Plantain mixed pasture: seasonality of herbage accumulation and potential for mitigating nitrous oxide emissions from cow urine patches

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Abstract

There is growing evidence that plantain (*Plantago lanceolata* L.) is recognised by dairy farmers to improve summer feed productivity and quality and to mitigate nitrogen (N) pollution from grazed pastoral soils. However, there is still limited information on the proportion of plantain required when it is mixed with perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture to optimise its yield stability and environmental benefits. The objectives of this thesis were to monitor seasonal changes in the contribution of plantain to perennial ryegrass/white clover pastures, and to evaluate the effect of incorporating plantain in the pasture diet of dairy cows on nitrous oxide (N_2O) emissions from urine patches during summer and autumn.

The field experiment was conducted over two growing seasons (2019/2020 and 2020/2021). The research site consisted of 20 experimental plots: each plot was 800 m^2 (20 m × 40m). There were four treatments, with different proportions of plantain (0%, 30%, 50% and 70%) in ryegrass/white clover pastures. Each treatment was replicated five times.

The proportion of plantain in the mixed pasture treatments reached a peak of 40% in the first growing season and of 50% in the second growing season during summer- autumn period. At the end of the first growing season following establishment of the treatments, the plantain proportion in the 50% and 70% plantain mixed pasture treatments were similar as around 50%. Plant density of plantain increased by 35% over the first growing season, but it decreased by 52–62% during the second growing season. Overall, the 30% and 50% plantain mixed pastures maintained more stable proportions of plantain than the 70% plantain mixed pasture treatment.

Including 30% and 50% plantain in the summer/autumn grazed diet of dairy cows reduced the urinary-N concentration to 5.40 and 4.40g N L^{-1}, respectively, compared to 6.15g N L^{-1} in urine from cows fed ryegrass and white clover. A lower N content in urine from cows grazing 50% plantain mixed pasture produced 39% less total N_2O emissions compared to ryegrass/white clover urine treatment, regardless of plantain treatments. Total N_2O emissions and the emission factor (EF_3) for plantain mixed pastures were 16% and 27%, respectively, lower than for ryegrass/white clover pasture. The effect of plantain swards on decreased N_2O emissions was linked to changes in soil N-cycling and water-filled pore space values. Plantain proportions of 30% to 50% in mixed pastures were relatively stable over two growing seasons and reduced the urinary-N concentration resulting in the subsequently decreased in N_2O emissions from urine patches in summer/ autumn season.

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1. Introduction

1.1 Introduction

Dairying is a major export earner for the New Zealand economy. In the year to June 2020, the revenue from dairy was NZ$19.7 billion, contributing 34% of New Zealand's total exported goods and services (Sense Partners, 2020). The country's predominantly perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture-based farming systems are one of the key competitive edges that enable New Zealand's dairy producers to obtain higher nutritional values in their milk products and meet consumer preferences for quality. These farming systems also provide a great level of adaptivity, cost-benefits and lower emissions compared with other dairy farm systems used in other countries (Sense Partners, 2020). However, these permanent pasture systems have poor tolerance of drought conditions which limits their herbage production and nutritive value and, in turn, can negatively affect grazing livestock performance (Kemp et al., 2010). Another main concern for dairy farmers relying on ryegrass–white clover (RWC) pastures is the environmental footprint of the deposited urinary nitrogen (N). The RWC swards contain approximately 3.5g N 100g^{-1} dry matter (DM) (range, 2.86–4.16g N 100g^{-1} DM) (Giltrap & McNeill, 2020), which is in excess of dairy cow requirements of approximately 2.6 g N 100g^{-1} DM (Selbie et al., 2015).

Grazing animals can excrete between 75% and 90% of ingested N (Whitehead, 1995). Excretion of urine by livestock results in patches in pastoral soils with extremely high N concentrations, with an average N loading rate of 613 kg N ha^{-1} reported in cattle urine patches (Selbie et al., 2015). This high N loading rate exceeds the capability of pasture N uptake, and the excess N is processed by rhizosphere microbes representing a significant cost to society through increased nitrate (NO_3^-) leaching and enhanced emissions of NO_x (pronounced "knox", the sum of NO and NO_2^-), ammonia (NH_3) and nitrous oxide (N_2O) (de Klein et al., 2003; Di & Cameron, 2002; Luo et al., 2007; Saggar et al., 2005; Selbie et al., 2015). These gaseous and leaching losses of N will create further problems in the future through their impacts on atmospheric, terrestrial and aquatic environments (Saggar et al., 2005). The introduction of environmental regulations to clean water and reduce greenhouse gas (GHG) emissions are the major challenge to farmers. Furthermore, in combination with global warming and increased incidences of unpredictable weather events, New Zealand's dairy farmers are faced with the dilemma of how to maintain sustainable pasture supply all year round versus (vs.) mitigating environmental impacts.

In response to this pressure, one of the most promising management strategies to reduce both leaching and gaseous losses of N appears to be the incorporation of alternative pasture species with high forage production and low environmental N footprint into conventional RWC pastures. Mixed-species pasture utilising multiple forages can extend the growing season, improving persistence and thereby providing higher DM production than standard RWC pasture over the summer months (Daly et al., 1996; Sanderson et al., 2005).

Narrow-leaf plantain (*Plantago lanceolata* L.) has received attention due to its drought tolerance, high forage production potential and low environmental impacts compared to RWC pastures. Under favourable conditions, pure plantain sward can yield up to 20t DM ha^{-1} $year^{-1}$, with a consistent DM production of high nutritive value during summer (Minneé et al., 2013; Reed et al., 2008; Stewart, 1996). Minneé et al. (2020) demonstrated that the N excreted in cow urine could be significantly reduced without any negative impacts on milk yield when dairy cows were fed a diet that included at least 30% plantain. Al-Marashdeh et al. (2021) observed that the inclusion of plantain in ryegrass swards on dairy farms provides similar DM yields with high nutritive value and reduced N leaching loss, while preserving milk production and farm profitability relative to traditional RWC sward. These findings suggested that integrating plantain into grazed dairy systems could fill summer feed gaps, maintain dairy production and mitigate N losses from cow urine patches.

Despite these benefits of plantain, adoption of such a mixed pasture has not been widely accepted by farmers due partly to insufficient evidence regarding the effective proportion of plantain needed to optimise farm productivity, enhance profitability and decrease environmental impacts. Focused research is required to provide more quantitative information on the proportion of plantain needed in a mixed swards to realise its benefits, particularly those related to their effects on N losses (leaching and gaseous), and how the proportion of plantain in a sward might vary across the seasons. This information will assist in the development of improved pastoral dairy systems in New Zealand.

The current research was a part of the NZ$22 million plantain research project funded by the New Zealand Government, which aims to reduce nitrate leaching from dairy farms into fresh water by using the herb plantain alongside grass and clover. The project was funded by the Ministry for Primary Industries (MPI) with the guidance of DairyNZ and the cooperation of Massey University and Manaaki Whenua – Landcare Research.

1.2 Objectives

The main objectives of this thesis are to understand seasonal changes in the growth of plantain mixed pasture over the year, and quantify N_2O emissions from urine patches of dairy cows grazing these pasture swards in late summer. These objectives can be achieved by quantifying the following:

(i) the seasonality of botanical composition and herbage accumulation of plantain mixed pasture.

(ii) the effect of plantain in mixed pasture and in the diet of dairy cows on late summer N_2O emissions from cow urine patches.

1.3 Thesis Structure

The outline of thesis chapters is described in Figure 1-1. Five chapters comprise the thesis, beginning with a general introduction (Chapter 1) that has described the importance of this research and its objectives. This chapter is followed by a literature review (Chapter 2), an overview of the current knowledge about plantain mixed pasture, which leads to the identification of a research focus. Chapter 3 describes field experiments conducted to assess the seasonal changes in botanical composition and plan density of plantain mixed pasture over time. In Chapter 4, the effects of plantain in the pasture sward and in cows' diet on N_2O emissions are studied. These experimental chapters consist of introductions, materials and methods, results, discussions, conclusions and references. Finally, Chapter 5 states the main findings and limitations of the research described here, proposes future research and ends with key conclusions.

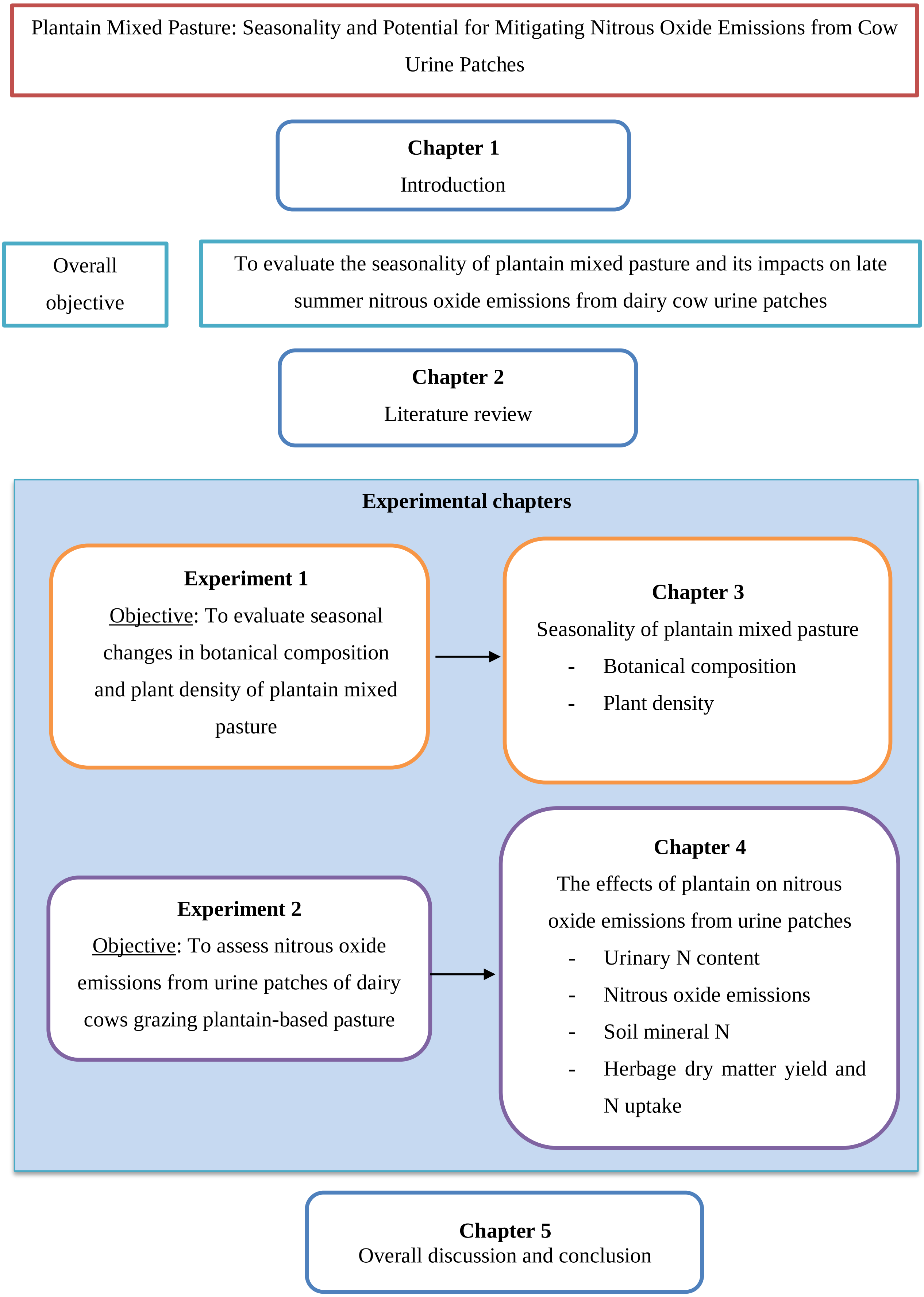

Figure 1-1 Outline of the thesis structure.

Abbreviation. N, nitrogen.

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2. Literature Review

2.1 The Importance of Plantain as a Pasture Specie

2.1.1 Plantain and Plantain Mixed Pasture

Narrow leaved plantain (*Plantago lanceolata* L.) is a rosette-forming perennial herb native to temperate Eurasia (Foster, 1988). This forage herb has lanceolate to oval lanceolate leaves, spreading or erect, and a short taproot followed by a deep, branching fibrous root system that allow plantain to persist in water stress conditions (Rumball et al., 1997). Plantain occurs naturally in pasture swards throughout temperate regions, so it has undergone a long history of use as minor forage plant (Foster, 1988).

Plantain is viewed as an occasional weed in temperate grasslands and crops, and also has a long history of cultivation as a medicinal plant (Stewart, 1996). The interest in plantain as livestock forage has been widespread since it became evident that it had potential to enhance the performance of grazing animals (Stewart, 1996). In New Zealand, this herb began to be cultivated for use on dairy farms in 1987 (Rumball et al., 1997). The first commercial cultivar released for pasture use was "Grasslands Lancelot", which was selected from germplasm in the Manawatū Region of New Zealand for its bushy, semi-erect growth habit, medium–large leaves and tolerance of close sheep grazing (Foster, 1988; Rumball et al., 1997). However, this cultivar was replaced by "Ceres Tonic" and other more recently released cultivars such as Boston, Captain, Ecotain, Oracle and Tuatara, on the market as new cultivars due to outstanding traits such as greater herbage production in autumn and summer.

Tonic plantain is the most popular cultivar due to its rapid establishment, lower cost, and reasonable persistence (Labreveux et al., 2004). Tonic plantain was selected from the common weed types in northern Portugal for upright growth habit, larger leaves, and improved winter activity. Tonic remains erect under diverse grazing conditions, while Grasslands Lancelot is likely to become prostrate under close grazing management (Stewart, 1996).

Plantain mixed pasture has been well documented as providing a balance between farm production, cost effectiveness and environmental benefits for pastoral dairy farms (Kemp et al., 2002). Mixed-pasture swards combine the desirable traits of multiple pasture species including grasses, legumes, and herbs, for growing season extension, additional forage, improved persistence and decreased environmental impacts (Kemp et al., 2002). Increased diversification of swards could offer improved and stable DM production, stable production over the year, greater persistence, and reduced weed invasion (Golding et al., 2011; Navarrete, 2015; Sanderson et al., 2005; Woodward et al., 2013). Mixing at least three pasture species could also increase biological N fixation (Goh & Bruce, 2005).

Under various grazing management and growing conditions, sward mixes including plantain produce higher or similar annual yields as standard RWC; especially notable is dry weight accumulation during summer and autumn (Daly et al., 1996; Goh & Bruce, 2005; Kemp et al., 2010; Sanderson et al., 2005; Somasiri, 2014). Furthermore, incorporating plantain into RWC swards improves herbage nutritive value, animal intake and animal production (Al-Marashdeh et al., 2021; Cranston, 2014; Somasiri et al., 2020; Woodward et al., 2013). Under various grazing regimes, plantain grown within multi-species swards is also more stable over the growing season than plantain grown in pure swards (Cranston, 2014), and the inclusion of plantain is a promising strategy for reducing the surplus N generated by dairy systems (Carlton et al., 2019; Rodríguez-Gelós, 2020; Simon et al., 2019; Totty et al., 2013). Al-Marashdeh et al. (2021) suggested that combining plantain with ryegrass on dairy farms provides similar annual production to RWC pastures, with high nutritive value and decreased N leaching losses, and similar milk production and farm profitability. These findings showcase the economic and environmental benefits for pastoral dairy farming of grazing mixed pastures containing plantain. Given the high potential of plantain, a great deal of scientific effort has been devoted to evaluating plantain as a pasture species in comparison with the standard RWC, which is discussed in Section 2.1.2.

2.1.2 Plantain Establishment, Growth and Persistence

2.1.2.1 Establishment.

Plantain can be adapted to a wide range of environmental conditions, but to ensure optimum yield, appropriate growing conditions and management practices are necessary. The germination of plantain seed is not affected significantly by fluctuating temperatures; seeds are still able to germinate after storage at 20°C (Thompson & Grime, 1983). Temperatures ranging from 20°C–25°C are favorable for the germination of plantain seeds (Pons & van der Toorn, 1988).

Plantain is also insensitive to darkness and leaf canopy interference, which benefits its emergence in established vegetation (Grey et al., 2019; Pons & van der Toorn, 1988). However, germination rates of plantain seeds in mixed swards can be constrained by intense competition from pre-existing plants and other grasses. It has been suggested that spraying herbicide onto existing grasslands before sowing, combined with drilling seed directly no deeper than 1cm, is the optimal establishment method for mitigating competition and ensuring plantain’s rapid emergence (Glassey et al., 2013; Sanderson & Elwinger, 2000a). Reducing competition in mixtures by combining plantain with slower establishing or less competitive species is another strategy to ensure plantain's establishment (Tiley & France, 1990). Thus, early grazing while plantain seedlings are still short enough to escape being grazed reduces competition from established pastures, thereby achieving desirable plant density and resultant pasture production (Bryant et al., 2019).

However, early grazing could truncate the winter survival of plantain sown in autumn when new plants are not ready to persist in winter, so timing the first grazing appropriately is essential. Plantain needs more than six fully expanded leaves and an adequate root system capable of supporting recovery from grazing and winter survival (Powell et al., 2007). In addition, regardless of the effects of grazing, plantain seedlings require at least two true leaves for winter survival in the US (Sanderson & Elwinger, 2000b).

Plantain can establish successfully when sown either in autumn or spring, but in New Zealand, autumn sowing is more common because it allows new plants to be ready for grazing before winter. Powell et al. (2007) suggested that sowing before mid-March in New Zealand is required to allow the plants to establish enough to persist through one grazing rotation before winter. In comparison with chicory, plantain establishes rapidly and is ready for its first grazing more quickly (Stewart, 1996).

2.1.2.2 Growth and Persistence.

After establishment in autumn, plantain grows at a slower rate through the winter months, but subsequently, the plant develops forage, roots, and seeds very quickly in spring, and continues to grow strongly through summer–autumn for at least 2 years (Navarrete, 2015). In herb–clover sward mixes, plantain growth starts earlier in spring and continues later in autumn than chicory and other species (Kemp et al., 2010). The reproductive stage of plantain begins in mid-spring, with more stems produced in the second year after establishment. Stem production accounts for 10%–48% of total above ground DM in year 2 under a range of management strategies (Lee et al., 2015). Reproductive stems allow plantain to reseed naturally in established swards, so that under suitable conditions, successful seedling recruitment in autumn contributes to high persistence of plantain pastures (Neal et al., 2007; Nie et al., 2008; Phillips et al., 2016). In winter, although the growth rate of plantain decreases to a lower level compared with ryegrass, plantain mixed pasture remains green and leafy (Stewart, 1996).

The persistence of plantain in either pure swards or mixed pasture under diverse grazing regimes ranges from 2–4 years, depending on grazing management (Stewart et al., 2014). Over the first grazing season, plantain density in pure swards decreases by 14% under sheep grazing (Powell et al., 2007), and decreases by 15%–40% under cow grazing (Glassey et al., 2013). Ayala et al. (2011) showed that, when sheep grazed plantain, plant density decreased by over 40% in the third year, regardless of grazing management techniques. However, when swards are harvested to two residual heights at different frequencies, plant populations of plantain remain stable during the first year of grazing and decline by around 40% after the second season (Lee et al., 2015).

Similar results were obtained under dairy cow grazing by Navarrete (2015) and Rodríguez-Gelós (2020). These studies found that, on dairy farms, plantain can persist into a third grazing season and that plantain has a greater tolerance of treading damage than many other species under several different grazing regimes. In mixed pasture grazed by cows, plantain density slightly increases in the first year, while it decreases by 55% in the second year compared to a 33% loss of pure plantain sward (Navarrete, 2015). The proportion of plantain in a pasture mixture is likely to decrease in summer but bounce back in late autumn (Rodríguez-Gelós, 2020; Woodward et al., 2013). Likewise, in grasslands, plantain rarely dominates, and naturally accounts for around 20% of the pasture, thus acting as a minor forage species (Stewart, 1996).

2.1.2.3 The Impacts of Environmental Conditions and Grazing Management.

Growth and persistence of plantain can be affected by environmental conditions and grazing management practices. In the Netherlands, Mook et al. (1989) demonstrated no negative impacts of drought on plantain growth due to its drought tolerance and high summer yields. Green-leafiness cover was found to be higher than other temperate pasture species, and similar to chicory (Nie et al., 2008). However, plantain growth slows faster that of chicory, lucerne and red clover during severe water deficits (Kemp et al., 2002; Li & Kemp, 2005; Navarrete, 2015), and Sanderson et al. (2003) demonstrated a 26% reduction in plantain DM production in non-irrigated summer pastures. Clearly, plantain has only moderate tolerance to water stress.

Growth of plantain is responsive to soil water content, nutrient availability and low temperatures (Stewart, 1996). Plantain can adapt to a wide range of soil acidity (pH 4.2–7.8), yielding various textures and organic matter levels (Mook et al., 1989; Stewart, 1996). Plantain tolerates low fertility and low nutritional levels of phosphorous (P) and potassium (K) (Cavers et al., 1980; Troelstra et al., 1992). In contrast, plantain responds strongly to N inputs, with an increase in leaf number, shoot mass and total biomass (Stewart, 1996). Under effects of high herbivory, N fertiliser application results in increased shoot mass but reduced nutritive value (Jarzomski et al., 2000). However, nutrient-rich conditions can reduce the competitive advantage of deeper-rooted plantain over shallow-rooted pasture species in pasture mixtures (Olff & Bakker, 1991).

Under low-fertility conditions and longer grazing intervals, plantain usually has a comparatively high nutrient concentration (Stewart, 1996). Furthermore, regulation of the supply of nitrate and other nutrients to the roots depends greatly on soil moisture. Plantain's nitrate utilisation may be limited by soil moisture stress, but immediately after soil water recovers, nitrate uptake can increase rapidly (Troelstra et al., 1992). In general, soil water deficits (SWDs) reduce the N fertiliser uptake by all pasture species (Shepherd et al., 2011). Kemp et al. (2010) showed that a decrease in crude protein (CP) content in a dry summer was greater than protein reductions in chicory and red clover. Also, plantain is highly responsive to soil moisture compared with chicory and clovers; irrigation can increase plantain yield by 15% in summer (Minneé et al., 2013).

Nevertheless, water saturation and high soil N content in winter can have a damaging impact on the growth of plantain (Mook et al., 1989; Thompson & Grime, 1983). Skinner and Gustine (2002) and Skinner (2005) also indicated that, in the US, high soil moisture and high N uptake reduce the freezing tolerance of plantain in winter, with only a 3% winter survival rate, whilst 46% of plantain experiencing summer drought survives the winter. A low survival rate under harsh winter conditions, which confirms plantain's poor freezing tolerance, has also been reported (Skinner & Gustine, 2002; Skinner, 2005).

Grazing management, frequency and intensity influence the proportion of reproduction stems and the nutritive levels of plantain-based pasture. Having moderate tolerance to treading damage (Stewart, 1996), plantain swards are suitable for rotational grazing (Kemp et al., 2002). Plantain is more resilient to defoliation than chicory, either in pure swards or in the mixes (Cranston, 2014; Navarrete, 2015). Longer intervals between defoliation events (e.g., grazing) can increase plantain production but decrease the quality of pasture and number of shoots per plant, partly due to a higher proportion of low-nutritive-value reproductive stems (Lee et al., 2015; Navarrete, 2015). Grazing intensity has less effect than grazing intervals on yield and the nutritive value of plantain. Lower residual plant height results in lower stem content, and hence higher quality, in herbage, but sward persistence decreases because of plantain's susceptibility to treading damage (Cranston et al., 2015; Lee et al., 2015). However, grazing frequency and intensity, as well as reproductive development, do not have significant effects on the nutritive value of plantain swards (Labreveux et al., 2006).

To strike a balance between quantity and quality of plantain-based pastures grazed by cattle, Lee et al. (2015) and Navarrete (2015) suggested that grazing to residual heights of

100mm, at 2–4 weeks intervals, constitute the optimum grazing regime for plantain persistence across at least two grazing seasons. Additionally, plantain is not a very winter-active pasture, so bad timing — late autumn and winter grazing — can severely compromise plantain persistence (Ayala et al., 2011; Cranston, 2014; Li et al., 1997). Clearly, plantain possesses better drought tolerance than RWC, reasonable freezing tolerance and flexibility to adapt to a wide range of environmental and grazing conditions, but defining appropriate soil moisture content, soil fertility and grazing management practices for this herb are necessary to maximise production, persistence, and quality.

2.1.3 Dry Matter Production

Plantain produces yields ranging from 7.5–20t DM ha^{-1} under various growing and grazing conditions. Powell et al. (2007) reported that, in the first year after establishment, DM yield was 17t DM ha^{-1}. Similarly, Lee et al. (2015) demonstrated that plantain swards defoliated at an extended leaf height of 450mm can produce 16.13t DM ha^{-1} in the first year, and 13.67t DM ha^{-1} in the second year. Under dryland conditions, plantain can produce up to 19t DM ha^{-1} during year 1, and about 15t DM ha^{-1} in year 2. In contrast, irrigation yields no significant difference to DM production over 2 years, with a range from 17–18.6t DM ha^{-1} (Minneé et al., 2013). Yet, irrigation can have a beneficial effect on annual yield and persistence of plantain, especially on DM accumulation during the summer–autumn period under both sheep and cattle grazing (Minneé et al., 2013; Reed et al., 2008).

In comparison with chicory, another summer-active pasture herb, plantain's annual herbage mass is higher, and its persistence is better; however, the herbage accumulation rate of chicory in mid-summer outstrips plantain (Lee et al., 2015; Minneé et al., 2013; Powell et al., 2007). Under cow grazing, the growth rate of plantain reaches a peak of 94kg DM ha^{-1} day^{-1} in December, the summer period in New Zealand (Navarrete, 2015). Pasture production can increase by 1.8t DM ha^{-1} in summer and 0.9t DM ha^{-1} in autumn when plantain is mixed with standard RWC pasture (Moorhead & Piggot, 2009). Similarly, the average yearly DM yield of pasture mixtures including plantain and chicory during the first two growing years can be 1.6t DM ha^{-1} higher than that of conventional RWC pasture (Nobilly et al., 2013).

The net herbage accumulation rate of plantain-based pasture can be significantly higher than that of RWC sward in late spring to autumn (Somasiri et al., 2020). Therefore, the very desirable growth rate of plantain in summer, and its persistence through the winter, allow plantain-based pastures to maintain feed supplies throughout the year. Plantain is at least as productive as standard pasture species, and more so during summer; therefore, adoption of plantain into dryland pastures can improve summer feed supply and provide sustainable, year-round pasture production.

2.1.4 Nutritive Composition

The nutritive values of pasture and animal intake are the other key factors determining animal performance. Higher biomass production enhances milk solid production in dairy cows if DM intake includes greater nutritive value per unit of DM (Hainsworth & Thomson, 1997). Compared to perennial ryegrass, plantain has a higher ratio of readily fermentable carbohydrates and soluble carbohydrates, and lower neutral detergent fibre concentrations. These differences cause higher degradation rates in plantain, which can increase DM intake (Lee et al., 2015; Swainson, 2006). Also, mixed swards containing plantain and clovers fix more biological N than standard ryegrass-based pastures, which contributes to greater animal production and reduced fertiliser application (Goh & Bruce, 2005).

Pure plantain swards have a low CP content, often less than 15% of DM, as opposed to approximately 20%–30% CP in standard RWC (Lee et al., 2015; Minnée et al., 2020). The CP concentration of plantain ranges from 120–280g kg^{-1} DM, with its lowest level evident in summer and under water-stress conditions, but the lower end of the range is unlikely to reduce milk production (Navarrete, 2015). The CP levels vary between vegetative and reproductive growth, and seasons. Fraser and Rowarth (1996) measured 202g CP kg^{-1} DM in plantain leaf, and only 138g CP kg^{-1} DM in stems, and in late summer and autumn, when stems accounted for 60% of the sward, the CP decreased significantly in comparison with other periods.

As opposed to CP content (which equates to N × a factor of 6.25), plantain and ryegrass are reported to have similar total herbage N content, about 3.1g $100g^{-1}$ DM, which is 30% lower than that of legumes (Martin et al., 2017; Minneé et al., 2019). Martin et al. (2017) showed that N-fertiliser application did not have any effect on the N content of legumes, while the N concentration of plantain and ryegrass increased linearly with an increase in N-fertiliser rates under irrigation. This partly explains the higher N content and greater risk of N losses in pasture swards that include legumes. Based on total herbage N figures available in the literature, the presence of plantain in a sward is unlikely to reduce grazing animals’ N intake. However, plantain contains consistently higher non-structural carbohydrates (NSCs) than ryegrass (Minneé et al., 2019). Consequently, with similar total N content, the ratio of NSCs to N in plantain is higher than in ryegrass. The NSC:N ratio influences N partitioning in grazing ruminants by increasing partitioning of N eaten to the faeces and other sinks such as milk, rather than urine, and improves N utilisation in ruminants (Edwards et al., 2007; Moorby, 2014).

Plantain is estimated to have a 30% higher water content than ryegrass. A diet containing 45% plantain provides 60% more water for cattle than a diet without plantain (Minneé et al., 2019; O'Connell et al., 2016). Consequently, urine output of dairy cows grazing plantain can be higher than the urine output produced by cows grazing RWC (Box et al., 2017; Mangwe et al., 2019).

When plantain enters its reproductive stage, neutral detergent fibre concentration increases, and metabolisable energy and CP reduce, so that the nutritive value of plantain swards tends to reduce during summer (Lee et al., 2015; Stewart, 1996). The green seed heads of plantain are eaten, but mature reproductive stems of plantain, because they are fibrous and less digestible, are normally avoided by grazing animals (Lee et al., 2015). Grazing before flowering and intensive grazing during the spring can therefore maintain the nutritive value of plantain (Labreveux et al., 2006). In short, the high DM yield of plantain is associated with its high nutritive value, which has been demonstrated to improve animal production and reduce dietary N intake, and consequently, urinary N excretion.

2.1.5 Secondary Compounds

Plantain produces numbers of bioactive secondary compounds divided into two main groups: (1) iridoid glucosides (aucubin, catalpol); and (2) phenylethanoid glucosides (acteoside), which have antimicrobial and antifungal effects (Bartholomaeus & Ahokas, 1995; Davini et al., 1986; Jiménez & Riguera, 1994). The concentrations of these bioactive compounds differ between plantain cultivars, and according to environmental conditions and leaf age (Stewart, 1996; Tamura & Nishibe, 2002). Aucubin and acteoside commonly present at high concentrations in plantain, while catalpol is detected at very low levels in wild varieties and the "Grasslands Lancelot" cultivar and is absent in the cultivar "Ceres Tonic". Acteoside content in plantain leaves ranges from 3.2mg g^{-1} DM to 41mg g^{-1} DM in the cultivar "Ceres Tonic", which is higher than that aucubin, which ranges from 7.9–13.1mg g^{-1} DM (Al-mamun et al., 2008; Navarrete et al., 2016; Tamura & Nishibe, 2002). Plantain attains its highest concentrations of secondary compounds in mid-autumn (Navarrete et al., 2016; Tamura & Nishibe, 2002). Rodríguez-Gelós (2020) found catalpol both in the root exudates and leaves of plantain, while aucubin was only found in the leaves. Miehe-Steier et al. (2015) showed that aucubin is present in the roots of plantain as well as the shoots. There is increasing evidence that these secondary compounds in plantain can reduce urinary N excretion in animals grazing plantain, and affect soil N dynamics and transformations, which are discussed in Section 2.2.3.

2.2 The Potential Effects of Plantain on Nitrous Oxide Emissions from Urine Patches

In addition to its drought tolerance and high forage production, plantain reduces cows' urinary N concentration, and hence, lowers N_2O emissions and NO_3^- leaching when dairy cows are fed a diet including plantain. The mechanisms of reduction in N losses via plantain pastures can be grouped into the "diet effect" on the urine N composition, and the sward's impacts on the microbial nitrification process and soil microclimate (de Klein et al., 2020). Section 2.2.1 briefly describes the N transformations in grazed pastures and subsequently reviews New Zealand-based literature pertaining to N_2O emissions from urine patches deposited in traditional grazed pastoral systems, with and without plantain.

2.2.1 Nitrous Oxide Emissions from Urine Patches

2.2.1.1 An Overview of the Nitrogen Transformations.

Nitrogen is the key nutrient responsible for the growth and development of plants and animals. Nitrogen in soils typically ranges from 0.1% to 0.6% in the top 15cm, equivalent to between 2,000 and 12,000kg N ha^{-1} (McLaren & Cameron, 1996). The majority (~90%) of N in soils is in the organic matter; a small amount of mineral N consists of ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-) (Cameron et al., 2013). Soil N accrues via fertilising and manure application, the biological fixation of atmospheric N and livestock excreta deposited during grazing (Bolan et al., 2004; Cameron et al., 2013; Saggar et al., 2009; Whitehead, 1995).

Substantial proportions of N inputs are recycled within the system through the deposition of animal excreta (Whitehead, 1995). Mineral N surplus to plant and soil microbial requirements is lost through gaseous emissions of N, or NO_3^- leaching. Among the sources of N, leaching/volatilisation of transformed N from animal urinary N deposition is the largest source of N loss in grazed pastoral systems (de Klein et al., 2010; Haynes & Williams, 1993). Transformations and loss mechanisms of N in legume-based pasture (soil/plant/animal system) are illustrated in Figure 2-1 (A modification of Cameron, 1992). These N transformations in soil include mineralisation (conversation of organic forms into plant-available forms via ammonisation and ammonification reactions); nitrification (biological conversion of NH_4^+ to NO_3^-); immobilisation (conversion of mineral N to plant-unavailable organic N); and denitrification (reduction of NO_3^-, which produces, in stages, NO_2^-, nitric oxide [NO], N_2O and finally, dinitrogen [N_2]). The processes are discussed in Bolan et al. (2004) in detail and are briefly described in Figure 2-1.

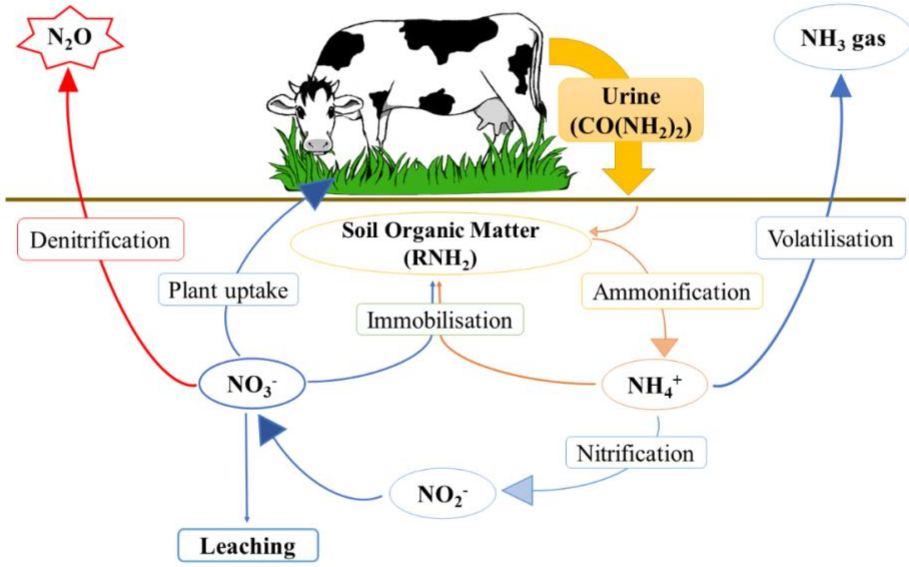

Figure 2-1 Schematic diagram of the N cycle in grazed pasture.

Note. Figure modified from Cameron (1992).

Abbreviations. N, nitrogen; NH_3, ammonia; NH_4^+, ammonium; NO_2^-, nitrogen dioxide; NO_3^-, nitrate; N_2O, nitrous oxide.

Ammonification is the biological process where urea, the predominant form of urinary N, is hydrolysed rapidly into ammonium carbonate and then dissociated to NH_4^+, NH_3 gas and hydroxide (OH^-) ions. The correlation of NH_4^+ and NH_3 gas is determined by the pH of the soil, where high soil pH conditions favour volatilisation of NH_3 (Sommer et al., 2004). After deposition of urine patches, the ammonification process rapidly increases soil pH and leads to volatilisation of NH_3 from the soil surface. The NH_3 volatilisation and subsequent deposition in ground and water constitute secondary sources of N_2O emissions (Haynes & Williams, 1993). The NH_4^+ ions can be absorbed by plants, adsorbed to the clay surface as organic matter or undergo nitrification.

Through the biological *nitrification* process, NH_4^+ is subsequently oxidised into NO_2^-, and then converted to NO_3^-, with the participation of two different groups of autotrophic bacteria. This process requires the presence of oxygen to occur. The NO_2^- is oxidised rapidly to NO_3^-, and hence, the accumulation of NO_2^- in soils is usually low (Cameron et al., 2013). As opposed to ammonification, which produces OH^- ions and so increases soil pH, nitrification releases H^+, and thereby reduces soil pH. The NO_3^- anion is highly mobile in most soils and cannot be fixed to clay because it is negatively charged, the same as clay particles. Therefore, if NO_3^- is not taken up by plants or immobilised or denitrified, this anion is easily lost into the environment through runoff or leaching. This form of N leaches to ground water, drainage and other water bodies and becomes an indirect source of N_2O

emissions (Intergovernmental Panel on Climate Change [IPCC], 2007). The nitrification process is highly responsive to soil conditions including soil moisture, pH, nutrient availability and soil temperature (McLaren & Cameron, 1996). Soil moisture content at "field capacity" is optimum for nitrification, while moisture content that is either higher or lower than field capacity can reduce nitrification rates (Haynes & Sherlock, 1986).

Immobilisation is a microbial process whereby NH_4^+ and NO_3^- are converted to organic forms not available for plant uptake. Conversely, the mineralisation turnover process releases N into the soil solution. These two processes occur concurrently, with the net process determined by the ratio of C:N in organic matter. When the C:N ratio is high, net immobilisation takes place, less N is released than N required, and therefore, the risk of N losses through leaching and gaseous emissions decrease (Sahrawat, 2008).

Under low oxygen availability conditions, facultative anaerobic bacteria use N oxides from NO_3^- as the terminal electron acceptor replacing oxygen. This process is called *denitrification*, and it represents not only an important loss of reactive N, but also a significant source of N_2O, a potent GHG, from the soil–plant system to the environment (Butterbach-Bahl et al., 2013). An estimated 20%–40% of urinary N is lost via denitrification (Bolan et al., 2004). This process occurs under low soil oxygen diffusivity, high respiration and not completely water-logged soil (Cameron et al., 2013); when soil temperature is too low for microbial activity, denitrification cannot occur (Saggar et al., 2009). The rate of denitrification depends on soil conditions such as moisture content, pH, N reductants, soil temperature and texture (Bolan et al., 2004). Denitrification rate normally peaks when soil moisture content is higher than field capacity (Müller & Sherlock, 2004; Saggar et al., 2009). Acidic soils and lack of available carbon (C) sources in the soil solution can also significantly reduce the rate of denitrification (Firestone, 1982; Saggar et al., 2009; Saggar et al., 2013). Scholar de Klein et al. (1996) demonstrated that, in grassland soils, an increase in soil temperature of 10°C–20°C improved the denitrification rate 10 times. Denitrification rates are more responsive to soil temperature in a rainfed paddock than in irrigated systems (de Klein et al., 1996; Dobbie & Smith, 2001).

2.2.1.2 Environmental Impacts of Grazed Pastoral Systems.

Traditional livestock grasslands in New Zealand are predominantly perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (Cunningham et al., 1994). The dominance of this sward type is partly due to the perception that they can provide desirable DM yields of high quality all year round (Kemp et al., 2002). Associated with its high quality, RWC pastures contain a high N concentration of approximately 3.2g N $100g^{-1}$ DM, which is far in excess of dairy cow requirements (Tamminga, 1992). Dairy cows can utilise only 5%–30% of the N available from RWC, which they turn into milk products, while 70%–95% of the ingested N is excreted in urine and dung, and of which 70% is in urine (Bolan et al., 2004; Oenema et al., 2005; Saggar et al., 2009; Selbie et al., 2015). Cow urine is deposited randomly on pasture in “patches”, which cover a surface area of from 0.24 to 0.68m^2 per patch (Selbie et al., 2015), accounting for 20%–30% of the total grazing area (Moir et al., 2011). Approximately 50%–80% of the N in urine is present in urea form, which is more readily available than organic forms of N in faeces (Bolan et al., 2004).

The N loading rate in urine patches has a strong correlation with dietary N and volumes of water intake (Dijkstra et al., 2013). Several studies have demonstrated that urinary N increases linearly with an increased N intake, as opposed to the amount of dung N excretion, which remains relatively constant (Dijkstra et al., 2013; Jarvis et al., 1995). Increased N content in the diet also increases the content of urine N present as urea (Bolan et al., 2004). In contrast, increased water consumption can result in N dilution in the urine, thereby reducing urinary N excretion (Ledgard et al., 2015). An estimated 25kg of dung and 21L of urine are deposited daily by an adult cow onto pasture, constituting over 12.8 dung patches and 10.2 urine patches (Haynes & Williams, 1993; Saggar et al., 2004b). Urinary N concentration is in the range of 200–2,000kg N ha^{-1} per urine patch (Selbie et al., 2015), which exceeds the capability of pasture to take up N. Consequently, surplus N is lost to the environment through volatilisation as ammonia (NH_3), nitrate (NO_3^-) leaching and gaseous forms of N, including nitrous oxide (N_2O) (Bolan et al., 2004; de Klein et al., 2010; Haynes & Williams, 1993; Kebreab et al., 2001; Saggar et al., 2005a).

Grazed pastoral systems are the major source of N_2O emissions in New Zealand, and are estimated to produce 10–12kg N_2O–N ha^{-1} $year^{-1}$ (Saggar et al., 2007). In managed, grazed dairy systems, in addition to deposition of grazing animal excreta, another sources of N losses arise from the application of fertiliser and the biological fixation of atmospheric N (Bolan et al., 2004; Whitehead, 1995). New Zealand dairy cows commonly graze outdoors on pasture throughout the year, with the addition of relatively low amounts of N fertiliser to the pasture (Lancashire, 1988). Consequently, the uneven deposition of animal urine and dung during grazing contributes to approximately 80% of total N_2O emissions in New Zealand (de Klein et al., 2003), while the application of N fertiliser is responsible for only 15.9% of the emitted N_2O (Ministry for the Environment, 2019b). Mineralisation of plant residue and organic matter is recognised as the third largest source of N losses in grazed pastoral systems (Ministry for the Environment, 2019b). Therefore, urine patches are primarily responsible for the vast majority of N losses in grazed dairy pastoral systems.

2.2.1.3 Nitrous Oxide from Urine Patches.

Nitrous oxide, a potent GHG, can remain in the atmosphere for more than 114 years, with a global warming potential nearly 265 times and 10 times higher than carbon dioxide (CO_2) and methane (CH_4), respectively (IPCC, 2014; Myhre et al., 2013). It also contributes heavily to stratospheric ozone depletion (Ravishankara et al., 2009). The concentration of N_2O in the air has increased by 23% since the pre-industrial era, to 333ppb in 2020 (National Oceanic and Atmospheric Administration [NOAA], 2020).

Nitrous oxide, a primary intermediary compound of nitrification and denitrification, can be lost as gas into the atmosphere from incomplete conversion of NH_4^+ to NO_3^-, or NO_3^- to N_2. Urine patches contain 2–3 times more N than soil microbes and pastures require for their maintenance and growth, making urine patches hotspots for N losses (Saggar et al., 2005b). Urine N unevenly deposited by grazing livestock contributes to estimated 64% of direct and indirect N_2O emissions (Ministry for the Environment, 2018).

The major pathways of urine N transformations in urine patches are presented in Figure 2-2 (adapted from Adhikari et al., 2021). Figure 2-2 shows that N_2O is mainly produced through aerobic autotrophic nitrification, an aerobic heterotrophic denitrification process, in addition to nitrifier denitrification and chemo denitrification.

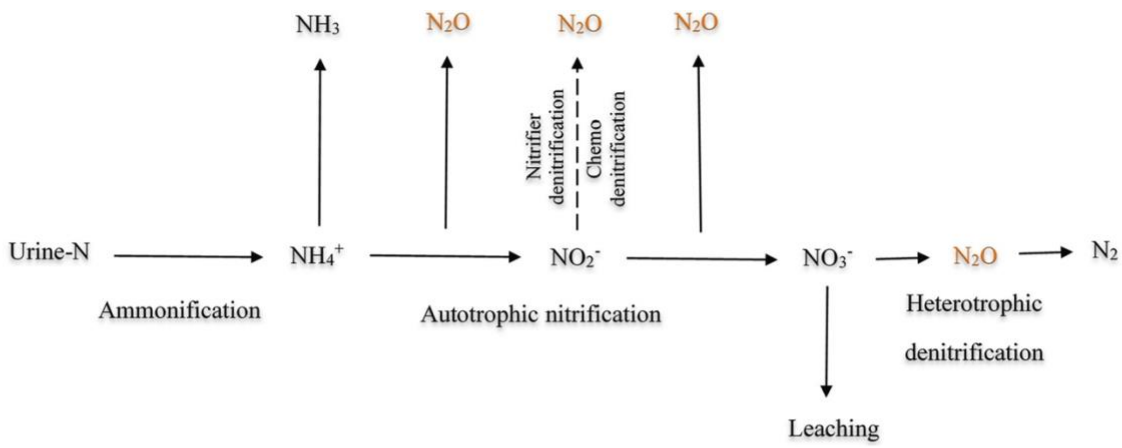

Figure 2-2 Schematic representation of the major urine N transformation pathways in urine patches.

Note. Figure adapted from Adhikari et al. (2021).

Abbreviations. N, nitrogen; N_2, nitrogen; N_2O, nitrous oxide; NH_3, ammonia; NH_4^+, ammonium ion; NO_2^-, nitrogen dioxide anion; NO_3^-, nitrate.

Cow urine patches add locally and readily available C and N in high concentrations, in addition to water content, into soils via the soil microbial processes of nitrification and denitrification, thus creating “hot spots” for on-farm N pollution (Bol et al., 2004; Luo et al., 2017; Saggar et al., 2004b). Selbie et al. (2015) estimated the proportion of N losses from deposited urine patches as 13% NH_3 volatilisation, 20% NO_3^- leaching, 2% N_2O emissions, 41% plant uptake and 26% immobilisation. The main N losses from urine patches present long-term threats to freshwater resources and the atmosphere. Therefore, the New Zealand Government and the dairy industry have identified the mitigation of N losses as a top priority for the sustainable development of dairy systems (DairyNZ, 2014).

Urine patches with high concentrations of soluble N and C, and a high volume of water, provide optimum conditions for the processes of nitrification and denitrification to occur, thus releasing N_2O into the atmosphere (Van der Weerden et al., 2017). Of these N_2O emissions, an estimated 70% are produced directly by the processes of nitrification and denitrification (Butterbach-Bahl et al., 2013; Saggar et al., 2004a; Selbie et al., 2015). The remainder come indirectly from secondary sources, including anaerobic NH_3 oxidation pathways involving volatilised N and leached inorganic N subsequently dissolved in water bodies (Haynes & Williams, 1993; Matthews et al., 2010). Additionally, chemical reactions between nitrous acid (HNO_2) and soil organic matter, NH_4^+, NH_2OH and amines in soil solution, can form N_2O gas (Cameron et al., 2013). Mathieu et al. (2007) observed that nitrification generates 60% of N_2O under unsaturated conditions, whereas in saturated conditions, 85%–90% of emitted N_2O is produced by denitrification. Parfitt et al. (2006) estimated that N losses from managed, grazed dairy farms in New Zealand caused by denitrification are around 10kg N ha^{-1} $year^{-1}$.

Immediately after the deposition of urine patches, N_2O losses increase (Yamulki et al., 1997), and during the first 24h, these losses contribute to an estimated 8% of the yearly N_2O emitted from grasslands (Williams et al., 1999). These high N_2O emissions are dominated by the process of denitrification when water-filled pore spaces increase rapidly after grazing. Most of the N_2O from urine patches is lost in the first 2 weeks after deposition, but it can continue to be emitted for more than 30 days after deposition (Baggs et al., 2000).

The availability of soil mineral N, soil microbial communities, temperature and water-filled pore spaces exert profound effects on N_2O fluxes from pastoral soils (Di et al., 2014; Dobbie et al., 1999; Rousset, 2021). Dobbie and Smith (2001) observed that N_2O emissions increased 12 times when the water-filled pore space value increased from 60% to 80% in grassland. Increasing temperature in grassland soils with the same water-filled pore space value resulted in greater N_2O losses (Dobbie & Smith, 2001). The highest N_2O emissions, which are 5–10-fold higher than in winter, were recorded 1 week following a heavy rain event after a long dry period (Saggar et al., 2009). Additionally, trampling caused by grazing animals can result in soil compaction and an increase in bulk density, consequently promoting denitrification rates and resultant N_2O emissions (Menneer et al., 2005).

Nitrous oxide emissions are compared as the emission factor (EF); the amount of N_2O–N emitted as a percentage of urine N applied, is calculated following the IPCC (2019) methodology, and using Equation 1:

$$EF_3 = \frac{N_2O\text{-N total (urine)} - N_2O\text{-N total (control)}}{\text{Urine N applied}} \qquad \text{Equation 1,}$$

where EF_3 is the emission factor; and where total treatment N_2O and total control N_2O are the cumulative N2O–N emissions from the urine-applied and control plots, respectively (kg N ha^{-1}); and where N applied is the rate of treatment N applied (kg N ha^{-1}). New Zealand employs the EF_3 values for urine ranging from 0.08%–0.98% based on livestock type and topography, a method developed by van der Weerden et al. (2020) to calculate direct N_2O emissions. Variability in the EF_3 is greatly dependent on soil drainage class, rainfall, weather conditions and the measurement period (de Klein et al., 2003). The urine EF_3 in free-draining soils is typically lower than in poor-draining soils (de Klein et al., 2014). Under dry and warm–temperate conditions, the EF_3 is significantly lower than the default value used in country-specific emission factor inventories (López-Aizpún et al., 2020).

It is evident from the literature review that the majority of N_2O emissions originating from grazed pastoral systems in New Zealand are regulated by soil and environmental conditions (pH, texture, moisture, soil mineral N). Given the greenhouse effect and ozone depletion potentials of N_2O, as well as its projected increases, the New Zealand Government has recently proposed the *Zero Carbon Bill* targeted to reduce N_2O emissions to net zero by 2050 (Ministry for the Environment, 2019a). While multiple N_2O loss reduction strategies targeting urine patches have been developed with the idea of using alternative feed supplies (de Klein et al., 2020), duration-controlled grazing (Christensen et al., 2012; Christensen, 2013) and the use of chemical and biological inhibitors (Adhikari et al., 2021; Di & Cameron, 2002; Luo et al., 2010) have also been proposed. In recent years, plantain has received the most attention as a promising option. The introduction of plantain into conventional pasture swards has been observed to:

- produce relatively high yields throughout the growing season that can maintain animal production during dry summer conditions
- produce low N concentrations in cow urine
- facilitate rhizospheric biological nitrification inhibition and
- reduce N losses from urine patches.

2.2.2 The Role of Plantain in Mitigating Nitrous Oxide Emissions

Reduction in N_2O emissions from urine patches by incorporating plantain into pasture systems, either in a lysimeter or at the field scale, can be significant. For example, Luo et al. (2018) obtained 28% reduction in N_2O emissions in winter from pure plantain swards than from RWC swards receiving same urine. Similarly, Rodríguez-Gelós (2020) showed 42% and 28% N_2O emissions reduction in plantain swards in spring from the urine of cows consuming plantain and RWC, respectively, compared to RWC swards. She also showed that a 50% reduction was achieved by plantain swards compared to RWC in a lysimeter study.

There is increasing evidence that increasing the amount of plantain in mixed swards and/or in animal diets can result in greater reductions of N_2O emissions (Pijlman et al., 2020; Podolyan et al., 2020; Simon et al., 2019). Pijlman et al. (2020) demonstrated a linear decrease in N_2O emissions as the proportion of plantain in mesocosms increased, but increasing plantain proportions in the mixture did not show a significant effect on N_2O in the field. In a lysimeter experiment with same urine type applied, a higher herbage proportion of plantain did not result in a greater decrease in N_2O emissions (Podolyan et al., 2020).

The ability of plantain to reduce N_2O emissions also varies with season. For example, it was observed that the effect of plantain on N losses more obvious during the late autumn and early winter period (Hainsworth & Thomson, 1997; Luo et al., 2018; Simon et al., 2019). In contrast, Podolyan et al. (2020) showed that plantain swards did not significantly reduce N_2O emissions in winter. This variability could be attributed to differences between soil water-filled pore spaces, weather conditions and pasture management.

Despite previous studies' variable results, the presence of plantain in the pasture and/or in the animal diet does reduce N_2O emissions from urine patches. Including plantain in pasture and in the diet can reduce the N content in excreted urine (de Klein et al., 2020; Luo et al., 2018; Simon et al., 2019) and/or the N_2O EF_3 of urine (Carlson et al., 2020; de Klein et al., 2020). The mechanisms for the effect of plantain on N_2O emissions was proposed to be related to increased partitioning of N excreted to dung and other N sinks rather than urine (Box et al., 2017; Minneé et al., 2020); the diuretic effects of secondary compounds and water content contained in plantain, in addition to the resultant higher urine volume and frequency (O'Connell et al., 2016); alternations in the soil microclimate (Simon et al., 2019); and biological nitrification inhibitor effects of secondary compounds from plantain root exudates and/or urine (Carlson et al., 2020; de Klein et al., 2020; Gardiner et al., 2018).

Simon et al. (2019) observed some positive effects of including plantain both in the diet and in the swards on urinary N content and soil microclimate. But, Podolyan et al. (2020)

suggested that lowered N_2O emissions are mainly due to decreased N loading rates in the urine of cows fed plantain, with no obvious link to plantain–soil interactions. However, the impacts of plantain on soil moisture and the nitrification processes were clearly demonstrated by other researchers to alter soil N cycling and soil moisture content (Carlson et al., 2020; Rodríguez-Gelós, 2020). These mechanisms of N dynamics could be classified into two major effects: the "diet effect" on urine-N composition and the "sward effect" on rhizosphere nitrification inhibition and the soil microclimate. Clearly, to widely adopt plantain as a N_2O mitigation option, it is necessary to understand the impacts of plantain on N_2O production processes, as elaborated in Section 2.2.3.

2.2.3 The Impacts of Plantain on Nitrous Oxide Emissions

2.2.3.1 The Diet Effect of Plantain on Urine Composition.

The presence of plantain in the animal diet could result in a lower urine N loading rate to the soil, which is the main driver of N_2O emissions from grazed pasture. The urine N content deposited onto soils depends on urinary N concentration and urine volume (Ledgard et al., 2009). The amount of N excreted in urine from cows grazing swards including at least 30% plantain was shown to be significantly lower than from cows consuming standard RWC (Box et al., 2017; Edwards et al., 2015; Minneé et al., 2020; Rodríguez-Gelós, 2020; Totty et al., 2013). A trend of lower urinary N content as the proportion of plantain in the diet increased has been observed (Rodríguez-Gelós, 2020). Although the amount of N consumed in a diet containing plantain is similar to the amount of N in diets without plantain, the N content in urine from cows grazing mixed pastures with various proportions of plantain actually decrease (Box et al., 2017; Minneé et al., 2020). This non-linear relationship between dietary and urinary N excretion means that the reduction in urinary N produced by cows fed plantain is attributable to a dilution effect, higher N partitioned into the faeces or a biological inhibitor effect of the secondary compounds, rather than N content in the plantain diet.

Plantain contains bioactive compounds grouped into iridoid glucosides (aucubin, catalpol) and phenylethanoid glucosides. These secondary compounds impact rumen fermentation, and ultimately, they improve rumen N utilise efficiency and mineral nutrition in ruminant livestock (Swainson, 2006). Consequently, the presence of antimicrobial compounds in plantain could reduce ammonia (NH_3) concentration in the rumen and reduce urinary N excretion (Navarrete et al., 2016; Stewart, 1996; Totty et al., 2013). Also, the diuretic effect of iridoid glucosides increases urination volumes (O'Connell et al., 2016; Tamura & Nishibe, 2002).

In addition to a higher concentration of bioactive compounds, plantain also has higher water content than RWC (Minneé et al., 2019). As a result, including plantain in the diet can cause diuresis and increased urine volumes (Minnée et al., 2020; O'Connell et al., 2016). Higher urine volume dilutes the amount of N excreted in each urine patch but does not increase urinary N content (Mangwe et al., 2019).

Furthermore, plantain has a higher ratio of NSCs:N relative to ryegrass, which leads to increased N content excreted in dung rather than urine (Moorby, 2014). Carulla et al. (2005) showed that condensed tannins in the diet increases N partitioning into faeces relative to urine. In fact, condensed tannins have been detected in plantain (Ramírez-Restrepo & Barry, 2005); hence, these compounds might contribute to higher dung N excretion and lower urinary N excretion when plantain is incorporated into animal feed. The faecal N of cows consuming a diet including plantain is, on average, 18% higher than excreta from cows eating a diet free of plantain (Dodd et al., 2019). As the plantain proportion in the pasture mixture increases, the amount of N partitioned to dung and milk is likely to increase (Minneé et al., 2020).

2.2.3.2 The Sward Effect of Plantain on Nitrification Inhibition and Soil Microclimate.

The reduction in N_2O emissions from plantain-containing swards is associated with the release of biological nitrification inhibitors (BNIs) from plantain, potentially via root exudates or plant litter decomposition. These BNIs are defined as organic molecules released naturally from some plant species that are able to derange the function and activity of nitrifying bacteria, and hence inhibit the nitrification process in soils (Abalos et al., 2014; Subbarao et al., 2007). As discussed in subsection 2.1.5, the secondary compound aucubin in plantain is a potential BNI suppressing N mineralisation, nitrification and resultant N_2O emissions (de Klein et al., 2020; Dietz et al., 2013; Luo et al., 2018; Simon et al., 2019). Secondary metabolites released from plantain in its root exudates concede the possibility of plantain inhibiting the nitrification process and interrupting N cycling in the soil, while the presence of aucubin in leaves could contribute to nitrification inhibition via leaf litter. Gardiner et al. (2018) observed that an extract of plantain leaf and aucubin solution reduced N_2O emissions from cow urine by 50% under field conditions. Similarly, the application of aucubin to RWC swards in lysimeters has resulted in a 36% decrease in N_2O emissions (Rodríguez-Gelós, 2020).

Plantain can suppress the number of nitrifying bacteria by 200 times when NH_4^+ fertiliser is applied to plantain-containing swards (Verhagen et al., 1995). Carlton et al. (2019)

demonstrated a lower population abundance of ammonia-oxidising bacteria (AOB), which generate the nitrification process, and resultant decreased NO_3^- eutrophication in the soil of summer pastures containing 30% plantain relative to soil under RWC swards.

Dietz et al. (2013) confirmed effective decreased soil mineralisation and nitrification by both aucubin and plantain leaf materials in a soil incubation experiment. In a mesocosm experiment, Pijlman et al. (2020) observed a 40% reduction in nitrification rates in pure plantain pasture, in comparison with RWC. Similarly, multispecies mixed pasture including plantain was demonstrated to slow down the nitrification rate, increase NH_4^+ fixed to clay and decrease the production of NO_3^- after application of urea–N fertiliser (Bracken et al., 2020). However, Podolyan et al. (2020) did not observe significant differences in AOB population abundance in winter plantain vs. conventional swards grown in a free-draining soil. This result may be due to the poor metabolic activity of plantain and low concentration of BNI exudates from the roots under lower winter temperatures. Additionally, urine collected from cows consuming plantain can reduce the rate of nitrification during the first month after application, relative to urine from cows grazing RWC pasture (Judson et al., 2019). This result suggests that the urine of cows fed plantain potentially contains nitrification inhibitors.

Because plantain has a deep-growing root system, its underground growth is likely to change the soil microclimate. Luo et al. (2018) reported a significantly fewer water-filled pore spaces under plantain swards compared with other pasture types in winter field conditions. In contrast, soil moisture under spring plantain and RWC pastures were not significantly different in the Canterbury region (Podolyan et al., 2020). Rodríguez-Gelós (2020) observed more water-filled pore spaces in soil under plantain than in soil under RWC swards in autumn/winter, leading to higher cumulative N_2O emissions and EF_3 from the plantain treatment.

To date, the effects of plantain on N content excreted in urine and soils, and the relationships between plantain proportions in mixed pastures and in animal diet with reductions in N_2O emissions, remain unconfirmed. In New Zealand, the impacts of plantain-containing pastures on N_2O emissions have been evaluated mostly during the winter/spring or autumn/spring periods. To the best of our knowledge, information about N_2O emissions from plantain swards in summer, when plantain grows actively, remains very limited.

2.3 Conclusions

Plantain’s moderate drought tolerance, sustainable DM production throughout the year and relatively high nutritive value have been well-documented. Incorporation of plantain into swards has a high potential to lengthen the growing season, fill the summer feed supply gap and enhance animal performance over summer. However, neither the most effective proportion, nor the optimum density, of plantain in pasture mixtures needed for a productive pasture under dairy cow grazing have been quantified.

Nitrous oxide is a potent air pollutant with serious environmental effects on global warming and the stratospheric ozone layer. The literature reviewed in this chapter suggests that the presence of plantain in animal diet and in pasture can reduce N_2O loss to the environment from cow urine patches, while increasing summer DM production and achieving desirable persistence in the sward. Thus, plantain has the potential for wider adoption by farmers. Yet, little information on the performance of plantain in mixed pastures through the growing seasons, or its effect on N_2O emissions in late summer, exists. The current work addresses the lack of published and/or unpublished domestic and international research on these topics. Chapters in this thesis describing related field work emphasise two objectives:

- Chapter 3 — Improve the understanding of seasonal changes in botanical composition of pasture species in plantain mixed pasture across two growing years.
- Chapter 4 — Enhance understanding of what effects various proportions of plantain in animal diet and in pasture mixtures have on N_2O emissions from dairy cow urine patches in summer.

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3 Seasonality of Plantain Mixed Pasture

3.1 Introduction

In New Zealand, the main feed source for dairy cows that has traditionally been relied on is permanent pasture mixtures of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) (Charlton & Stewart, 1999; DairyNZ, 2016). Perennial ryegrass and white clover (RWC) pastures are considered to provide a sustainable pastoral supply for dairying due to their great persistence, high DM yield and high nutritive value (Kemp et al., 2002). Perennial ryegrass is easily established and managed, highly competitive and very persistent under grazing (White & Hodgson, 2000). White clover can biologically fix N_2 and produce high-quality forage, thereby improving grazing animal production (Caradus et al., 1995).

In recent years, narrow-leaf plantain (*Plantago lanceolata* L.), a summer-active pasture species incorporated in swards, has been widely adopted by farmers to enhance feed availability and quality during summer and autumn in drought-prone regions without irrigation. Plantain can establish rapidly, adapt to various soil conditions and is responsive to N availability and cool temperatures (Stewart, 1996). Plantain has moderate drought tolerance and maintains its green leafiness over summer (Nie et al., 2008). As discussed in Chapter 2, plantain pasture herbage DM accumulation and quality is greater than that of RWC in summer and autumn (Navarrete, 2015; Nobilly et al., 2013; Totty et al., 2013). Therefore, the inclusion of plantain in pastures can increase summer feed supply and improve animal performance.

The benefits of multi-species swards have been reported as increased DM yield, lengthened pasture growing seasons, improved pasture stability and reduced invasion of weed and insects (Kemp et al., 1999; Woodward et al., 2013). However, the proportion of pasture species with different growing characteristics in a multi-species pasture can change over the growing season, with species composition varying according to plant competition, grazing impacts and weather conditions. Ryegrass is more competitive and tolerant of treading damage and soil compaction than white clover and plantain (Stewart et al., 2014). When ryegrass is sown with other pasture species in a pasture mixture, ryegrass is therefore likely to dominate the sward (Anderson, 2015; Stewart et al., 2014). However, during the summer–autumn period, due to poor drought tolerance, RWC growth tends to slow down (Kemp et al., 2010; Lee et al., 2013), while plantain grows strongly by utilising its deep roots to access water deeper in the soil (Nie et al., 2008). Therefore, the botanical composition of pasture mixtures tends to change significantly over the years, impacting on total productivity and quality of swards.

Determination of effective proportions and seasonality of plantain mixed pastures could assist in the wider adoption of plantain in New Zealand's dairy pastoral systems. The objective of the current experiment was to evaluate the persistence of plantain under dairy cow grazing when included in RWC pastures. To meet this objective, a field study was conducted to monitor the seasonality of botanical composition and plant density in RWC pasture containing different proportions of plantain (0%, 30%, 50% and 70% plantain) in the sward managed under a dairy cow grazing regime through two growing seasons (2019/2020 and 2020/2021).

3.2 Materials and Methods

3.2.1 Experimental Site and Design

A field study was established in a 6.6 ha rain-fed paddock at Massey University's Dairy No. 4 Farm, Palmerston North, New Zealand. The experiment evaluated four pasture treatments: ryegrass/white clover (RWC), and RWC mixed with 30% (P30), 50% (P50) and 70% (P70) of plantain under grazing by dairy cattle. The experimental area was set up with mole channel and pipe drain system in each replicate plot. Plant species, cultivars and sowing rates are presented in Table 3-1.

As per the New Zealand soil classification system, the only soil type at the experimental site is classified as a poorly drained Tokomaru silt loam soil, termed as Argillic-fragic Perch-gley Pallic soil (Hewitt, 2010), and classified as Alisol following Food and Agriculture of the United Nations Scientific and Cultural Organization (FAO–UNESCO) (2008) soil classification guidelines. Before sowing, existing vegetation was eradicated by the application of 540g L^{-1} glyphosate herbicide (4L ha^{-1} of Polaris® 540), thifensulfuron-methyl (Harmony® SG at 30g a.i. ha^{-1}) and 100mL $100L^{-1}$ ha^{-1} Pulse® penetrant (organosilicone). No fertiliser was applied before sowing or at the establishment stage. The experimental area was drilled and sown directly on the 5th of April 2019.

Table 3-1 Species and sowing rate for each pasture treatment

		Pasture treatments (kg ha^{-1})			
Species	**Cultivar**	Ryegrass/ white clover (RWC)	30% Plantain (P30)	50% Plantain (P50)	70% Plantain (P70)
Ryegrass	One50-AR1	20	15	10	5
White clover	Emerald	3	3	3	3
Plantain	Agritonic	0	4	7	10

Notes. Sowing rates are shown in kg ha^{-1}. Table author's original work.

Pasture treatments were arranged in a randomised block design, with five replicates per treatment. In total, the research site was divided into 20 experimental plots of 800m^2 (40m × 20m) each and four adaptation paddocks of 1ha per paddock, representing each pasture treatment (Figure 3-1). Replicate plots and adaptation paddocks were individually fenced off by electric fence for grazing.

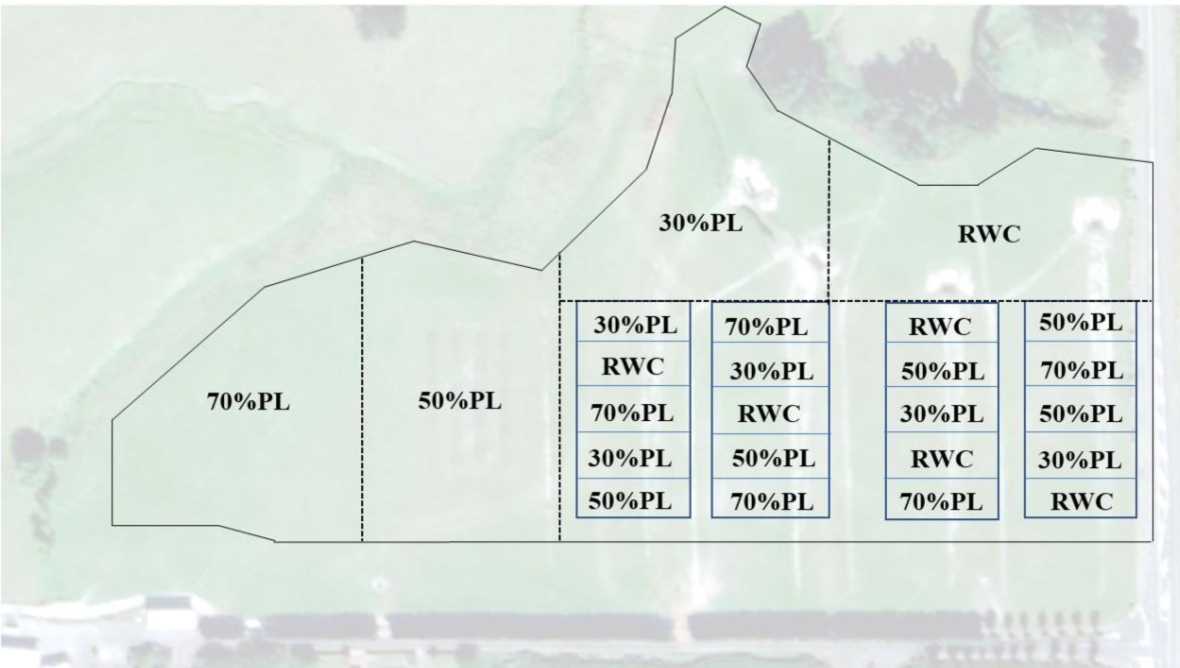


Figure 3-1 Experimental site at Massey University's Dairy No.4 Farm, Palmerston North, New Zealand.

Note. Figure author's original work.

Abbreviations. PL, *Plantago lanceolata*; RWC, ryegrass/white clover.

3.2.2 Grazing and Pasture Management

The experimental site was grazed by dairy cows on the 18th of June 2019 (12 weeks after sowing), nine times from August 2019 to July 2020, during the 2019/2020 grazing season (year 1), and eight times from September 2020 to May 2021, in the 2020/2021 grazing season (year 2). In the two grazing seasons, the grazing interval (3–5 weeks) was decided according to pre-grazing masses targeting a cover ranging from 3,600–3,800kg DM ha^{-1}. For each grazing, 80 dairy cows were selected from the farm herd and separated into four groups (n=20) to graze in the adaptation paddocks. Each group grazed in one treatment adaptation plot for 6 days, and then, the cows were moved to the experimental plots to graze for 2–3 days, with four cows in each replicate plot. Dairy cows grazed the plots after milking in the morning (at around 07:00hrs) and again in the afternoon (at around 14:30hrs).

The experimental plots received urea fertiliser at 50kg N ha^{-1} in October, December and February of the 2019/2020 grazing season. The pasture treatments were mown after the January 2020 grazing to control seed heads, favour leaf development and control weed invasion. In March 2020, plantain was re-sown in plantain plots to maintain the proportion of plantain, with the sowing rate at 3, 6 and 9kg ha^{-1} in the P30, P50 and P70 plots, respectively.

In the 2020/2021 season, urea fertiliser at 50kg N ha^{-1} was applied in September and November, and 35kg N ha^{-1} was applied later in April to all the pasture treatment blocks. The plots were mown, and all cut herbage was removed from the plots immediately after the October and December 2020 grazing rotations. In autumn 2021 (the 20th of April), the P70 treatment plots were sprayed with SeQuence (240g L^{-1} clethodim) at 0.5L ha^{-1} and Bonza (450g L^{-1} paraffinic oil) at 1L 100L^{-1} to remove ryegrass. One week later, these plots were re-drilled at a sowing rate of 10kg plantain ha^{-1}, 3kg ryegrass ha^{-1} and 3kg white clover ha^{-1}.

3.2.3 Sward Measurements

3.2.3.1 Botanical Composition.

The botanical composition of the pasture treatments was monitored throughout the two growing seasons. In the first growing season, the botanical composition (percent of total DM) was evaluated pre-grazing in the following periods: September 2019 (spring); December 2019 (summer); March 2020 (autumn); and July 2020 (winter). During the second growing season, botanical samples from the pasture treatments were collected in September 2020 (spring); October 2020 (early summer); November 2020 (summer); February 2021 (early autumn); and May 2021 (late autumn). In May 2021, due to the application of herbicide to remove ryegrass for the reestablishment of the P70 plots, botanical samples from these plots were not taken.

In all instances, botanical samples were taken the day before grazing by cutting herbage to ground level from 10 random spots along a diagonal line within each replicate plot. The herbage was bulked in one sample per plot, sub-sampled (~100g fresh weight per sub-sample) and manually separated into the following categories: ryegrass, white clover, plantain (leaf and reproductive stems), other grasses, weed and dead material. Each separated category was individually oven-dried at 70°C for 48h and weighed to determine botanical composition as the percentage of each component in the total DM sub-sample.

Pre-grazing herbage was collected at the same time as botanical composition samples, while post-grazing samples were taken immediately after grazing. Pre- and post-grazing herbage mass was determined by cutting three herbage samples within 0.1 m^2 quadrats to ground level at random sites within each plot. The herbage was bulked, the soil was removed and the sample was oven-dried at 70°C for 48h. The DM yield at each sampling date was determined as the pre-grazing herbage mass minus the post-grazing/post-mowing herbage from the prior grazing round (Hodgson, 1979). This value was multiplied by the proportion of each category to estimate component yield accumulated between grazing events.

3.2.3.2 Plant Density.

Following the sampling for botanical composition, plant densities (plants/m^2) of plantain in the P30, P50 and P70 pasture treatments were measured pre-grazing by counting the number of plants in a quadrat in each plot. Each plantain plant can have several shoots, but only the number of plants within the quadrat was counted towards an estimate of plant density. On the 11th of September 2019, before the first grazing took place, plantain density in the P30 and P50 plots was counted by locating 0.1m^2 and 0.25m^2 quadrats at 10 random sites in a plot to determine quadrat size and sample size needed to obtain a 95% confidence level. The plantain densities calculated from the 0.1m^2 quadrats were significantly higher than those from the 0.25m^2 quadrats in both pasture treatments evaluated (Table 3-2).

Table 3-2 Plant density of plantain sampled from two quadrat sizes in September 2021

Treatment	**Sample size**	**Plant density** (plants/m^2)		***P*-value**
		0.25m^2	**0.1m^2**	
30% Plantain (P30)	10	58	77	0.0060
50% Plantain (P50)	10	81	106	<0.0001

Notes. Two different quadrat sizes were sampled, 0.25m^2 and 0.10m^2 quadrats. Table author’s original work.

In both pasture treatments (P30 and P50), confidence intervals (CIs) and standard errors (SEs) remained relatively constant across more than five sampling times, regardless of quadrat size (Figure 3-2). This result indicated the number of plants from at least four random sites required to estimate plant density in an 800m^2 plot. Therefore, in this experiment, plant density was counted within 5 × 0.25m^2 quadrats along a diagonal line in each replicate plot.

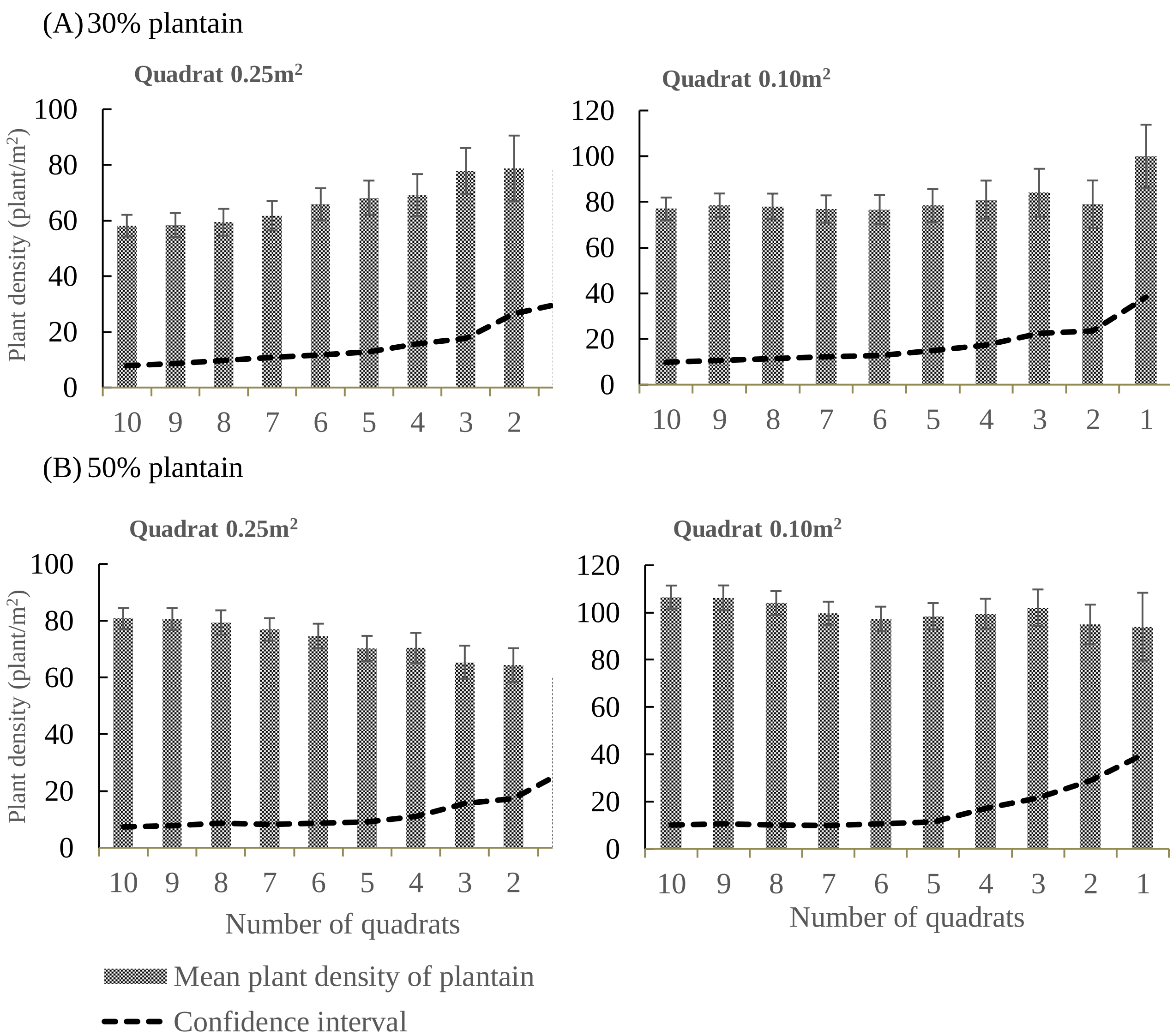


Figure 3-2 Plant density of plantain in 30% plantain (A) and 50% plantain (B) sampled from 0.10m^2 and 0.25m^2 quadrats with different sample sizes (1–10) in September 2019.

Notes. This evaluation occurred in September 2019. Bars represent the mean of plant density; error bars indicate standard errors (SEs) of the mean (SEMs) (n=1–10); and the dashed lines represent confidence intervals (CIs). Figure author's original work.

3.2.4 Climatic and Soil Moisture Conditions

Weather data for the duration of this study were taken from a nearby meteorological station, the climate database of the National Institute of Water and Atmospheric Research (NIWA)/AgResearch Grasslands weather station, Palmerston North (network no. 21963). This station is located at a latitude of –40.381, longitude 175.60915, 21m above sea level (ASL) and an estimated 1km from the experimental site. Daily soil water deficit (SWD) was calculated as evapotranspiration minus rainfall. Evapotranspiration was determined using the Penman–Monteith equation suggested by Allen et al. (1998). The cumulative SWD was determined as the sum of each water deficit event; water deficit was assumed as zero when soil was at field capacity and drainage events occurred.

3.2.5 Statistical Analysis

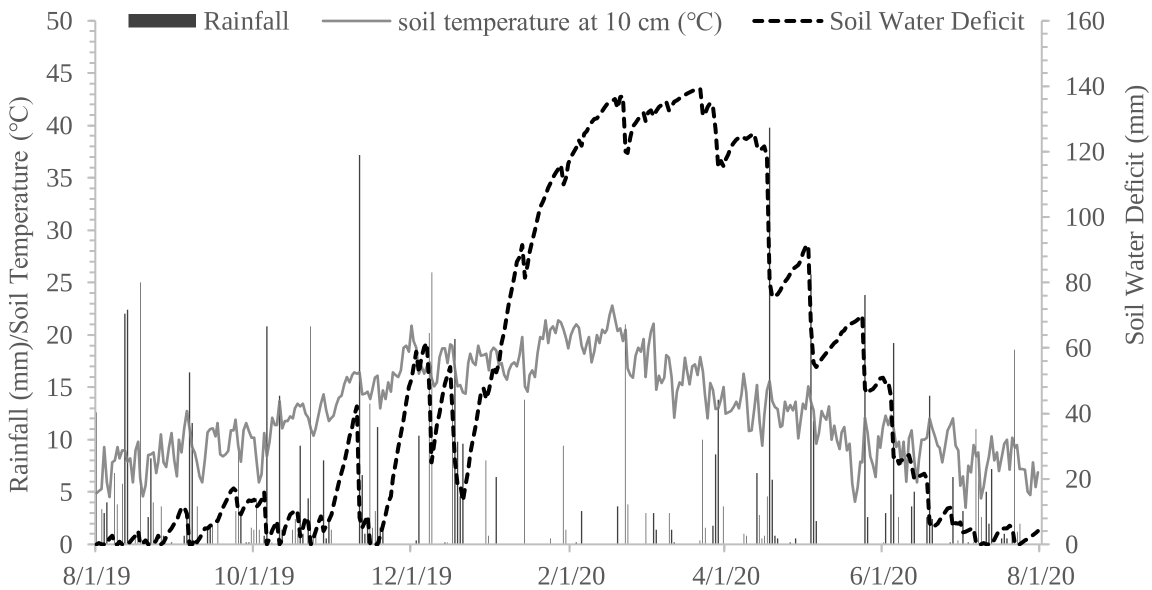
All data were analysed using SAS version 9.4 via mixed models for a completely randomised design. The two grazing seasons (years) were analysed separately. Botanical composition, component DM yield and plant density data for each season were analysed as repeated measures. The mixed model included the fixed effects of pasture treatments (RWC, P30, P50, P70), seasons and the two-way interactions between pasture treatment and season, and the random effect of plot. Statistical differences between treatments were analysed by one-way ANOVA. The significant difference of the means was declared at $p< 0.05$. The relationship between the proportion and plant density of plantain in the two growing years was determined by linear regression analysis using SAS. The RWC treatment was not included in the statistical analyses for plantain proportion, DM yield and plant density.

3.3 Results

3.3.1 Climatic Conditions and Soil Water Deficit

The daily rainfall, soil temperature and SWD during the 2019/2020 and the 2020/2021 growing seasons are presented in Figure 3-3. During the first growing season (August 2019 to July 2020), the total rainfall was 867.4mm, and it varied throughout the growing seasons, leading to SWD events. The SWD started in December 2019 and lasted for 7 months. Sustained low rainfall in summer resulted in a high level of moisture stress in summer, from January 2020 to April 2020. Soil water deficits were approximately zero during both winter–spring periods. Soil temperature at a 10cm depth was, on average, 12.9°C, ranged from 5°C–21°C.

(A) Year 1 (2019/2020)



(B) Year 2 (2020/2021)

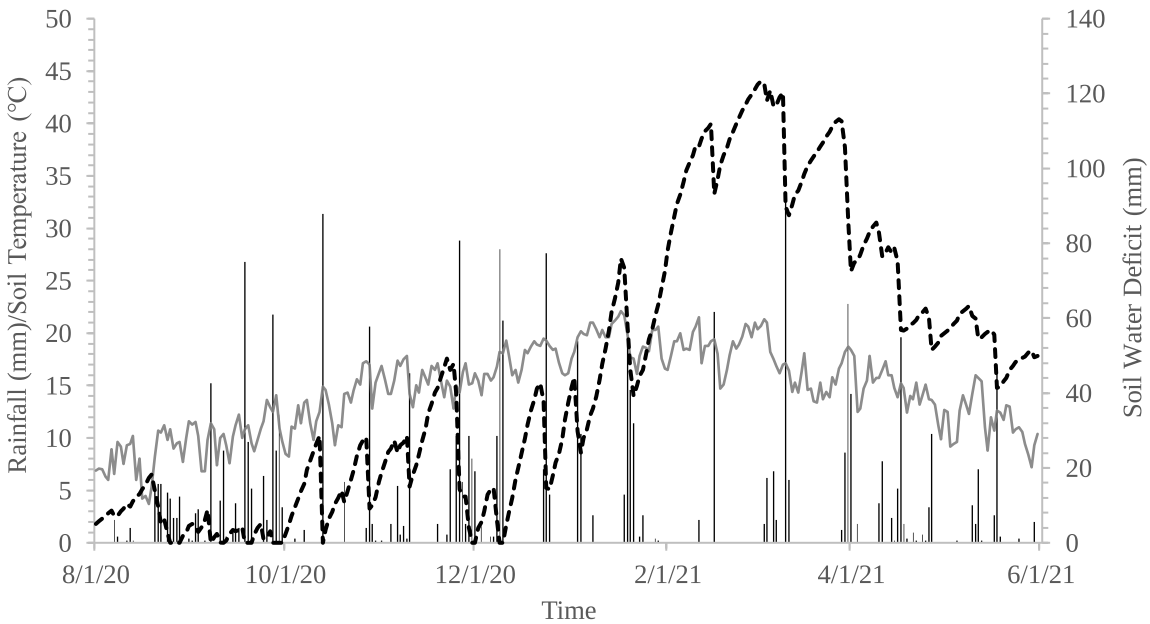


Figure 3-3 Daily rainfall (mm), soil moisture deficit (mm) and soil temperature at a 10cm depth (0°C) in the 2019/2020 grazing season (A) and the 2020/2021 grazing season (B).

Note. Figure author's original work.

The total rainfall in the second growing season (September 2020 to May 2021) was 756mm. The total rainfall during spring was lower than in the previous year, but the rainfall during summer in year 2 was higher than that of year 1. Consequently, SWDs in year 2 were higher in spring, but lower in summer than in year 1. Soil temperature in the second year did not include winter data and were in the range of 4°C–21°C.

3.3.2 Botanical Composition

Figure 3-4 shows the botanical composition of the four pasture treatments over two growing seasons. During the summer–autumn period of both growing seasons, the proportion of plantain obtained 40%–50%, while the proportion of ryegrass steadily reduced in all pasture treatments. In the summer of the second growing season, reproductive stems of plantain accounted for up to 20% botanical composition, which was significantly higher than in the first year in all plantain-mixed pasture treatments. The proportion of white clover in the second growing season was higher by around 10% than in the first season, regardless of pasture type. In both growing seasons, the proportion of dead material in all pasture types was highest in early autumn, ranging from 41%–52% in the first year, and from 22%–38% in the second year.

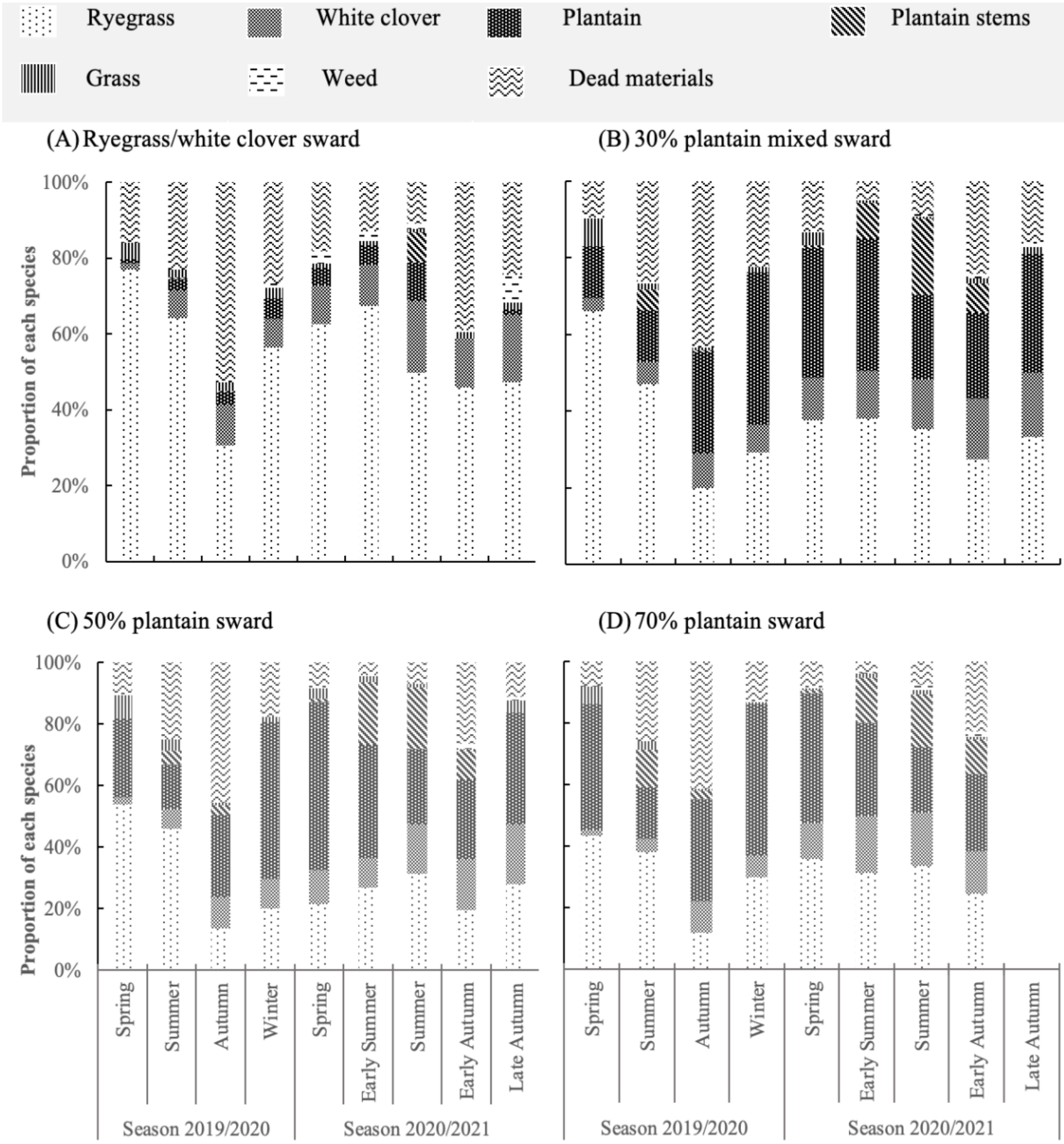


Figure 3-4 Botanical composition (percent of DM) in (A) RWC; (B) 30% plantain; (C) 50% plantain; and (D) 70% plantain mixed pasture at each grazing time in the 2019/2020 and the 2020/2021 growing seasons.

Note. Figure author's original work.

Abbreviations. DM, dry matter; RWC, ryegrass/white clover.

In the first growing season, the proportion of ryegrass in the RWC pasture treatment decreased from 77% in spring to 31% during late summer–autumn. The proportion of ryegrass recovered in winter to around 60% and remained that way until the following spring. In the second growing season, the ryegrass composition (45%) in late autumn was approximately 22% lower than at the beginning of the growing season. The proportion of white clover increased throughout the experiment and was highest in autumn, at 10.7% in year 1 and 19% in year 2. The unsown species (other grasses and weeds) content increased through the trial period but was consistently low, comprising up to a 7% of the DM in the second growing season. In both seasons, a small proportion of plantain and its stems appeared in the RWC treatment during the summer–autumn period (7% in year 1 and 16% in year 2).

In the P30 pasture treatment, the proportion of plantain increased over the first growing season from 14% in spring 2019 to 39% in winter 2020. In contrast, the percentage of ryegrass declined from 66% in spring to 29% at the end of the season. The proportion of white clover remained consistent at around 5%–10% in year 1, increasing to approximately 15% in year 2. At the beginning of the trial, the highest proportion of the sward was ryegrass, but at the end of year 1, plantain contributed a significantly greater proportion of total DM than other species in the pasture. The P30 pasture contained 20% productive plantain stems in the 2020/2021 season, which was 15% higher than in the 2019/2020 season.

During the first (2019/2020) season, in the P50 pasture treatment, the proportion of plantain (leaf) in the sward was 26% in spring, but reduced to 14% in summer, increasing in autumn and winter to 26% and 50%, respectively. The ryegrass content decreased by 40% in autumn, while the proportion of white clover increased over time to nearly 10% at the end of year 1. In the second grazing season, plantain content decreased to 25% during the summer/early autumn, along with an increased plantain stem content. In late autumn, the proportion of plantain, ryegrass and white clover increased to 36%, 27% and 19%, respectively.

In the first growing season, the P70 pasture treatment had 41% plantain in spring, which then decreased to 17% in summer, after which it recovered and reached 49% in winter. The proportion of ryegrass was similar during the spring–summer period, but declined to only 12% in autumn. In the second season, the proportion of plantain decreased over the season, ranging from 25%–30% of plantain in the sward. The percentage of ryegrass and white clover remained stable at around 33% and 17%, respectively, during summer, but fell to 24% and 13%, respectively, in early autumn.

The proportions of ryegrass between pasture treatments were significantly different, while all pasture treatments contained similar white clover proportion throughout two growing seasons (Table 3-3). Pasture treatments had significant effects on plantain proportion in spring of both growing seasons. The proportion of plantain in P70 treatment was significantly higher than in P50 and P30 in spring of the first growing season, whilst in spring of the second growing season, P50 treatment had a 13–20% higher plantain proportion than that of P70 and P30 treatments. Plantain stems presented in the sward from late spring to early autumn and accounted for a similar proportion across the plantain mixed pasture treatments except early summer of the second growing season. The proportion of plantain stems in P50 treatment was significantly higher by 6–10% than in P70 and P30 treatments. RWC treatments had significant higher proportion of unsown species than plantain mixed pasture treatments in autumn of the first growing season and during summer and autumn period of the second growing season. Additionally, dead material content in RWC pasture was significantly higher than in plantain containing pastures over the two growing seasons except spring of year 1 and summer of both growing years.

Table 3-3 Statistical significance for seasonal botanical composition of pasture species between four pasture treatments: RWC, P30, P50, P70 in the 2019/2020 growing season and the 2020/2021 growing season

Growing season	Season	Ryegrass	White clover	Plantain	Plantain stems	Other grasses	Weed	Dead materials
2019/ 2020	Spring	**	n.s.	**	-	n.s.	n.s.	n.s.
	Summer	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Autumn	**	n.s.	n.s.	n.s.	**	-	*
	Winter	***	n.s.	n.s.	-	n.s.	n.s.	**
2020/ 2021	Spring	***	n.s.	*	n.s.	n.s.	n.s.	***
	Early summer	***	n.s.	n.s.	*	n.s.	***	***
	Summer	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Early autumn	**	n.s.	n.s.	n.s.	n.s.	n.s.	*
	Autumn	**	n.s.	n.s.	-	n.s.	**	**

Note: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n.s., non-significant
Note. Figure author's original work.

The seasonal accumulated DM yield of individual pasture species over two growing seasons are presented in Table 3-4. During the first growing season, ryegrass DM production was reduced, with increasing proportions of plantain ($p< 0.05$) evident in spring and summer. Ryegrass DM in the P50 and P70 treatments were similar (93 kg DM ha^{-1}) in autumn, but lower than in RWC and P30 pasture treatments. In winter, the component yield of ryegrass was similar ($p> 0.05$) in all four pasture types. The contribution of ryegrass to DM production in autumn was significantly lower than in the other seasons of year 1. In year 2, during the spring to early autumn period, the DM yield of ryegrass in the P50 treatment was significantly lower ($p< 0.01$) compared to the other pasture treatments (Table 3-4A, year 2). The contribution of ryegrass to DM yield decreased significantly ($p < 0.05$) during the summer to early autumn period. In late autumn, there were no differences ($p > 0.05$) between the pasture treatments in terms of ryegrass DM production.

There was no effect ($p > 0.05$) on the component yield of white clover by pasture treatments in both years and pasture treatment by season interaction in year 1 (Table 3-4B). White clover DM yields in summer and late autumn were significantly higher ($p < 0.05$) than for other periods/seasons. The contribution of white clover to DM yield differed significantly across seasons in both year 1 and 2.

Table 3-4 Component DM yields across two growing seasons (years 1 and 2) for the four pasture treatments: ryegrass and white clover (RWC), 30% plantain mixed with RWC (P30), 50% plantain mixed with RWC (P50), and 70% plantain mixed with RWC (P70).

(A) Ryegrass

	Ryegrass yield (kg DM ha^{-1})				***p*-value**		
	RWC	**P30**	**P50**	**P70**	**Treatment**	**Season**	**Treatment × season**
Year 1 (September 2019–July 2020)							
Spring	2,937 (246)	2,640 (296)	1,924 (291)	1,705 (228)	<0.0001	<0.0001	0.0023
Summer	1,846 (168)	1,265 (87)	1,274 (104)	1,040 (76)			
Autumn	229 (59)	168 (42)	93 (20)	94 (16)			
Winter	350 (150)	222 (34)	190 (46)	357 (76)			
Year 2 (August 2020–May 2021)							
Spring	809 (170)	503 (44)	308 (37)	510 (50)	<0.0001	<0.0001	0.0020
Early summer	1,705 (240)	882 (57)	678 (48)	926 (202)			
Summer	1,193 (157)	789 (81)	664 (87)	765 (100)			
Early autumn	579 (110)	384 (43)	250 (55)	301 (44)			
Late autumn	582 (97)	489 (46)	498 (30)	-			

(B) White clover

	White clover yield (kg DM ha^{-1})				***p*-value**		
	RWC	**P30**	**P50**	**P70**	**Treatment**	**Season**	**Treatment × season**
Year 1 (September 2019–July 2020)							
Spring	60 (17)	132 (34)	78 (16)	73 (20)	0.6300	0.0100	0.8200

Summer	200 (44)	166 (51)	178 (44)	119 (38)			
Autumn	92 (31)	71 (24)	103 (42)	86 (26)			
Winter	65 (26)	69 (25)	141 (70)	75 (16)			
Year 2 (August 2020–May 2021)							
Spring	112 (10)	166 (65)	171 (54)	170 (21)			
Early summer	270 (35)	294 (52)	252 (53)	546 (143)			
Summer	448 (83)	289 (16)	352 (38)	394 (33)	0.3800	< 0.0001	0.0054
Early autumn	145 (27)	256 (74)	219 (35)	172 (15)			
Late autumn	211 (35)	244 (12)	355 (36)	-			

(C) Plantain

	Plantain yield (kg DM ha^{-1})				***p*-value**		
	RWC	**P30**	**P50**	**P70**	**Treatment**	**Season**	**Treatment × season**
Year 1 (September 2019–July 2020)							
Spring	-	534 (160)	882 (100)	1,639 (316)			
Summer	-	520 (119)	527 (82)	838 (218)	0.0009	< 0.0001	0.0227
Autumn	-	277 (73)	246 (74)	305 (55)			
Winter	-	344 (89)	530 (171)	558 (78)			
Year 2 (August 2020–May 2021)							
Spring	-	481 (82)	811 (63)	624 (88)			
Early summer	-	1,036 (100)	1,460 (122)	1,267 (51)			
Summer	-	929 (90)	957 (127)	867 (60)	0.0070	<0.0001	0.0758
Early autumn	-	469 (75)	468 (76)	461 (84)			
Late autumn	-	458 (59)	659 (78)	-			

(D) Dead material

	Dead material (kg DM ha^{-1})				***p*-value**		
	RWC	**P30**	**P50**	**P70**	**Treatment**	**Season**	**Treatment × season**
			Year 1 (September 2019–July 2020)				
Spring	590 (33)	382 (52)	372 (68)	311 (64)			
Summer	663 (135)	712 (47)	692 (47)	712 (27)	0.5000	<0.0001	0.6900
Autumn	397 (93)	403 (121)	366 (127)	342 (52)			
Winter	176 (84)	174 (36)	166 (48)	151 (25)			
			Year 2 (August 2020–May 2021)				
Spring	232 (43)	181 (28)	114 (12)	117 (15)			
Early summer	332 (55)	114 (24)	104 (13)	114 (27)			
Summer	249 (20)	194 (52)	144 (25)	175 (35)	<0.0001	<0.0001	0.7000
Early autumn	483 (89)	364 (43)	327 (9)	298 (73)			
Late autumn	287 (31)	237 (23)	197 (23)	-			

Notes. A) ryegrass, (B) white clover, (C) plantain, and (D) dead material in RWC, 30% plantain mixed pasture (P30), 50% plantain mixed pasture (P50), and 70% plantain mixed pasture (P70). Numbers in parentheses show SEM values for each treatment (n=5). Table author's original work.

Abbreviations. DM, dry matter; RWC, ryegrass/white clover; SEM, standard error of the mean.

Pasture treatments and season had an effect ($p < 0.01$) on DM yield of plantain in the pasture treatments including plantain (Table 3-4C). In year 1, increasing the plantain proportion in the sward significantly increased the DM yield from plantain in the pasture, both in spring ($p < 0.01$) and summer ($p < 0.05$). During autumn, there was no difference in DM production from plantain between the pasture treatments ranging from 246 to 305 kg DM ha^{-1}. However, in winter, the DM yield of plantain in the P50 and P70 treatments (530–558 kg DM ha^{-1}) was greater ($p < 0.05$) than the P30 pasture treatment (344 kg DM ha^{-1}). The DM yield of plantain in spring was from 40%–70% higher ($p < 0.05$) than in the other seasons. During the second year, component dry matter (DM) yield of plantain reached a peak of from 867 to 1,460 kg DM ha^{-1} in summer. In spring and early summer, the DM production of plantain growing in the P50 pasture treatment area was significantly greater than the DM production evident in the P30 and P70 treatments in spring, early summer and summer. The contribution of plantain to DM yield was highest in the early summer, associated with the presence of reproductive stems.

Dead material content in pasture treatments was affected by seasons in both years (Table 3-4D). The DM yield of all pasture treatments contained the highest amount of dead material during summer to early autumn, relative to other periods. In early autumn of year 2, including plantain in pasture significantly reduced the proportion of dead material compared to RWC pastures ($p<0.05$, a 30% reduction). Visually, standard RWC swards evidenced higher dead material yield than the three plantain mixed-pasture treatments (Figure 3-5).

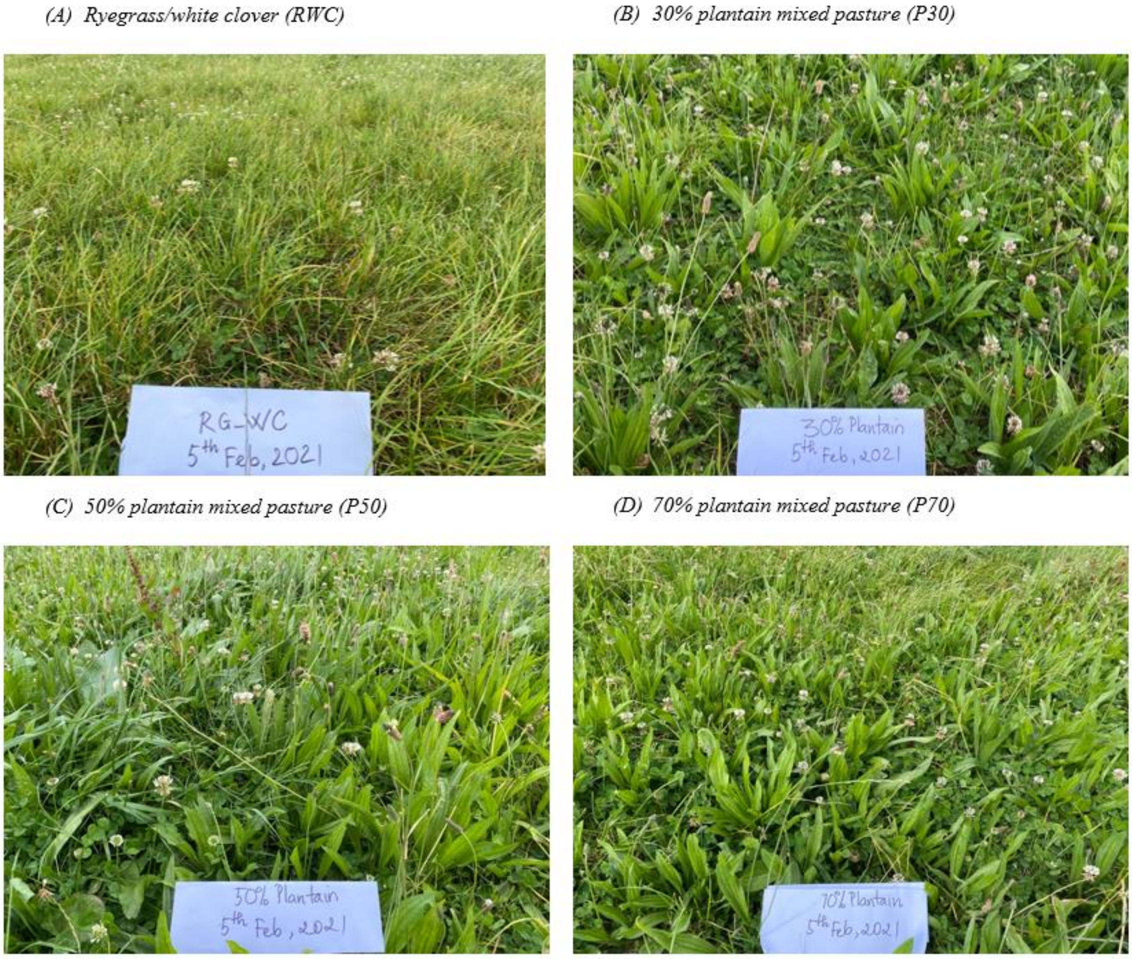


Figure 3-5 Ryegrass/white clover sward (A); 30% plantain mixed pasture (B); 50% plantain mixed pasture (C); and 70% plantain mixed pasture (D) in summer of the second growing season (2020/2021).

Note. Photos taken by author.

3.3.3 Plant Density

Plant density of plantain in pasture treatments incorporating plantain is presented in Figure 3-6. When first measured (spring 2019), plantain density in the P70 pasture treatment was, on average, 140 plants per square metre compared to 86 plants per square metre and 64 plants per square metre in the P50 and P30 pasture treatments, respectively. Through the first growing season, the plantain population increased from spring to autumn. In winter, plantain density declined by 20% in the P70 treatment, while plant density increased 10% in the P50 treatment and remained stable, with a plantain population of 104 plants per square metre, in the P30 treatment. As a result, the plantain population in the P70 treatment was higher ($p<0.01$) than in the P50 treatment area until autumn 2020, but from winter of the first season until the end of the second season, the plantain densities in the P70 and P50 treatments were similar ($p>0.05$). At the end of the first grazing season (winter 2020), there was approximately a 40% increase in plantain populations in the P30 and P50 treatments compared to initial plant densities (spring 2019), while in the P70 treatment, the plantain density increased by 7%.

In year 2, the plant densities of plantain in the P70 and P50 pasture treatments were higher than in the P30 treatment. The plantain density tended to decline ($p=0.07$) in all pasture types, with plant loss ranging from 52%–62% of the plant density evidenced at the beginning of the season (spring 2020). In late autumn, plantain populations in the three pasture treatments were similar ($p>0.05$). There was no interaction between pasture treatment and season with regards to plant density of plantain during the second growing season ($p=0.63$).

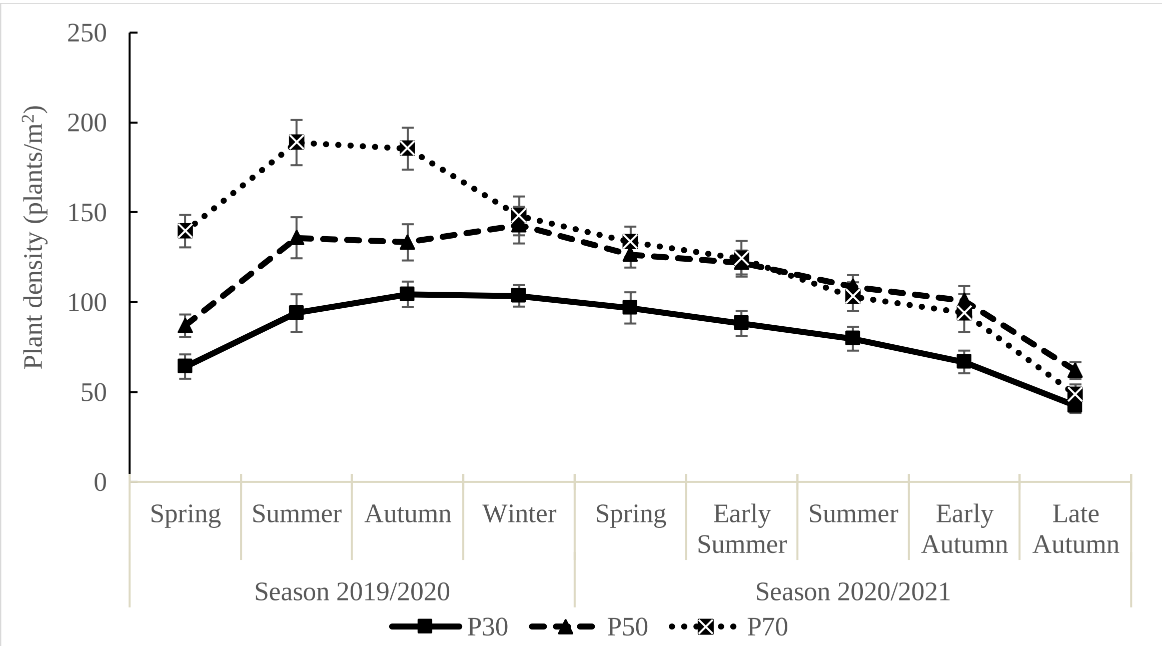


Figure 3-6 Plant density of plantain in 30% plantain mixed pasture (P30), 50% plantain mixed pasture (P50) and 70% plantain mixed pasture (P70) throughout the first growing season (2019/2020) and the second growing season (2020/2021).

Notes. Plant density is measured as plants per square metre (m^2). Error bars represent plant density standard errors for each treatment (n=5). Figure author's original work.

Figure 3-7 shows the relationship between plant density and the proportion of plantain in pasture across two growing seasons (years 1 and 2). When plantain density increased, the proportion of plantain in pasture increased, but the relationship was weak. The relationship in year 2 was stronger than in year 1. The proportion of plantain in pasture in year 2 was higher than in year 1, with the same plant density.

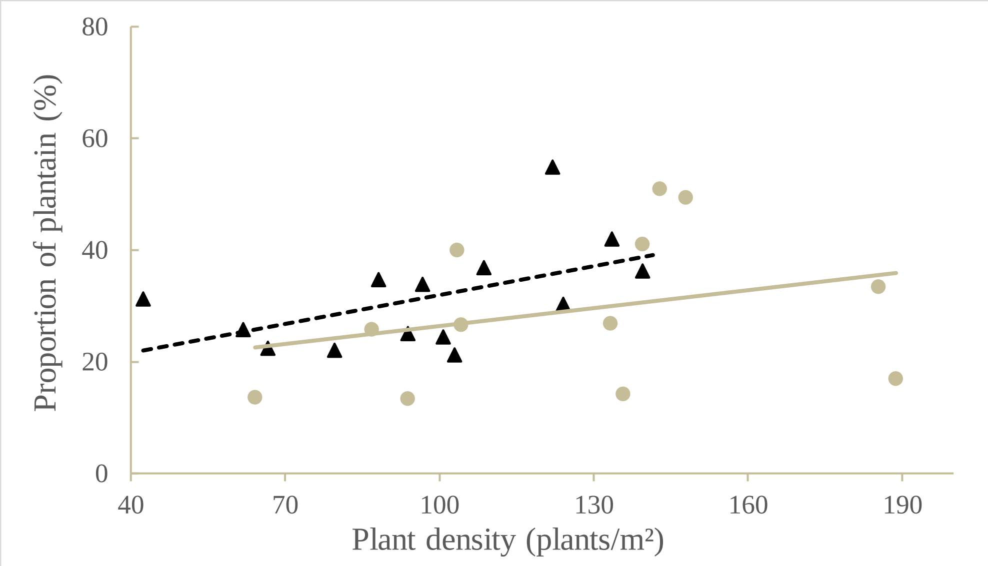


Figure 3-7 The relationship between plant density (for plantain) and the proportion of plantain in pastures during the first growing season

Notes. For the first growing season, represented by the solid, taupe-coloured line and circles, y= 0.11x + 15.68, R^2 = 0.29; for the second growing season, represented by the dashed, black line and triangles, y= 0.17x + 14.67, R^2 = 0.51. Figure author's original work.

3.4 Discussion

The evaluation of botanical composition in pasture mixtures can detect the effects of season, management and pasture types on total production and persistence of swards across growing seasons (Whalley & Hardy, 2000). Seasonal changes in plantain proportion and plant density in mixed pasture are the major factors influencing the DM contribution and persistence of plantain in the sward. In the current field experiment, the proportion of plantain in all plantain mixed-pasture treatments reached 50% in autumn across the two growing seasons. Plant loss of plantain in the mixed-pasture treatments occurred in the second growing season.

During the first growing season, the higher sowing rate of plantain resulted in an increase in the proportion of plantain in the P30, P50 and P70 mixed pastures. Plantain contributed to up to 50% of the DM in the P50 and P70 pasture treatments during late summer and autumn, the dry period in both growing seasons. Similarly, Lambert (1963) observed an increase in the proportion of plantain in mixed pasture under water-stress conditions. In the current work, the increase in plantain proportion facilitated improved summer DM yields in mixed pasture under dry conditions when ryegrass growth was slow. The incorporation of plantain into the mixed pastures increased summer DM yield by about 3-400kg DM compared to the RWC pasture treatment in the second growing year. These results concur with other published studies that showed how the incorporation of plantain in swards can fill NZ's summer feed gap (Navarrete, 2015; Moorhead & Piggot, 2009; Reed et al., 2008). After the first growing season, in spring of the following year, the P50 pasture treatment contained higher the plantain proportion than that of the P70 treatment. The proportion of plantain across pasture treatments were similar throughout the year 2 but in early summer, with the presence of reproductive stems, P50 treatment contained a significant higher plantain content than that of P30 and P70 treatments. A decrease in DM contribution of plantain in plantain mixed pasture treatments is probably associated with plant loss observed at the beginning of the second growing seasons. This is in line with previous findings that stability of herbage production largely depends on plant density (Neal et al., 2009; Nie et al., 2008).

The highest proportion of plantain leaf evident across two seasons was 54% in the P50 pasture in the spring of year 2. Plantain leaf content reduced in summer with increases in the presence of plantain stems in the sward. This result suggests that plantain would likely not contribute more than 50% to the botanical composition in mixed pastures, even when sown at a high rate. Stewart (1996) indicated that plantain naturally represents up to 20% of a productive pasture and typically acts as minor forage in swards. Bryant et al. (2019) also observed that plantain contributed less than 30% of DM in established pastures. Similarly, in pure swards and in herb and legume mixed pastures, the proportion of plantain leaf reduces over time, with higher plantain stem content reported in the second growing season (Lee et al., 2015; Navarrete, 2015). In this study, plantain produced more reproductive stems in year 2 than in year 1, with the proportion in the range of 9% to 20% of total DM in year 2. The presence of plantain in RWC plots in the second growing season was probably due to seedling recruitment from plantain reproductive stems produced during the previous season, which was also observed by Ayala et al. (2011) and Navarrete (2015).

Plant density and plantain loss in mixed pasture are the major factors predicting pasture persistence and yield stability (Tozer et al., 2011). Pasture's poor persistence can be associated with a low rate of germination from the seed sown, a low survival rate under grazing conditions and the impact of weed invasion (Parsons et al., 2011; Tozer et al., 2011). In the present field trial, the ability of plantain to establish and germinate well in pasture mixtures was demonstrated by an initial plant density ranging from 64–139 plants/m^2. Plantain density increased by around 35% in autumn, at the end of the first growing season, but started to decline at the beginning of year 2. This reduction could be, in part, due to higher competition from ryegrass and white clover in spring, because those plants have higher cool-temperature tolerance than plantain (Stewart et al., 2014), and the susceptibility of plantain to winter grazing (Skinner, 2005). Plant loss of plantain in the P30, P50 and P70 pasture treatments occurred throughout the second growing season, similar to the findings of Lee et al. (2015) and Navarrete (2015). Plantain is generally regarded as a short-lived pasture species with persistence ranging from 2–4 years, depending on grazing management (Stewart et al., 2014). By the end of the second growing season (May 2021), plantain density in the P50 treatment was higher than in the P30 and P70 pasture treatments. The similar trend in seasonal changes between plant density, proportion and DM contribution of plantain observed in this study is in supporting with the earlier findings about the close relationship of DM yield and plant density of pasture species (Navarrete, 2015; Neal et al., 2009). Seedling recruitment of plantain by reseeding from reproductive stems did not seem to increase plant density in mixed pasture in year 2, which might have been due to the low proportion of plantain stems in the early summer of the year 1, or insufficient germination sites within the dense ryegrass and white clover in the sward in year 2. The higher reproductive stem content in year 2 would have provided plantain seed that could potentially reseed and provide additional plants in year 3 (Nie et al., 2008). Management techniques that encourage establishment of plantain plants within existing pastures and maintain plantain at the optimum proportion in mixed pastures require further exploration.

The decrease in plant density and contribution to DM production of plantain over time could be due, in part, to plantain size. Navarrete (2015) observed that plantains are at their largest at approximately two shoots/plant in the autumn of the second growing year, and then, size reduces. Plantain tends not to develop further after reaching a certain size, whilst the grazing tolerance and tillering ability of ryegrass allows this grass to increase its size and dominate grasslands over time (Neuteboom & Lantinga, 1989). Therefore, plantain is unlikely to generate the majority of DM yield in mixed pastures.

Additionally, plantain requires enough time to recover the carbohydrate storage of its roots and for regrowth after grazing (Kemp et al., 2010). This herb has moderate tolerance to treading damage (Stewart, 1996); consequently, under rotational grazing with dairy cows, which are heavy animals, plant loss in plantain is unavoidable (Navarrete, 2015). Furthermore, in the current work, it appears that an increase in unsown species content in the second season may contribute to reduction of plantain density in pasture. The higher proportion of volunteer grass species and weed can lead to poor persistence of pasture (Brouwer et al., 1994). Overall, plantain proportions, including leaf and stems, were stable throughout the growing seasons. The observed yield stability of plantain supports previous findings that plantain is more tolerant of seasonal variation and grazing by cows than chicory and lucerne (Navarrete, 2015; Skinner & Gustine, 2002; Woodward et al., 2013).

The similar densities observed in three plantain mixed pastures in autumn 2021 were probably due, in part, to that the P30 and P50 pastures were broadcast with plantain seed at the end of year 1. The relationship between plantain density and botanical composition in pasture mixtures in year 2 was stronger than in year 1. A higher plantain proportion in year 2, but with the same plant density, can be attributed to increases in shoots per plant and plant size during year 2 (Navarrete, 2015). This result suggests that plantain might persist longer in pasture mixtures and maintain high herbage production when its seed is re-sown after every growing season, and when grazing management encourages shoots per plant to increase (Navarrete, 2015). According to the relationship of plantain composition to plant density, it is likely that at least 130 plant m^{-2} in the first growing season, and 90 plant m^{-2} in the second growing season, is needed to achieve more than 30% plantain composition in mixed pastures. In the current study, plantain maintained its 30%, or greater, contribution in competition with ryegrass and white clover.

In the current work, botanical composition of ryegrass and white clover were highly responsive to season, while plantain composition, unsown species and weed content were more affected by pasture treatments. At the beginning of the experiment in the spring of year 1, the botanical composition of pasture treatments reflected the sowing rates of the species. As expected, the RWC pasture was dominated by ryegrass, compared to the mixed pastures that included plantain. Ryegrass had similar seasonal trends over the two growing seasons. The proportion of ryegrass was relatively well maintained during spring and early summer. Drought conditions at the experimental site occurred during the period from December to April. Therefore, the lowest proportion of ryegrass, with the highest amount of dead material, was observed in early autumn. Reductions in the proportion of ryegrass under water deficit stress is well reported (Anderson, 2015; Kemp et al., 2010; Woodward et al., 2013). Additionally, increased competition with plantain, a summer-active species, can reduce the amount of ryegrass in mixed-pasture treatments (Woodward et al., 2013). Ryegrass in the current study recovered in winter and comprised up to 67% of RWC pasture, and up to 38% of plantain mixed pastures. This recovery by ryegrass was probably due to its greater cool-temperature tolerance and higher competitiveness (Stewart et al., 2014).

White clover content in P30, P50 and P70 pasture treatments were similar to that of the RWC pasture throughout two growing seasons. This was contrary to the findings of Woodward et al. (2013), who observed a lower white clover content in plantain mixed pasture than in RWC swards. White clover has been widely adopted in dairy farming systems due to the benefits of biological N_2 fixation and forage quality (Caradus et al., 1995). The similar proportions of white clover in pasture mixtures as compared to standard pasture can benefit forage quality and decrease the amount of fertiliser required. In the current work, white clover botanical composition in all pasture types in year 2 was greater than in year 1. A similar increase in the proportion of white clover in the second year has been observed in herb–clover mixed pastures (Anderson, 2015; Navarrete, 2015; Rodríguez-Gelós, 2020).

The proportions of unsown species and dead material in mixed pasture containing plantain was lower than in RWC pasture in both years. Reductions in unsown species content have been linked to increased species diversity (Navarrete, 2015; Tracy & Sanderson, 2004; Woodward et al., 2013). In the present research, dead material content in early autumn was the result of ryegrass' low survival rate in summer; therefore, including summer-active species such as plantain in the pasture mix could reduce dead material content. Plantain usually stays green over summer and rarely suffers a high death rate during droughts (Mook et al., 1989; Nie et al., 2008). This means that plantain mixed pasture can provide higher quality forage than RWC during summer and autumn.

3.5 Conclusion

Clearly, variations in the botanical composition of RWC and plantain mixed pastures are influenced by seasonal variations and plantain treatments. In the present field experiments, ryegrass content was reduced during autumn in both year 1 and 2, regardless of plantain treatment, whilst the proportion of white clover increased over time. Plantain mixed pasture reduced the proportion of unsown species and dead material relative to standard RWC swards. The P30 and P50 mixed swards maintained more stable plantain compositions and densities than the P70 mixed pasture over two growing seasons when plantain seed was broadcast at the end of the first growing season. Plantain is unlikely to dominate the pasture mixture as it is likely to decrease in proportion after the first growing season, even when it accounted for 70% of initial sowing rate. However, at rates of 30% and 50% of the sward, plantain mixed pasture can relatively well persist. Broadcasting plantain seed after every growing season is needed to ensure the ongoing viability of plantain in mixed pastures. Therefore, sowing seed mixtures with 30%–50% of plantain, along with the broadcasting of fresh seed at the end of each growing season, could maintain a sufficient proportion of plantain in pasture mixes to extend the benefits of plantain into a third growing season.

3.6 References

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4 The Effects of Plantain on Nitrous Oxide Emissions from Urine Patches

4.1 Introduction

Grazed dairy pasture systems are the biggest source of N_2O emissions in New Zealand (Saggar et al., 2007), with the majority of these emissions being associated with the N excreted by dairy cattle in urine. As discussed in Chapter 2, this high urinary N input is far above the N requirements of pasture plants and rhizosphere biota, and is prone to being lost to the environment via leaching or gaseous losses of N.

In response to the growing environmental concerns, a great deal of scientific effort has been devoted to developing strategies for reducing N losses, such as nitrate leaching and gaseous emissions of NH_3 and N_2O, from grazed pastoral systems. There is increasing evidence that the introduction of plantain in pastures and the animal diet can reduce N losses, including N_2O emissions from urine patches, as described in Chapter 2. The N_2O emitted from plantain swards were found to be 28% to 74% lower than those from RWC swards (Luo et al., 2018; Rodríguez-Gelós, 2020). Increasing the proportion of plantain in the pasture mixture and/or in the diet has resulted in greater reductions in N_2O emissions (Simon et al., 2019).

Plantain plays a dual role in reducing N_2O emissions in dairy grazed pastures first by reducing the N content in urine from cows consuming plantain (Box et al., 2017; Luo et al., 2018; Minnée et al., 2020; Simon et al., 2019); and second by biologically inhibiting the nitrification of urine-N through producing of secondary compounds (Carlson et al., 2020; de Klein et al., 2020; Gardiner et al., 2018). The effects of plantain and resultant N_2O emissions can vary between the seasons and pasture management conditions. For example, Luo et al. (2018) observed plantain-soil interactions and reductions in N_2O emissions from plantain pasture only in winter but not in summer, whilst Podolyan et al. (2020) reported plantain pasture did not affect N_2O emissions in winter which he proposed was due to low concentration of active secondary metabolites under low temperature conditions. Most of the earlier studies were conducted in winter and spring when nitrate is at high risk of leaching. Limited information exists on the effects of urine of cows fed plantain and plantain pastures on N_2O emissions during summer and autumn when the ryegrass/white clover growth slows down, and plantain dominates the pasture and the feeding diet.

The present field study quantified N_2O emissions and emission factors (EF_s) from urine of dairy cows grazing three pasture types during summer and autumn: ryegrass/white clover mixed pasture with no (0%) plantain (RWC), and 30% plantain (P30) and 50% plantain (P50). The objectives of this study were to (i) evaluate the effects of different proportions of plantain in the mixed pasture-diet on reduction in N_2O emissions over summer and autumn, and (ii) to determine the optimum proportion of plantain in the mixed pasture-diet required to maximise the reduction of N_2O emissions. It was hypothesised that N_2O emissions may decline as the proportion of plantain in the sward increases; and pastures containing plantain will reduce N_2O emissions via effects on the N-loading rate and the N-cycling in the soil and soil microclimate.

4.2 Materials and Methods

4.2.1 Trial Description

4.2.1.1 Experimental Site and Treatments.

This study of N_2O emissions and emission factor values from plantain pastures was undertaken at Massey University's No 4 Dairy Farm in the established experimental site described in Chapter 3. According to the results of the experiment on botanical composition of plantain mixed pasture presented in Chapter 3, the proportion of plantain in the P70 and p50 plots were similar in the 2020/2021 season. Therefore, the 70% plantain plots were excluded in this experiment. Gas measurement chambers were set up in selected pasture plots from summer to late autumn 2021 (the 15th of February to the 1st of June 2021).

Ryegrass and white clover mixed with different proportions of plantain: 0% (RWC), 30% (P30) and 50% (P50) in the swards were selected as three pasture treatments. Sowing rates for the species in the pasture treatments are presented in Chapter 3. Pasture treatments were arranged in a randomised block design, with five replicates per treatment. In total, there were fifteen experimental plots of $800m^2$ each (40m × 20m) presented as Figure 3-1 without the 70% plantain mixed pasture plots. Urine was collected from cows grazing pasture treatments: RWC, P30 and P50, resulting in three urine treatments: URWC, U30 and U50.

In each replicate plot, a representative area (2m × 5m) was fenced off and excluded from grazing on the 10th of December 2020, nine weeks before the experiment started, to avoid interference from fresh dung and urine inputs, and to reduce spatial variability from the previous N fertiliser inputs (and uneven deposition of dung and urine). The designated areas were cut to 5cm above ground level, and the herbage was removed before the start of gas emission measurements.

On the 15th February 2021, urine (URWC, U30 and U50) treatments were applied to the corresponding pasture treatments. The RWC and P50 plots received urine collected from both the cows grazing P50 and RWC plots. A control treatment, which did not receive any urine (NoU), was also included. In total, eight combined treatments consisted of:

ryegrass/white clover treated with ryegrass/white clover urine (RWC+URWC), with 50% plantain urine (RWC+U50) and without urine (RWC+NoU); 30% plantain pasture treated with 30% plantain urine (P30+U30) and without urine (P30+NoU); 50% plantain pasture treated with 50% plantain (P50+U50), with ryegrass/white clover urine (P50+UWRC), and without urine (P50+NoU). The treatments were arranged in randomised pasture plots with five replicates.

Two modified polyvinyl chloride (PVC) gas sampling chambers (240mm diameter × 150mm deep) were set up within the fenced off 2m × 5m area in each of the P30 plots; three chambers were set up in each of the RWC plots and P50 plots (Figure 4-1). Each chamber had a removable lid with one port connected to a 3-way stopcock through a tubing (3.2 mm diameter; 100 mm length) for sampling N_2O emissions. Gas chambers were inserted 50–100mm into the soil 1 week before application of urine. One chamber was allocated to each treatment category, with five replicates per treatment. The gas chambers were installed in chosen areas representing the proportion of plantain treatments to minimise the variation between replicate plots. There was an area of 0.25m^2 (0.5m × 0.5m) for soil moisture, mineral-N (ammonium and nitrate) measurements adjacent to each gas chamber.

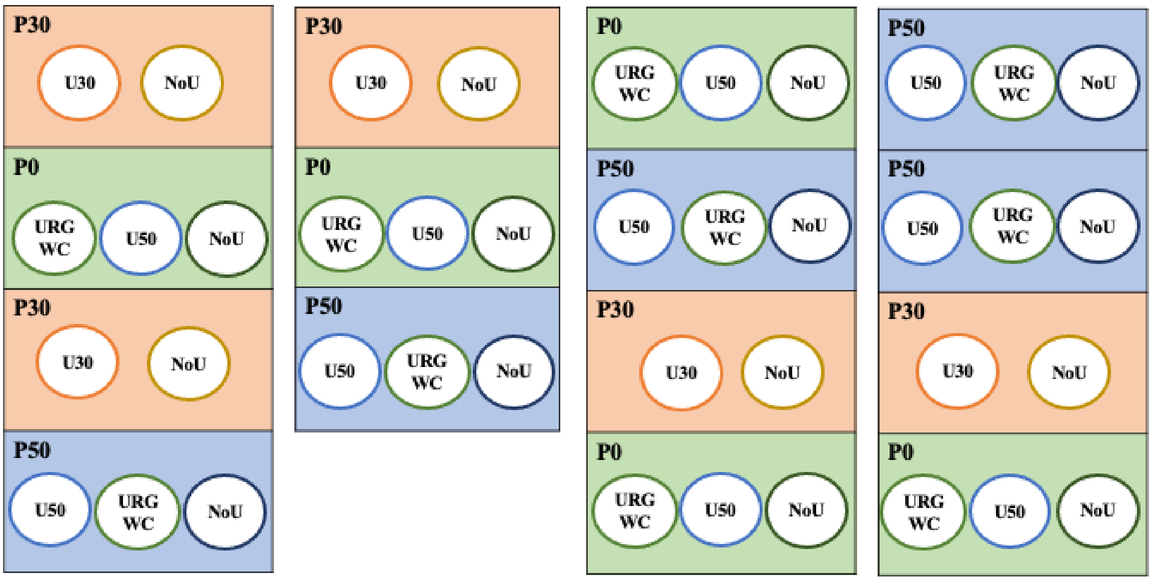


Figure 4-1 The layout of plots showing plantain and urine treatments in the field experiment.

Notes. RWC, RWC pasture; P30, 30% plantain mixed-species pasture; P50, 50% plantain mixed-species pasture. NoU, control treatment receiving no urine; URWC, urine collected from cows grazing RWC; U30, urine collected from cows grazing mixed-species pasture containing 30% plantain; U50, urine collected from cows grazing mixed-species pasture containing 50% plantain. Figure author's original work.

Abbreviation. RWC, ryegrass/white clover.

4.2.1.2 Cow Urine Collection, Analysis and Application.

Urine was collected from cows grazing the experimental plots in the field study. Animal ethics for this study was approved by Massey University Animal Ethics Committee (application No. 19/54). On days 7 and 8 of the grazing period (10th and 11th February 2021), fresh urine samples were collected from individual dairy cows grazing RWC, P30 and P50 pastures. The urine samples were bulked and stored at 4°C to avoid urea hydrolysis. Sub-samples, 35mL of each urine type, were taken immediately for total N, urea and ammonia analysis. Total N content in urine was analysed by the Dumas method (AOAC 968.06, 2000). Urea concentration was determined by kinetic UV assay and ammonia was analysed by using the colorimetric method in the Nutrition Laboratory, Massey University.

Urine was sprayed uniformly in the chambers at the equivalent rate of 10 L m^{-2}. This application rate is similar to a typical urination volume of 2 L (Doak, 1952) deposited onto an average urine patch area of 0.2 m^2 from dairy cows (Haynes & Williams, 1993). Therefore, 0.452 L urine was applied to the circular gas chamber of 0.0452 m^2 area and 2.5 L to the soil measurement area of 0.25 m^2 (Figure 4-2). The control treatments received the equivalent volume of water. Urine types, total urinary-N content and application rate expressed on a total N in kg ha^{-1} are presented in Table 4-1.

(A) (B)

Figure 4-2 Urine application to gas chambers (A) and soil treatment areas (B).

Note. Photo taken by author.

Table 4-1 Treatments, urinary N and application rates

Treatment name	**Pasture type**	**Urine type**	**Urinary-N concentration** (g N L^{-1})	**Rate of urinary N applied** (kg N ha^{-1})
RWC+URWC	0% plantain	0% plantain	6.15	615
RWC+U50	0% plantain	50% plantain	4.40	440
P30+U30	30% plantain	30% plantain	5.40	540
P50+U50	50% plantain	50% plantain	4.40	440
P50+ URWC	50% plantain	0% plantain	6.15	615
RWC+NoU	0% plantain	N/A	N/A	N/A
P30+NoU	30% plantain	N/A	N/A	N/A
P50+NoU	50% plantain	N/A	N/A	N/A

Notes. RWC, RWC pasture; P30, 30% plantain mixed-species pasture; P50, 50% plantain mixed-species pasture. NoU, control treatment receiving no urine; URWC, urine collected from cows grazing RWC; U30, urine collected from cows grazing mixed-species pasture containing 30% plantain; U50, urine collected from cows grazing mixed-species pasture containing 50% plantain. Table author's original work.
Abbreviation. N/A, not applicable; RWC, ryegrass/white clover.

4.2.2 Nitrous Oxide Measurements

Nitrous oxide emissions were measured by using the non-vented closed chamber method, following the N_2O chamber methodology guidelines on gas measurement described by the Global Research Alliance on Agricultural Greenhouse Gases (GRA) (Grace et al., 2020). Background emission samples were taken 2 days before treatment application to determine the variability in background N_2O fluxes between replicate plots and to aid interpretation of N_2O fluxes between chambers after urine application. The N_2O flux measurements were carried out at 4, 24 and 72h after urine application, twice per week in the first 4 weeks, weekly in the following 5 weeks and then, fortnightly, until gas flux was equal to the background level. During weekly phases of N_2O flux measurements, additional samples were collected as soon as practical following rainfall events greater than 10mm of rain in the previous 24h period. In total, N_2O fluxes were measured 19 times over 102 days.

For the N_2O flux samples, the gas chambers were closed with a single-port lid (Saggar et al., 2004a). Gas sampling was carried out between 10am and 12pm, the period that represents the daily average flux, without bias (Van der Weerden et al., 2013). Three headspace samples (25mL per sample) were taken from each chamber by using 60mL and 35mL plastic syringes, and then injected through a septum into evacuated 5.6mL glass vials during a cover period of 80min at three intervals of t0, t40 and t80. On each sampling day, three background atmosphere samples were also taken. After sampling, N_2O concentration in the samples was analysed via gas chromatography using a Shimadzu Nexis 2030 at the Biosphere Lab in the Manaaki Whenua — Landcare Research complex at Palmerston North, New Zealand.

On each sampling day, chamber temperatures were recorded at the three points of the cover period (t0, t40 and t80), and the average of the three readings was used as the chamber temperature for calculating the gas flux.

The hourly N_2O fluxes (mg N m^{-2} h^{-1}) were calculated based on the slope of the linear increase (R^2>0.90) in N_2O emissions within the chamber headspace, collected at different times of the sampling time (t0, t40 and t80) as described by de Klein et al. (2003) and using Equation 1:

$$N_2O \text{ flux} = \frac{\delta N_2O}{\delta T} \times \frac{M}{Vm} \times \frac{V}{A} \qquad \text{Equation 2,}$$

where δN_2O is the increase in N_2O in the headspace over the cover period (µL/L); δT is the enclosure period (hours); M is the molar weight of N in N_2O; Vm is the molar volume of gas at the sampling temperature (L mol^{-1}); V is the headspace volume (m^3) and A is the area covered (m^2).

The hourly flux data for each chamber were assumed to represent the mean daily flux. The cumulative emissions of N_2O over the experimental period were estimated by integrating the daily fluxes from each chamber on set measurement dates. The N_2O EF_3 and N_2O–N emitted were expressed as a percentage of N applied for the whole trial period, and were calculated using Equation 2:

$$EF_3 = \frac{N_2O\text{-N total (urine)- } N_2O\text{-N total (control)}}{\text{Urine N applied}} \qquad \text{Equation 3,}$$

where N_2O-N total (urine) and N_2O–N total (control) were the cumulative N_2O–N emissions over the measurement period from the urine-treated and control chambers, respectively (kg N ha^{-1}), and urine N applied was the rate of urine N applied (kg N ha^{-1}).

4.2.3 Soil Sampling and Measurements

During the N_2O measurement period, soil samples were collected from the designated soil areas at 1, 4, 9, 16, 23, 38, 52, 84 and 105 days after urine application. At each sampling, two soil cores (25mm diameter) were taken at two depths, 0–50mm and 50–100mm. After collecting the soil cores, sealed PVC tubes were placed into the remaining soil holes to minimise any impacts on the existing anaerobic conditions of the rest of the plot. Two soil cores were then bulked, plant roots were removed, and the soil was sieved through a 4mm sieve for moisture content and mineral N content analysis.

4.2.3.1 Soil Mineral Nitrogen.

A 5g sub-sample of field-moist soil was taken and extracted with 30mL of 2M KCl solution by shaking on an end-over-end shaker for 1h (1:6 soil:extractant ratio). Then, the extract was filtered through a Whatman no. 41 filter papers prior to calorimetric determination for NO_3^- and NH_4^+ content using a Technicon AutoAnalyser (Blakemore, 1987).

4.2.3.2 Soil Water-filled Pore Space.

Another 5g sub-sample of moist soil was oven-dried at 105°C for 24h to measure gravimetric soil water content using Equation 3.

$$\text{Soil gravimetric water content (\%)} = \frac{\text{Weight of water in moist soil (g)}}{\text{Weight of oven-dried soil (g)}} \times 100\% \qquad \text{Equation 4,}$$

On the 25th of March, 2021, a soil bulk density ring was taken from two depths, 0–50mm and 50–100mm, in each pasture treatment plot. In total, 15 rings at each depth were collected to determine the soil bulk density of the surface soil on each plot. The bulk density rings were then oven-dried at 105°C for 24h, and bulk density was calculated using Equation 4:

$$\text{Bulk density (g cm}^{-3}\text{)} = \frac{\text{Weight of oven-dried soil (g)}}{\text{Volume of soil (cm}^3\text{)}} \qquad \text{Equation 5,}$$

where volume of soil was 90 cm^3.

The particle density was assumed to be 2.65mg m^{-3}. Total porosity and soil volumetric water content were calculated using Equations 5 and 6 (Gradwell & Birrell, 1972):

$$\text{Total porosity (\%)} = 1 - \frac{\text{Bulk density of soil (g cm}^{-3}\text{)}}{\text{Particle density of soil (g cm}^{-3}\text{)}} \times 100\% \qquad \text{Equation 6,}$$

$$\text{Soil Volumetric water content (\%)} = \frac{\text{Gravimetric water content (\%)} \times \text{Bulk density of soil (g cm}^{-3})}{\text{Density of water (g cm}^{-3})}$$

Equation 7,

where density of water was assumed to be 1g cm^{-3}.

Soil water-filled pore space (WFPS) was calculated using the volumetric water content and total porosity (Equation 7):

$$\text{WFPS (\%)} = \frac{\text{Soil Volumetric water content (\%)}}{\text{Total porosity (\%)}} \times 100\%$$

Equation 8.

4.2.4 Pasture Sampling

Pasture from each gas chamber was harvested at 39 and 102 days after treatment application to simulate grazing by cows for the purpose of estimating DM yield and N uptake. The herbage was cut to a 5cm height and oven-dried at 65°C for 48h and weighed for total DM yield. The dried forage was finely ground, and total N content was determined using the Kjeldahl analysis method (McKenzie & Wallace, 1954). The total cumulative DM yield and N uptake during the experimental period were calculated as the sum of results from each harvest. Pasture on soil measurement areas, and fenced off areas, were cut at the same time, and the herbage was removed from the plots immediately.

4.2.5 Statistical Analysis

Data were analysed using the mixed procedure of SAS version 9.4. The effect of pasture and urine treatments on cumulative N_2O, EF_3, cumulative DM yield and plant N-uptake data were analysed in a mixed model that included the fixed effects of pasture treatments, urine treatments and two-way interaction between pasture and urine treatments and the random effect of replicate. Differences between treatments were analysed by one-way ANOVA followed by Least Significant Differences (LSD) test. The significant difference of the means for all analyses was established at $p < 0.05$.

4.3 Results

4.3.1 Weather and Soil Moisture Conditions

Over the entire experimental period (105 days), the total rainfall was 228.6mm, and average soil temperature at a 100mm depth was 14.7°C (Figure 4-3). Soil water deficits occurred throughout the period of study but were greater during the beginning of the experiment. The SWD values ranged from 93–122mm during the first 14 days of the trial, and gradually decreased to approximately 40mm at 93 days after application (DAA).

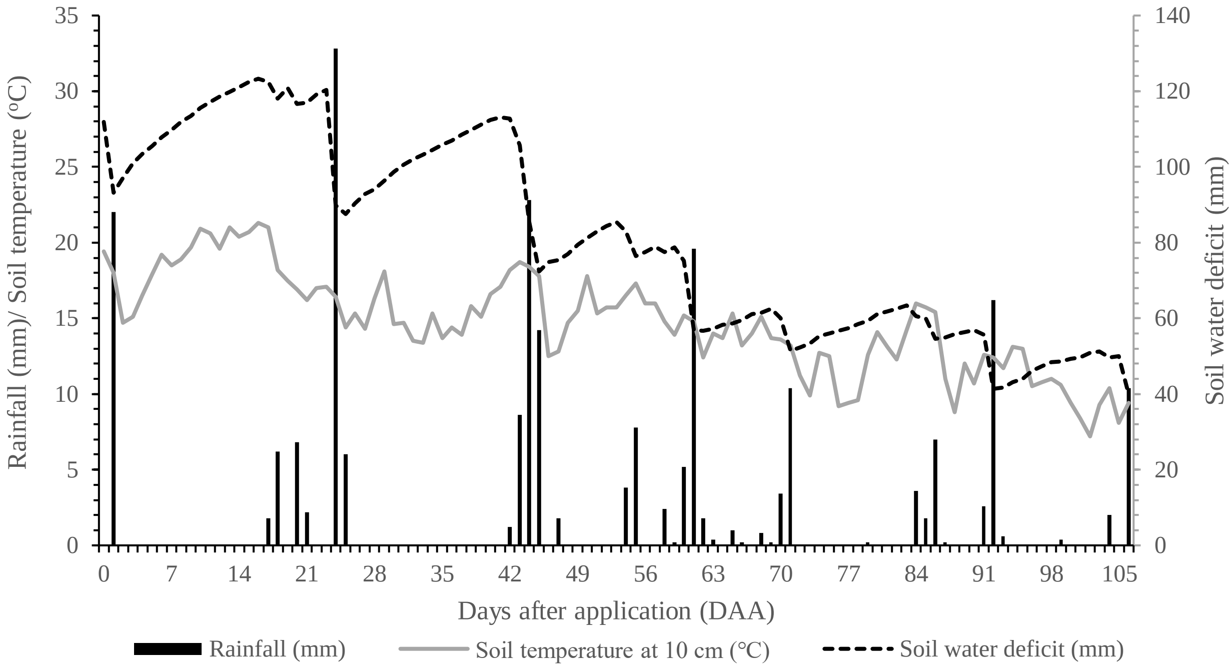


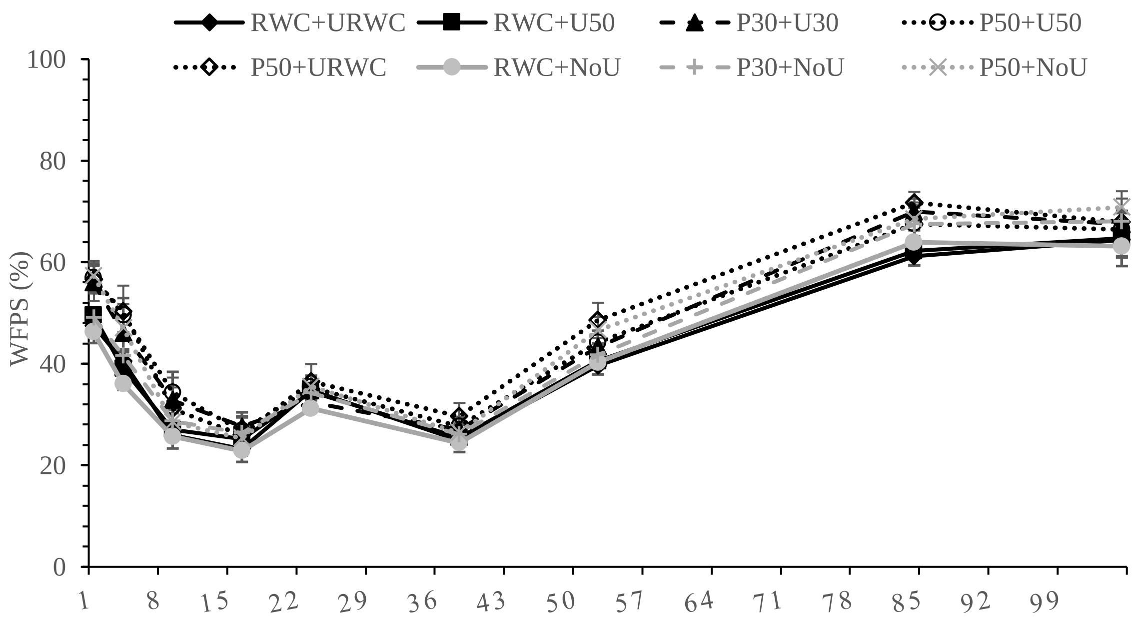
Figure 4-3 Daily rainfall (mm), SWD (mm) and soil temperature at 10cm depth (°C) over the 105-day field experiment.

Note Day 0 started from 15th February 2021

Abbreviation. SWD, soil water deficit.

The soil WFPS percentage (WFPS %) at the two depths fluctuated between 22% and 75% over the trial period (Figure 4-4). The lowest WFPS % values were observed from 16–38 DAA, and these were attributed to low rainfall during this period. Following this period, the WFPS % values increased to 70% by the end of the trial. The WFPS % values at both depths in P30 and P50 pastures including plantain, regardless of applied urine types, were significantly higher than for soil under standard RWC during the first 4 weeks of the experiment ($p<0.05$).

(A) 0-50 mm depth



(B) 50-100 mm depth

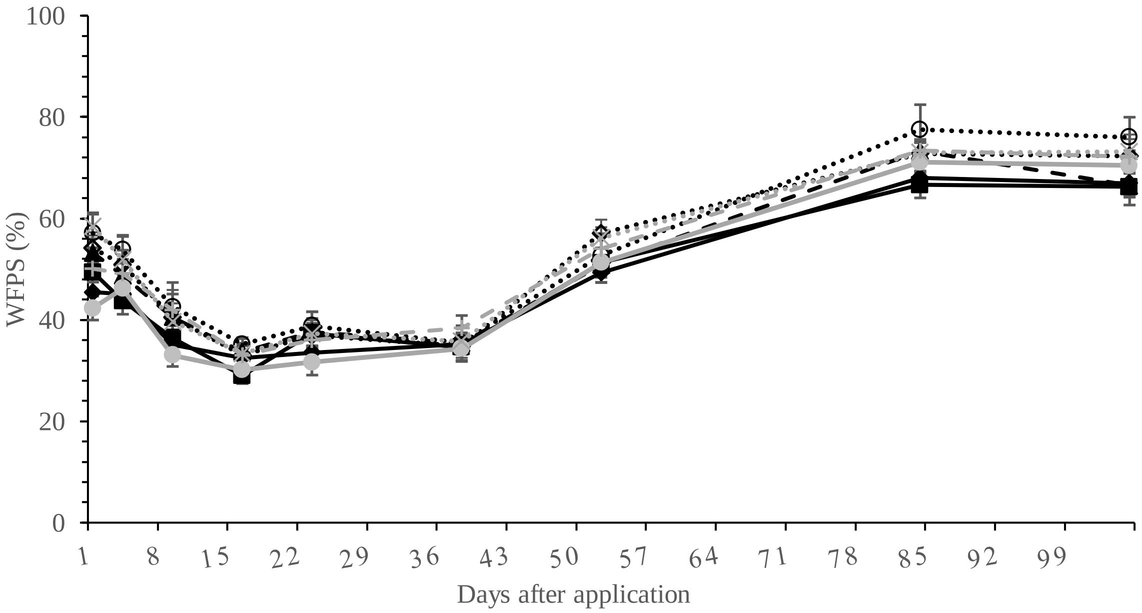


Figure 4-4 Soil water filled-pore space (WFPS, %) at 0–50mm (A) and 50–10 mm (B) depths in RWC mixed with 0% plantain (RWC), 30% plantain (P30) and 50% plantain (P50) pasture where different urine types were applied: ryegrass/white clover urine (URWC), 30% plantain (U30) and 50% plantain (U50) and water as a control (NoU).

Note. Error bars represent the SEM for each treatment (n=5).

Abbreviations. RWC, ryegrass/white clover; SEM, standard error of the mean.

4.3.2 The urine composition

The urinary-N concentration in urine collected from cows grazing RWC, P30 and P50 treatments were 6.15, 5.40 and 4.40g N L^{-1}, respectively (Table 4-2). The total urine-N concentration decreased linearly with increasing proportion of plantain. Urea-N accounted for 80-85% of the total urine-N in plantain treatments, while 92% of the N content of RWC urine was in urea form. In contrast, the ammonia content in U50 treatment was approximately 40% higher than in U30 and URWC in range of 61–66mg L^{-1}. The remaining proportions (7, 13.8 and 16.3%) of urinary-N that are unaccounted for this analysis represented other forms of N.

Table 4-2 Chemical composition of urine from cows grazing ryegrass/white clover mixed with 0% plantain (URWC), 30% plantain (U30) and 50% plantain (U50)

Urine type	**Total N content** (mg N L^{-1})	**Urea** (mg L^{-1})	**Urea proportion of the total N** (%)	**Ammonia** (mg L^{-1})	**Ammonia proportion of the total N** (%)
URWC	6150	5684	92	61	1
U30	5400	4592	85	66	1.2
U50	4400	3545	80	167	3.7

Notes. URWC, urine collected from cows grazing RWC; U30, urine collected from cows grazing mixed-species pasture containing 30% plantain; U50, urine collected from cows grazing mixed-species pasture containing 50% plantain. Table author's original work.

4.3.3 Nitrous Oxide Emissions

The hourly N_2O fluxes during the 102-day gas measurement period are presented in Figure 4-5. Background pre-treatment N_2O emissions were low and in the range of 0.006–0.030mg N m^{-2} hr^{-1}. Urine application sharply increased N_2O flux from all pasture treatments. At 4 hours after application, N_2O emissions from the ryegrass/white clover pasture were significantly lower ($p< 0.05$) than from for the P50 mixed pasture, but continued to increase until they peaked on the following day when they reached 0.5mg N m^{-2} h^{-1}. At 4 hours, urine from P30 and P50 pastures produced the highest peak at 0.55 and 0.95mg N m^{-2} h^{-1}, respectively, followed by a decline to around 0.3mg N m^{-2} h^{-1} in both pasture treatments.

The emissions from all the treatments declined after day 1 and remained low between day 3 and day 37 due to summer dry conditions, but small peaks occurred following rain events. The larger peaks were observed at day 44 with the greatest N_2O fluxes from RWC+URWC (0.51mg N m^{-2} h^{-1}), followed by P50+U50 (0.34mg N m^{-2} h^{-1}), RWC+U50 (0.26mg N m^{-2} h^{-1}), P30+U30 (0.2mg N m^{-2} h^{-1}) and P50+U50 (0.14mg N m^{-2} h^{-1}). The emissions from RWC+URWC were significantly higher ($p< 0.05$) than from P30+U30 and P50+U50 at day 25 and 44. N_2O emissions from plantain pastures reached the background level at 88 DAA while ryegrass/white clover swards obtain the background emission level at day 102. The fluxes from the control treatments had a similar trend to the urine treated treatments throughout the duration of the trial.

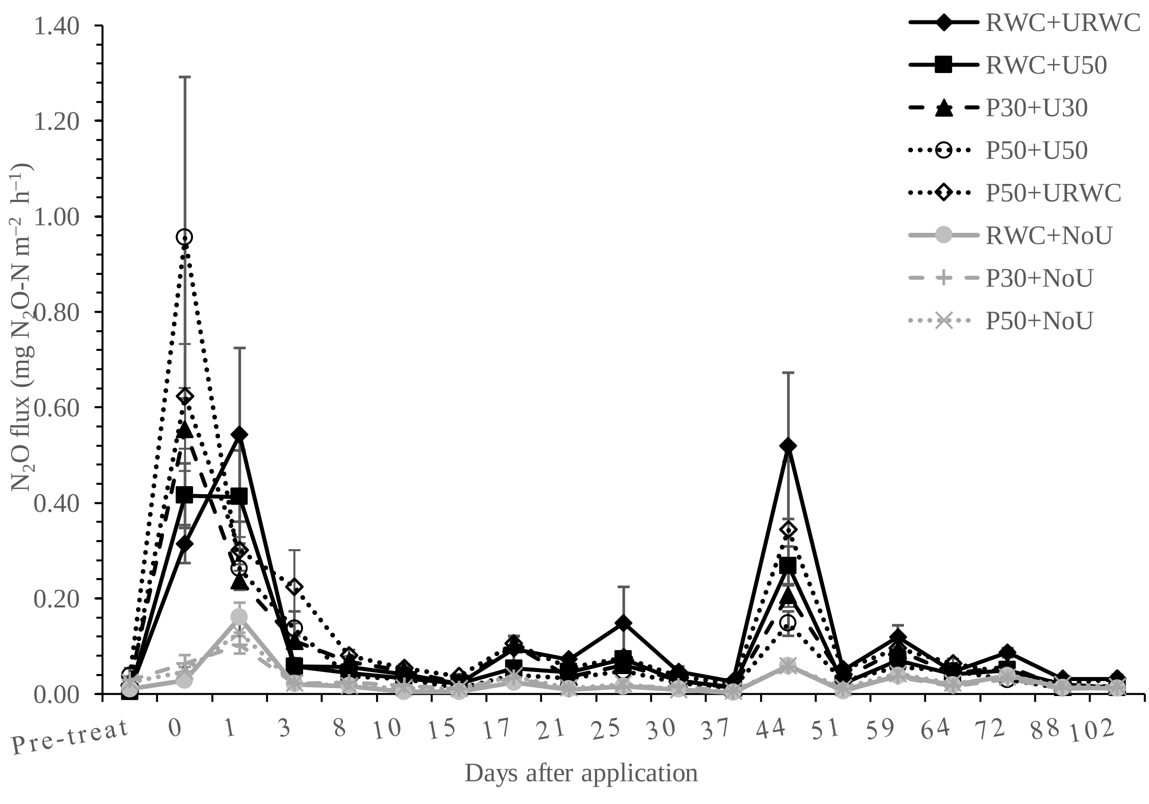


Figure 4-5 Hourly N_2O fluxes from urine patches in 0% plantain (RWC), 30% plantain (P30) and 50% plantain (P50) pasture, where different urine types from cows grazing RWC (URWC), 30% plantain (U30) and 50% plantain (U50) were applied; water (NoU) was applied as the control. Error bars show N_2O–N fluxes' SEM (n=5).

Abbreviations. N_2O, nitrous oxide; RWC, ryegrass/white clover; SEM, standard error of the mean.

Pasture and urine types significantly affected ($p< 0.05$) on the total N_2O emissions and the emission factors (Table 4-3). The higher loading rate of 615kg N ha^{-1} in ryegrass urine type significantly increased (*p*< 0.01) the cumulative N_2O–N fluxes from RWC and P50 pasture treatments. The total N_2O emissions from 28% lower N loading rate in the U50 treatment were around 39% less than the emission from the URWC treatment regardless pasture types. In contrast, there was no significant differences (*p*> 0.05) in the cumulative N_2O emissions between the N-application rate of 540kg N ha^{-1} and 440kg N ha^{-1} when these urine types were applied to the corresponding pasture types (1.27kg N-N_2O ha^{-1} from P50+U50 treatment and 1.51kg N-N_2O ha^{-1} from P30+U30 treatment). No statistical difference (*p*> 0.05) was observed between RWC and P50 pasture treatments treated at the same urine loading rate. The P30 and P50 pasture treatments resulted in lower cumulative N_2O emissions by 16% when URWC was applied and by 38–48% when U30 and U50 were applied compared to RWC pasture. The total N_2O emissions from the control treatments did not significantly differ ranging from 0.53 to 0.6 kg N ha^{-1}.

The highest emission factor values were observed in RWC+URWC (0.31%), followed by P50+URWC (0.23%) and RWC+U50 (0.22%). Urine application at higher N-loading rate of 615kg N ha^{-1} from RWC urine did not have significant effects (*p*> 0.05) on the EF_3 values. The EF_3 in P50 and RWC pasture treatments were similar (*p*> 0.05) with the same N-application rate of either 615kg N ha^{-1} or 440kg N ha^{-1}. EF_3 values in P30+U30 treatment and P50+U50 treatment were 42–51% lower than the RWC+URWC treatment, and this difference was significant (*p*< 0.05). There was an insignificant difference in emission factors between P30 and P50 pastures. This result showed that the increasing the plantain content in the pasture and in the animal diet, i.e. going from P30 and P50, did not affect the emission factor.

Table 4-3 Cumulative N_2O emissions, emission factor of the applied urine N emitted as N_2O (EF_3), change in cumulative N_2O emissions and EF_3 compared to from ryegrass/white clover pasture applied the corresponding urine type during the measurement period.

Treatments	**Urine rate** (kg N ha^{-1})	**Change in urine rate** (%)	**Cumulative N_2O emissions** (kg N ha^{-1})	**Change in N_2O** (%)	**Emission factor** (EF_3, %)	**Change in EF_3** (%)
RWC+URWC	615	N/A	2.43 (0.26)$_a$	N/A	0.31 (0.04)$_a$	N/A
RWC+U50	440	-28%	1.48 (0.16)$_b$	-39%	0.22 (0.01)$_{ab}$	-30%
P30+U30	540	-12%	1.51 (0.11)$_b$	-38%	0.18 (0.02)$_b$	-42%
P50+U50	440	-28%	1.27 (0.16)$_b$	-48%	0.15 (0.02)$_b$	-51%
P50+URWC	615	N/A	2.05 (0.25)$_a$	-16%	0.23 (0.03)$_{ab}$	-24%
RWC+NoU	N/A		0.53 (0.02)$_c$			
P30+NoU	N/A		0.53 (0.05)$_c$			
P50+NoU	N/A		0.60 (0.04)$_c$			
***p*- Value**						
Pasture type			0.0070		0.0220	
Urine type			<.0001		0.0054	
Pasture × urine			0.3014		0.8731	

Notes. Values sharing the same subscript letter do not differ significantly (p>0.05). The SEM applied to five treatments (n=5)

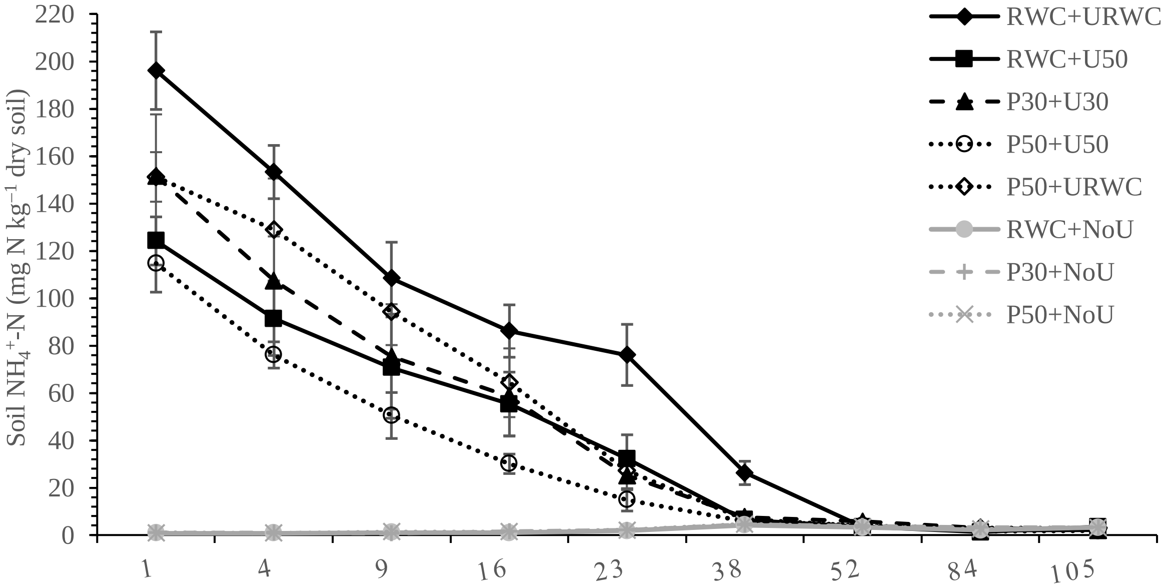
Abbreviations. EF_3, emission factor; N, nitrogen; N/A, not applicable; N_2O, nitrous oxide; SEM, standard error of the mean.

4.3.4 Soil Mineral Nitrogen

4.3.4.1 Ammonium.

The soil ammonium (NH_4^+) concentration at the 0–50mm depth in all treatments peaked at 24h after treatment, reaching 196mg N kg^{-1} in RWC+URWC, followed by P50+URWC (151mg N kg^{-1}) (Figure 4-6A). These treatments retained a significantly higher NH_4^+ content than P50+U50 until 38 DAA (p< 0.05). After peaking at 24h after application, the NH_4^+ concentration steadily decreased to similar levels as the control treatments by 38 DAA, for pastures receiving U30 and U50, and at 52 DAA when URWC was applied to pastures.

(A) 0–50 mm depth



(B) 50–100 mm depth

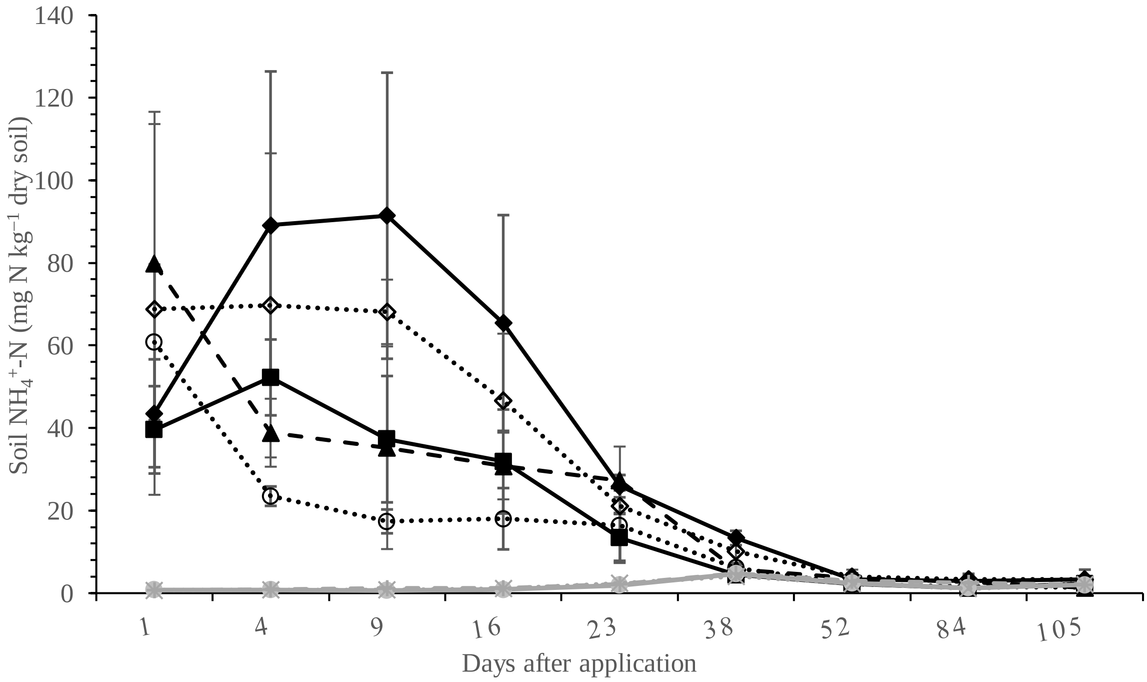


Figure 4-6 Soil ammonium N concentration (mg N kg^{-1} dry soil) at 0–50mm depth (A) and 50–100mm depth (B) during the experimental period (105 days).

Note Error bars present the SEM (n=5). Pasture with no (0%) plantain (RWC), 30% plantain (P30) and 50% plantain (P50), where different urine types from cows grazing RWC (URWC), 30% plantain (U30) and 50% plantain (U50) were applied; water (NoU) was applied as the control.

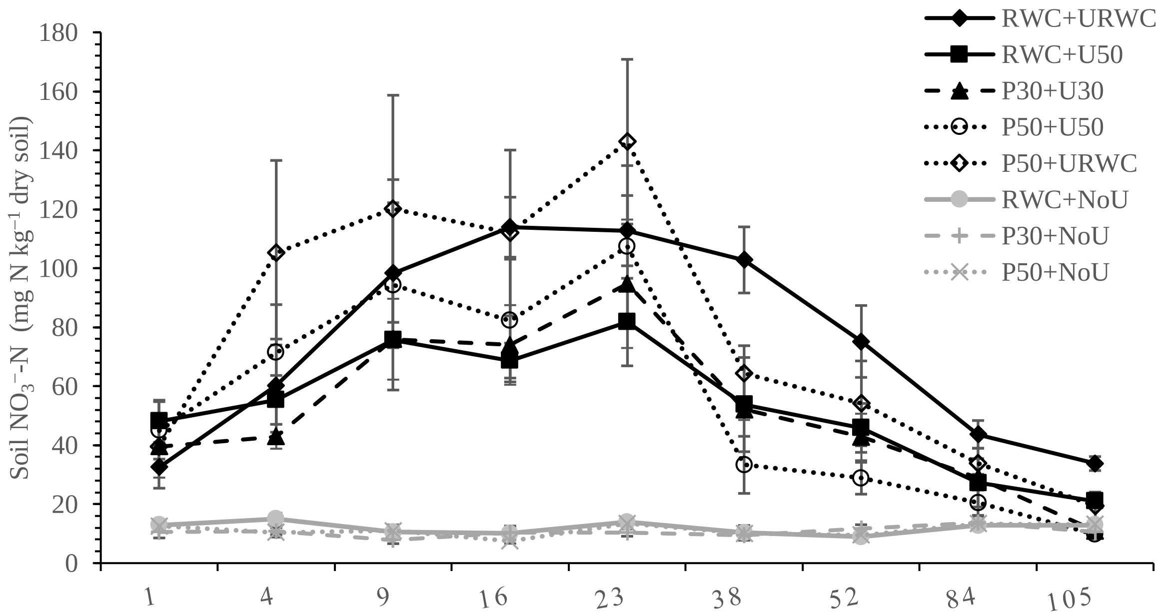
Abbreviations. N, nitrogen, NH_{4+}, ammonium; RWC, ryegrass/white clover; SEM, standard error of the means.

At 50–100 mm depth, the P30 and P50 pasture treatments reached the peak in soil NH_4^+ concentration of ranging from 60 to 80mg N kg^{-1} at 1 DAA (Figure 4-6B). In contrast, RWC+U50 and RWC+URWC treatments obtained the highest NH_4^+ values at 4 DAA and 9 DAA, reaching 52mg N kg^{-1} and 91mg N kg^{-1}, respectively. The NH_4^+ concentrations were significantly higher in RWC+URWC and P50+URWC ($p < 0.05$) compared to other treatments at 4, 9 and 16 DAA. After peaking, the soil NH_4^+ concentration from plantain urine decreased faster than NH_4^+ concentrations derived from RWC urine and reached the background level at 38 DAA while the lowest NH_4^+ content under RWC urine occurred at day 52. There was a significant trend of decreased soil NH_4^+ concentration with the lower N-loading rate ($p < 0.05$). From 4 to 23 DAA, soil NH_4^+ under URWC treatment was about 35% and 45% higher than under U30 and U50 treatments in the 0–50 mm and 50–100 mm depths, respectively. Overall, pasture types did not affect soil NH_4^+ concentration at either depth.

4.3.4.2 Nitrate.

Figure 4-7 shows the soil NO_3^- concentration at both the 0–50mm and 50–100mm soil depths for the duration of the experiment. At both depths, the soil NO_3^- concentration in all treatments gradually increased and peaked at 23 DAA. Soil NO_3^- concentration in RWC pasture, applied with either plantain urine or RWC urine treatments, remained at a high level longer than in the plantain mixed pasture treatments. In the 0–50mm depth, at 105 DAA, soil NO_3^- concentrations in RWC+URWC and RWC+U50 were 34mg N kg^{-1} and 19mg N kg^{-1}, respectively. These treatments had significantly higher NO_3^- content than P30 and P50 pastures, which declined to the background level at 105 DAA.

(A) 0 – 50 mm depth



(B) 50 – 100 mm depth

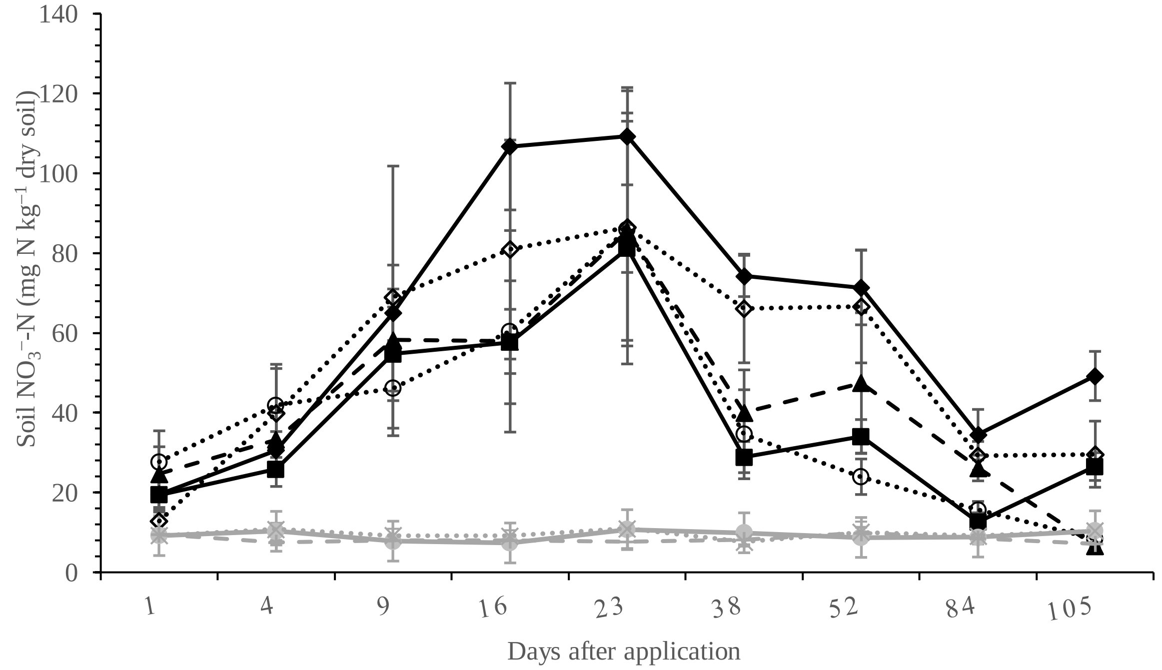


Figure 4-7 Soil nitrate N concentration (mg N kg^{-1} dry soil) at 0–50mm depth (A) and 50–100mm depth (B) during the experimental period.

Notes. Pasture with no (0%) plantain (RWC), 30% plantain (P30) and 50% plantain (P50), where different urine types from cows grazing RWC (URWC), 30% plantain (U30) and 50% plantain (U50) were applied; water (NoU) was applied as the control. Error bars present the SEM (n=5).

Abbreviations. N, nitrogen, RWC, ryegrass/white clover; SEM, standard error of the means.

At the 50–100mm depth, soil NO_3^- concentration varied throughout the experimental period and peaked at 23 DAA, and again at 52 DAA. After decreasing at 84 DAA, NO_3^- concentration in P30+P30 and P50+U50 samples reached the background level at 105 DAA, whilst NO_3^- concentration in RWC pastures recovered at 105 DAA, reaching 50mg N kg^{-1} in RWC+URWC and 30mg N kg^{-1} in RWC+U50. Treatment P50+URWC maintained NO_3^- concentration at 30mg N kg^{-1} at 105 DAA.

The effect of urine types and the N-loading rates on the soil NO_3^- concentration was not observed during the first 9 days after application. However, from day 16 to 105, the URWC treatment at the higher N-loading rate of 615kg N ha^{-1} resulted in 30% higher soil NO_3^- content compared to the U50 treatment at both depths. There were no significant differences in NO_3^- concentrations between the U30 and U50 urine treatments applied to the corresponding pasture types.

At the 0–50 mm depth, at day 4 and 9, the soil NO_3^- concentration in P50 pasture tended to be higher than in RWC pastures applied at the same urine types. However, at the end of the experiment, plantain pasture reduced ($p < 0.05$) NO_3^- concentration at 38 and 105 days compared to ryegrass/white clover pasture when the same urine type was applied. At 50–100 mm depth, pasture types did not have significant effect on NO_3^- concentration throughout the experimental period except day 105. Soil NO_3^- concentration in P50 pasture treatment were 47-60% lower than in RWC pasture receiving the same urine treatment.

4.3.5 Herbage Nitrogen Uptake and Dry Matter Yield

Urine types, pasture treatments and their interactions did not have significant effects on pasture DM yield and N uptake (Table 4-4). The DM yield for all treatments fell within the range of 4,819–7,441kg DM ha^{-1}, and the N uptake varied from 62–102kg N ha^{-1}.

Table 4-4 Cumulative DM yield (kg DM ha^{-1}) and N uptake (kg N ha^{-1})

	Cumulative DM (kg DM ha^{-1})	**SEM**	**N uptake** (kg N ha^{-1})	**SEM**
RWC+URWC	5,691.15	577.33	88.60	11.58
RWC+U50	6,263.27	875.61	70.97	9.24
P30+U30	7,282.74	566.81	95.55	13.59
P50+U50	7,441.15	919.55	101.92	13.67
P50+URWC	6,491.59	1,260.97	101.47	17.46
RWC+NoU	4,819.91	371.27	69.87	15.30
P30+NoU	5,999.11	767.89	77.15	9.65
P50+NoU	4,620.80	840.67	62.01	6.29
***p*-value**				
Pasture type	0.3645		0.2638	
Urine type	0.0577		0.1199	
Pasture × urine	0.6851		0.6516	

Notes. Values sharing the same subscript letter do not differ significantly ($p>0.05$). The SEMs applied to five treatments (n = 5).

Abbreviations. DM, dry matter; N, nitrogen; SEM = standard error of the means.

4.4 Discussion

This study highlighted that the lower N-loading rate onto soil from plantain derived urine is the major effect resulting in the reduction of total N_2O emissions. The urinary-N concentration, urea-N content and subsequent N-loading rate onto soil decreased when mixed pastures with 30% and 50% of plantain are part of the diet of cows, supporting previous studies which demonstrated lower N content in urine of cows grazing pastures containing plantain compared to ryegrass/white cover (Box et al., 2017; Di et al., 2016; Minnée et al., 2020; Podolyan et al., 2020; Rodríguez-Gelós, 2020; Totty et al., 2013). Minnée et al. (2020) suggested that at least 30% plantain in the cow's diet is needed to reduce urinary-N content without any negative effects on milk production. In this study, including 30% plantain in the cow diet resulted in 13% lower urinary-N and 20% lower urea-N content compared to urine from cows fed ryegrass/white clover.

The effects of plantain on reduced urinary N excretion were most likely associated with increased N partitioning into dung and other sinks rather than urine (Minnée et al., 2020; Navarrete, 2015; Totty et al., 2013). Marshall et al. (2021) demonstrated that 100% plantain diet increased N partitioning to faeces and reduce total N excreted resulting lower urinary N concentration. Higher N excretion into dung rather than urine can assist in the reduction of total on-farm N losses, as faecal N is much less susceptible to gaseous losses than urinary N (van der Weerden et al., 2020). The urea-N concentration in urine of cows grazing plantain was also observed to be 40% lower than in urine from cows grazing ryegrass by Rodríguez-Gelós (2020). In this study, the urea-N in plantain urine treatments decreased by 20-38% relative to in ryegrass/white clover urine. The effect of plantain on the urea content of urine has been related to the presence of secondary compounds in plantain which can reduce the rumen's ammonia production resulting in lower urea-N concentrations (Navarrete, 2015). Additionally, a reduction in urinary-N concentration evident in the current work can be also a consequence of a N dilution with higher urine volume when cows are fed a that includes plantain (Di et al., 2016; O'Connell et al., 2016). Plantain contains 30% more water content than ryegrass (Minneé et al., 2019), consequently, higher water consumption from the herbage can cause diuretic effects in cows and dilute N content in urine via increased urination volume and frequency (Mangwe et al., 2019; O'Connell et al., 2016). More frequent urination may result in a greater surface area being wetted by urine and to the extent that denitrification is a surface-soil phenomenon, this may undermine some of the advantages of plantain. Balancing the effect of plantain on reductions in N_2O emissions per typical urine patch versus its effect on N_2O production from multiple urine patches was beyond the scope of this study. Some authors claimed that N_2O emissions are directly linked to total urinary-N excreted (de Klein et al., 2020), which is beyond the scope of this study without the information on excreted urine volume. The urinary-N concentration and the decrease in N_2O emissions measured in this study then can contribute to determination of total urinary-N excreted on the paddock and farm scale.

The WFPS values at 0–100mm soil depth in plantain mixed pastures were significantly higher than RWC pastures during the first 16 DAA, when rainfall was nearly zero. Similarly, Rodríguez-Gelós (2020) observed higher WFPS values in plantain pasture than in ryegrass swards in autumn/winter on Tokomaru silt loam soil, the same soil types used in the current study. In contrast, in winter/spring, Luo et al. (2018) reported a lower WFPS values in plantain pasture, while Podolyan et al. (2020) did not observe any significant difference in WFPS in plantain compared to ryegrass/white clover in free-draining soil. The higher WFPS values in plantain plots might be related to the architecture and growth behaviour of plantain roots. Plantain has a truncated tap-root with thick, fibrous side roots, a high root density in the 0–100mm depth and an average rooting depth of 0.97m (Nie et al., 2008). In contrast, an estimated 75% of ryegrass roots are present in the 0–70 mm depth, making them unlikely to survive under water stress conditions (Wedderburn et al., 2010). Deeper root systems provide plantain access to water in deeper soil layers and greater tolerance to water stress conditions. Therefore, plantain roots may extract water more uniformly down the soil profile while ryegrass removes most of its water requirement from the surface soil, particular in early summer. Therefore, further research on water extraction patterns under plantain pastures is needed.

The N_2O emissions from plantain pastures with either ryegrass urine or plantain urine applied were higher than for RWC pastures at the start of the trial, and this was presumably associated with the higher WFPS values in plantain pasture during this period. A higher WFPS value can increase the rate of the denitrification process and result in greater N_2O emissions (Saggar et al., 2013; Di et al., 2014). In this trial, N_2O fluxes peaked following every significant rain event with the largest increases recorded in RWC pastures, regardless of urine type applied. In fact, N_2O emissions following heavy rainfall in summer were shown to be 5-10 times higher than in winter after grazing (Saggar et al., 2004b). At the end of present experiment, when rainfall and WFPS values increased, N_2O emissions from RWC pasture were significantly higher than for plantain pastures. These results support previous findings about the positive relationship between soil moisture and the denitrification process and N_2O emissions (Müller & Sherlock, 2004; Saggar et al., 2009).

In this experiment, urine collected from cows grazing plantain reduced ($p< 0.05$) total N_2O emissions from urine patches over time. This reduction in N_2O emissions is mainly associated with the decrease in the total N in plantain derived urine and subsequently lower N-loading rate of 440 and 540kg N ha^{-1} onto soil. Lower N-loading rates have been shown to reduce mineral-N content in soil and consequently, result in lower N losses to the environment via leaching and gaseous losses of NH_3 and N_2O (Di et al., 2016; Monaghan & De Klein, 2014; Podolyan et al., 2020; Simon et al., 2019). In contrast, Carlson et al. (2020) did not observe an effect of lower urinary-N content on a reduction in N_2O emissions in a poorly-drained soil. de Klein et al. (2014) also indicated that N_2O emissions were not reduced by the lower N-loading rate in poor draining soils with high N_2O emissions rates. The presence of secondary compounds in urine of grazing animals is another mechanism that has been proposed to explain decreases in N_2O emissions from plantain urine type (Gardiner et al., 2018; Keir et al., 2001). However, two studies found that when urine from the plantain diet was adjusted to the same N concentration as urine from ryegrass diet, N_2O emissions from the two different urine types did not differ at the same N loading rate (Rodríguez-Gelós, 2020; Simon et al., 2019). Therefore, it would appear that lower N concentrations in urine and resultantly lower N loading rate is the main factors resulting in the reduction of N_2O emissions from urine of cows fed plantain.

However, lower N loading in U50 treatment applied to either RWC or P50 did not have a significant effect on the emission factors compared to the addition of RWC urine. Similarly, Marsden et al. (2016) did not observe any effect of urine N loading rate on the emission factor from sheep urine patches. These results suggest that reduction in emission factor is likely associated with other factors such as low soil moisture, limited C content or plants and soil microbes competition for N (de Klein et al., 2020; Kim et al., 2013), rather than the loading N input onto soil.

Over the 102 DAA of urine, the total N_2O emissions from P50 and RWC pasture treatments receiving the same urine type were not significantly different. This experiment observed that both urine and pasture types affected the emission factor value. The summer/autumn EF_3 values in this study ranged from 0.15 to 0.31%, which were significantly lower than the annual EF_3 value of 0.98% for cattle urine in New Zealand (Ministry for the Environment, 2019). Similarly, smaller EF_3 values have been recorded in other studies under warm and dry conditions and in well-drained soil (Carlson et al., 2020; López-Aizpún et al., 2020; Podolyan et al., 2020). These results confirmed that EF_3 is likely to be much lower than the default NZ specific value under dry conditions. A significantly lower EF_3 from P30+U30 and P50+U50 treatments compared to RWC+URWC observed in this study could be attributed to the release of biological nitrification inhibition by plantain as proposed by de Klein et al. (2020). Plantain contains secondary compounds acting as nitrification inhibitors which can reduce the nitrification rates, mineral-N content and resultant N_2O emissions (Dietz et al., 2013; Luo et al., 2018; Rodríguez-Gelós, 2020). In this trial, the soil NO_3^- concentration in the P50 treatment at 38 and 105 days were around 40% lower than in RWC pasture when URWC was applied at the loading rate of 615kg N ha^{-1}. The lower NO_3^- concentration could be related to lower population of nitrifying bacteria resulting in decreased soil nitrifying activity with the presence of plantain (Carlton et al., 2019; Verhagen et al., 1995). Additionally, Bowatte et al. (2018) proposed that lower N_2O emissions was related to higher plant N uptake efficiency. However, this study did not observe any effect of plantain on plant N uptake.

Contrary to the first hypothesis, this study observed similar N_2O emissions and EF_3 values between P30+U30 and P50+U50 treatments and so, in terms of N_2O emissions, there did not appear to be any advantage to P50 over P30. Similarly, Pijlman et al. (2020) did not observe any effects of increasing plantain proportions in mixed pastures on reduction of N_2O emission in their field experiment. The lack of effects of increased plantain proportions on N_2O emission could be related to the fluctuation in plantain composition in mixed pasture over time (see Chapter 3 for details). The less-than-obvious effects of plantain proportions on N_2O emissions also may be associated with the size of the gas chambers used i.e. they may not have been large enough to cover representative proportions of plantain for each pasture treatment. These findings suggest that at least 30% of plantain in mixed pasture and in the cow's diet could effectively reduce N_2O emissions and EF_3 from individual urine patches.

The N loading rate had a significant effect on NH_4^+ concentration during the first 16 DAA. Lower urine loading rate in U30 and U50 treatments reduced by 35–45% soil NH_4^+ concentration. Soil NH_4^+ concentration in P50 and RWC pasture treatments receving the same urine type were similar throughout the experimental period. For the 50–100mm soil depth, RWC obtained its peak later than plantain pasture, which was likely due to the high root density of ryegrass in the top-soil, from 0–50mm (Wedderburn et al., 2010).

Soil NO_3^- concentration when urine from animals on a diet of plantain and RWC was applied did not statistically differ during the first 23 DAA. However, after day 23, the effect of higher loading rate of 615kg N ha^{-1} from URWC resulted in significantly higher (p >0.05) soil NO_3^- concentration compared to from U50 treatment in both depths. Soil NO_3^- concentration in RWC pasture recovered after heavy rainfall at 105 DAA while the values in plantain pastures reached the background concentration, regardless of urine types applied. These findings suggest that the processes of nitrification and denitrification process in RWC pasture occurred slower than in plantain pastures. Further studies are required to quantify plantain-soil interactions providing understanding of effect of plantain on soil N dynamics in the soil.

Soil NH_4^+ concentration in P30+U30 was significantly higher than in P50+U50: this effect was associated with the urine N loading rate. However, NO_3^- concentration in these two treatments were not significantly different. This result might suggest the possible effect of secondary metabolites produced by plantain on reducing nitrification rates and consequently, NO_3^- concentration and N_2O emission.

Urine and pasture types did not have significant effects on DM yield and plant N uptake. Although applied at a high N loading rate, N uptake in all treatments were similar as the control treatment. The similar N content in herbage from plantain mixed pasture and ryegrass/white clover indicated that lower N_2O emission were associated with other mechanisms rather than plant N uptake.

4.5 Conclusions

The inclusion of 30% and 50% of plantain in ryegrass/white clover (RWC) pastures reduced N_2O emission from cow urine patches in summer/late autumn mostly due to lower urine N-loading rate and possibly the effect of secondary metabolites produced by plantain in the rhizosphere. The urinary-N and urea-N concentrations were reduced by 12% and 28%, and by 19% and 38%, respectively with 30% and 50% of plantain in the animal diet. Lower N-loading rate from 50% plantain urine type reduced the N_2O emissions from RWC by 39%. Cattle urine from plantain in the pasture diets (i.e., P30+U30 and P50+U50) resulted in a significant decrease (42 to 51%) in the emission factors compared to urine from RWC pasture diet. Increasing proportions of plantain from 30% to 50% in mixed pasture did not decrease N_2O emissions significantly. The results of this study demonstrated that compared to standard RWC pasture, including at least 30% plantain in the pasture and in the diet of dairy cows may effectively reduce N_2O emissions from dairy grazed cow urine patches.

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5 General Discussion and Conclusions.

The aim of this chapter is to summarise the key findings of experimental chapters relating to the seasonality of plantain mixed pasture and the effects of plantain on mitigating N_2O emissions from urine patches. The aim is also to discuss the implications of plantain mixed pastures as a promising alternative pasture type in relation to traditional RWC swards in New Zealand.

5.1 Overall Thesis Objective

Dairy farmers are increasingly incorporating plantain into pastures due to its drought tolerance and environmental benefits of reducing on-farm N losses. Several studies have demonstrated greater DM production in plantain-containing swards during summer and autumn compared to RWC swards (Minneé et al., 2013; Moorhead & Piggot, 2009; Navarrete, 2015). The environmental advantages of plantain in mitigating N_2O emissions from cow urine patches have also been documented (Carlson et al., 2020; Luo et al., 2018; Podolyan et al., 2020; Rodríguez-Gelós, 2020; Simon et al., 2019). The quantity of plantain in a pasture can affect its efficacy in reducing N losses to the environment. Minnée et al. (2020) suggested that including at least 30% plantain in a cow's diet is needed to reduce the N content excreted in urine, which subsequently results in lower N losses to the environment without negative effects on milk production. However, there is still limited information available on the optimal plantain mixed-pasture type that enable maintenance of herbage production and persists across growing seasons, while effectively reducing N losses from grazed pastoral systems.

This thesis reports on two field experiments conducted to monitor seasonal changes in the botanical composition of plantain mixed pastures and to evaluate the effectiveness of including plantain in mixed pastures and in the cow's diet to reduce N_2O emissions from cow urine patches. The results were presented and discussed in Chapters 3 and 4. Chapter 3 evaluated botanical composition of plantain mixed pasture and plant density of plantain across two growing seasons. Following this, chapter 4 assessed the effects of plantain mixed pasture on N_2O emissions and studied the diet effect and sward effect of plantain on reductions in N_2O emissions.

5.2 Main Findings

5.2.1 The Seasonality of Plantain Mixed Pasture

Chapter 3 highlighted that plantain generated the highest proportion in mixed pastures, of up to 50%, during late summer and autumn and plantain proportion declined throughout the growing season. In pure plantain swards or in herb–clover mix swards, plantain proportion was demonstrated to decrease over time (Navarrete, 2015; Stewart, 1996). In the current work, plant density of plantain in all plantain containing pasture treatments increased by around 35% in the first growing season, but plant loss of plantain occurred throughout the second growing season. At the end of the second season, plantain population had declined by 52%–62% compared to the initial plant population at the beginning of the season. It was suggested that plant loss of plantain is unavoidable due to low germination rate in established sward, weed invasion and grazing impacts (Lee et al., 2015; Navarrete, 2015). The proportions and plant density of plantain in the 70% plantain mixed-pasture treatment were similar to that in the 50% plantain mixed-pasture treatment throughout the second year. Decreased plant density of plantain contributed to a decline in DM contribution of plantain in the second year, which was due to the positive relationship between DM yield of pasture species and their plant density (Neal et al., 2009). These results suggest that sowing plantain at rates of 30% and 50% in the seed mixture and then broadcasting seed at the end of the growing season can maintain the proportion of plantain in pasture, and obtain yield stability.

The DM contribution of ryegrass declined significantly in late summer and early autumn and recovered in winter and the next spring. Consequently, dead material and unsown species content in the RWC pasture treatment was significantly higher than in the plantain mixed-pasture treatments. Similar to previously published findings, the proportion of white clover increased over time and reached its highest value, ranging from 14%–19%, at the end of the second growing season (Figure 3-4). The observed botanical composition showed the advantage of plantain mixed pastures: greater DM yield and quality of herbage during summer and autumn periods compared to RWC pasture.

5.2.2 The Effects of Plantain on N_2O Emissions

Following the results presented in Chapter 3, Chapter 4 described the field experiments conducted to evaluate N_2O emissions from RWC, 30% plantain and 50% plantain mixed-pasture treatments when different urinary N loading rates were applied. The sward effect and urine effect of plantain on the reduction of N_2O emissions were observed in the current study. The urinary-N concentration and urine N loading rates reduced with an increasing proportion of plantain in the cow’s diet. The urea-N concentration was 38% lower in 50% plantain urine treatment than in RWC urine, while higher proportion of N content in U50 type was in ammonia form than in RWC urine. The N and urea-N concentration excreted to urine is in close relationship with the dietary N supply (Kebreab et al., 2001). However, this study observed the similar herbage N content between RWC and plantain mixed pastures. Rodríguez-Gelós, 2020 showed a similar N intake for cows grazing pure plantain and ryegrass/white clover pastures. Therefore, the plantain including diet reduced the total N and urea-N concentration excreted to cow urine by increasing N partitioning into dung, improving rumen N efficiency and diluting N concentration in urine rather than reducing N intake.

Plantain urine reduced by 39% the total N_2O emissions through a lower urine N loading rate onto soil of 440kg N ha^{-1} (Table 4-3). The lower N loading rate from plantain urine resulted in 40% lower soil NH_4^+ concentration, but did not affect soil NO_3^- content during the first 16 DAA. After day 23, RWC urine with its higher N loading rate increased soil NO_3^- content by an estimated 30% compared to U50 treatment. The lower urine N rate was demonstrated as the key factor to reduce N losses from urine patches to the environment (Monaghan & De Klein, 2014; Podolyan et al., 2020). Therefore, this thesis highlighted that the efficacy of plantain on mitigating N_2O emissions is largely associated with the reduction of the urinary N content and subsequent N loading rate onto soils.

When the same urine type was applied, the cumulative N_2O emissions and EF_3 value from P50 treatment were lower by around 16% and 27%, respectively, than from the RWC treatment. The observed decreases in N_2O emissions and EF_3 value were probably associated with effects of plantain sward on soil N dynamics and microclimate. Luo et al. (2018) observed lower WFPS values in plantain pasture in winter on well-drained soil. However, in summer, the current work showed higher WFPS values in plantain mixed pasture treatments than in RWC (Figure 4-4). These results suggested that the effect of plantain on soil microclimate contributed to changes in N_2O emissions. Additionally, plantain mixed pastures did not affect soil NH_4^+ content but reduced approximately 35% NO_3^- content compared to RWC at the end of the trial. Some field experiment did not observe any effects of plantain swards on soil mineral N in spring and poor-draining soils (Rodríguez-Gelós, 2020; Simon et al., 2019). However, application of plantain leaf material and presence of plantain in pasture were demonstrated to reduce abundance of nitrifying bacteria and affect mineralisation and nitrification process resulting in lower NO_3^- content under laboratory condition or in well-drained soils (Carlton et al., 2019; Dietz et al., 2013; Verhagen et al., 1995). These results indicate that the effects of plantain swards on soil N dynamics and N_2O emissions vary depending on soil and weather condition. The effects of plantain swards on soil mineral-N and N_2O emissions are likely to be more obvious in well-drained soil condition.

Soil NO_3^- content in RWC at 105 DAA continued increasing following significant rainfall, whilst soil NO_3^- concentration in P30 and P50 treatments reached the background. The present study suggests that the rate of soil nitrification and denitrification processes in RWC were lower than in plantain mixed pastures through the experimental period. This is in part due to the positive relationship between WFPS values and the rate of nitrification and denitrification processes (Saggar et al., 2013). Therefore, the presence of plantain in swards can reduce N_2O emissions by affecting on soil N dynamics and transformations as well as soil microclimate via production of secondary metabolites and root growth behaviors.

The current work did not observe the effect of increasing plantain proportions in the diet and in the sward on N_2O emissions and EF_3 value. This result is in line with the earlier field study conducted under nutrient rich soil conditions by Pijlman et al. (2020). The lack of differences in N_2O emissions between P30 and P50 pasture treatments is likely associated with high fluctuations in the quantity of plantain over time under the large-scale field conditions, as highlighted in Chapter 3. The results from Chapters 3 and 4 suggest that including 30% plantain in the ryegrass/white clover pasture and in the cows' diets can obtain stability in plantain proportions across the growing seasons, while effectively reducing N_2O emissions from urine patches.

5.3 Limitations and Future Research

This field study assessed the botanical composition and the persistence of plantain mixed pastures over two growing seasons. However, further work evaluating stability of yield and long-term persistence of plantain mixed pastures in subsequent growing seasons is needed before promoting this pasture type widely as an alternative option to perennial RWC sward.

Poor persistence and low germination rate of plantain within established pastures are potential problems associated with the use of plantain mixed pastures, and these issues could limit the wider adoption of plantain by farmers. Therefore, it is important to assess optimal management options for maintaining plantain DM contribution in mixed pastures and encouraging the establishment of plantain in existing pastures grazed by cattle.

This thesis assessed N_2O emissions in mixed pasture treatments when the corresponding urine was applied. Future research is required to evaluate the effects of different proportions of plantain on N_2O emissions when the same urine N loading rate is applied. This knowledge can enable a comprehensive understanding of different plantain proportions in swards on the reduction of N_2O emissions.

Future research is needed to quantify more clearly the presence of plantain and secondary metabolites from plantain on soil microclimate, and on the rate of nitrification and denitrification processes. This knowledge can contribute to a better understanding of the sward effect of plantain on N losses across seasons and soil types.

This study demonstrated the effect of plantain on soil moisture content. Soil moisture content is a prerequisite for deciding the rate of denitrification and N_2O emissions. Future studies on the effects of plantain on water extraction patterns may be required. There is also a need to evaluate the effects of plantain mixed pasture on the other types of N losses to the environment, such as nitrate leaching and ammonia emissions.

5.4 Main Conclusions

This thesis determined the most efficacious proportion of plantain in mixed pasture for achieving a stable proportion across two growing seasons and persistence into the third growing season, while mitigating N_2O emissions from dairy cow urine patches relative to RWC pasture under field conditions. This thesis showed that:

- the highest contribution of plantain to pasture yield was 50% of DM in pastures mixed with RWC during late summer and autumn, even when plantain was sown with a seed mixture for 70% plantain.
- plantain density increased by approximately 25% at the end of the first growing season. In the second year, plant populations of plantain steadily decreased over time.
- the inclusion of plantain in mixed pastures increased DM production and quality of pasture relative to RWC pastures during summer and autumn, when the survival rate of ryegrass decreased under water stress conditions.
- proportions of 30% and 50% plantain mixed with RWC with additional plantain seed broadcast in autumn provided a stable proportion of plantain over two years, and the plantain persisted to the third year.
- increasing plantain proportions in cows' diets reduced the N and N-urea concentration in cow urine linearly.
- both the application of urine collected from cows grazing plantain and the presence of plantain in pastures had positive effects on the reduction of N_2O emissions from urine patches.
- diet effect of plantain resulted in reduction of N_2O emissions by lower urinary-N content and subsequently lower N loading rate onto soil.
- plantain swards possibly affected N_2O emissions through plantain-soil interactions and changes in soil microclimate.
- more than 30% of plantain in mixed pasture effectively reduced the urinary N concentration, total N_2O emissions and EF_3 compared to RWC pastures.

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6 Appendix

Table 6-1 Botanical composition (% of DM) of pasture species in plantain mixed pasture over 2019/2020 and 2020/2021 growing seasons.

Pasture species	The growing season	Season	Ryegrass/ white clover	30% Plantain	50% Plantain	70% Plantain	*P*-value
Ryegrass	2019/2020	Spring	77.01(3.41)	66.15(7.00)	53.95(6.15)	43.33(5.84)	0.005
		Summer	64.26(3.54)	46.98(4.19)	46.10(3.51)	37.86(3.75)	0.001
		Autumn	30.53(2.31)	19.83(4.39)	13.72(2.21)	11.91(2.39)	0.002
		Winter	56.33(3.47)	29.16(3.07)	20.22(1.86)	29.81(3.78)	< 0.000
	2020/2021	Spring	62.58(3.25)	37.63(4.44)	21.39(1.74)	35.85(2.01)	< 0.000
		Early summer	67.32(3.25)	38.04(3.00)	26.72(1.74)	31.21(5.23)	< 0.000
		Summer	49.78(3.58)	35.32(2.50)	30.98(4.57)	33.46(4.31)	0.012
		Early autumn	45.97(4.84)	27.48(5.53)	19.51(3.48)	24.39(3.56)	0.003
		Autumn	47.35(4.19)	33.33(2.95)	27.88(1.54)	-	0.002
White clover	2019/2020	Spring	1.58(0.46)	3.37(0.88)	2.26(0.47)	1.88(0.55)	0.224
		Summer	7.12(1.68)	5.97(1.73)	6.51(1.63)	4.38(1.47)	0.674
		Autumn	10.72(1.56)	9.06(3.44)	9.90(2.42)	9.98(1.80)	0.970
		Winter	7.54(2.17)	7.25(1.55)	9.51(3.38)	7.06(1.59)	0.865
	2020/2021	Spring	10.39(2.45)	11.15(3.42)	11.02(2.97)	11.78(0.82)	0.985
		Early summer	10.96(1.30)	12.44(1.99)	9.75(1.55)	18.52(4.45)	0.130
		Summer	18.96(2.95)	13.10(0.95)	16.16(1.17)	17.44(1.64)	0.189
		Early autumn	13.27(3.73)	15.71(3.62)	16.70(1.77)	13.96(1.73)	0.826
		Autumn	17.66(2.36)	16.59(0.38)	19.67(1.41)	-	0.412
Plantain leaves	2019/2020	Spring	-	13.59(4.23)	25.69(3.75)	40.97(6.70)	0.008
		Summer	-	13.32(1.65)	14.15(1.45)	16.92(2.05)	0.341
		Autumn	-	26.61(4.17)	26.76(3.29)	33.34(1.04)	0.258
		Winter	-	39.95(6.65)	50.95(1.65)	49.30(4.60)	0.251

	2020/2021	Spring	-	33.89(4.16)	54.80(3.25)	42.00(2.51)	0.003
		Early summer	-	34.68(2.52)	36.78(2.15)	30.24(5.11)	0.432
		Summer	-	22.03(1.08)	24.37(2.04)	21.17(3.46)	0.631
		Early autumn	-	22.38(2.59)	25.73(2.63)	24.98(3.38)	0.698
		Autumn	-	31.17(3.94)	36.25(1.93)	-	0.280
Plantain stems	2019/2020	Spring	-	-	-	-	-
		Summer	-	5.46(2.75)	4.68(1.03)	11.99(3.97)	0.179
		Autumn	-	0.52(0.34)	3.26(1.74)	2.89(1.14)	0.273
		Winter	-	-	-	-	-
	2020/2021	Spring	-	0.52(0.52)	1.17(0.5)	1.04(1.04)	0.804
		Early summer	-	9.19(3.05)	20.26(2.88)	14.69(1.58)	0.033
		Summer	-	19.93(3.55)	19.65(4.42)	17.10(1.98)	0.818
		Early autumn	-	7.73(2.46)	9.71(1.54)	11.29(3.61)	0.651
		Autumn	-	-	-	-	-
Other grasses	2019/2020	Spring	4.37(2.45)	7.10(3.23)	7.50(3.25)	5.59(1.49)	0.836
		Summer	2.05(0.75)	1.61(0.46)	3.47(0.80)	2.89(1.09)	0.388
		Autumn	2.28(0.53)	0.66(0.20)	0.73(0.34)	0.41(0.18)	0.005
		Winter	2.88(1.21)	1.29(0.87)	1.69(0.90)	0.47(0.41)	0.320
	2020/2021	Spring	1.01(1.01)	3.58(1.09)	3.27(1.24)	0.61(0.50)	0.113
		Early summer	1.10(0.77)	0.59(0.45)	1.99(1.08)	1.24(0.97)	0.712
		Summer	0.92(0.57)	0.35(0.35)	1.11(0.98)	1.48(1.26)	0.824
		Early autumn	1.26(0.92)	1.46(0.90)	0.40(0.37)	0.93(0.72)	0.773
		Autumn	1.87(0.81)	1.75(0.90)	3.75(2.12)	-	0.546
Weed	2019/2020	Spring	0.19(0.15)	0.27(0.27)	0.19(0.19)	0.24(0.17)	0.991
		Summer	0.08(0.08)	0.36(0.36)	0	0	0.483

		Autumn	0	0	0	0	-
		Winter	1.07(0.94)	0.03(0.03)	0	0.40(0.31)	0.413
	2020/2021	Spring	3.16(2.56)	0.17(0.08)	0.39(0.25)	0.23(0.23)	0.322
		Early summer	2.40(0.50)	0.37(0.22)	0.46(0.16)	0.27(0.19)	< 0.000
		Summer	1.28(0.32)	0.74(0.21)	0.21(0.12)	1.75(0.75)	0.098
		Early autumn	0.55(0.36)	1.15(0.13)	1.45(0.54)	1.66(0.73)	0.435
		Autumn	7.44(2.09)	0.83(0.37)	1.39(0.51)	-	0.005
Dead materials	2019/2020	Spring	15.83(1.57)	9.53(1.15)	10.41(1.73)	7.99(1.71)	0.14
		Summer	22.97(3.53)	26.31(1.98)	25.08(1.69)	28.91(5.61)	0.765
		Autumn	52.82(2.94)	43.32(2.05)	45.63(2.67)	41.48(0.83)	0.014
		Winter	26.72(1.17)	22.33(4.07)	17.63(2.07)	12.98(1.43)	0.007
	2020/2021	Spring	18.30(1.04)	13.06(1.29)	7.96(1.01)	8.48(1.38)	< 0.000
		Early summer	13.06(1.27)	4.68(0.77)	4.04(0.28)	3.83(0.74)	< 0.000
		Summer	10.91(1.53)	8.54(1.95)	6.78(1.32)	7.60(1.31)	0.300
		Early autumn	38.95(3.17)	24.09(1.59)	26.52(3.16)	22.80(4.43)	0.011
		Autumn	24.35(2.51)	16.33(1.96)	11.05(1.33)	-	0.001

Note Numbers in parentheses show standard error of the mean for each treatment (*n* =5)