the plant canopy, thereby potentially increasing mineralisation and nitrification activity. We were not able to find any evidence in the literature supporting or contradicting this second possible explanation.

When roots are present, root morphology can affect soil structure and hydrology (Gregory 2006). Increasing root length and biomass is closely related to increasing organic matter inputs and soil aggregate stability (Francis et al. 1999). An improved soil structure and reduced soil moisture content may influence conditions that govern the reduction of N_2O to N_2 and diffusion of gases to the soil surface (Chapuis-Lardy et al., 2007). However, there is little evidence to support plant species indirectly influencing N_2O emissions by altering soil moisture content. Dijkstra et al. (2010) observed a significant albeit weak positive relationship between soil moisture content and N_2O emissions from soils supporting five different semi-arid grassland species. However, the species composition effect on N_2O emissions could not be explained by species composition effects on soil moisture.

Plants may alter other soil conditions such as soil pH, particularly in the rhizosphere. Legumes generally have a greater effect on rhizosphere pH compared to non-legumes (Maltais-Landry 2015; Wang et al. 2016). Wang et al. (2016) examined pH changes in the rhizosphere of chickpeas, field peas, wheat and white lupin and observed that only chickpeas acidified its rhizosphere. It was thought the difference was due to chickpea's apparent excess uptake of cations over anions during N_2 fixation. In contrast, Li et al. (2011) studied wheat and peas, and showed that pH in rhizospheric soil was approximately one unit lower than that in the bulk soil for both plant species. While legumes generally have a greater effect on rhizosphere pH compared to non-legumes (Maltais-Landry 2015), they explore a smaller soil volume than monocotyledons with extensive adventitious root systems. Therefore, it could be argued that rooting systems of legumes have a limited impact on the pH of the bulk soil, thereby restricting its effect on N_2O producing processes.

4.2.4.2 Effects of plant canopy on leaf N uptake and urine distribution to soil

The size and shape of plant canopies may influence the amount of urine being intercepted by plants, thereby affecting the amount reaching the soil surface. Interception of a proportion of the urine volume may result in direct uptake of urine-urea by pasture herbage. Research with wheat and pasture plants has shown that plant uptake of foliar-applied liquid urea does occur (Powlson et al. 1989; Dawar et al. 2012). However, in these studies N application rates were restricted to between 20 and 40 kg N ha^{-1} per application; in the case of wheat this was to avoid the risk of foliage burn. Urine deposition by sheep and cattle results in greater N loads, equivalent to between 300 and 1000 kg N ha^{-1}. If it is assumed that the potential uptake by pasture foliage is a maximum of 40 kg N ha^{-1}, it is unlikely that plant interception of urine would have a significant effect on urine-derived N_2O emissions from the soil surface.

Plant canopy size and shape may also influence the spread of urine when voided by livestock, with increased dispersion of urine reducing the N load per unit of soil surface area. For instance, a plant with a large, near-horizontal leaf structure may act as a ‘splash-plate’, spreading urine over a larger soil area compared to an upright, narrow-leafed grass species. However, given the strength of an urination stream, any potential ‘splash-plate’ effect offered by a pasture-based plant canopy may be limited.

4.2.4.3 Effects of root N uptake and N cycling

Biomass production has been found to influence N_2O emissions (e.g. Abalos et al. 2014). A mesocosm study comparing N_2O emissions following urine deposition on contrasting plant communities found lower emissions from communities with greater total biomass productivity (Abalos et al. 2014). The most productive species in their study was perennial ryegrass, which dominated the various mixed plant communities: the presence of this species was found to be a determining factor in lowering N_2O emissions. This species has been found to have the least impact on soil microbial activity, measured by basal respiration (Innes et al. 2004), which may partly explain the observed decrease in N_2O emissions (Abalos et al. 2014). Abalos et al. (2014) propose that differences in the composition of nitrifying and denitrifying microbial communities, possibly due to different plant species, may influence N_2O emissions. These workers suggest long term experiments are required to elucidate these effects due to the composition of plant-specific microbiological communities varying over time.

Plant species diversity and interactions also influence the magnitude of N_2O emissions (Abalos et al. 2014; Niklaus et al. 2016). Abalos et al. (2014) found that combining two grasses, perennial ryegrass (*L. perenne*) with rough stalked meadow grass (*Poa trivialis*), which is a high fertility responsive grass similar to perennial ryegrass (Lambert et al. 1986), produced the greatest amount of biomass and also the lowest N_2O emissions (Abalos et al. 2014). They suggest this was due to the complementarity of the root foraging strategies of these two species, where *P trivialis* may access N hotspots not previously emptied by *L. perenne*. This combination, together with its very high total root biomass, is thought to increase mineral N uptake, thereby lowering soil nitrate content and subsequent N_2O emissions (Abalos et al. 2014). Indeed, increasing plant species richness from 1 to 16 grassland species has been found to reduce N_2O emissions in the absence of N fertiliser (Niklaus et al. 2016). This has been related to more efficient soil inorganic N uptake and therefore lower soil nitrate concentrations in more species-rich communities where legumes are absent. This pattern was not observed when the diversity includes a large proportion of legumes (Niklaus et al. 2016).

Pasture species also affect N leaching losses. In studies of animal urine application in early winter, leaching losses decreased with increasing root mass and plant N uptake (Moir et al. 2013; Malcolm et al. 2014; Woods et al. 2016). High plant winter activity is more important than specific root architecture (e.g. deep roots) to reduce N leaching losses from late autumn-applied urine (Malcolm et al. 2014, 2015; Woods et al. 2016). Malcolm et al. (2014) found that winter-active pasture species such as an Italian ryegrass/white clover mix produced the lowest N leaching losses compared to a perennial ryegrass/white clover mix, tall fescue/white clover mix and a diverse pasture containing perennial ryegrass/Italian ryegrass/white clover/red clover/chicory/plantain. Woods et al. (2016) compared N leaching and N uptake from urine applied to three contrasting swards: perennial ryegrass/white clover, Italian ryegrass and lucerne. They observed winter-active Italian ryegrass having the greatest N uptake and lowest N leaching, whereas the opposite was found for the tap-rooted lucerne. The high N leaching losses from lucerne in this study were attributed to poor winter herbage growth and the limited depth of the lysimeters (0.7 m) used for this deep rooting species. Reduced N leaching due to increased N uptake by Italian ryegrass pasture will result in a reduction in indirect N_2O emissions from receiving water environments such as rivers and estuaries (Ministry for the Environment 2016). However, it is unclear whether the increased N uptake may subsequently lead to increased N excretion by grazing livestock, ultimately leading to greater direct N_2O emissions compared to pasture species where N leaching is greater.

5. Conclusions

Table 5 provides a summary of the key findings of the NZAGRC programme (2014-2017) and an assessment of possible mechanisms and explanations, based on the literature review. The results to-date are clearly highlighting the potential of plant species to reduce N_2O emissions, with plantain and fodder beet showing the greatest potential. Plant species that reduce the N concentration of urine are also likely to reduce N_2O emissions, even if the total amount of urine N is not reduced, as the N_2O emission factor for urine was lower at a lower urine N loading rate. None of the New Zealand studies has provided conclusive evidence of any mechanisms by which these plants reduce N_2O emissions. However, based on the evidence to-date and the results from published studies, the key mechanisms with which forage species such as plantain and fodder beet decrease N_2O emission include regulation of N cycling processes and reduction in the total urinary output of the grazing animals.

We suggest the following key hypothesis for future N_2O research:

- Plantain and fodder beet have root exudates that inhibit nitrification and/or increase initial nitrogen immobilisation by increasing available C near roots.

Other hypotheses that could be tested are that plantain and fodder beet reduce soil moisture content and/or increase soil pH, resulting in reduced N_2O emissions. However, the review of the international literature suggested that there is limited evidence to support these.

Table 5: Summary of findings and possible mechanisms that could explain them.

Key finding of N_2O programme	**Commentary/potential mechanism**
1. Soil with plants had lower N_2O emissions compared to bare soil.	A limited number of studies suggest that the presence of plants reduces N_2O emissions from soils, compared with bare soil. This could be due to plant N uptake, and/or modifications in the biological or abiotic environment, but none of the studies has given any conclusive evidence about the potential mechanism.
2. Different plant species resulted in different N_2O emissions from urine applied to soil.	N_2O emissions over a 6-week period following urine application varied with plant species (ranging from 0.7 kg N_2O-N ha^{-1} to 3.2 kg N_2O-N ha^{-1}). Plants with high N uptake had low emissions, but the majority of N_2O emissions occurred in the first two weeks following urine application when soil mineral N was in excess of plant N demand. High N uptake *per se* may therefore not be the driver for low emissions. Instead, there was some evidence that the effect of plant species on soil nitrification potential could be an important factor for the observed differences in N_2O emissions.
3. For some plant species, different cultivars of the same species had different N_2O emissions from urine applied to soil (e.g. Italian ryegrass cultivar Moata had significantly lower emissions than cultivar Tama).	We found no other published studies comparing N_2O emissions from different cultivars of the same species. It is unclear what the underlying mechanism is for the observed differences.
4. Lower N loading in individual urine patches resulted in lower N_2O emissions and emission factors.	There have been some conflicting results on the effect of N loading rate on N_2O emission factors. Some studies have found no effect, while others have found a positive effect. However, on balance, the evidence points towards a reduced N_2O emission factor with reduced urine N loading rates.
5. 'Diverse' pastures (including perennial ryegrass, white clover, plantain and chicory) had lower N_2O emissions from urine applied at 500kg N ha^{-1} compared with standard pasture receiving urine at 700kg N ha^{-1}. There were no differences in N_2O emissions between diverse and standard pasture at the same urine-N loading rate.	These results underpin the conclusion that N_2O emission factors are lower with lower urine N loading rates. Therefore, plants species that reduce the urine N loading rate can reduce total N_2O emissions, even if the total amount of urinary N is not reduced. If, in addition, the total urinary N output is also lower, then N_2O emissions will be further reduced. Plantain does not appear to

	reduce animal N intake compared to ryegrass, but there is some evidence that the observed reduction in urine N concentration from animals on plantain may be due to a diuretic effect.
6. In a field experiment with plant monocultures, plantain had significantly lower N_2O emissions following urine application, compared with ryegrass (and lucerne) monocultures that received the same urine application.	As the same standard urine was used in these monoculture trials the observed difference are plant-induced, rather than urine-induced. The literature suggested that plantain does not appear to reduce animal N intake compared to ryegrass, but there was some evidence that the observed reduction in urine N concentration from animals on plantain may be due to a diuretic effect. There also was some evidence in the international literature that plantain can produce biological nitrification inhibitors.
7. ‘Winter-active’ species had no effect on N_2O emissions, but reduced N leaching and thus indirect N_2O emissions.	Urine deposited onto ‘winter-active’ Italian ryegrass either in autumn or spring did not reduce N_2O emissions compared with urine deposited to standard ryegrass pasture. The literature showed that Italian ryegrass can reduce N leaching, due to higher winter plant growth rates and thus greater uptake of N during the high leaching risk period of late autumn and winter. The increased plant N uptake was not due to root architecture.
8. N_2O emissions and emission factors from urine applied to land planted in fodder beet were 39% lower than from urine applied to land planted in kale.	In this study, fodder beet-specific urine was applied to fodder beet, and kale-specific urine was applied to kale. The observed differences in N_2O emissions can therefore be a plant-effect, a urine composition-effect, or a combined effect. There is no evidence in the literature on the potential mechanism for lower emissions from fodder beet.
9. Plant metabolites *glucosinolate (GLS)* and *aucubin* reduced N_2O emissions in laboratory studies but these results were not always observed under field conditions.	Studies on the effects of plant metabolites GLS and aucubin on N_2O emissions were inconclusive. The literature review indicated that both GLS and aucubin exhibited biological nitrification inhibition (BNI) properties. However, there are no published studies on the effect of these compounds on N_2O emissions. The reasons for the inconclusive results from the New Zealand field studies are unclear.

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