Nitrous oxide emissions – can they be abated by forages in ruminant agricultural systems?

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Abstract

Nitrous oxide (N_2O) emissions account for approximately 10% of global greenhouse gas emissions, with about 90% of these emissions derived from agricultural practices. In New Zealand, the single largest source (60%) of agricultural N_2O is animal excreta (urine and dung) deposited during grazing while nitrogen fertiliser use currently directly contributes only 2.7% of agricultural emissions. Currently available options to reduce N_2O emissions (e.g., dietary interventions or using enhanced fertilisers), are more suited to more-intensive animal production while fewer options are available for more extensive grazing systems. The aim here is to examine possible forage solutions that might reduce N_2O emissions from grazed pasture systems. Plant-based options that could lead to reduced N_2O emissions include using plants with chemistry that divert excess nitrogen into faeces rather than urine and/or by influencing soil N transformation processes. Faeces degrade more slowly than urine and as a result the slow mineralisation of organic N results through a slow supply of ammonium results in a reduced rate of N_2O emissions. The challenge will be to improve the agronomic characteristics of these plants which currently limits their usefulness. This could be approached through plant breeding and/or use of forb-based pastures. This thought piece on how New Zealand grassland farmers can reduce N_2O emissions without having to disrupt current management systems will seek to identify practical opportunities.

Keywords forbs, nitrogen, polyphenols, secondary metabolites, tannin

Background

Nitrous oxide (N_2O) emissions account for 7 to 10% of global greenhouse gas emissions, with ~90% of these emissions derived from agricultural practices (Smith et al. 2007; US EPA 2012). Nitrous oxide emissions from agricultural soils are expected to grow by 35% by 2030 compared with 2005 (US EPA 2012). In New Zealand, agricultural N_2O contributes 11% of the nation's total net greenhouse gas emissions (Ministry for the Environment 2025). Nitrous oxide in soils is produced largely by the microbial process of denitrification and to a lesser extent by nitrification (Eckard et al. 2010). The importance of each of these processes is climate specific with denitrification the dominant process in NZ temperate grassland soils while nitrification is considered the dominant process in Mediterranean soils/climates (Barnard et al. 2006; Jha et al. 2012). Nitrification is an aerobic process that oxidizes ammonium to nitrate, with N_2O as a by-product, whereas denitrification is an anaerobic process that reduces nitrate to dinitrogen, with N_2O as an obligatory intermediate (de Klein and Eckard, 2008).

In New Zealand, 17% of gross greenhouse gas emissions from agriculture are N_2O (Ministry for the Environment 2026) with the single largest source of N_2O emission being from animal excreta deposited during grazing (60% of agricultural N_2O emissions (de Klein et al. 2001)), while nitrogen (N) fertiliser use directly contributes only 2.7% of agricultural emissions (Ministry for the Environment 2025 – refer page 199). Currently available options (e.g., dietary interventions, manure management, enhanced fertilisers and nitrification inhibitors (de Klein et al. 2026)), to reduce N_2O emissions are generally more suited to more-intensive animal production with fewer options available for more extensive grazing systems (Eckard et al. 2010). Indeed, de Klein and Eckard (2008) concluded that technologies available at that time could deliver up to 50% reduction in N_2O emissions from an animal housing system but only up to 15% from a grazing system. These authors also concluded that application of nitrification inhibitors to pastures offered significant potential to reduce N_2O emissions from urine patches. However, in 2013, the main nitrification inhibitor dicyandiamide (DCD) was voluntarily withdrawn, as a precaution, from the market as trace residues were discovered in milk (Ministry for Primary Industries 2013). Since then, research has focussed on developing alternative N_2O mitigation options (Ag Emissions Centre nd.).

High animal N intake has been shown to result in high level of N excretion (Kebreab et al. 2001) which leads to proposing that reducing level of N consumed by ruminants would reduce N_2O emissions from excreta

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(Schils et al. 2013). However, feeds most likely to result in lower urinary-N excretion would have characteristics with <12% crude protein (Dalley et al. 2017), which is well below the optimal dietary requirement of lactating dairy cows which require a crude protein content of 16%–20% (de Klein and Eckard 2008; Dalley et al. 2017). Therefore, offering low N forages or feeds may not be viewed as a practical or attractive long-term option for reducing N_2O emission. Additionally, high-producing pasture in New Zealand has herbage N concentrations of about 3% N or 19% protein (de Klein et al. 2020).

Nitrogen inputs are essential for agricultural production and improving nitrogen-use efficiency has been considered a key N_2O mitigation strategy while satisfying the need for increased food demand (Reay et al. 2012). In pastoral systems, measures that reduce the amount of excreta N or reduce the proportion of excreta N that is lost as N_2O are important strategies (de Klein and Ledgard 2005). These authors also indicated that faecal N is mainly in the organic form and is thus less volatile, whereas urinary N is largely urea and is therefore more rapidly converted to nitrate and is vulnerable to N_2O emission as well as leaching. Indeed, the New Zealand average N_2O emission factor value for urine on flat-land ranges between 0.5 and 0.98% of the N deposited, while for dung it is only 0.12% of the N deposited (van der Weerden et al. 2020). Others have shown N_2O emissions from urine patches can range from two (Murphy et al. 2022) to 11 times (Voglmeier et al. 2019) that which can occur from dung patches.

Forage options that can influence the physiology of digestion in the rumen so that excess N consumed from grazed pasture ends up in the dung rather than urine could be considered a potentially effective method of reducing N_2O emissions. Additionally, some plants may have an ability to influence soil N dynamics through root structure and/or root exudates that then leads to a reduction in N_2O emissions from soil N. This review examines possible forage solutions that might reduce N_2O emissions in grazed pasture system by achieving lower urine N concentrations and/or by influencing soil N processes. It then identifies issues that need to be addressed for their practical use on farm, for ensuring that reductions in N_2O emissions are not offset by unwanted increases in ammonia, methane (CH_4) or carbon dioxide emissions (Schils et al. 2013).

Approach

A literature search was performed using Google Scholar in February and March 2026, using the initial search term of 'nitrous oxide' with 'abatement', 'effect of plant secondary metabolites', 'mitigation' and 'implication of pasture plants'. Further searches were undertaken:

1. On specific secondary metabolites that influence nitrous oxide production, and
2. To identify issues, related to agronomic value and use, associated with specific plant species identified as contributing to reducing nitrous oxide emissions.

Results and Discussion

Nitrous oxide is largely emitted from soil systems and therefore either reducing the amount of mineral N in the soil or manipulating/inhibiting the conversion of soil ammonia to nitrate and subsequently N_2O, are plant strategies associated with, or could be used in New Zealand pastoral systems (de Klein et al 2020). Plant root exudates can also profoundly modify soil microbial communities that play the dominant role in cycling nitrogen and in doing so influence their N transformations (Coskun et al. 2017).

A review of the available literature highlighted several possible forage strategies that could be employed in grazed pasture systems to reduce N_2O emissions including:

1. Introducing plants into grazed pasture swards, that contain secondary metabolites that a) divert excess N from urine into faeces or b) whose root exudates contain compounds that inhibit nitrification, reducing N_2O emissions by limiting the conversion of ammonium to nitrate in the soil (Subbarao et al. 2007; Gardiner et al. 2016; Clough et al. 2020).
2. Increasing the metabolisable energy (e.g., carbohydrate and lipid) of pasture which leads to more animal product per unit of feed consumed (Waghorn and Clark 2004). High metabolisable energy in some forms (e.g., lipid) may directly reduce methanogenesis (Grainger and Beauchemin 2011) and possible N loss into urine (based on a negative correlation between digestible organic matter and urinary N concentration (Giraldez et al. 1997)), and decrease N_2O from dung/manure (Machmüller et al. 2006).
3. Incorporating pasture species with root systems that have matching and/or high rates of N uptake from soil, particularly at lower temperatures, resulting in lowered soil nitrate content and subsequent N_2O emissions (Abalos et al. 2014). For example, the ability of grasses (e.g., Italian ryegrass) to absorb N at lower winter temperatures reduces N leaching (Cameron et al. 2013; Maxwell et al. 2019) and concomitantly indirect N_2O emissions from rivers and estuaries (Reading et al. 2020).

In addition, strategies that improve animal N-use efficiency and thereby reducing N excretion (Reay et al. 2012) or replace high N content grass with low

Table 1 An overview of secondary metabolites that may reduce N_2O emissions by A. diverting nitrogen away from urine, or B. reducing soil nitrification rates. Their mode of action and species known to contain the specific metabolite are also presented.

Plant secondary metabolite	Mode of action	Example species that express the metabolite	References
A. Diverting N from urine into faeces			
Condensed tannins	Flavonoids that protect protein and carbohydrates from microbial digestion in the rumen	Lotus	Foo et al. 1996 and 1997; Meagher et al. 2004; Jonker and Yu 2017
Hydrolysable tannins	Flavonoids that precipitate proteins and alkaloids and effective antagonists against rumen microbes	Salad burnet (*Sanguisorba minor*)	Stewart et al. 2019; Verma et al. 2021 and 2022
Oxidative polyphenols	Polyphenol oxidase to protect proteins and glycerol-based lipid in the rumen	Red clover (*Trifolium pratense*) and sub clover (*T. subterraneum*)	Sakakibara et al. 2003; Lee et al. 2009
Saponins	Structurally and functionally diverse glycosides that can cause cell lysis of rumen protozoa	Lucerne (*Medicago sativa*) and white clover (*T. repens*)	Wina et al. 2005; Ahmad and Zeb 2021; Tava et al. 2022
Glucosinolates	Glucosinolate and its metabolites may negatively impact crude protein digestibility	Brassica	Gao et al. 2022
B. Nitrification inhibition			
Glucosinolates	Hydrolysis products isothiocyanates, thiocyanate and nitriles can be toxic to microbes	Brassica	Brown and Morra 1997; Collett et al. 2014
Aucubin	An iridoid glycoside inhibiting microbial function	Plantain (*Plantago lanceolata*)	Massaccesi et al. 2015; Rahamouz-Haghighi et al. 2023
Catalpol	An iridoid glycoside inhibiting microbial function	Plantain	Rahamouz-Haghighi 2023
Acteoside	A phenylpropanoid glycoside possibly with some microbial impacts	Plantain	Navarrete et al. 2016; Laanet et al. 2024

N content high-energy feeds (Clark et al. 2005; Gao and Zhao 2022) could also reduce N_2O emissions. However, these are relatively impractical to achieve while attempting to maintain adequate production in pasture-based systems and are therefore not considered further in this review.

Plants with secondary metabolites that influence N cycling and N_2O emissions

The key mechanisms with which plant secondary metabolites can influence the N cycle and reduce N_2O emissions from grazed pastures are by diverting more N into dung rather than urine, or by influencing soil N processes (particularly nitrification). summarises the key plant metabolites, their primary mechanism and their mode of action.

Plant secondary metabolites that divert excess nitrogen from urine into faeces

Some species used in grazed pastures produce secondary metabolites that either affect microbial function in the rumen or protect protein and carbohydrate from breakdown by rumen microbes which results in less loss of N to urine (A). However it needs to be noted that leguminous forages will have naturally higher N contents which may lead to increased urine N concentrations, thereby potentially negating any potential decrease in N_2O emissions related to secondary metabolites.

Condensed tannins

Condensed tannins are known to bind protein and restrict their digestion in the rumen but then with the lower more acidic pH in the abomasum the protein is released and utilised by the animal (Barry and McNabb 1999; Min et al. 2003; Naumann et al. 2017). Condensed tannin in plants consumed by ruminants has been well documented as diverting excess N away from urine to faeces (Carulla et al. 2005; Molle et al. 2009; Patra and Saxena 2011; Grosse Brinkhaus et al. 2016;

Table 2 Plants species capable of inclusion in grazed mixed species pasture that express condensed tannins (CTs) in leaf tissue.

Plant species	Level of condensed tannin	Agronomic suitability	Integration into farm systems	Comment	References
More suitable for mixed species pastures					
Big trefoil (*Lotus pedunculatus*)	High	Suited to low fertility; does not compete well with ryegrass. Suited to wet soils.	May need specialist management	Lower CT containing germplasm has been developed	Foo et al. 1997; Jonker and Yu 2017
Birds foot trefoil (*L. corniculatus*)	Moderate to good depending on tannin levels	Slow to establish from seed; does not compete well with ryegrass. Suited to dry soils	May need specialist management	Breeding programmes for improved persistence have occurred in Australia. May need consistently higher tannin levels	Foo et al. 1996; MacAdam et al. 2006
Narrowleaf birdsfoot trefoil (*L. tenuis*)	Low to moderate	Adapted to low fertility, waterlogged and saline soils	May need specialist management	Consistently higher levels of tannins required	Sivakumaran et al. 2006
Other Lotus species	Generally low – although 7 had soluble CTs above 2% in spring	Unknown – some may be new to NZ	Unknown		Ross and Jones 1974; Sivakumaran et al. 2006
White clover (*Trifolium repens*)	Moderate	Potentially reduced agronomic fit – but needs testing	Possible	Genetically modified	Hancock et al. 2012; Roldan et al. 2020
Haresfoot trefoil (*T. arvense*)	High	Adapted to infertile, sandy soils; possibly not suited to competition; annual legume	Naturalised in dry waste places, riverbeds, modified tussock grasslands	Unlikely to be useful in competitive grass swards	Jones et al. 1973
Other Trifolium species	Low if at all, except for *T. affine* which has high CT levels in leaves, and possibly *T. angustifolium*.	Largely unknown	Largely unknown		Jones et al. 1973; Kamalak and Canbolat 2010
Chicory (*Cichorium intybus*)	Possibly moderate, but CT presence seems to vary with germplasm and can be zero in some	Used now in mixtures and as pure sward	Routinely used in pure or mixed swards to provide forb-based pasture mixtures	Perennial herb with numerous pharmacological and nutritional benefits; will need selection for adequate CT levels	Terrill et al. 1992; Waghorn et al. 2002; Seeno et al. 2022
Dock (*Rumex obtusifolius)*	Moderate	Considered a weed	Possible		Waghorn and Jones 1989
More suitable as monoculture forages or grazing shrubs; often mechanically harvested					
Lucerne (*Medicago sativa*)	Moderate	Unknown, but likely reduced yield	Possible	Genetically modified	Hancock et al. 2012
Sulla (*Hedysarum coronarium*)	Very high	High yields when grown a pure species sward; worth persevering with in NZ mixed species pastures	Possible, but possibly needs lax management in autumn; may be best grown as a monoculture specialist crop	Cultivars are available and limited seed sold in NZ; Seed production can be seen as a challenge due to dehulling of seed; Can also contain saponins	Waghorn et al. 2002; Tibe et al. 2011
Arrowleaf clover (*T. vesiculosum*)	Very low	Unknown; an annual legume	Unknown		Evans and Mills 2008; Li et al. 2025

Plant species	Level of condensed tannin	Agronomic suitability	Integration into farm systems	Comment	References
Serradella (*Ornithopus compressus* and *O. sativus*)	May contain low levels of both hydrolysable and condensed tannins	Suited to deep, acidic and sandy soils; used in mixed grazed pastures across eastern NSW; annual legume	Possible; while naturalised it is not used in NZ	An annual legume	Terrill et al. 1992; Li et al. 2025
Sainfoin (*Onobrychis viciifolia*)	Very high	Failure to persist in damp soils and limited cool season growth, and poor tolerance to grazing; perennial legume	Unlikely to persist in mixed species grazed pasture, but could be grown as a specialist monoculture crop		Li et al. 2014; Stewart et al. 2019
Crown vetch (*Coronilla varia*)	Moderate	Unknown; perennial often viewed as an aggressive weed	Unknown		Terrill et al. 1992
Sericea lespedeza (*Lespedeza cuneata*)	Very high	Perennial subtropical legume that is productive on acid and infertile soils; used in erosion control	Unknown; but should be fed in a mix	Selection for lower CT levels is possible but also results in lower yield	Naumann et al. 2014
Stylo (*Stylosanthes*)	Moderate	Unknown - subtropical short lived perennial legume	Unknown	Present in New Zealand	Pereira et al. 2018
Dorycnium species	Possibly very high	Adapted to light, infertile and sandy soils. Grown in semi-arid regions of South Island	Should be fed in a mix	Herbaceous leguminous perennials and deciduous sub-shrubs	Terrill et al 1992
Tagasaste (*Chamaecytisus proliferus / C. palmensis*)	Possibly high	Grazing shrub	As a grazing shrub	Shrub legume – found naturalised on some roadsides. Low levels of CTs but high levels of hydrolysable tannins	Seidavi et al. 2020

Ghelichkhan et al. 2018; Stewart et al. 2019) which then degrades more slowly (Chadwick et al. 2018).

Another study indicated that while more N (15%) (P=0.038) ended up in faeces of dairy cows fed sainfoin (*Onobrychis viciifolia*) (which can contain a high level of condensed tannin) silage as 50% of the diet, urine-N concentrations were not significantly different. However, N retention (which is N intake minus all N in faeces, urine and milk) was more than twice as high for cows fed on the 50% sainfoin diet compared with animals fed a mixture of grass silage, corn silage, concentrate, and linseed (Huyen et al. 2016). Ammonia emissions from slurry of dairy cows fed diets in which the forage legume component was lucerne or birdsfoot trefoil with a low or high concentrations of condensed tannins, respectively, showed that emissions were consistently lower for the birdsfoot trefoil with lucerne (Misselbrook et al. 2005).

Pasture plants that express condensed tannins are summarised in Table 2, outlining their agronomic strengths and weaknesses. Grain of some cereals, notably sorghum (*Sorghum bicolor*) (Terrill et al. 1992), also contains condensed tannins. The limitation to increased use of pasture species containing condensed tannin in grazed mixed species sward is their relatively poor competitive ability on high-fertility soils and a lower nett dry matter yield than some grass-dominant pastures (Waghorn et al. 1998).

It has been observed that condensed tannins are not degraded by rumen microbes (Makkar et al. 1995a and b) and as a result could end up deposited in dung (Makkar 2003a), further slowing the release of N as ammonia, nitrate and N_2O.

Hydrolysable tannins

Hydrolysable tannins precipitate proteins and alkaloids (Haslam 1989) and are effective antagonists against viruses, bacteria and eukaryotic microorganisms (Buzzini et al. 2008). However, unlike condensed tannins they have been rarely researched due to having been considered toxic to livestock and causing death if consumed in large quantities (Makkar 2003b), although they may be

more effective than condensed tannins in reducing methanogenesis (Loza et al. 2021). Hydrolysable tannins such as tannic acid can reduce N_2O emissions in three ways (Gao and Zhao 2022): (a) by moving N excretion from urine to faeces (Lou and Kelliher 2010), (b) by increasingthe urinary excretion of hippuric acid which could inhibit the nitrification process in soil (Kool et al. 2006), and (c) tannic acid can be hydrolysed in the rumen (Singh et al., 2001) forming pyrogallol and resorcinol which when excreted into the urine of ruminants inhibit the urine N_2O formation (Bao, 2019, unpublished results – reported by Gao and Zhao 2022). Despite this, there are few studies on use of plants expressing hydrolysable tannins in grazed mixed species pastures.

Oxidative polyphenols

Oxidative polyphenols are found in many plant species used in grazed pasture and catalyse phenolics into quinones that can complex proteins in feed resulting in reduced protein digestibility in the rumen and subsequently increase undegraded dietary protein flow to the small intestine (Lee 2014). For example - red clover has been shown to reduce rumen protein degradation *in vitro* (Marković et al. 2021) and ruminants seem therefore able to capture these proteins more efficiently into meat and milk. However, red clover polyphenol oxidase activity which could protect proteins and glycerol-based lipid in the rumen need oxygen to be effective (Lee et al. 2009), which may occur during regurgitation and chewing of cud. The rumen being anaerobic limits the potential for polyphenol oxidase activation and activity can really only occur during the period of mastication. Lee et al. (2009) concluded that effective protection of glycerol-based lipid could only be achieved with a prior conditioning (wilting) of the red clover.

Saponins

Saponins are amphipathic glycosides which can also complex with protein and have been linked with decreased CH_4 production, although the results are variable (Kingston-Smith et al. 2010 and reviewed in Niderhorn and Baumont 2010). Few studies have explored the impact of saponins in the diet of ruminants on N retention or excretion, although they have been recommended due to the "beneficial effect of saponins on N metabolism, their use to overcome problems associated with inefficient/poor N retention and utilisation in ruminants" (Kholif 2023). One *in vivo* study using lambs (Sliwinski et al. 2002a) comparing high and low saponin diets indicated a weak potential for small doses of extracts rich in saponins to modify N turnover favourably in the rumen, in the metabolism of the animal and in manure during storage. This study also included a comparison with low and high condensed tannin extracts in feed and showed a largely similar result to the saponin extracts. They also noted inconsistency in results between *in vitro* (Sliwinski et al 2002b) and *in vivo* methanogenesis data. Saponins are found in lucerne and white clover and their impact on N flow in the rumen and soil are yet to be fully understood. As mentioned, the high N content of these legumes may nullify the possible benefit of saponins.

Glucosinolates

A trial using rapeseed cake fed to cattle up to 8% of the total dietary dry matter (contained 18 nmol/g of glucosinolates) had no effect on urinary N excretion compared with cattle fed a diet without rapeseed cake, but faecal N excretion increased linearly with the increasing levels of rapeseed cake in the rations, thus decreasing crude protein digestibility (Gao et al. 2022). The authors suggested that the preparation of the rapeseed cake by hot-pressing 'over-protected' the crude protein or that glucosinolate and its metabolites negatively impacted on crude protein digestibility. Other authors have also found that dietary glucosinolate can reduce crude protein digestibility (Rinne et al. 2015; Dillard et al. 2018).

Plant secondary metabolites that induce biological nitrification inhibition

The adaptive advantage for plants to produce root exudates expressing biological nitrification inhibitors leads to improving the plant's ability to retain nitrogen in the ammonium form, which is less prone to leaching compared to nitrate, thus improving nitrogen use efficiency (Subbarao et al. 2013). A recent review has determined that biological nitrification inhibition reduces net nitrogen loss and increases plant nitrogen uptake in most conditions (Kuppe and Postma 2024). Biological nitrification inhibition decreased nitrogen uptake when there were "(1) low ammonium concentrations, (2) high ammonium adsorption to the soil, (3) rapid nitrate- or slow ammonium uptake by the plant, and (4) a slowly growing or (5) fast-declining nitrifier population. "This raises the question whether plant root biological nitrification inhibition becomes ineffective in high nitrogen urine patches? However, recent evidence indicates that plantain can reduce N2O emissions from urine patches even under high soil N concentrations (Simon et al. 2026).

While biological nitrification inhibition is well documented for some tropical grasses and crops (Byrnes et al. 2017; Subbarao et al. 2013b), it is less obvious in temperate grasses and crops. Compounds found in root exudates that can act as biological nitrification inhibitors include, from sorghum, sorgoleone, sakuranetin, and methyl 3-(4-hydroxyphenyl) propionate which block nitrification enzymes (ammonia monooxygenase and hydroxylamine oxidoreductase) (Subbarao et al. 2013a). For the tropical garss Brachiaria, brachialactone

isanother nitrification enzyme blocker (Subbarao et al. 2009). Nitrification inhibitors can also be found in plant tissues (Coskun et al. 2017). It has been proposed that expression of nitrification inhibitors from tropical species could either be managed and/or introduced into pastures/crops via genetic improvement and therefore combine high productivity along with some capacity to regulate soil nitrification process (Subbarao et al. 2007).

A meta-analysis of 173 observations from 42 articles evaluating global patterns of principal factors controlling N_2O emissions in the presence of root exudates and extracts (Lu et al. 2024) indicated that primary metabolites such as sugars, amino acids, and organic acids in root exudates can result in a significant increase in N_2O emissions, particularly in aerobic conditions, by accelerating the microbial denitrification process (Langarica-Fuentes et al. 2018), while secondary metabolites, such as phenolics, terpenoids, and flavonoids, can significantly reduce N_2O emissions through functioning as either biological nitrification or biological denitrification inhibitors (Bardon et al. 2017).

In New Zealand, some evidence exists that plant secondary metabolites in brassica crops and plantain can affect N cycling and potentially N_2O emissions (e.g., Balvert et al. 2017; Simon et al. 2019; Gardiner et al. 2020).

In brassica crops, glucosinolate and its hydrolysis products (e.g., isothiocyanates, thiocyanates, and nitriles) (Blažević et al. 2015) have been linked to specific inhibition of soil nitrifying bacteria and hence a reduction in N_2O emissions (Bending and Lincoln 2000; Brown and Morra 2009; Balvert et al. 2017; Piumi et al. 2026), but results have been inconsistent. In one study, N_2O emissions from urine of steers fed rapeseed cake with high levels of glucosinolates were higher than from urine of steers fed on a diet without rapeseed cake in the diet (Gao et al. 2022). The authors found a close correlation between the measured N_2O emissions and the concentration of the glucosinolate-derivative, thiocyanate, in urine. They concluded that thiocyanate excreted in urine could be a major factor leading to increased N_2O emissions (Gao et al. 2022). But in another study another glucosinolate derivative, allyl isothiocyanate, from *Brassica juncea* showed a marked suppression of N_2O emission by a facultative anaerobe denitrifier *Pseudomonas* sp. in the culture-based bioassay (Nishiyama et al. 2016). A comparison of a range of brassica crops with wheat in a laboratory trial indicated strong negative correlations between N recovery and both glucosinolate concentration and lignin:N ratio (Piumi et al. 2026). However, this was observed using finely ground plant material that was well mixed into the soil, which raised the question of whether this suppression would be sustained under field conditions where glucosinolate hydrolysed products can be more dissipated and often where whole plant material is incorporated. In field trials incorporating three brassica tissues (kale (*B. oleracea*), turnip (*B. rapa*) bulb, and turnip leaf and stem) into soil with and without added urea N solution, urea-derived N_2O emissions relative to ryegrass (*Lolium perenne*) tissues was reduced, but examination of the mineral N and microbial community data could not attribute this reduction to inhibition of nitrification (Balvert et al. 2018). Indeed, total N_2O emissions increased after incorporation of all tissue residues into soil (Balvert et al. 2018), which were carbon rich and could have resulted in some priming of the soil microbial community (Kuzyakov and Bol 2006). It has been noted that "accumulation of mineral N in soil collected after harvest of canola crops and incubated in the laboratory was double that of soil collected after non-brassica crops (Ryan et al. 2006). However, this occurred only in the top 5cm of soil and did not persist beyond week 3 of the incubation. Changes in the composition of the soil microbial community and differences in the chemical constituents of root tissues were considered the drivers of this effect.

Another study, reporting on three field trials, showed significantly higher emissions from urine of animals fed forage rape compared with pasture (average N_2O emissions of 1.54% vs. 1.20% of animal urine applied, $P = 0.03$). However, no significant difference in emissions were observed when comparing forage rape urine deposited onto forage rape soil compared with pasture urine deposited onto pasture soil (Hoogendoorn et al. 2016). Urine or dung from sheep fed forage rape or ryegrass, compared with a control without dung or urine applied showed that in terms of per unit of N intake, a similar amount of N was excreted in urine from sheep fed either forage rape or ryegrass, but less dung N was excreted from sheep fed forage rape than ryegrass (Lou et al. 2015). However, N_2O emissions for urine from sheep fed forage rape were lower than from urine from sheep fed ryegrass possibly due to transfer of glucosinolates in forage rape to urine affecting soil N transformation processes. It has recently been proposed that "the inconsistency in the results of different experiments [with brassica] could have resulted from the differences in urinary components of the animals in different experiments" (Gao et al. 2022). This conclusion is similar to one made a decade ago that "there is insufficient quality data available on the effect of brassicas on N_2O emissions from grazing systems. Reported experiments have confounding variables, such as the application of urine (or faeces) from brassica-fed animals on soils growing conventional pasture (or vice versa of urine from pasture-fed animals applied to soils

growing brassicas)" (Thomson et al. 2016). It would appear little has changed as research investment into the effects of brassica has been limited.

The inclusion of plantain in grazed pasture systems has been identified as a potential tool for mitigation of N losses to the environment, including reduced N_2O emissions (Judson et al. 2018a, b; de Klein et al. 2020; Minnée et al. 2020; Pinxterhuis et al. 2024). The effect of plantain on N_2O emissions can be due to lower urinary N content compared to cows on a grass-only diet (Chen et al. 2025) and/or due to the effect of the plantain plant on soil N processes (de Klein et al. 2020).

In feeding or grazing trials, plantain-based diets reduced urinary N excretion by stimulating a greater number of urine patches with lower concentrations of urinary N (Minnée et al. 2020; Navarrete et al. 2022). Urine collected from animals on diets with increasing proportions of plantain applied to swards with the same proportion of plantain significantly reduced total emissions of N_2O (Simon et al. 2019). When applying standard urine (from animals fed ryegrass/white clover) to swards with increasing proportion of plantain a smaller but still significant reduction in N_2O emissions was measured (Simon et al. 2019) thus confirming that plantain can have both an effect on N excretion and on soil N processes. Using standard urine, another study showed that an increase of proportion of plantain in the pasture dry matter decreased cumulative N_2O emissions in an allophanic soil but not in a gley soil (Ding et al. 2025). Soil type differences could be due to differential effects on nitrification inhibition by plantain. Over a 148-day mesocosm experiment a monoculture of plantain had a 40% lower nitrification potential compared with a monoculture of ryegrass, although no effects on soil nitrate and ammonia concentrations were observed (Pijlman et al. 2020). These authors also found that, under field conditions, N_2O fluxes were also nearly 40% lower in the presence of plantain, irrespective of the amount of plantain present above 33% of the sward (which was the lowest presnce above zero in the trial). Other studies have confirmed the reduction in soil nitrification rate with urine from both cattle (Judson et al. 2019) and sheep (Judson et al. 2018b) fed plantain. The length of time nitrification inhibition is effective after grazing plantain most likely depends on plantain cultivar type, the rate of degradation of the inhibitory compounds by soil microorganisms, and soil and climatic conditions (Peterson et al. 2022; Hammond et al. 2026).

It is suggested that the ability of plantain to induce biological nitrification inhibition (BNI) is caused by secondary metabolites exuded by the roots. The most common BNI compound of plantain is aucubin (Gardiner et al. 2016; Navarrete et al. 2016). Aucubin is an iridoid glycoside inhibiting microbial function (Massaccesi et al. 2015) and, is a potential nitrification inhibitor that could also be excreted in urine by animals grazing plantain (Dietz et al. 2013; Gardiner et al. 2020). Aucubin may degrade in a urine patch to its active aglycone, aucubigenin, which might inhibit nitrification (Gardiner et al. 2016). Studies have shown that aucubigenin, or its derivatives might have an active inhibitory effect for 5–17 days after urine deposition to soil but over a 35 day experiment this inhibition was not sufficient to reduce overall N_2O emissions (Gardiner et al. 2020).

Other potential nitrification inhibitors produced by plantain include catalpol (another iridoid glycoside) and the phenylpropanoid glycoside acteoside (Gardiner et al. 2016) with the latter associated with reduced rumen ammonia production, a major source of urinary N (Swainson and Hoskin 2006; Navarrete et al. 2016). Catapol had little effect on ammonia production in soil relative to no inhibitor and nitrate levels may increase (Dietz et al. 2013), suggesting it is not as effective as aucubin in reducing N_2O emission. However, other studies have indicated that catalpol, rather than aucubin, was the secondary metabolite exudated by plantain roots (Rodriguez et al. 2023). The effect of aucubin is therefore most likely due to its presence in excreted urine although this has not been confirmed. Acteoside, alone or with aucubin has been shown to promote propionate production, an alternative hydrogen sink (to CH_4), increase gas production in rumen fluid by up to 13%, and decrease the CH_4 proportion in gas by 5−15% (Sivanandarajah et al. 2025). There is evidence that soil microorganisms can induce an increase of aucubin concentrations in the roots, whereas soil nematodes lead to an increase of aucubin and catalpol concentrations in root exudates of plantain (Wurst et al. 2010). Additionally, arbuscular mycorrhizal fungi, such as *Glomus intraradices* and *Gigaspora margarita*, may modify N cycling microbial rhizosphere communities of plantain (Veresogloua et al. 2012) leading to an increase in root aucubin concentration in some genotypes (de Deyn et al. 2009).

Secondary metabolites with nitrification inhibition capacity in plantain are present in both shoots and roots (Marak et al. 2000; Dietz et al. 2013; Box and Judson 2018). A recent study concluded (Sivanandarajah et al. 2025) that "the potential of plant secondary metabolites to mitigate CH_4 emissions and reduce N losses from dairy cows, warrants *in vivo* evaluation and targeted breeding of plantain pastures with increased plant secondary metabolites content." The estimated realised heritability (±SE) for selection of the constitutive level of iridoid glycosides aucubin and catalpol in the leaves of plantain over four generations were 0.23±0.07 and 0.23±0.04 for upward and downward selection, respectively (Marak et al. 2000). While genetic

improvement for these metabolites would be possible a heritability of 0.23 is generally considered low-to-moderate with most of the impact on the trait being caused by environmental factors, non-additive gene interactions, or measurement errors. Concentration in plantain leaves of catalpol (1 to 2%), aucubin, and acteoside were often highest in mid-autumn and can vary between cultivars (Tamura and Nishibe 2002). Catalpol was present at relatively low concentration between 1 and 2% of dry matter with no obvious seasonal effect; aucubin concentration increased from 2.1% to 4.8% in Grasslands Lancelot and from 1.0% to 2.7% in Ceres Tonic through to mid-summer; and acteoside concentration increased from 3.4% to 7.1% in Grasslands Lancelot and from 1.5% to 4.1% in Ceres Tonic over the course of the growing season, although in the summer it declined steadily to lows of 2.5% in Grasslands Lancelot and 1.9% in Ceres Tonic (Tamura and Nishibe 2002).

Forage plantains contain not just iridoids but also polyphenols and flavonoids (Laanet et al. 2024). In some species of *Plantago* polyphenolic compounds include the non-volatiles plantamayoside, ferulic and chlorogenic acid (Khortetska et al. 2020), but also the volatiles β-caryophyllene, D-limonene, and α-caryophyllene (Laanet et al. 2024). The biological function of many of these is not well understood.

The use of forb-based pastures combining a diverse diet of chicory (*Cichorium intybus*), plantain, lotus (*Lotus pedunculatus*), high-sugar ryegrass and white clover provided a 17%–19% decrease in urinary-N content compared with ryegrass and white clover diets alone (Totty et al. 2013). Cows grazing a forb-based pasture composed of chicory, plantain, and white clover had substantially less ($P < 0.01$) N in urine, milk, and blood compared with cows grazing the other pastures, and a larger ($P < 0.01$) faecal N content, indicating a shift of N from urine to faeces (Wilson et al. 2020). Additionally, some chicory cultivars do have levels of soluble and total condensed tannins reaching 1.7% and 2.4% on a dry matter basis, respectively (Seeno et al. 2022).

However, while plantain is important in sward mixtures based on a survey of 59 paddocks on 21 dairy and dry-stock farms across New Zealand it was determined that for grass-based pastures, plantain content declined with age after sowing with few paddocks having more than 20% of their available dry matter as plantain after three years (Dodd et al. 2019). They concluded that while plantain content tended to be highest when sown without grass, and established by direct drilling and on clay soils. A study to identify optimal sowing rates for successfully integrating plantain into mixed species swards reported that increasing plantain sowing rate from 4 to 7 and then 10 kg seed per ha with a concomitant reduction in amount or perennial ryegrass (*Lolium perenne*) seed of 15, to 10 and 5 kg seed per ha, while keeping white clover sowing rate at 3 kg seed per ha, gave an average plantain content (including leaves and reproductive stem) was 32, 44, and 48%, respectively (Nguyen et al. 2022). Although plantain content increased over the first 15 months it then decreased rapidly to 20 to 30% after about 2 years from sowing. Investigations into management interventions to sustain plantain content beyond two years from sowing was recommended (Nguyen et al. 2022). Key actions for improving plantain performance in grazed mixed species swards and aspects of plantain management requiring further research have been recently discussed (Dodd et al. 2025).

Increasing plant metabolisable energy

Rate of feed intake and quality can be a critical factor for improving animal productivity, feed efficiency and reducing emissions of both CH_4 and N_2O. Feed of high digestibility can result in a depression in the amount of CH_4 produced per unit of feed consumed and in the amount of CH_4 produced per unit of product because the ruminant animal requires less feed to meet their energy requirements (Beauchemin et al. 2011; Hristov et al. 2013). Additionally, significant improvements in the incorporation of N from feed into milk and meat have been observed when feeding ruminants with grasses containing increased concentrations of carbohydrate, concomitantly resulting in reduced N excretion to urine by 29% (Miller et al. 2001). Increasing dietary carbohydrate intake has also been shown to increase N excretion in faeces by 76%, most likely as microbial N, because post-ruminal microbial synthesis is greatly dependent on energy availability (Dijkstra et al. 2013; Spek et al. 2013). Increasing the ratio of water-soluble carbohydrate to crude protein in diets produces more milk urea-N and less urine-N (Edwards et al. 2007). A diverse diet with lower crude protein and higher water-soluble carbohydrates will likely lead to higher rumen N use efficiency (Totty et al. 2013). However, there is a consideration that while an increased ratio of water-soluble carbohydrate to crude protein may lead to reduce N excretion to urine that effect is dominated by lower total N intake rather than improving the synchrony of energy with crude protein supplied to the rumen *per se* (Gardiner et al. 2016)

A consideration is whether an increased concentration of lipid in plants can also have the same effect on N_2O emissions as increased concentaraions of carbohydrate knowing that compared with a diet containing 3% lipid (which is at the top end for perennial ryegrass which has between 1.5% and 3.4% lipid on a dry matter basis (Elgersma et al. 2003; Morgan et al. 2020)) the average

decrease in CH_4 with 4.5% lipid is 16% for sheep and 7.1% for cattle; at 6% lipid it is 32% and 14%; and at 8% lipid it is 53% and 24%, respectively (Grainger and Beauchemin 2011).

Increasing lipid concentrations in perennial ryegrass leaves has been achieved using genetic modification by modifying two genes involved in lipid biosynthesis and storage (Beechey-Gradwell et al. 2020) and referred to as high-metabolisable energy (HME) perennial ryegrass. Leaf fatty acid concentration of 5% of dry weight were achieved compared to a 'wild type' untransformed genotype with levels of 3% growing a glasshouse (Beechey-Gradwell et al. 2018). When glasshouse grown leaf material was ensiled the HME silage consistently retained elevated fatty acid content of 4.8% of dry weight compared with null controls with 2.7% of dry weight, and higher gross energy (18.1 vs. 17.5 MJ kg−1 DM, respectively) (Winichayakul et al. 2026). Mesocosms were used to compare the genetically modified HME perennial ryegrass line and a wild type untransformed ryegrass and identified differences in plant soil microbial interactions between the two sward types which resulted in significantly ($P < 0.03$) lower total N_2O emissions (38% lower over four weeks) from the HME sward when cattle urine was applied at a rate of 420 kg N ha^{-1}, equivalent to 10 L m^{-2} (Bowatte et al. 2023).

Combinations of pasture plants species with complementary root morphology

Reducing soil N through increased plant root absorption could lead to reduced N_2O emissions (de Klein et al. 2020). It has been concluded that the complementarity of the root foraging strategies of rough bluegrass (*Poa trivialis*) and perennial ryegrass may explain why when grown together they produced the greatest amount of biomass and also reduced N_2O emissions by 44% compared with perennial ryegrass alone from applied urine (Abalos et al. 2014). In addition to root mass, the ability of grasses to absorb N at lower winter temperatures such as may occur with Italian ryegrass (*Lolium multiflorum*) compared with perennial ryegrass has also been shown to reduce N leaching losses (Wood et al. 2016) and concomitantly indirect N_2O emissions from rivers and estuaries into which nitrates may have leached (de Klein et al. 2020).

Practical implications

Seeking forage based solutions to reduce N_2O emissions for situations where ruminants are grazing outdoors year-round is an important goal for New Zealand. This could be achieved through identifying plants (a) with secondary metabolites that move excess N from urine to faeces, (b) have root exudates containing chemistry that inhibit nitrification in soil,(c) have high metabolisable energy for improving animal productivity, feed efficiency and reducing emissions resulting in reduced N excretion to urine and/or, (d) have root systems that have high soil N uptake rates through increased root biomass and complementarity with other species present,. There is no easy and ready to use solution and while some opportunities have been identified there are challenges to overcome to ensure effective outcomes resulting in reduced N_2O emissions from grazed mixed species swards can be achieved (Table 3). Previous reviews have also attempted to identify attributes and managements of resilient pastures that deliver improved environmental outcomes including reducing N_2O emissions (de Klein et al. 2021). They concluded that while "there are numerous attributes and managements …. none are likely to deliver major improvements on their own at the scale of a whole-farm system; progress will instead be more likely achieved through incremental improvements associated with the implementation of a range of these attributes and actions."

Ranking of each opportunity (Table 3) was based on the degree of substantiated proof of the likely benefit and the degree of difficulty to include plants exhibiting the trait of benefit in grazed pasture. This took into account a combination of Reach (how widespread can the option be adopted), Impact (how effective is its N_2O reduction potential), Confidence (degree of confidence as to whether the option can be adopted), and Effort (how much work is required to get to the stage of adoption) (based on an initiative from ProductPlan nd.). The best opportunity would involve using forage and pasture plants which express condensed tannins in their leaves leading to redirection of excess N to dung, away from urine when consumed by ruminants. However, those pasture species that do express condensed tannins are agronomically inferior in mixed species grazed swards although some as monocultures, such as sulla and sainfoin, could have merit, assuming they can be integrated into current management systems. Breeding programmes to improve establishment and tolerance to competition and grazing might increase the value of species such as *Lotus corniculatus*, and possibly *L. pedunculatus*. The use of white clover expressing foliar condensed tannins would require regulatory approval as it is genetically modified. Condensed tannins also have several other benefits that can lead to improve on farm production and profitability (Mueller-Harvey et al. 2019; Besharati et al. 2020).

Some potential opportunities rated highly include use of forb-based pastures, plants with biological nitrification inhibitors and plants with compounds directing N to urine, all of which reference the use of plants such as plantain. The integration of forb-based pastures into grazing systems would be a reasonably

Table 3 Summary of potential opportunities and challenges for their successful use in grazed pasture systems to reduce nitrous oxide emissions.

Potential opportunity		Significance of potential benefit	Proposed priority ranking	Key issues to confirm assessment
Trait or system	**Plant species examples**			
Condensed tannins	*Lotus corniculatus*, sulla, sainfoin, white clover	High – directing excess N to urine	1	Agronomic suitability in mixed species grazed swards; regulatory hurdle for condensed tannins in white clover
Hydrolysable tannins	Small Burnet (*Sanguisorba minor*)	High – directing excess N to urine	6	More research needed to identify forage species with hydrolysable tannins
Polyphenols	Red clover	Moderate – need confirmation of activity	10	Identify which polyphenols are most beneficial and determine how to capture the benefit
Saponins	Lucerne, white clover	Low – need confirmation of activity	11	Determine if saponins are beneficial and then identify how to capture the benefit
Glucosinolates	Brassicas	Uncertain, possibly moderate – need confirmation of activity	8	Confirm benefit and systems to capture the benefit
Acubin, catapol, acteoside	Plantain	High – directing excess N to urine and can inhibit nitrification	4	Definitive research required to confirm when and how benefits can be maximised, and how these can be captured
Biological nitrification inhibitors	Sorghum, plantain	High – root exudates	3	Identify how these can be employed effectively in pasture and forage species
Forb-based pasture	Combinations of chicory, plantain and legumes	High – directing excess N to urine, and root exudates with nitrification inhibitors	2	Gaining farmer acceptance to integrate these pastures into their management systems
High carbohydrate	High sugar grasses	Moderate – directing excess N to urine	9	Determine actual level of benefit and how this can be delivered in major pasture species
High lipid levels	High metabolisable energy ryegrass	Moderate – directing excess N to urine	5	More research needed to capture the benefit and due to being genetically modified is subject to regulation
High root biomass	Rough bluegrass (*Poa trivialis*) and perennial ryegrass	Moderate – reduce soil N levels and indirectly N_2O emissions	7	Methods of measurement and selection for this trait required so it can be delivered in major pasture species

straightforward development but would depend on the acceptance of the farmer for their adoption. Depending on the species incorporated into forb-based pastures a reduction in N_2O emissions could be through redirected excess N into dung (away from urine) and nitrification inhibition from root exudates. The use of species such as plantain in mixed species swards or sorghum as a monoculture could lead to reduced N_2O emissions through nitrification inhibition activity in root exudates.

While there are no perfect options in using pasture and forage plants to reduce N_2O emissions from grazed pastures there are imperfect options that require further research and development for effective on farm use. The motivation for pursuing these will be driven by recognition of the responsibility to reduce greenhouse gas emissions, a concomitant increase in productivity and/or regulations that demand compliance.

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