Investigating the impact of abiotic stresses on the growth and persistence of plantain (*Plantago lanceolata* L.) within grazed mixed pastures

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Abstract

The New Zealand dairy industry has identified plantain (*Plantago lanceolata* L.) as a tool to mitigate nitrogen losses from perennial ryegrass (*Lolium perenne* L.) based pastures, however reports of variable pasture plantain contents (dry matter basis) and poor persistence within grazed mixed pastures have led to industry concern over its viability. There is anecdotal evidence that the persistence of plantain within dairy pastures is negatively impacted by waterlogging and treading damage from livestock, however there is little scientific literature relevant to these issues. This research aimed to answer the following research question: What are the impacts of waterlogging and treading damage on plantain growth and survival within mixed dairy pastures? This thesis includes a series of experiments conducted between 2021 and 2024.

In a glasshouse, the performance of plantain under waterlogging was evaluated against perennial ryegrass. Plantain growth was particularly sensitive to waterlogging, however the survival of plantain under waterlogging suggested that plantain possesses some waterlogging tolerance. In contrast, perennial ryegrass proved to have an enhanced ability for coping with, and recovering from, waterlogging stress. These findings suggest that while waterlogging may cause limitations to plantain growth, there is no evidence to suggest that waterlogging alone could cause a major decline in plantain density within mixed pastures. Rather, waterlogging could undermine the ability of plantain to compete with perennial ryegrass in mixed pastures.

The impact of treading damage on plantain regrowth and survival was investigated in two field experiments. In a small-plot study, treading damage was simulated on separate plantain and perennial ryegrass pastures using a novel treading device. Damage by the treading device, in late spring, immediately reduced the herbage accumulation rate of both plantain and perennial ryegrass pastures by 30%, however neither plantain content nor density were reduced as a consequence of the damage. In a larger, two-year experiment, on a mixed plantain + perennial ryegrass pasture, early spring treading damage by dairy cows reduced pasture growth by 50% and 75% during the early spring periods of year one and two, respectively. Plantain content tended to be lower in damaged plots during early spring in both years, before recovering throughout summer, although this effect was more pronounced during year one. Treading damage caused a reduction in plantain shoot yield (leaf + petiole) and density in year one, however the negative effects of the damage on plantain growth were short-lived and subsided throughout the first year of the experiment. Treading damage in early spring in year two did not significantly impact plantain content or density. An observed reduction in plantain shoot density in late spring in year two was possibly caused by shading from perennial ryegrass. Taken together, these results suggest that treading damage in early spring is unlikely to be a primary cause of plantain content decline within mixed pastures, although it might contribute to temporary reductions in plantain content during spring, particularly in newly established swards. It is possible that treading damage could reduce the ability of plantain to compete with perennial ryegrass in mixed pastures.

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Chapter 1 Introduction

1.1 Background

The loss of nitrogen (N) from grazed pastoral systems poses a significant threat to ground and surface water quality and has the potential to contribute substantially to agricultural greenhouse gas (GHG) emissions (Cameron et al. 2013). The level of N in New Zealand waterways has increased rapidly over the past few decades, coinciding with the intensification of a large proportion of low-producing pastoral land into highly productive dairy systems. Data from Land and Water Aotearoa (LAWA) shows that the proportion of monitored pasture catchment sites exceeding national guidelines for water N concentration (which was 0.44 mg/L) in New Zealand waterways increased from 40% in 1990, to 65% in 2011 (Joy 2015). Excess nutrient flows within waterways pose significant threats to present ecosystems as oxygen is often depleted to the level where most life forms are lost (Joy 2015). Additionally, the pollution of human drinking water resources with N is of major concern, with an estimated 15% of New Zealanders drinking water with potentially hazardous nitrate (NO_3^-) concentrations (Schullehner et al. 2018; Richards et al. 2022).

The N content of a typical New Zealand perennial ryegrass (*Lolium perenne* L.)/ white clover (*Trifolium repens* L.) dairy pasture far exceeds the N requirement of grazing animals (de Klein et al. 2020). It is estimated that around 60-90% of the N ingested by grazing livestock is excreted and returned to the soil, primarily in the urine (Haynes & Williams 1993; Cameron et al. 2013), which is concentrated in small patches ($0.4m^3$) (Moir et al. 2011). Pasture plants are only able to assimilate a small fraction of this N before it is lost to deeper parts of the soil profile, and eventually to freshwater systems as NO_3^-, or to the atmosphere as nitrous oxide (N_2O) (Cameron et al. 2013).

The potential impact of rural and urban land use on the natural environment has been of increasing societal concern in New Zealand, prompting the proposal of new national regulations for nutrient losses from dairy farming (Doole et al. 2021). Recent economic analysis (Doole 2019) has shown that the proposed N-loss targets will likely lead to reduced production and profit for the dairy sector if no low-cost, N-loss mitigation strategies are identified.

One potential low-cost tool is the inclusion of plantain (*Plantago lanceolata* L.) in perennial ryegrass based pastoral systems. Losses of N from dairy pastures have been reduced with the inclusion of plantain (Luo et al. 2018; Rodriguez et al. 2020). Navarrete et al. (2018) found that under dairy grazing during autumn, the quantity of NO_3^- leached from plantain and plantain + clover pastures was 90 and 85% lower respectively than from perennial ryegrass + white clover pasture. Simon et al. (2019) showed that N_2O emissions were reduced linearly with increasing proportions of plantain in the sward. The N loss mitigation ability of plantain is attributed to a number of mechanisms, most of which, are associated with specific bioactive compounds found in relatively high concentrations in plantain plant tissue (Navarrete et al. 2016; Peterson et al. 2022).

However, there is anecdotal and scientific evidence that plantain does not persist well under common pasture management strategies (Ayala et al. 2011b) and has been generally considered as a short lived forage species (Kuiper & Bos 1992). Data from commercial farms in New Zealand suggests that high plantain contents (>30% on a biomass basis) are attainable in the first two years following pasture renewal but cannot be maintained (Dodd et al. 2019). This decline is a significant issue as the efficacy of plantain for reducing N losses from pastures is strongly associated with the proportion of plantain in the diet of cows (Minnée et al. 2020; Navarrete et al. 2022). Competition from other pasture species, treading damage, compaction and over-grazing are likely contributing factors to declines in plantain density in mixed pastures (Bryant et al. 2019).

There is also some evidence that plantain does not cope well in wet soil conditions which occur on many New Zealand pastoral farms during winter and early spring. Wet soil conditions may introduce additional stresses to plantain in the form of waterlogging, caused by prolonged periods of excessively high soil moisture content, or treading damage, which is caused by livestock grazing pasture while soil moisture contents are sufficiently high to reduce the structural resistance of soil (Climo & Richardson 1984). Previous findings from ecological studies are conflicting, with some having suggested that plantain is largely tolerant of waterlogging (van Tienderen 1992; Grimoldi et al. 2005) and treading damage (Sagar & Harper 1964), while others suggested that waterlogging (Van Groenendael 1985; Mook et al. 1989) and treading damage (Chappell et al. 1971; Blom 1979) are likely to cause plant death and reduce plantain population density. However, literature detailing the effects of wet soil conditions on plantain in an agricultural setting are limited. Furthermore, studies which have investigated the impact of wet soil conditions on plantain in grazed perennial ryegrass-based pastures are extremely scarce.

Given the importance the industry is placing on plantain for reducing N losses from dairy farm systems, it is therefore important that we consider the impact of wet soil conditions on the growth and survival of plantain within grazed perennial ryegrass-based pastures.

1.2 Research questions

1. Is waterlogging likely to impact the growth and survival of plantain within mixed dairy pastures?
2. Does treading damage by grazing livestock impact the growth and survival of plantain within mixed dairy pastures?
3. Is there likely to be an impact of an interaction between waterlogging and treading damage on plantain growth and survival within grazed dairy pastures?
4. Are there any effects of an interaction between waterlogging, treading damage and other stresses which occur within mixed dairy pastures on plantain growth and survival within these pastures?

1.3 Thesis structure and experimental chapters

The thesis is structured according to a PhD by publication format.

Chapters 1 and 2 include an introduction to the topic, a review of current literature relating to the research objectives and identifies current knowledge gaps.

Chapter 3 investigated the impact of waterlogging on plantain and perennial ryegrass growth and physiology in a glasshouse.

Chapter 4 relates to two field studies that investigated the impact of treading damage on plantain regrowth and survival within plantain-dominant and perennial ryegrass-dominant pastures, as well as the productivity of those pasture types. Additionally, a novel technique for simulating treading damage on pasture is evaluated.

Chapter 5 is a methods chapter which covers the development and assessment of a novel treading technique for simulating treading damage on pasture.

Chapter 6 covers both years of experimentation from the latter of the two studies mentioned in chapter 4. This chapter investigates the long-term impact of treading damage on plantain growth and survival within a plantain + perennial ryegrass pasture and also the productivity of that pasture. Evidently there is considerable overlap in data between chapters 4 and 6.

Chapter 7 is concerned with the growth and survival of individual plantain plants within a plantain + perennial ryegrass pasture over two years following treading damage from dairy cows during early spring in both years. Some of the data presented and discussed in this chapter has been repeated from chapters 4 and 6.

Chapter 8 is a general discussion of research findings and considers the implications of those findings for the effective management of grazed pastures that include plantain.

Chapter 2 Review of literature

2.1 Plantain (*Plantago lanceolata* L.) agronomy

Plantain (*Plantago lanceolata* L.) is a perennial herbaceous species that has successfully colonised grasslands throughout the temperate world (Stewart 1996). Also known as English plantain, narrow-leaf plantain, ribwort plantain and ribgrass, plantain is a widely distributed throughout Europe, northern Africa, South Africa, West Asia, North and South America, Canada and Australasia (Sagar & Harper 1964). Plantain is one of the world's 12 most successful colonising weed species (Grime et al. 1989). Its natural habitat ascends to 2320m in the Bernina alps (Sagar & Harper 1964) and it is commonly found on riverbanks (van Tienderen 1992) and sand dunes (Page et al. 1985). Some plantain genotypes exhibit a potential lifespan of more than 12 years (Grime et al. 1989).

Morphology of the shoot

Commercially bred plantain has erect, broad leaves that extend from a rosette, produced from a crown (Stewart & Charlton 2006). Plantain has pronounced ribs or veins, with 3-5 veins per leaf (Sagar & Harper 1964). Early breeding strategies selected types with larger, more erect growth habits than its relative broad-leaved plantain (*Plantago major* L.) (Stewart 1996; Rumball et al. 1997). Grazing strategies can induce plastic changes between erect and prostrate shoot growth, which is thought to depend largely on the height of surrounding, competitive plants (Sagar & Harper 1964). Dense swards induce erect growth via linear lanceolate leaves, while close grazing results in the formation of prostrate rosettes with short ovate leaves (Grime et al. 1989). The erect leaves are typically narrowed gradually to the leaf base, where they meet a petiole (Sagar & Harper 1964). Recently bred cultivars such as Grasslands Lancelot were bred for their prolific tillering capabilities under close grazing by sheep (Rumball et al. 1997). Plantain produces erect reproductive stems with flowers throughout spring and summer, which are both wind-pollinated and frequented by insects (Grime et al. 1989). Plantain seeds have no well-defined dispersal mechanism (Grime et al. 1989) and anecdotal evidence suggests seeds may survive animal ingestion. Plantain also forms a buried seed bank (Grime et al. 1989). Additionally, plantain reproduces vegetatively through new buds, which can form ramets (secondary shoots), which eventually become new plants. While plantain has a limited capacity for vegetative spread, it possesses much more flexibility for sexual reproduction (Grime et al. 1989). Figure 2-1 shows the important anatomical features of plantain. This diagram provides a reference for all plant structures mentioned in this thesis.

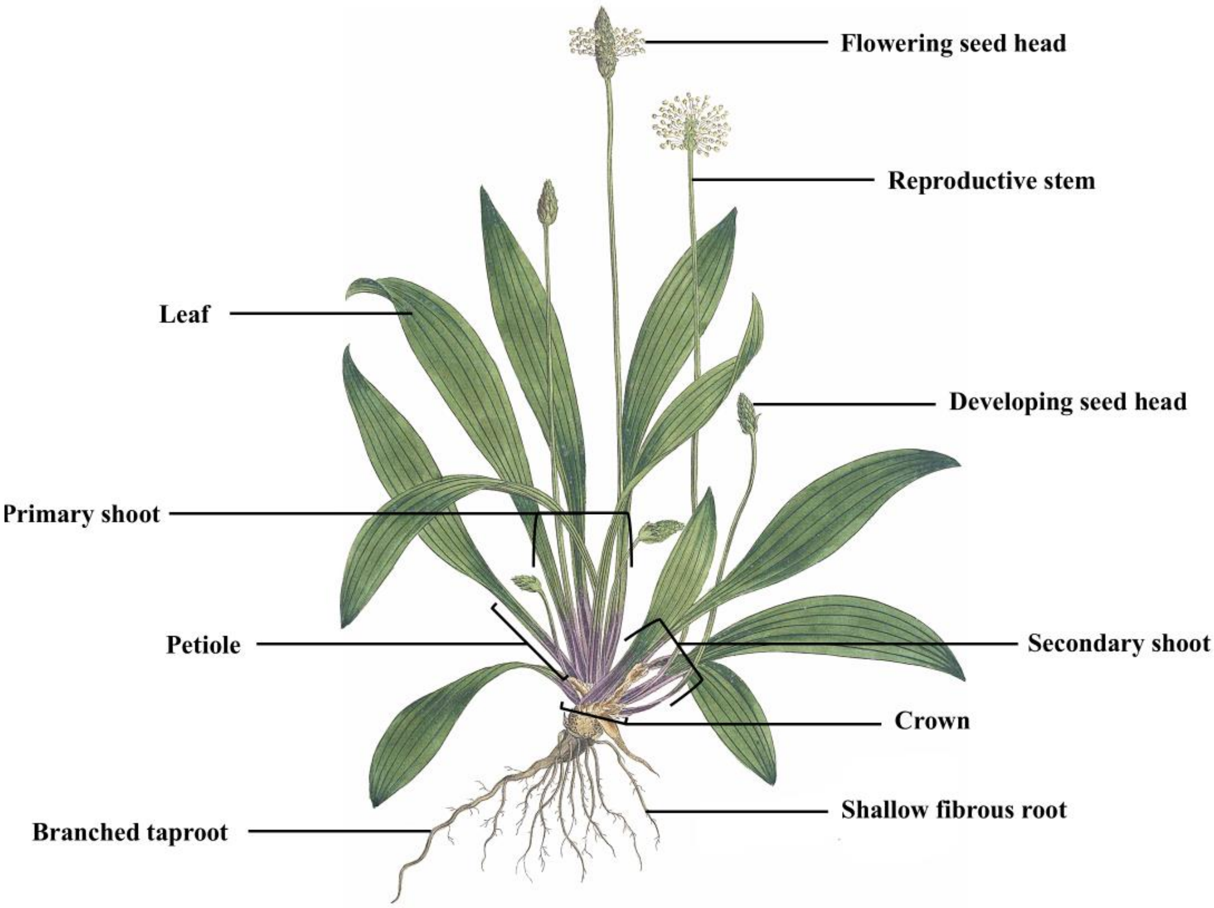

Figure 2-1. *Plantago lanceolata* L. morphology. Drawing taken from Curtis (1777). Anatomical labels have been added by the current author.

Morphology of the root

The taproot of plantain is smaller than that of chicory (*Cichorium intybus* L.) (Yu et al. 2008; Cranston et al. 2015a), with the lateral roots of plantain forming a fibrous network (Cranston 2014; Cranston et al. 2016). Pastures containing plantain typically out-perform ryegrass-based pastures over the summer period (Kemp et al. 2010b), likely due to its roots being able to access water deeper in the profile (Grime et al. 1989). Plantain possesses some degree of plasticity with regards to root structure in response to abiotic stresses. Plantain root structure may change in response to waterlogging, with an increase in the production of specialised adventitious roots (Banach et al. 2012) and potentially the formation of aerenchyma within the root cortex (Mook et al. 1989; Grimoldi et al. 2005; Striker et al. 2007; Banach et al. 2012). Plantain is capable of regeneration from root fragments, although the importance of this in the field is not clear (Grime et al. 1989).

Soil requirements

Plantain exists naturally over a wide-range of soil types, textures, acidities and organic matter contents (Grime et al. 1989; Stewart 1996). It is largely considered tolerant of mild soil moisture deficits and is unsusceptible to most common pasture pests (Stewart & Charlton 2006). It is a natural resident of low fertility grasslands (Grime et al. 1989) due in part to its adaption to low phosphorus (P) and potassium availability (Troelstra & Brouwer 1992). For this reason, plantain has previously offered the greatest contribution to pasture-based animal diets in low fertility dryland swards (Grime et al. 1989; Stewart 1996). In contrast, a recent study (Dodd et al. 2000) found greater plantain contents within hill country pastures on high fertility (Olsen P >30) slopes than low fertility slopes, suggesting than plantain contribution to dry matter (DM) could be increased with an increase in soil P availability.

Establishment

The establishment of plantain is generally considered rapid (Charlton & Stewart 1999), with emergence nearing that of perennial ryegrass (Blom 1978). Plantain generally establishes well when sown at 3-5kg/ha in pasture mixes, but perennial ryegrass sowing rates need to be low (<10kg/ha) in these mixes to prevent it out-competing plantain early in establishment (Kemp et al. 1999). Plantain requires sufficient thermal time to develop a minimum of six leaves before the first grazing, to build up root reserves for survival post-grazing (Powell et al. 2007). It is partly because of this that plantain requires a longer time to the first grazing than perennial ryegrass (Stewart 1996).

Since conventional cultivation and drilling methods are relatively costly, farmers have opted for single pass practises such as direct drilling or broadcasting to introduce plantain into their systems. The latter options have resulted in variable rates of success for plantain establishment on-farm and thus have become the focus of some research. Plantain establishment has been found to be improved following direct drilling, in comparison with broadcasting methods (Glassey et al. 2013; Bryant et al. 2019). In a study by Glassey et al. (2013), plantain plant densities and DM growth in the first 201 days were improved following the spraying-out of existing pasture with herbicide and direct drilling, in comparison with not spraying and broadcasting. Those results also agree with findings by Bryant et al. (2019) who found that direct drilling was more effective for establishing plantain following grazing than broadcasting plantain seed before or after grazing. That experiment also showed that grazing the pasture, above the height of seedlings, three weeks after drilling improved plantain establishment by reducing competition for light from other pasture species. However, it is suggested that paddocks are treated with a molluscicide prior to direct drilling plantain, since plantain emergence may be severely impacted by slug herbivory (Ferguson et al. 2019), which is usually mitigated by cultivation (Barker 1986).

Varieties

The early varieties, developed before 1996, were selected from productive wild types. Grasslands *Lancelot*® was selected by AgResearch Grasslands from productive North Island pastures based on its high productivity and ability to tiller well under close grazing by sheep (Rumball et al. 1997). Ceres *Tonic*® was selected from germplasm thought to have originated in Portugal and was chosen based on its increased erectness and very large leaves (Stewart 1996). *Tonic*® remains erect under a wide range of conditions, while *Lancelot*® is more likely to form a prostrate growth habit (Stewart 1996). Later, the cultivar *Agritonic*® was developed as a more agronomically suitable cultivar as its improved tolerance of 2,4-D type herbicides allowed for a better control of broadleaf weeds in swards containing plantain (Shrivastav et al. 2023).

Currently there several plantain cultivars on the market in New Zealand, although the number of cultivars that have been scientifically proven to provide N-loss mitigatory effects ("Ecotains") are limited (Judson et al. 2018). These cultivars include *Toni*c® and *Agritonic*®, as well as other breeding material that has genetic relationships with *Tonic*® and *Agritonic*® (Judson et al. 2018).

2.2 Plantain productivity and animal performance

Herbage production and seasonality

Plantain is capable of producing similar herbage yields to perennial ryegrass and white clover pastures, when sown as a pure stand. Powell et al. (2007) found that in Manawatu, plantain (cv. *Tonic*) produced 17t DM/ha/year, while in dryland Canterbury plantain (cv. *Tonic*) produced 8.4t DM/ha/year (Stewart 1996). Although plantain may produce at least 7.5t DM/ha/year (cv. *Lancelot*) in the first year in a mixed sward (Rumball et al. 1997), plantain is likely to contribute only a small proportion (5-15%) of the total DM yield after three growing seasons, if persistent grasses and clovers are present (Stewart 1996; Dodd et al. 2019).

Plantain typically produces the greatest proportion of its total annual DM yield over the summer months (Cranston 2014). Owing to the moderate drought and heat tolerance of plantain, pastures that contain plantain commonly out-perform perennial ryegrass pastures over the summer period (Kemp et al. 2010b). Plantain generally exhibits lower winter activity than common New Zealand pasture species, although there are differences between cultivars (Stewart 1996).

Nutritive value

Compared with perennial ryegrass plantain foliage has a lower proportion of cell wall, less cellulose, less neutral acid detergent fibre, less crude protein, less water-soluble carbohydrate and more lignin. (Stewart 1996; Navarrete et al. 2022). The palatability of plantain is contested. In some studies, it was found to be highly palatable to cattle and sheep in mixed swards (Milton 1933), but more recent work has suggested that plantain may be less palatable than chicory and white clover (Fraser & Rowarth 1996). Cave et al. (2015) found that ewe lambs preferentially grazed plantain and chicory during early spring, when given the choice between plantain, chicory, red clover (*Trifolium pratense* L.) and white clover. Pain et al.

(2015) also found that lambs consumed plantain at a similar rate to perennial ryegrass in a feed-stall experiment. That study also showed that plantain was much more acceptable to stock during spring than summer. The quality of plantain forage has been reported by some to be difficult to maintain over the summer months, particularly where it is established as a pure stand (Stewart 1996). Additionally, once plantain herbage yield reaches a maximum point older leaves begin to senesce, reducing the palatability and digestibility of plantain feed (Ayala et al. 2011b). However (Cave et al. 2015) found that ewe lambs displayed no preference for plantain swards when given the choice between plantain that had been grazed 3, 6, 10 or 16 weeks prior, during late summer. That suggests that palatability of plantain was not affected by an accumulation of older leaf material in infrequently grazed swards.

It is important to note that summer conditions (low soil moisture content, high temperature) that induce a drop in the forage quality of perennial ryegrass + clover mixtures, don’t necessarily cause a loss of plantain forage quality. In a study by Navarrete et al. (2022) the metabolisable energy content of plantain pasture was higher than that of perennial ryegrass pasture during late summer, due to the lower fibre content and greater non-structural carbohydrate content of the former during that period. However, management practices such as grazing rotation length will have a significant impact on the forage quality of plantain during this period, since older leaves are generally less palatable to stock (Stewart 1996; Ayala et al. 2011b).

Animal performance

The milk production of dairy cows grazing mixed pastures that contain plantain is equal to or greater than the milk production of cows grazing perennial ryegrass + white clover pasture (Totty et al. 2013; Navarrete et al. 2020). There are contrasting animal performance results where plantain is the dominant species a pasture mix (Fraser & Rowarth 1996; Moorehead et al. 2002; Golding et al. 2008), which could largely be dependent on the ability of the forage to provide adequate levels of protein for growth and milk production. This may be because of poor fertility soils or the reduction in nutritive value associated with the production of reproductive plantain stems over the summer period (Fraser & Rowarth 1996; Stewart 1996). Experiments have shown that plantain + clover pastures can support higher live weight gains in weaned lambs than perennial ryegrass + white clover pastures (Moorehead et al. 2002; Golding et al. 2008; Kemp et al. 2010a).

There have been suggestions of plantain possessing anthelmintic properties (Rumball et al. 1997; Cooledge et al. 2022). While there is limited evidence to confirm these claims, it is possible that the iridoid glycosides aucubin, catalpol and acteoside may contribute to this effect, as well as the upright structure of plantain swards limiting the migration of parasite larvae through the plant profile, and reducing parasite survival (Alomar et al. 2018). The plantain cultivar *Tonic*® has also been shown to reduce the prevalence of dags in lambs (Turner 1999).

Management and use

In dairy production systems, plantain has typically been included as a component of a ryegrass-based pastures because of its ability to increase summer forage quality and herbage production (Dodd et al. 2022). Dairy pastures are usually rotationally grazed down to a set herbage mass to maximise animal intake and pasture DM accumulation. This system is often employed by dairy farm managers year-round, meaning that pastures are grazed intensively, even during late winter, during and post-calving. Plantain is usually a small component of these pastures, typically contributing between 5-30% to total pasture biomass (Stewart 1996).

New Zealand sheep and beef finishing systems are likely to use plantain as a specialist forage crop for maximising live weight gains of terminal stock (Cranston et al. 2015b). The plantain in these crops is usually accompanied by red or white clover and sometimes chicory. It is normally rotational grazed or set-stocked. Plantain + chicory + clover pastures have been shown to increase milk production in ewes and liveweight gain in ewes and lambs during spring, in comparison with perennial ryegrass + white clover pastures (Hutton et al. 2011), indicating that it is suitable for lambing ewes onto.

2.3 The effects of plantain on the dairy system

Nitrogen losses from dairy production systems

The major source of N loss from dairy pastures is the urine patch (Di & Cameron 2007; Cameron et al. 2013; Ravera et al. 2015; Luo et al. 2018; Judson et al. 2019; Simon et al. 2019). The N content of a typical New Zealand dairy pasture far exceeds the N requirement of grazing animals (de Klein et al. 2020). Around 60-90% of the N ingested by grazing dairy cows is excreted and thus returned to the pasture (Haynes & Williams 1993; Cameron et al. 2013). This excess N is returned to the soil primarily in the urine (Cameron et al. 2013), which is concentrated in small patches ($0.4m^3$) (Moir et al. 2011). Within the urine patches of high-producing dairy pastures, the urea loading rate may be between 700-1200kg N ha^{-1} (Cameron et al. 2013). Pasture plants are only able to assimilate a fraction of this N (usually less than 600kg N ha^{-1} (Moir et al. 2007)) before it is lost to deeper parts of the soil profile as NO_3^- or to the atmosphere as N_2O (Cameron et al. 2013).

Nitrate leaching from dairy production systems

NO_3^- leaching losses from the soil solution to ground water represent both a loss of soil fertility as well as a threat to the natural environment and to human health (Cameron et al. 2013). NO_3^- leaching and subsequent degradation of natural and recreational water bodies in New Zealand has been closely associated with the dairy sector (Box et al. 2017; Doole et al. 2021). Regional councils have implemented regulatory limits for NO_3^- leaching from agricultural land as a consequence.

The amount of NO_3^- loss from a given soil depends on the concentration of NO_3^- in the soil solution, as well as the amount of drainage that occurs within a period of time (Cameron et al. 2013). NO_3^- is produced through the process of nitrification, which is the conversion of ammonium to NO_3^- . The reaction occurs in two stages: The oxidation of ammonium to nitrite

(NO_2^-), which is mainly carried out by ammonia-oxidising bacteria (AOB) (1) and the oxidation of NO_2^- to NO_3^- , which is conducted by *Nitrobacter* (2). This reaction is typically rapid, and thus NO_2^- rarely accumulates in soil. The rate at which ammonium can be converted to NO_3^- is dependent on soil moisture content, pH, nutrient status, and temperature. The rate of nitrification slows when the soil temperature falls below 5°C, which typically happens during winter months in New Zealand.

NO_3^- has a negative charge and so is repelled by cation exchange sites (which also have a negative charge) (McLaren & Cameron 1996), causing it to remain in the soil solution and therefore in potential drainage water. NO_3^- leaching usually occurs when the amount of rainfall exceeds the amount of water used by plants, which in New Zealand usually occurs during late autumn, winter and early spring (McLaren & Cameron 1996). The uptake of NO_3^- during these periods is also low, and so NO_3^- may be present in large concentrations within the soil solution, exacerbating NO_3^- leaching potential. Dry summers also contribute to high a NO_3^- leaching risk because of reduced plant N uptake, leaving NO_3^- to be leached during the subsequent wet season. Soil texture and structure affect NO_3^- leaching potential, with poorly structured and/or sandy soils tending to pose a higher risk for NO_3^- leaching than poorly-drained, clay-based soils (Cameron et al. 2013). Recorded NO_3^- leaching losses from New Zealand dairy pastures have been as high 110kg N ha^{-1} $year^{-1}$ (Ledgard et al. 1996), but vary strongly with climate, soil properties and the level of N inputs.

Nitrous oxide emissions from dairy production systems

N_2O is an intermediary product of the conversion of NO_3^- to dinitrogen (N_2), a process also known as denitrification (Smith & Tiedje 1979). N_2O is an extremely potent GHG with a warming potential between 265 and 298 times greater than carbon dioxide (CO_2) (Myhre et al. 2013). N_2O is also an important depleting substance of stratospheric ozone (O_3) (Ravishankara et al. 2009). Agricultural soils are the primary contributor of N_2O emissions globally (Sutton et al. 2011). According to the New Zealand Ministry for the Environment, agricultural soil accounts for 94% of all N_2O emissions (MFE 2018). N_2O emissions have the potential to become significant in pastoral dairy systems where cows graze pasture in-situ, year-round (de Klein et al. 2001). N_2O losses make up 20% of New Zealand's annual greenhouse gas emissions currently, up from 15.7% in 1990 (Ministry for the Environment 2022). N_2O emissions from agricultural soils are the largest contributor to increased greenhouse gas emissions in New Zealand between 1990 and 2020, with a 48.7% increase, thought largely to be a result of increased applications of synthetic N fertilisers.

Biological denitrification occurs in poorly drained soils where there are anaerobic soil conditions, characterised by low oxygen availability and low redox conditions. Under such conditions, facultative anaerobic bacteria use NO_3^- as the terminal electron acceptor during respiration, instead of oxygen (Cameron et al. 2013). This causes NO_3^- to become reduced, producing in order; nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2). Although denitrification is generally greatest under anaerobic conditions, it can also occur in soils that are only periodically waterlogged, where it may occur inside soil aggregates with low oxygen availability (Cameron et al. 2013). Denitrification is usually highest in soils where there are high concentrations of NO_3^- in soil solution, an adequate supply of carbon and a temperature that facilitates adequate microbial activity (McLaren & Cameron 1996).

Plantain’s ability to reduce nitrogen losses from grazed pastoral systems

The inclusion of plantain in the diets of grazing ruminants, particularly dairy cows, has been shown to significantly reduce losses of NO_3^- to ground water and stifle N_2O emissions (Luo et al. 2018; Carlton et al. 2019; Simon et al. 2019). Plantain reduces N loss via multiple mechanisms: Plantain increases urine volume (1) (de Klein et al. 2020; Minnée et al. 2020; Navarrete et al. 2020) and reduces urine N concentration (2) (Totty et al. 2013; Box et al. 2017; Cheng et al. 2017; Simon et al. 2019; Navarrete et al. 2020), leading to a greater number of ‘dilute’ urine patches (animal effect). Plantain alters N use and partitioning within the grazing animal, leading to a greater proportion of N being partitioned to faeces, as opposed to urine (de Klein et al. 2020). Metabolites present in the plantain foliage consumed by livestock, are returned to the soil in urine, where they slow the nitrification process (3) (metabolite effect) (Judson et al. 2018; Judson et al. 2019; Gardiner et al. 2020b, a). Plantain releases metabolites as root exudates that further suppress nitrification (4) (Luo et al. 2018; Carlton et al. 2019; Simon et al. 2019) (sward effect). It is likely that a combination of these effects provides the reduction in N losses observed in field-scale studies.

N losses from dairy pastures have been reduced with the inclusion of plantain (Luo et al. 2018; Rodriguez et al. 2020). Navarrete et al. (2018) found that under dairy grazing, the quantity of NO_3^- leached from plantain and plantain + clover pastures was 90 and 85% lower respectively, than from perennial ryegrass + white clover pastures. Similarly, (Rodriguez et al. 2020) showed that NO_3^- leaching was lower from plantain pastures than perennial ryegrass + white clover pastures in successive lactation seasons. Simon et al. (2019) showed that N_2O emissions were reduced linearly with increasing proportions of plantain in the sward. That author attributed the reduction to a combination of the lower N loading on soil (through a reduced urinary N concentration) as well as biological nitrification inhibition caused by the presence of plantain in the sward. Plantain pastures have been found to have lower N_2O

emissions than perennial ryegrass pastures, following an autumn application of cow urine (Luo et al. 2018).

Animal effects

Urine N concentration is reduced directly, by an increase in urine volume. Urine volume is regulated predominantly by the amount of water excreted within urine (Spek et al. 2012). The increase in the urine water volume of animals grazing plantain may be due, to an increase in water intake (Mangwe et al. 2019; Minnée et al. 2020), as well as the potential diuretic effects of plantain's unique metabolites or mineral composition (Cheng et al. 2017; de Klein et al. 2020). Navarrete et al. (2020) estimated that cows grazing plantain or plantain + clover pastures produced 22% and 24% more urine than cows grazing a perennial ryegrass + white clover pasture. Similar findings were demonstrated by (Minnée et al. 2020), where cows grazing a pasture with 45% plantain produced around 25% more urine than cows grazing a perennial ryegrass + white clover pasture, or pasture containing only 15-30% plantain. An increase in urine volume results in an increased urination frequency, (Mangwe et al. 2019; Minnée et al. 2020) leading to more urine patches per given area of pasture, effectively distributing urine N more uniformly over the paddock area.

There is evidence that the reduction in urine N concentration may also partly be a consequence of N being partitioned away from the urine, into dung, milk, or other places withing the animal (Totty et al. 2013; de Klein et al. 2020). Totty et al. (2013) suggested that an increase in the milk N output of cows grazing chicory and plantain, could explain a reduction in urine N concentration. Potential explanations for this could be an increase in the proportion of digestible rumen undegradable protein, the bioactivity of secondary metabolites, or an improved ratio of water-soluble carbohydrates to crude protein. Plantain may also enhance the partitioning of N into dung because of its condensed tannins content (de Klein et al. 2020). Misselbrook et al. (2005) showed that the feeding of supplements or forages with a high condensed tannins content, increased the amount of N excreted in the dung of dairy cows. The culmination of these effects is a reduction in the N loading rate within urine patches. Pasture plants are then capable of utilising a greater proportion of the incident N before it can be lost from the system.

Metabolite effect

Plantain foliage contains potent metabolites that act as biological nitrification inhibitors (BNIs), when added to the soil within urine from grazing animals (Gardiner et al. 2020b, a). The metabolites include the iridoid glycosides aucubin and catalpol and the phenylpropanoid glycoside, acteoside (Gardiner et al. 2016; Navarrete et al. 2016). Soil incubation experiments have shown that plantain leaf constituents, as well as synthetic aucubin, can potentially reduce N mineralisation and nitrification (de Klein et al. 2020; Gardiner et al. 2020a). Urine from sheep (Judson et al. 2018) and cattle (Judson et al. 2019) grazing plantain, have been found to have nitrification inhibitory properties. In a 35-day soil microcosm incubation experiment, nitrification rate was initially slower in microcosms treated with urine from plantain-fed cows, than those treated with urine from ryegrass, white clover-fed cows (Judson et al. 2019). The largest difference in total NO_3^- between the two treatments occurred 21 days after urine application. Similarly, Gardiner et al. (2020b) showed a brief reduction in soil nitrification rate following the application of cow urine, with a synthetic aucubin component, to a perennial ryegrass + white clover pasture.

Field-scale data showing a reduction in nitrification rate, NO_3^- leaching or N_2O emissions, exclusively as a result of aucubin presence in cow urine, is limited. Simon et al. (2019) compared the N_2O emissions from soils with a perennial ryegrass + white clover pasture that received urine from cows with a diet comprising of either 45% plantain or no plantain. The

N_2O emissions and N_2O emission factors were similar for each treatment, indicating that there was no significant effect of the secondary metabolites present in the urine. Gardiner et al. (2020b) also concluded that the inhibitory effects of aucubin applied to the soil are not sufficient to change overall, long-term, urine patch N_2O emissions. The contrast between soil incubation experiments and field trials may be a result of differences in soil moisture, soil pH or ammonia oxidising bacteria (AOB) (Gardiner et al. 2020b).

Sward effect

Plantain may release root exudates which also act as BNI's in the soil. In a small-plot study Simon et al. (2019) found that N_2O emissions were lower in grass-based swards with a higher plantain content following the application of a standard urine (610kg N ha^{-1}) derived from cows fed a perennial ryegrass + white clover pasture. Similarly, Carlton et al. (2019) found that NO_3^- leaching from lysimeters containing plantain, perennial ryegrass and white clover was significantly lower than from lysimeters containing only perennial ryegrass and white clover, following the application of standard urine (700kg N ha^{-1}) from cows grazing a perennial ryegrass + white clover pasture. It is currently unknown how large the influence of the 'sward effect' might be on N losses from actual grazed pastures, however results from these experiments suggest that it could be substantial.

Proportion of plantain in cows diet required to reduce nitrogen losses

Currently there is contention over the proportion of plantain required in a cow's diet to reduce N loses from grazed pastoral systems. One recent metabolism stall study (Minnée et al. 2020) found that cows consuming a diet with at least 30% plantain (on a DM basis) had lower urine N concentrations than cows consuming only ryegrass. Furthermore, cows consuming a diet comprising of 15% plantain had similar urine N concentrations to those grazing perennial ryegrass only. That suggests that cows may need at least 30% plantain in their diet for the full N loss mitigation abilities of plantain to be realised. However, given that a reduction in urine N concentration is one of several modes of action for plantain in reducing N losses from grazed pasture, the actual proportion of plantain required in the sward to reduce N losses might be less than 30%. In a field study (Nguyen et al. 2023), cows grazing a pasture with 25% plantain had a higher urine volume, lower urine N concentration and had more urination events than cows grazing a perennial ryegrass + white clover pasture. Additionally, cows grazing a pasture containing 18% plantain had a urine N concentration that was 18% lower than that of cows grazing a perennial ryegrass + white clover pasture. These results suggest that cows may need 25-30% plantain in their diet for the system to gain meaningful reductions in N losses, but lower proportions of dietary plantain may also have some mitigatory effect on N losses from pastures. It is not currently clear what proportion of plantain is required in the sward to reduce N losses via BNI at field scale.

2.4 The effects of the dairy system on plantain

Plantain persistence and grazing tolerance

There is anecdotal and scientific evidence that plantain does not persist well under grass-based pasture management strategies (Ayala et al. 2011b) and has been generally considered as a short lived forage species (Kuiper & Bos 1992; Labreveux et al. 2004). Data from commercial farms in New Zealand suggests that high plantain contents are attainable in the first years following pasture renewal but cannot be maintained (Figure 2-2) (Dodd et al. 2019). Of the paddocks older than two years in that study (36 out of 49), only three clover-based mixes and two grass-based mixes, maintained plantain contents of 30% or more.

Competition from other pasture species, treading damage, compaction and over-grazing are likely contributing factors to declines in plantain density in mixed pastures (Bryant et al. 2019). Grasses have a particular competitive advantage over plantain, owing to their rapid recovery following disturbances such as trampling, their competitive advantage for light through their efficient leaf morphology (Stulen et al. 1992) and their enhanced ability to respond to fertiliser applications (Stewart 1996).

Plantain persistence may be reduced following winter grazing (Ayala et al. 2011b) and usually struggles to compete for essential resources when paired with a winter-active grass species in a pasture (Cranston et al. 2015b). In work by Ayala et al. (2011b), plantain plots grazed twice by sheep during winter (July and August) had 32% fewer plants than un-grazed plots in March of the following year (Figure 2-3).

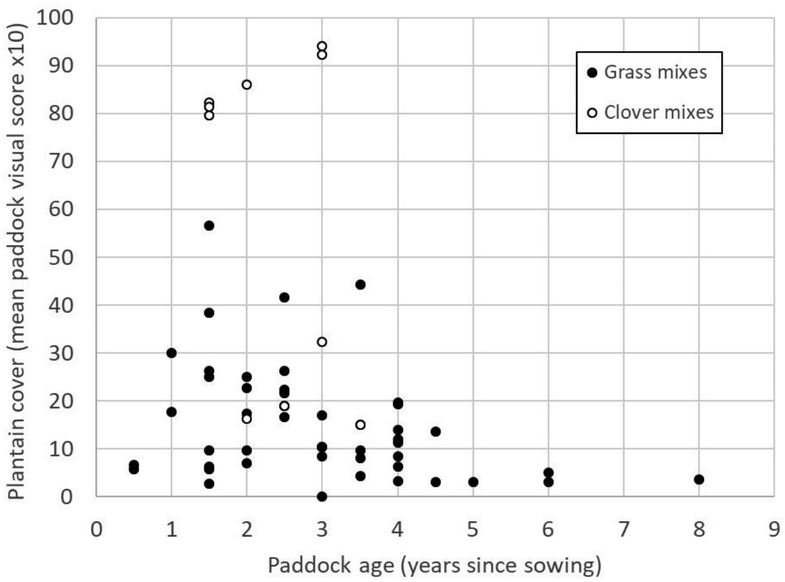

Figure 2-2. Plantain cover versus years since sowing for 49 grass-based mixes and 10 clover-based mixes, from (Dodd et al. 2019). Of the paddocks older than three years, only three clover-based mixes and two grass-based mixes had 30% or more plantain.

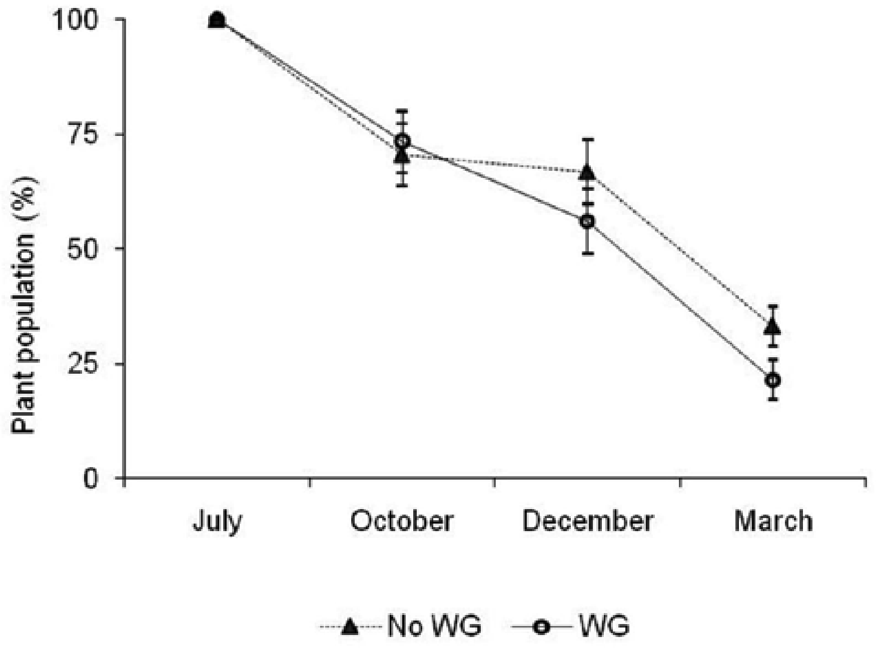

Figure 2-3. Change in plantain population density from July to March (expressed as a percentage of initial plant population in July (96 plants/m^2=100) under two winter grazing managements (Ayala et al. 2011b).

Plantain is susceptible to changes in the frequency (days between grazing events) and intensity (residual height) of defoliation (Ayala et al. 2011b). Plantain herbage production is generally promoted with a grazing interval of 21-35 days (Labreveux et al. 2004) and a residual height of 4cm (Cranston et al. 2015a), similar to that of perennial ryegrass (Lee et al. 2008). Plantain density is likely to be reduced more rapidly under close grazing however, with the compounding effects repeatedly reducing plant size and crown weight (Ayala et al. 2011b, a). The attributes of the root and crown system of plantain suggest there is limited capacity for carbohydrate storage in comparison with other crown forming species (Ayala et al. 2011b). This probably contributes to reduced regrowth and persistence under intensive grazing or other limiting conditions.

Competition with companion species

There is no shortage of anecdotal evidence when discussing the lack of competitiveness of plantain when incorporated in high-performing perennial ryegrass-based pastures. Some research has shown that the productivity of plantain is reduced following winter grazings (Ayala et al. 2011b) and therefore has particularly poor persistence when paired with a winter active species such as perennial ryegrass (Cranston et al. 2015b). In such mixes, plantain is also likely to encounter strong competition for light whenever conditions favour high perennial ryegrass growth rates, which typically occur between spring and early summer (Kemp et al. 1999).

Shading has been shown to have a significant effect on plantain morphology (Teramura 1983). Plantain plants found naturally growing in shaded environments have been shown to have fewer shoots than those plants growing in full sunlight (Teramura 1983). That work also showed that plantain plants exposed to high irradiance in a glasshouse had a greater leaf elongation rate than those under low irradiance. The reduction in the capacity of plantain plants to regrow under low-light conditions, such as those that occur beneath a perennial ryegrass canopy, would likely contribute to a reduction in plantain content in high-biomass grass swards. Additionally, the limited capability of plants to produce new vegetative shoots under such conditions could lead to a reduction in plantain shoot density over time.

Pest and disease tolerance

Plantain is generally considered tolerant of several typical New Zealand pasture pests and diseases. Some leaf-feeders have been found on plantain, including weevils, gall midges, flea beetles and moths. Dodd et al. (2024) reported that damage by plantain moth (*Scopula rubraria and Epyaxa rosearia*) is common in pure plantain swards in the northern regions of New *Zealand*. However, damage by caterpillars in late summer is usually largely aesthetic and presents no cause for concern. (Stewart 1996). Below ground herbivory has been shown to negatively impact juvenile plantain populations (35 days old or younger), whereas older plantain populations (45 days old) were not affected (Tsunoda et al. 2014). Early literature reported that plantain has good tolerance of the native New Zealand grass grub (*Costelytra zealandica*) (Cockayne 1920), however it is currently understood that this pest is extremely detrimental to plantain populations (Kelly & Foley 2019; Dodd et al. 2024). Older plantain plants may also exhibit infection from *Aschocyta* leaf spot, Ramularia *leaf spot*, and *Rhizoctonia* root rot (Stewart 1996; Kelly & Foley 2019).

Drought tolerance

Plantain has a good tolerance of soil moisture deficit (Cranston et al. 2016), thanks largely to its deep rooting structure being able to access water deep in the profile (Grime et al. 1989). Plantain also possesses a good degree of summer heat tolerance, evidenced by its distribution in some sub-tropical grasslands (Sagar & Harper 1964). There are multiple accounts of plantain improving summer feed quality and quantity, as well as increasing the live weight gain of lambs in comparison with lambs grazing perennial ryegrass-based pastures, in areas that are prone to seasonal soil moisture deficit (Moorehead et al. 2002; Macfarlane et al. 2015; Spall et al. 2019).

Cold tolerance

Plantain is not likely to persist in regions that typically encounter prolonged periods of winter freezing. In an experiment by Skinner and Gustine (2002), plantain was found to have extremely low survival rates when subjected to temperatures of -12 to -14 degrees Celsius. While this finding is significant for countries that may experience these conditions, low freezing tolerance is unlikely to be a major factor limiting the survival and persistence of plantain in New Zealand pastures. Plantain DM growth is usually reduced during winter in New Zealand, in comparison with other common pasture species (Stewart 1996), and the management of pure plantain swards usually reflect this (Powell et al. 2007; Ayala et al. 2011b). Nighttime temperatures of 11℃ have been shown to cause low daily leaf elongation rates in plantain (Teramura et al. 1981) suggesting that even in mild winters, plantain leaf growth is likely to be diminished as a direct result of low temperatures.

2.5 Waterlogging and livestock treading damage may be responsible for poor plantain persistence in New Zealand pastoral systems

2.5.1 Waterlogging

The availability of molecular oxygen is required to sustain the metabolism and growth of higher plants (Zhang et al. 2019). Waterlogging can be characterized by a depletion of soil oxygen caused by a high water-table or impeded drainage within the soil profile (McLaren & Cameron 1996; Irving et al. 2007). As a result of waterlogging, water fills soil pores and capillaries and reduces the normal processes of gaseous exchange between the soil and the atmosphere, leading to a net decrease in oxygen diffusion (Janiesch 1991). The oxygen concentration in waterlogged soil (around 10 ppm) is significantly lower than the oxygen concentration of the atmosphere (around 200,000 ppm) (Taiz et al. 2015). The immediate consequence is that the soil environment becomes anaerobic.

Effect of waterlogging on pastoral systems

There have been several studies that have investigated the impact of waterlogging on pasture grass growth and survival (Lee et al. 2013). Perennial ryegrass photosynthetic rates were reduced after three weeks of waterlogging (at 20°C), leading to a reduction in leaf and root biomass (McFarlane et al. 2003). Dunbabin et al. (1997) also showed a 14% reduction in DM production from a perennial ryegrass + white clover pasture frequently subjected to 24 hours of waterlogging, in comparison with the same pasture subjected frequently to 4 hours of waterlogging. Waterlogging could also lead to the death of some grasses and legumes. Woods et al. (1993) showed that typical pasture species such as perennial ryegrass, cocksfoot (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* S.), white clover may only survive total submergence in water for 6-9 days.

Pasture species vary in their tolerance of waterlogging and thus waterlogging has the potential to influence the botanical composition of a pasture. Work by Grieve et al. (1986) showed that the botanical composition of periodically waterlogged pastures in the Murray valley, Australia, favoured *Paspalum dilatatum* Poir. (a waterlogging tolerant grass) over the sown pasture species perennial ryegrass and white clover. Grasses have been shown to be more tolerant of waterlogging than legumes (Bolton & McKenzie 1946), suggesting that pastures that experience waterlogging may often have a reduced legume component.

Waterlogging effect on soil

There are a series of flow-on effects from waterlogging and the imminent reduction in oxygen concentration, including changes to the soil redox potential and availability of plant nutrients (McLaren & Cameron 1996). The first measurable event is the lowering of the soil redox potential, which when measured gives an excellent indication of the intensity of waterlogging (Janiesch 1991). The pH of most soils tends towards neutrality once conditions become anaerobic, with the pH of acidic soils increasing and the pH of alkaline soils decreasing. There are a number of phytotoxic compounds that may accumulate in waterlogged soil. Once all available oxygen and NO_3^- has been consumed, anaerobic bacteria use oxidized soil components such as hydrous oxides of manganese (Mn_4^+), iron (Fe_3^+) and sulfate, converting them to their counter forms Mn_2^+, Fe_2^+ and sulfide respectively (Janiesch 1991; McLaren & Cameron 1996). Since plant species vary in their ability to cope with these toxic compounds, waterlogging induced toxicity may be responsible for determining the botanical composition of some grasslands (Janiesch 1991).

Waterlogging effects on plants

Prolonged waterlogging can cause widespread damage to important plant structures and have profound effects on plant respiratory systems, leading to plant death (Turkan et al. 2013).

Anaerobic respiration within the plant leads to the production of ethanol, causing cellular and membrane injury, impairing nutrient uptake and enabling pathogen infection (Phillips & Greenway 1998). The initial effect of a lack of soil oxygen on plant morphology is usually a loss of root mass (Drew 1997). Pasture grasses cocksfoot, and prairie grass (*Bromus catharticus* V.) were shown to have a loss of root mass following defoliation, under waterlogging stress (Ploschuk et al. 2017). The loss of root mass is most likely to be a result of root tissue senescence, following a cessation of cellular respiration (Ploschuk et al. 2017).

The loss of leaf mass will almost certainly accompany a loss of root mass. In barley (*Hordeum vulgare* L.) seedlings subjected to three weeks of waterlogging, both shoot and root growth were negatively affected by waterlogging stress (Pang et al. 2004). Growth was probably inhibited further by a reduction in the photochemical capability of the shoot. Leaf biomass was reduced in *Paspalum* accessions under waterlogging, resulting from a lower leaf elongation rate during the waterlogging stress (Beloni et al. 2017). Perennial ryegrass plant height was significantly reduced under short-term (7days) submergence in water, reflecting a loss of leaf chlorophyll content and substantial reductions in the water soluble carbohydrate content of both shoot and root material (Liu & Jiang 2015).

One response of plants to waterlogging stress is a reduction in stomatal conductance through stomatal closure in leaves (Sojka 1992; Zhao et al. 2021). Stomatal closure is associated with an accumulation of abscisic acid (ABA) in leaves, which acts to impair the accumulation of potassium ions by guard cells, and thus prevent them becoming turgid enough to open stomata (Sojka 1992; Pirasteh-Anosheh et al. 2016; Pan et al. 2021). The role of ABA accumulation in leaves during conditions which lower plant water potential, such as drought are well researched (Pirasteh-Anosheh et al. 2016), and is commonly understood as a mechanism by which plants conserve water under such stresses (Taiz et al. 2015). However, the closure of stomata results in a reduction in leaf gas exchange with the atmosphere, which reduces the supply of CO_2 for use in the light independent reactions of photosynthesis (Baker 2008), which ultimately reduces carbohydrate production and thus the potential DM yield of plants (Sojka 1992).

Waterlogging stress increases the production of reactive oxygen species (ROS) such as super oxide (O^{2-}) and hydrogen peroxide (H_2O_2), which can cause lipid peroxidation (Mittler 2002). Plant physiological processes are adversely affected by waterlogging, including reductions in chlorophyll content, phytochemical efficiency and net photosynthetic rate (Zhang et al. 2019). The subsequent energy depletion causes reductions in protein synthesis, shoot and root growth and final yield (Pang et al. 2004). Anaerobic stress can cause cell death within hours or days (Taiz et al. 2015).

Plant photosynthesis (net CO_2 assimilation rate) may be reduced as a result of waterlogging induced ROS activity. The net CO_2 assimilation rate of flooding-intolerant brachiaria grass was significantly reduced after 14 days under flooded conditions (Dias-Filho & De Carvalho 2000). Similarly Zhang et al. (2019) found that 10 days of waterlogging had significantly reduced the net CO_2 assimilation rate and the photochemical efficiency (Fv/Fm) of lucerne seedlings. Those authors found evidence of cell lipid membrane peroxidation in waterlogged plants and suggested ROS are capable of breaking down chlorophyll and damaging chloroplasts. This most likely contributed to the loss of photochemical efficiency observed in the waterlogged lucerne plants. Photosynthetic rate was also reduced in lucerne varieties subjected to waterlogging in a glasshouse study (Irving et al. 2007). The authors noted reductions in the carboxylation efficiency and in the operating efficiency of photosystem II (ΦPSII) in the intolerant variety in comparison with the tolerant variety.

Plant morphological responses to waterlogging stress

Plant responses to waterlogging stress can be divided into two strategies: Avoidance (through the alteration of morphological structure to restore oxygen supply), or tolerance (typically through a physiological modification to improve function under limited oxygen supply).

The capability of plants to adapt to excess water conditions is vital for the survival of those plants growing in areas where frequent flooding stress may occur (Liu & Jiang 2015). Plants may use adaptive changes to improve gas exchange and maintain energy production to cope with the effects of waterlogging on root growth and development (Armstrong et al. 1994). Typical alterations to root morphology are an increase in the production of adventitious roots and, depending on the species, the formation of specialised air transport pores called aerenchyma (Pang et al. 2004; Mui et al. 2021). Pang et al. (2004) found that aerenchyma formation in some barley types, may have contributed to improved performance under waterlogging and Mui et al. (2021) found increased adventitious root and aerenchyma development in waterlogged tall fescue and cocksfoot plants.

Plant physiological responses to waterlogging stress

The employment of growth-dependent escape mechanisms to avoid anaerobic conditions such as the aeration of submerged organs may not be feasible for plants exposed to waterlogging in periods outside of the normal growing season (Crawford 2003). The formation of aerenchyma requires carbohydrate reserves to be expended so that the plant may be able to resume normal gas exchange with the atmosphere and thus continue to grow. When plants undergo flooding outside of optimal growing seasons, while temperatures are low and solar radiation is limited, the rates of growth and development will be reduced. Therefore, the benefit of producing aerenchyma is likely minimal. Thus typical plant responses to waterlogging during winter, favour tolerance mechanisms, instead of escape mechanisms

(Crawford 2003). Common strategies of plants possessing improved waterlogging tolerance are the increased production of antioxidants (Liu & Jiang 2015) and the conservation of carbohydrates by down-regulating metabolic activity (Crawford 2003).

One of the major metabolic changes under waterlogging is antioxidant metabolism (Liu & Jiang 2015). Plants have evolved enzymatic defence systems to protect cells against oxidative injury by removing or scavenging reactive oxygen species (ROS). In this system, superoxide dismutase (SOD) plays a central role in catalysing the dismutation of O^{2-} and H_2O_2 (Bowler et al. 1992). Liu and Jiang (2015) suggested that higher root SOD activity may have contributed to improved waterlogging tolerance in some cultivars of perennial ryegrass. Increased root SOD activities have also been correlated with enhanced waterlogging tolerance in kentucky bluegrass (*Poa pratensis* L.) (Wang & Jiang 2007).

Membrane lipid peroxidation is a major damaging effect of ROS (Liu & Jiang 2015). Usually, membrane lipid peroxidation is detected by measuring malondialdehyde (MDA) in plant tissue. Studies have shown increased MDA concentration in plants under waterlogging (Tang et al. 2010; Simova-Stoilova et al. 2012; Liu & Jiang 2015). Liu and Jiang (2015) showed that the MDA concentration in perennial ryegrass leaf tissue increased following a period of waterlogging stress. The author suggested that the minimization of lipid peroxidation, characterised by low MDA concentrations under stress, could contribute to the waterlogging and submergence tolerance of perennial ryegrass.

Some plants possess metabolism regulation strategies that enable them to conserve energy and survive for extended periods under anoxic conditions. Carbohydrate levels are affected by the balance between the production of carbohydrates by photosynthesis (and the access to storage) and the consumption of carbohydrates in respiration or fermentation (Schlüter et al. 1996). Schlüter and Crawford (2003) showed that under waterlogging oxygen consumption, total CO_2 production and total non-soluble carbohydrate concentrations in mature cranberry leaves fell rapidly, but then stabilised between 21-28 days after then onset of anoxia. This indicated a reduction in respiration rate which likely contributed to their survival for six months under water. Some plants accumulate carbohydrates when subjected to anaerobic conditions, as a result of reduced energy demands. Schlüter et al. (1996) showed that the accumulation of water-soluble carbohydrates in the root and shoot of plants following waterlogging, was probably related to a reduction in carbohydrate demand from the root, which exhibited lower growth rates under hypoxia.

2.5.1.1 The effect of waterlogging on plantain

Literature detailing the effects of waterlogging on plantain, in an agricultural field setting, are scarce. Mook et al. (1989) compared the demography of eight plantain populations, across several grassland habitats, with ranging soil types and soil moisture availability. Winter plantain mortality was most affected by a high soil moisture content, that occurred during the second winter of the experiment. This effect was also found in plantain in a fen habitat, where young plants in particular, exhibited a high winter mortality, even in mild winters (Van Groenendael 1985). There are also industry recommendations that suggest plantain may not persist, or maintain high DM production when subjected to waterlogging during winter (DairyNZ 2013). Interestingly, in a 2018 survey of 59 farms across New Zealand, a higher plantain content was observed in paddocks with a clay soil type, than those with sandy or silty soil types (Dodd et al. 2019). This finding may suggest that plantain is well suited to soils with a higher water holding capacity, which, by definition have the potential to experience wet soil conditions more frequently than soils with lower water holding capacities. Therefore, the susceptibility of plantain to wet soil conditions may not relate to soil texture alone. However, that study did not assess the likelihood of paddocks being waterlogged, which is also likely to be affected by regional and seasonal effects.

There is some work on the effects of flooding on natural communities of plantain (van Tienderen 1992). Plant populations were observed along a natural topographical gradient, with four distinct environments; The dry top of a riverbank, the former riverbank and two ‘wetter’ low-lying zones (former riverbed), separated into wet-rough and wet-low areas. The lower lying zones were typically waterlogged for several months during winter and were often submerged for weeks at a time. The highest density of plantain was found at the wet-low site and the lowest density of plantain at the dry-top site. This indicates that some plantain types are capable of surviving multiple, prolonged periods of waterlogging. It is important to note that soil moisture became limited at the dry-top site during summer, which could partly explain the lower plantain density at that site. Grime et al. (1989) showed that plantain is unlikely to be found at sites which are almost always waterlogged, and is much more commonly found at periodically waterlogged sites, or sites that are seldom waterlogged (Figure 2-4).

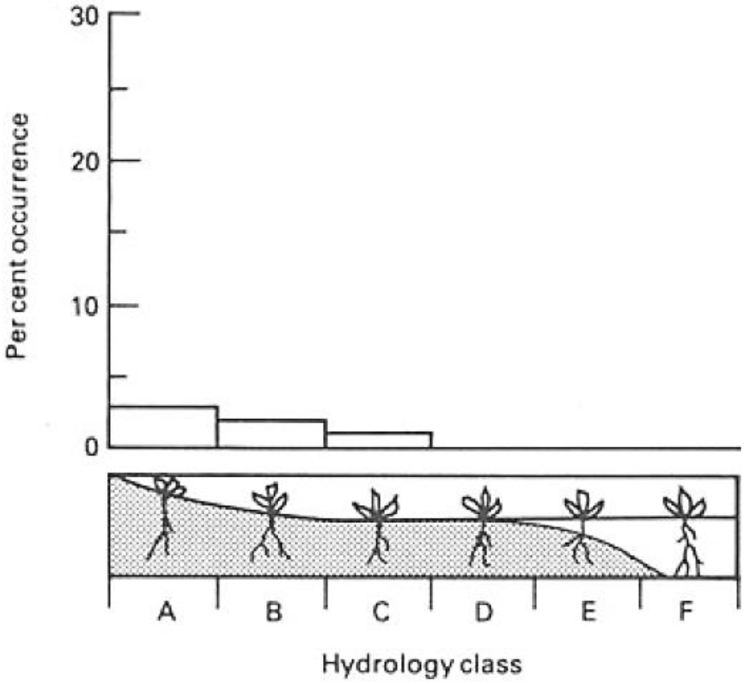

Figure 2-4. Percentage occurrence of plantain in quadrats in British wetlands, along a gradient from sites that are seldom waterlogged (hydrology class A) to permanently waterlogged sites (hydrology class F) taken from (Grime et al. 1989).

Controlled experiments have shown that plantain has some important waterlogging tolerance features (Grimoldi et al. 2005; Striker et al. 2007). Grimoldi et al. (2005) found that plantain possesses the ability to respond readily to flooding conditions. Plantain was able to increase root porosity through the generation of lysigenous aerenchyma, where adjoining cells in the root cortex were senesced to create lacunae (intercellular spaces for air transport). Striker et al. (2007) showed an evident formation of aerenchyma by plantain under waterlogging, characterized by an increase in root diameter and porosity. An increase in root porosity was closely associated with improved flooding tolerance across multiple species of a flooding pampa grassland in Argentina (Grimoldi et al. 2005).

Pasture species differ in their tolerance of saturated soil conditions (Rumball 1977; Ordóñez Vásquez 2020). Some grasses are better able to tolerate prolonged waterlogging, while other pasture components may exhibit poor growth and/or survival rates. Perennial ryegrass has relatively good waterlogging tolerance (Ordóñez Vásquez 2020) and is able to survive prolonged bouts of flooding (28 days) (McFarlane et al. 2003). In pastures that are prone to seasonal waterlogging, tolerant species could be expected to provide the bulk of the forage biomass following wet periods (Grieve et al. 1986). In contrast, plant populations of intolerant species may be reduced. This may be an important dynamic involved in the loss of plantain density that occurs in ryegrass-based pastures. The waterlogging tolerance of plantain is largely unknown, although the natural occurrence of plantain in riverbeds prone to periodic waterlogging may suggest that it possesses some degree of waterlogging tolerance (van Tienderen 1992). Given the importance the industry is placing on plantain for reducing the environmental footprint of New Zealand dairying systems, it is therefore important that we consider the responses of plantain and its most important companion species, perennial ryegrass, to waterlogging stress.

2.5.2 Treading damage

Treading damage is a general term employed when referring to a loss of plant productivity or degradation of soil structure as a direct consequence of grazing animals exerting compressive forces on pasture, during in-situ grazing. The term encompasses two main effects: The physical injury of plants, via a destruction or burying of plant organs by direct hoof contact, and the compaction or deformation (pugging) of soil as a result of soil structural failure (Howes 2019). Treading damage is regarded as inevitable on many New Zealand dairy farms (Drewry et al. 2000), particularly those in regions with a high annual rainfall, or where a large proportion of rainfall occurs during winter. Treading damage has been shown to significantly reduce pasture productivity and persistence (Pande et al. 2000; Nie et al. 2001) and the effects of significant damage are often evident months later.

Causes of treading damage

Grazing livestock often exert ground pressure similar to, or greater than that of farm machinery. The pressures exerted by dairy cows on pasture during grazing are typically between 130-360kpa, with a stationary, standing pressure of 220kpa (Scholefield & Hall 1986; Di et al. 2001). The amount of pressure is a function of the mass of the animal and the surface area of its hooves that are in contact with the ground (Howes 2019), which for a walking dairy cow includes at least two hooves.

The capacity for soils to resist penetration by animal hooves is heavily influenced by soil moisture content (Climo & Richardson 1984). Saturated, or near-saturated soils are generally susceptible to treading damage regardless of soil texture or structure. For instance, the type and extent of damage to any given soil during winter and spring is largely governed by soil moisture content during that period (Howes 2019). The relationship between soil moisture content and soil strength was explained by Climo and Richardson (1984).

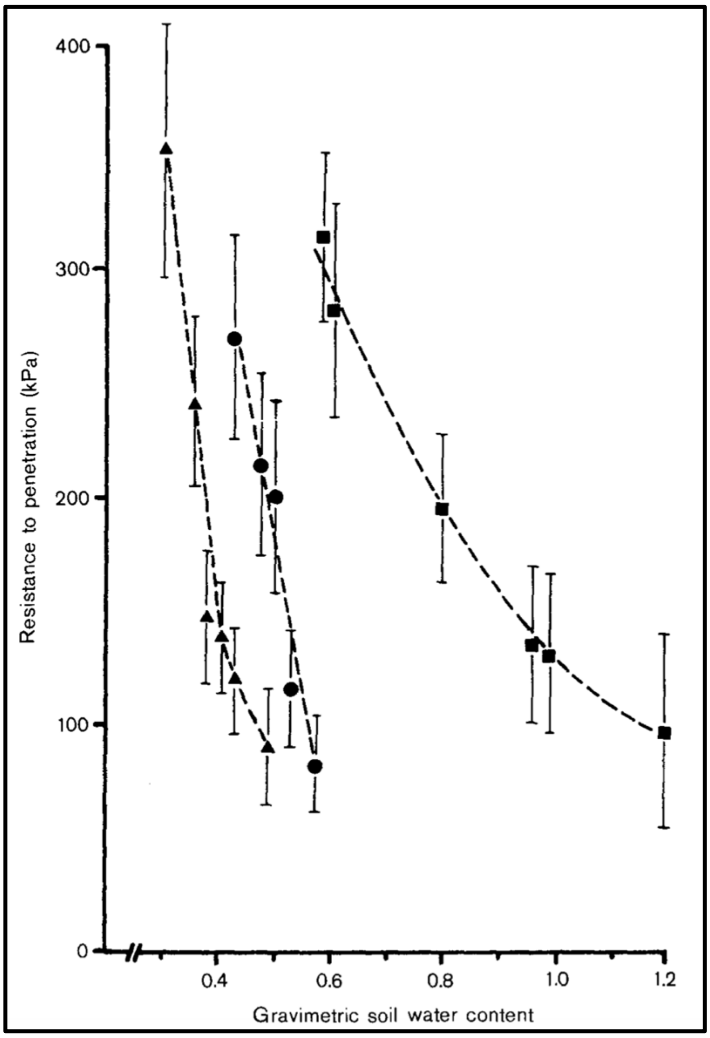

Figure 2-5. Relationship between resistance to penetration and soil water content for three soils in Manawatu. Ramiha silt loam (squares), Manawatu fine sandy loam (triangles) and Tokomaru silt loam (circles) taken from (Climo & Richardson 1984).

When the soil moisture content nears field capacity, the soil loses frictional resistance, meaning that soil particles move more freely past each other and are thus susceptible to deformation. This has the potential to result in compaction of the soil under cattle grazing. When soils approach saturation, plastic deformation is more likely to occur than compaction. In Figure 2-5, Climo and Richardson (1984) showed the relationship of soil moisture content and penetration resistance (soil strength) for three soil types. The graphic illustrates the rapid loss of soil strength, with increasing soil moisture. It also shows the contrasting abilities of different soil types to resist treading damage. The Ramiha silt loam is much freer draining than the Manawatu and Tokomaru silt loams, meaning that excess soil moisture does not persist in the topsoil, where penetration resistance is most vital. For the Tokomaru silt loam, a high clay content results in poor drainage through the profile, meaning that successive rainfall events during seasons of low evapotranspiration (winter and spring) cause a prolonged period of heightened susceptibility to treading damage.

Soil compaction is possibly the most commonly associated, long-term consequence of treading damage, and the central focus of a number of recent studies (Crush & Thom 2011; Hu et al. 2018; Thomas et al. 2019; Hu et al. 2021). It occurs when the pressure of a grazing animal exceeds the load-bearing capacity of a soil (Bilotta et al. 2007). The predominant effects of soil compaction are a reduction in hydraulic conductivity, macropore volume, air permeability and soil strength, and an increase in soil bulk density (Thomas et al. 2019).

Effect of treading damage in grazed pastoral systems

There is ample literature detailing the effects of cattle treading on pasture production and botanical composition (Edmond 1964; Brown & Evans 1973; Betteridge et al. 1999; Menneer et al. 2001; Nie et al. 2001; Pande 2002; Betteridge et al. 2003; Drewry 2003). Pande et al. (2000) found that a single treading event on winter-wet hill country reduced spring pasture growth by 7kg DM/ha/day, in comparison with untreaded pasture. Similarly, on a poorly drained Tokomaru silt loam at Massey University’s Dairy 4 unit, a 44% reduction in pasture growth rate was observed in damaged plots in the period between 33 and 48 days after treading compared to undamaged pasture (Pande et al. 2000). Nie et al. (2001) found that pugging by cows at a stocking rate in excess of 133 cows/ha in wet winter soil resulted in a loss of spring pasture yield by up to 40-42%, and a reduction in perennial ryegrass tiller density of 39-54%.

Following treading damage events, pasture botanical composition is likely to favour species with greater treading tolerance. In work by Edmond (1964) perennial ryegrass was found to be more tolerant of sheep treading than other pasture species, including cocksfoot, white clover, and red clover. This agrees with work by Menneer et al. (2005) who showed that following treading damage, the white clover content of a perennial ryegrass pasture decreased significantly, while the perennial ryegrass content increased.

The majority of the studies that have quantified treading damage on pasture have involved a grass-dominated pasture, that typically being perennial ryegrass. Perennial ryegrass based dairy pastures may contain in excess of 5000 tillers/m^2 (Matthew et al. 1996), and seldom leave patches of bare ground. The structural support provided by such a high number of growing points alone is likely to provide some resistance to treading damage of any given soil type or ground condition. Pastures that have a lower number of tillers and/or growing points, (potentially including those with a high plantain component) may naturally have more bare ground area. These areas are more susceptible to treading damage than areas with high plant density (Betteridge et al. 1999).

An increase in the area of bare ground within a pasture may be caused directly from treading damage, where plant physical damage and burial results in the death of plants. Therein lies a self-perpetuating problem, where greater treading damage to a pasture results in more bare ground area, and an elevated susceptibility to treading damage in the future. Drewry (2003) showed that the bare ground area within pasture following treading may be as high as 48% on a clay soil. Bare ground area may also increase as a result of plant-pulling, a phenomenon that affects pasture plants during summer grazings where a compacted soil surface layer results in poor plant anchorage (Crush & Thom 2011).

The effect of treading damage on forb pastures is not well researched and literature detailing the direct effect of treading damage on plantain is scarce. Herb species may be particularly sensitive to livestock treading (Li et al. 1997a). Jones and Haggar (1994) showed that treading damage, particularly in wet soil conditions, could be a potential cause of plant death in chicory. This was thought to be caused by damage to the crown and shoot buds, which increased the susceptibility of the plant to fungal diseases such as *Fusarium* spp.

Effect of treading damage on plant morphology and physiology

Treading damage is capable of causing immediate and long-term plant stresses. The immediate effect of treading is plant tissue injury, in both the shoot and the root, and the long-term effect of treading damage is soil compaction, which leads to a suppression of water and air flow within the root layer of soils (Asady & Smucker 1989).

Direct plant injury, as a result of livestock treading damage, induces a sequence of plant stress responses within surviving plants, as well as potentially causing plant organ (e.g., leaf or root) abscission by the cutting force of the animal’s hoof. The latter ending with the death of the removed plant tissue. In work by (Han et al. 2008) the treading injury of turf grasses resulted in reductions in leaf relative water content, leaf chlorophyll concentration and leaf antioxidant activity. The treading injury caused an increase in cell membrane permeability, characterised by an increase in electrolyte leakage. That work found that tolerance to treading stress by turf grasses may be related to improved antioxidant activity in injured leaves.

2.5.2.1 Effect of treading damage on plantain

Plantain is generally considered as less-tolerant of the treading damage and soil compaction than perennial ryegrass (Stewart 1996). Treading damage has been cited as a potential cause of long-term reductions in plantain density in grazed pastures and is often advised as a situation to avoid (DairyNZ 2013; Lee et al. 2015a; Bryant et al. 2019). Conversely, naturally observed populations of *Plantago lanceolata* L. appear to have good tolerance of treading by livestock and other human activities as they tend to be found growing on well-worn tracks and pathways (Sagar & Harper 1964). However, this contrasts observations by Chappell et al. (1971), who found plantain at a considerably higher density in moderately trampled areas (subject to light trampling and without rank growth), than well-worn areas (areas commonly trampled and used by vehicles).

Previous research has also suggested that plantain leaves could be particularly susceptible to damage under trampling due to their fragile leaf morphology (Sun & Liddle 1993). One important factor believed to contribute to the improved treading tolerance of perennial ryegrass is a high cellulose content within leaves (Evans 1967). Plantain leaves have a lower proportion of cell wall and less cellulose than perennial ryegrass leaves (Stewart 1996; Navarrete et al. 2022), which means they don't provide the same cushioning effect for plant growing points in the crown. Furthermore, Sun and Liddle (1993) suggested that erect herbaceous species with broad leaves are particularly susceptible to treading damage since a greater proportion of their leaves were damaged under trampling, than grass species with narrow leaves. In a study of a related species, Ikeda and Okutomi (1995) found that the leaf length of asiatic plantain (*Plantago asiatica* L.) was significantly reduced following trampling damage, probably as a result of physical injury to growing leaves (Sun & Liddle 1993). Treading damage has also been shown to reduce photosynthetic activity in plantain leaves. Mills et al. (1996) observed a 62% reduction in photosynthesis in trampled plantain leaves 24 hours after trampling. Measurement of thylakoid activity in trampled leaves revealed that approximately half of the reduction in photosynthesis was caused by an inhibition of primary photosynthetic apparatus, which includes photosystem II (PSII). The author concluded that inhibition of photosynthesis in plantain leaves was complex but may contain a large contribution from increased stomatal or mesophyll resistance (Mills et al.

1996). Taken together this literature suggests that plantain leaves are not well suited for coping with treading pressure. However, the response of plantain leaves to treading damage by dairy cows, is largely unresearched under field conditions.

Blom (1979) compared root lengths of *Plantago* species in compacted and non-compacted soils. The reduction in root system length between non-compacted and compacted soils was larger for plantain (*Plantago lanceolata*) than for *Plantago major*, *Plantago media* or *Plantago coronopus*. When plantain was trampled lightly in loose soils (trampled once per day by a trampling machine), total root biomass was reduced by 60%, which contributed strongly to an increase in the shoot-root ratio of trampled plants.

Striker et al. (2007) investigated the trade-off between root porosity and root mechanical strength in plantain. Increases in root porosity are associated with a reduced mechanical strength (Engelaar et al. 1993). It has also been reported that flooding induces increases in root diameter during the processes of cell division, expansion, separation, and lysis that precede aerenchyma formation, in other species (Visser et al. 2000; Grimoldi et al. 2005). Striker et al. (2007) showed that under normal growing conditions there was no correlation between root diameter and root strength in plantain, probably because aerenchyma lacunae were not developed. Under flooded conditions however, greater plantain root diameter and porosity, brought about by the formation of lysigenous aerenchyma (channels formed by cell lysis), seemed to weaken root structure.

When soil pore diameters are smaller than the root diameter, roots will have difficulty penetrating the soil and expanding their root system (Engelaar et al. 1993bb). The findings by Striker et al. (2007) indicate that the ability of plantain roots to penetrate soil under waterlogged conditions will be particularly sensitive to the resistance of soil particles to displacement. Furthermore, Blom (1979) showed that the root systems of four *Plantago* species (including *P. Lanceolata*) were shorter following compaction by trampling, suggesting that *Plantago* species may have limited capacity for root growth in dense soils.

These works suggest that waterlogging and soil density may have a combined, and confounding effect on plantain root development in wet soils. Grazed pastures often experience a number of events that alter soil density over the course of a season (Di et al. 2001). Soil compaction may result as a consequence of animal treading damage, farm vehicle pressure and soil wetting and drying cycles. As alluded to earlier, compaction reduces soil macroporosity and increases soil bulk density (Hu et al. 2018). These factors combine to make it harder for roots to expand through the soil volume (Engelaar et al. 1993b). Compaction and waterlogging may occur simultaneously, e.g., in a wet winter and spring period, where pastures are grazed while the soil is susceptible to treading damage. The earlier results suggest that the deleterious effects of compaction on plantain roots which arise from treading damage, may be exacerbated by a preceding period of waterlogging stress. Therefore, there could be a trade-off for plantain between producing aerenchyma (to survive a long-term waterlogging event) and maintaining the mechanical strength required to produce an efficient root system in a compacted soil.

Plantain plant organs may also be wounded directly by animal hooves and the displacement of soil around the roots (Blom 1979). These injuries may have sudden effects and could result in the death of plants or growing points. The wounding of roots has also been shown to cause the release of ethylene, which induces the development of broader and shorter roots (Barley 1976). Since plantain root mechanical strength may be reduced following a prolonged waterlogging event, direct tissue damage as a consequence of treading damage could be more life-threatening, in soils prone to periodic waterlogging.

Plantain seedling establishment, and thus the ability to persist via seedling recruitment, may also be reduced in compacted soils. It has been suggested that improving plantain persistence could be achieved by broadcasting new seed to an existing sward or by simply allowing plantain plants to complete their reproductive lifecycle and set their own seeds. These methods have had mixed success (Bryant et al. 2019) and one potential reason for the poor establishment of new seedlings in an existing plantain sward may be the reduced ability for plantain seedling emergence in compacted soil. Blom (1979) showed that plantain emergence was significantly reduced in compacted soils in growth cabinets (Figure 2-6) and field plots. The author concluded that in relation to seedling emergence, plantain is poorly adapted to soils with high mechanical resistance.

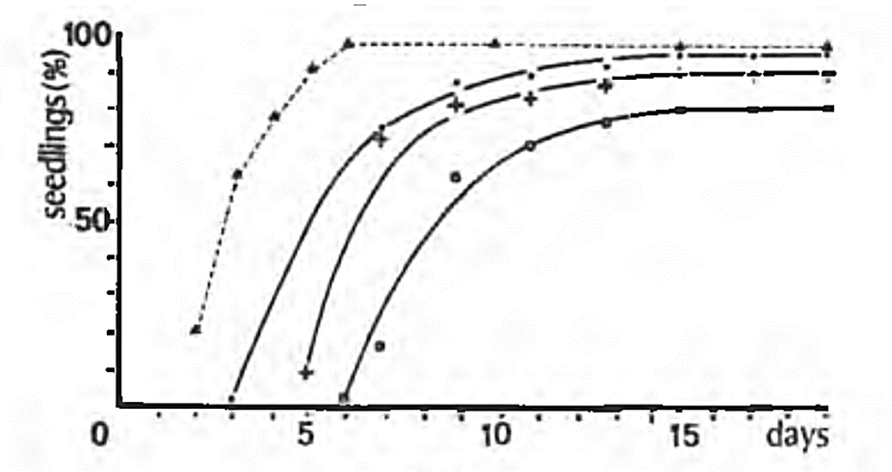

Figure 2-6. The influence of soil compaction at an optimal soil-moisture level on seedling emergence from buried seeds of *Plantago lanceolata*, in a growth cabinet. Triangles: control (germination on wet filter paper), closed (small) circles: loose soil, crosses: moderately compacted soil, open (large) circles: compacted soil. Taken from (Blom 1979).

2.5.3 Climate change forecasts for New Zealand and their consequences for waterlogging and treading damage

Recent climate change forecasts have detailed a change in precipitation pattern for some regions of New Zealand. Below, Figure 2-7 shows the percentage change in rainfall for New Zealand projected by climate models under the highest CO_2 concentration scenario (RCP 8.5). Summer rainfall is generally unchanged, however slight reductions are projected for most northern and eastern regions. The west coast of the South Island and parts of Canterbury may receive more rainfall during summer. Changes to winter precipitation are most obvious in the west and the south of the country, where increases could be as high as 40% for the west coast of the South Island. This reflects an increase in prevailing westerly quarter winds. Reductions in winter rainfall would be felt primarily by the Hawkes Bay and Northland regions. Some important dairying regions (Taranaki, Manawatu-Whanganui, Southland, and the West coast of the South Island) are also expected to experience more annual rainfall, with potentially more flooding events (Mullan et al. 2016).

That suggests that a large proportion of dairy pastures could receive a greater volume of rainfall with increased frequency, particularly during winter months. Without any interventions such as improvements to drainage, there is therefore the potential for these pastures to become waterlogged more often. Aside from the increased challenge of meeting animal demand during late winter and early spring due to potentially lower pasture growth, these conditions could also reduce the performance of pasture species with less waterlogging tolerance. Furthermore, since the chief driver of treading damage is an elevated soil moisture content (Howes 2019), it is also likely that a greater proportion of land area within dairy farms will be subjected to treading damage under this scenario. Without the implementation of techniques designed to limit treading damage such as targeted duration-controlled grazing

(Howes 2019), pasture growth will likely be dependent on pasture species with improved treading damage tolerance, such as perennial ryegrass (Edmond 1964).

Given that the incidence of waterlogging and treading damage is likely to increase on some New Zealand dairy farms over the coming decades, it is important that we consider the effects of these stresses on the growth and survival of plantain within mixed pastures, to ensure that plantain is implemented and managed effectively in affected regions.

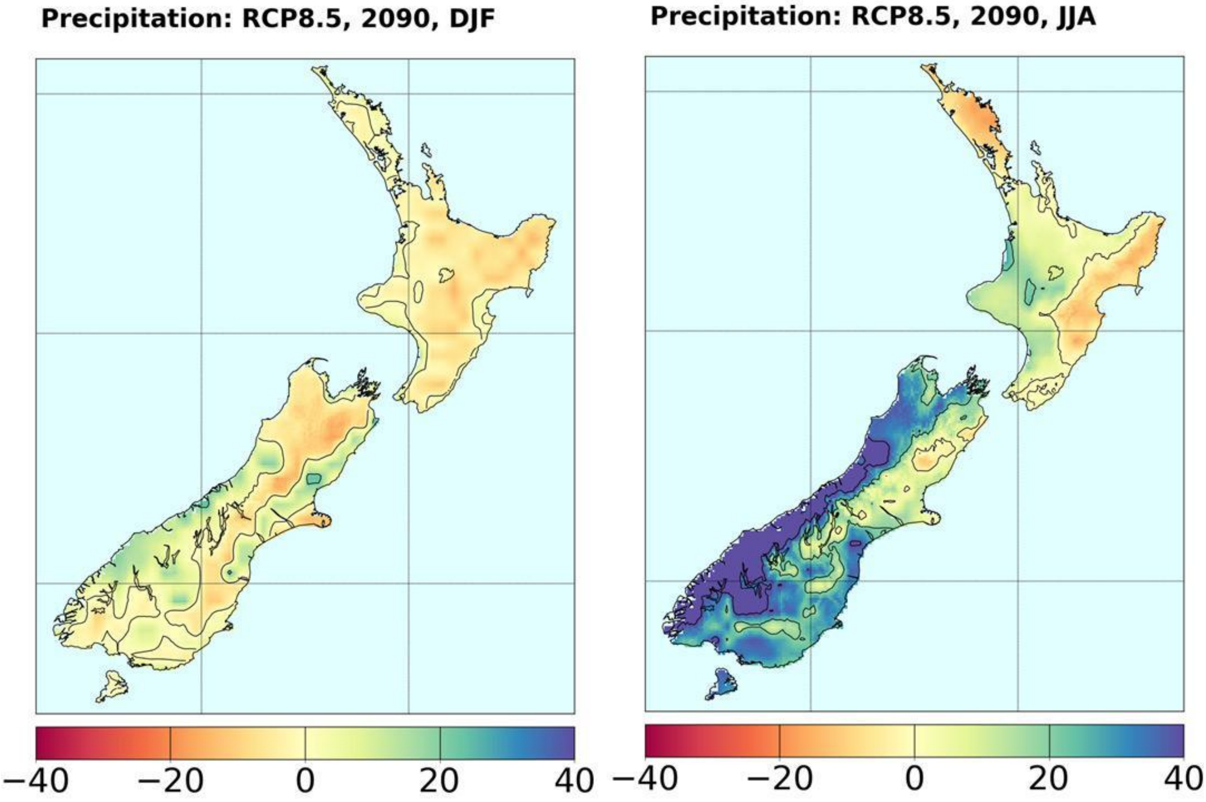

Figure 2-7. Projected changes in precipitation (in %) for summer (Dec-Jan-Feb; left) and winter (Jun-Jul-Aug; right) by the end of the 21st century, for the ensemble-mean of 6 climate models under the highest CO_2 concentration scenario RCP8.5 from the IPCC 5th Assessment (Mullan et al. 2016).

2.6 Review of mechanical treading devices

The treading damage caused by livestock is typically spatially random and can result in a large variability of treading pressure (Di et al. 2001). Thus, the use of grazing livestock for implementing treading damage in small-plot experiments, or in experiments that require uniform treading damage is impractical. In such experiments, a mechanical device capable of producing repeatable and life-like treading damage is desired.

There have been several attempts at manufacturing such a device, however they appear to have come at a high cost for investigators and/or have required proper technical aptitude. The following section provides a brief review of some of the existing treading-simulation devices. The information collected here will assist in the development of a novel treading device, so that treading damage may be studied in small-plot experiments, at a low cost.

2.6.1 The recording penetrometer

The recording penetrometer, devised by Scholefield and Hall (1986) was evaluated against force patterns obtained with the use of a Kistler multicomponent force-platform, which measured forces exerted on the ground by a 530kg walking dairy cow. With a hoof surface area of 100cm^2 the average vertical force imposed over one hoof step was 250kpa; the average vertical stress by a standing, stationary cow is 132.5kpa. This instrument was able to test the strength of soil, by directly measuring the displacement of the mechanical limb. The hoof prints produced by the machine were shallow compared with those produced by live cattle, however a reduction in soil treading resistance correlated well with an increase in SMC.

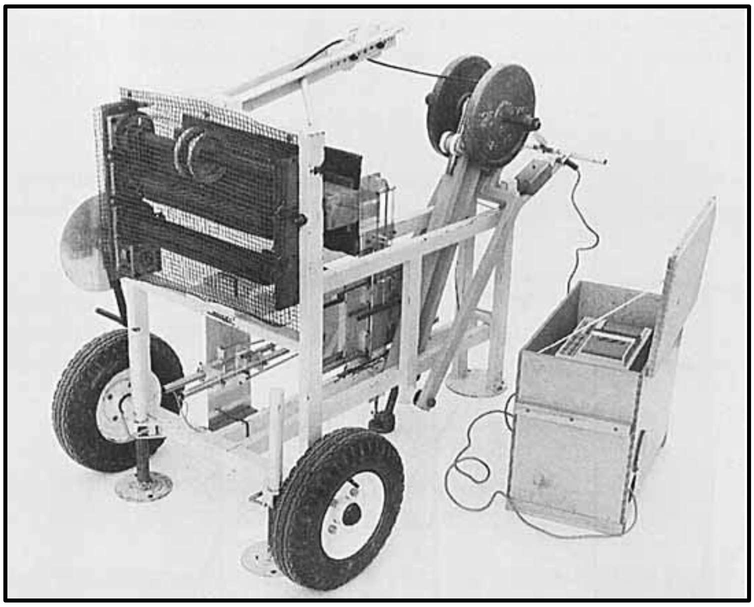

Figure 2-8. The recording penetrometer (Scholefield & Hall 1986).

2.6.2 The artificial pivot-hoof

The artificial hoof created by Abdel-Magid et al. (1987) was cast of bronze from the mould of a steel hoofprint. The surface area of the hoof was 85cm^2. The hoof was suspended from a long, weighted metal handle, and when lowered onto the soil, delivered a static load of 118kpa. The instrument was able to influence the soil bulk density, water infiltration rates and shoot biomass of samples. This design was simple and effective for use on soil in pots or lysimeters, but would have limitations for field use, given its fixed position.

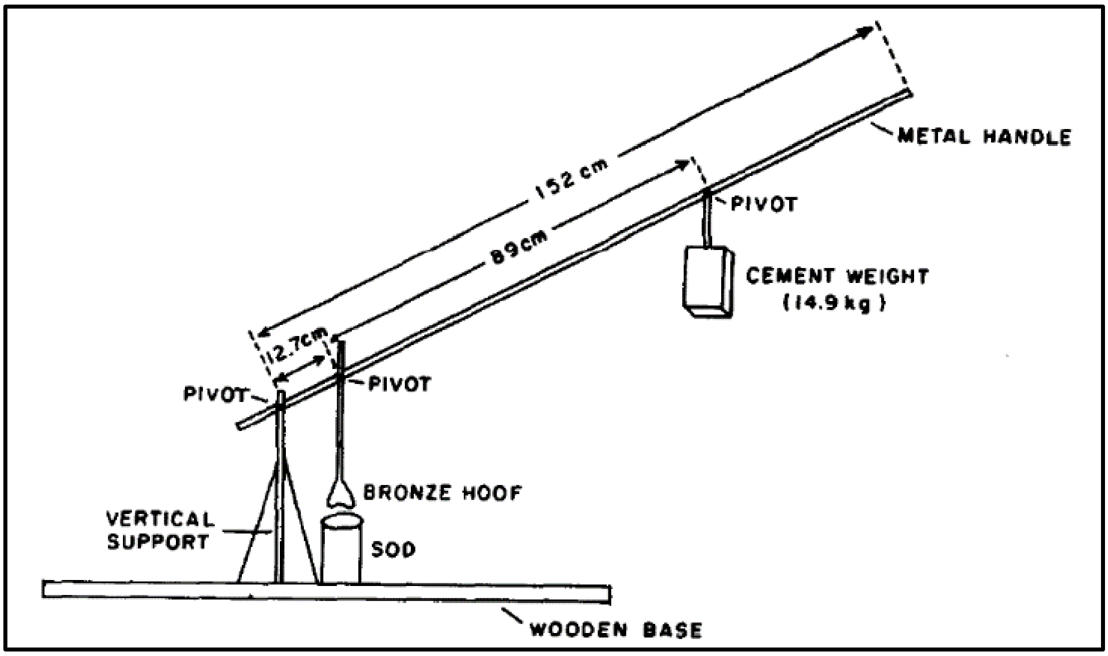

Figure 2-9. Schematic diagram of the artificial hoof apparatus (Abdel-Magid et al. 1987).

2.6.3 The mechanical hoof

The mechanical hoof devised by Di et al. (2001) comprised of an artificial hoof mounted onto a compressed air ram system on wheels. The steel hoof was modelled on an adult Friesian cow. The hoof was split into two halves, which were joined by a pivot in the middle, allowing some independent movement by each half, just as the halves of a cow’s hoof. The surface area of the steel hoof was $90cm^2$ and delivered a pressure of 220kpa. Treading with the mechanical hoof on pasture caused increases in soil bulk density and reductions in soil microporosity and pasture DM yield. This design was efficient and closely replicated the vertical action of a cow’s hoof, providing an excellent simulation of cattle treading to small plots and lysimeters. However, given the total weight of the device was 450kg, it was probably difficult to transport and physically difficult to manoeuvre.

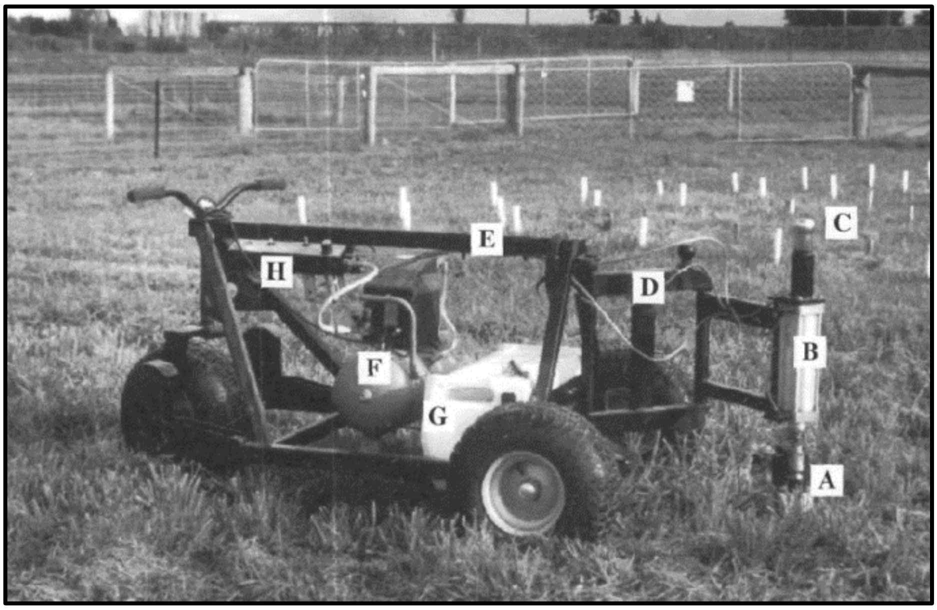

Figure 2-10. The entire mechanical cow. A, mechanical hoof; B, compressed air ram; C, release button to initiate treading; D, bi-folding arm; E, main body frame; F, air compressor; G, counter weights; H, control panel for treading pressure, speed and duration of hoof-soil contact (Di et al. 2001).

2.6.4 The hoofinator

The ‘hoofinator’ developed by Hu et al. (2018) was constructed by the New Zealand Institute for Plant and Food Research Limited. Its intended application was the simulation of compaction effects that result from the treading of soil by livestock. It was able to apply a range of pressures (147-261kpa) to the soil and the treading covered 100% of the soil surface area. Hydraulic rams with steel plates on the base were forced down onto the soil to create the compaction. Compaction with the hoofinator reduced soil macroporosity and saturated hydraulic conductivity. This device recreated the compaction effects of cow treading well, however failed to cause the lateral damage and smearing of soil associated with cattle treading. The equipment was also highly specialised and thus required sufficient technical competence and a significant amount of work and likely a large cost to construct.

Figure 2-11. Left: The hydraulic rams and treading plates of the hoofinator (Photos: Richard Gillespie, New Zealand Institute for Plant and Food Research, Lincoln). Right: Treading damage to un-tilled pasture plots by the hoofinator at 261kpa (Hu et al. 2018).

2.7 Gaps in the literature

Significant quantities of research have been conducted on the use of plantain for improving summer pasture yield and quality for a range of farming systems, along with its use as a low-cost N loss mitigation tool for pastoral dairy farming. To date, it has been established that including plantain in a dairy pasture can reduce N losses from the system whist maintaining or boosting milk production. However, observations of poor plantain persistence within ryegrass-based pastures have highlighted the existence of significant knowledge gaps within the agronomical understanding of plantain. In particular, there is a lack of understanding of factors that consistently cause the sharp decline in plantain content and plant density in ryegrass-based pastures during the second and third year after sowing.

It has been suggested that winter abiotic stresses such as waterlogging and treading damage play a role in the loss of plantain density in mixed pastures, but there is little scientific evidence to prove it. There is minimal information regarding plantain’s tolerance of waterlogging, particularly in the context of grazed pastures. There is also little research quantifying the effects of livestock treading damage on plantain plants or pastures containing plantain. In fact, there is a lacking of studies that have investigated the impact of treading damage on herb-based forages in general. Furthermore, it is unknown how waterlogging and treading damage stress would interact with the grazing management of mixed pastures that contain plantain.

Currently there is little knowledge on which to base agronomical strategies for improving the resilience of plantain within grass-based pastures in general, not to mention those that commonly experience waterlogging and/or treading damage. It appears that a large proportion of the understanding of factors that influence plantain proficiency within ryegrass-based pasture is anecdotal, which, given that it has only been commonplace in pastoral farming in New Zealand for the last 20 years, is probably to be expected. There is much work to be done in this space, given the importance the industry is placing on plantain for reducing the environmental footprint of current and future New Zealand dairy farm systems.

The current PhD research will attempt to fill literature gaps, particularly those pertaining to the performance of plantain under abiotic stress conditions. Focus will be placed on the mechanisms responsible for declines in plantain content under such conditions, as well as on the identification of strategies that might lead to improvements in plantain performance within perennial ryegrass-based pastures.

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Name/title of Primary Supervisor:	Danny Donaghy

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Date:	11-Jul-2024
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Wilson, S.; Donaghy, D.; Horne, D.; Navarrete, S.; Kemp, P., & Rawlingson, C. 2023. Plantain (Plantago lanceolata L.) leaf elongation and photosynthesis rates are reduced under waterlogging. Presented at: 3rd International Electronic Conference on Agronomy, MDPI biology and life sciences forum, Basel, Switzerland, Pp 6.

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Chapter 3 Plantain (*Plantago lanceolata* L.) growth is limited under waterlogging.

To determine the likelihood of waterlogging negatively impacting the growth and survival of PL within RG-based dairy pastures, in this chapter, the growth and physiology of PL under waterlogging was evaluated under controlled conditions in a glasshouse. The growth and physiology of RG was evaluated under the same waterlogging conditions so that the performance of the two species under waterlogging stress could be compared. This then allowed for inferences to be made on the interaction between waterlogging and interspecific competition between PL and RG in grazed mixed swards.

This chapter has had two published proceedings papers:

Wilson, SS., Donaghy, DJ., Horne, DJ., Navarrete, S., & Kemp, PD. 2023. Plantain (*Plantago lanceolata* L.) growth is limited under waterlogging., in *Proceedings of the 25th International Grasslands Congress*. Kentucky, USA.

Wilson, S.; Donaghy, D.; Horne, D.; Navarrete, S.; Kemp, P., & Rawlingson, C. 2023. Plantain (*Plantago lanceolata* L.) leaf elongation and photosynthesis rates are reduced under waterlogging. Presented at: *3rd International Electronic Conference on Agronomy, MDPI biology and life sciences forum*. Basel, Switzerland. Pp 6.

3.1 Abstract

Plantain (*Plantago lanceolata* L.) is becoming an increasingly important component of New Zealand dairy pastures because of its ability to reduce nitrogen losses to the environment. However, widespread suggestions of its poor persistence have led to industry concern over its suitability for dairy systems. Anecdotal evidence suggests that the persistence of plantain is negatively affected in waterlogged soil, yet there is little scientific literature relevant to this issue. Thus, the aim of the present study was to investigate the impact of waterlogging stress on plantain growth and survival. In a glasshouse, the performance of plantain under waterlogging was evaluated against perennial ryegrass (*Lolium perenne* L.), the predominant grass species in New Zealand dairy pastures. Three watering regimes, defined by soil volumetric water content (VWC) were applied to plantain and perennial ryegrass plants in plastic pots: Soil kept at 23% VWC (control), soil kept at 31.5% VWC (wet) and soil kept at 37% VWC by maintaining the water table 50mm below the soil surface (waterlogged). Field capacity was determined to be 31% soil VWC. Treatments were implemented for 39 days, before 27 days of recovery under control watering. Plant dry matter growth, shoot and root morphology, and plant photosynthetic capacity were measured throughout the experiment. The waterlogging stress reduced the leaf dry matter growth of plantain by 37% and 38% during the stress and recovery periods, respectively. The reduction in leaf growth was likely underpinned by the reduction in the number of leaves per shoot, leaf elongation rate, photosynthesis, the operating efficiency of photosystem two, and possibly specific leaf area observed in plantain plants under the waterlogging treatment. These results suggest that plantain growth was particularly sensitive to waterlogging under the conditions of the present experiment. However, the survival of plantain under the waterlogging stress and the recovery of growth and photosynthesis following the removal of stress, suggest that important physiological functions remained intact under waterlogging, possibly due to tolerance

mechanisms. In contrast, perennial ryegrass proved to have an enhanced ability for coping under waterlogging stress, in comparison with plantain, as it produced 28% and 45% more leaf dry matter under waterlogging than plantain, during the stress and recovery periods, respectively. These findings suggest that while waterlogging may cause limitations for plantain growth, there is no evidence to suggest that it alone could cause irreversible damage to plants and thus prevent their recovery. Rather, waterlogging stress could undermine the ability of plantain to compete with the more waterlogging-tolerant perennial ryegrass under such conditions, should they co-exist in a mixed sward.

3.2 Introduction

Plantain (*Plantago lanceolata* L. – PL) is becoming an increasingly important component of New Zealand dairy pastures because of its ability to reduce nitrogen (N) losses. Plantain has secondary metabolites that, when included in a cow's diet, can increase urine volume (Minnée et al. 2020; Navarrete et al. 2020), reduce urinary N concentration (Totty et al. 2013; Box et al. 2017), and suppress soil nitrification (Judson et al. 2019; Gardiner et al. 2020b). These attributes, along with a high nutritive value, have made it a suitable, low-cost tool for reducing nitrate (NO_3^-) and nitrous oxide (N_2O) losses from highly productive, perennial ryegrass (*Lolium perenne* L. – RG) based dairy systems (Doole et al. 2021).

However, there is growing anecdotal and scientific evidence that PL does not persist well under conventional pasture management (Ayala et al. 2011b), and thus, PL is generally considered a short-lived forage species (Kuiper & Bos 1992). Data from commercial farms in New Zealand suggests that high PL contents (>30% on a biomass basis) in mixed species pastures are attainable in the first two years following establishment, but cannot be maintained (Dodd et al. 2019). This is a significant issue given that the level of N-loss mitigation provided by PL is strongly associated with the PL content in a pasture and/or in a cow's diet (Minnée et al. 2020).

There is anecdotal evidence that PL does not persist in wet or waterlogged soils. An increased frequency and intensity of rainfall events, as well as fine-textured soil types, combine to create wet conditions which typically occur from autumn to spring in many of the dairying regions of New Zealand. Literature detailing the effects of waterlogging on PL, in an agricultural field setting, is scarce. Mook et al. (1989) compared the demography of eight PL populations, across several grassland habitats, and winter PL mortality was most affected by a high soil moisture content. This effect was also found in PL in a fen habitat, where young plants in particular, exhibited a high winter mortality, even during mild winters (Van

Groenendael 1985). However, glasshouse experiments have shown that PL possesses important waterlogging tolerance features. For example, Grimoldi et al. (2005) found that PL possesses the ability to respond to flooding conditions by increasing root porosity through the generation of lysigenous aerenchyma (intercellular spaces for air transport).

Pasture species vary in their tolerance of waterlogging and thus waterlogging has the potential to influence the botanical composition of a pasture (Bolton & McKenzie 1946; Grieve et al. 1986). Perennial ryegrass can tolerate extended periods of high soil moisture (McFarlane et al. 2003; Ordóñez Vásquez 2020; Di Bella et al. 2022), potentially allowing it to out-compete less tolerant species, (such as PL), during wetter seasons. This difference may be an important dynamic involved in the loss of PL density that occurs in RG-based pastures.

Given the importance that the New Zealand pastoral industry is placing on PL for reducing N losses from farms, it is therefore important that we consider the responses of PL and its most important companion species, RG, to waterlogging stress. The current study aimed to investigate the effect of waterlogging on the regrowth and survival of PL and RG under glasshouse conditions.

3.3 Materials and methods

3.3.1 Experimental set up

The experiment was conducted in a temperature-controlled glasshouse (range of 12-25°C), under ambient light, at Massey University's plant growth unit in Palmerston North, between March and August 2021. The experiment consisted of 60 plastic pots with a volume of 8.96L (soil surface area of 471.44 cm^2). The pots were filled with dried soil in a 2:1 mix of Manawatu fine sandy loam and common builder's sand as well as slow release Osmocote® (NPK:18,9,10), added at a rate of 100g/100L of soil during soil mixing.

Nitrogen fertility was maintained throughout the experiment by replacing the average amount of N removed from all pots during each dry matter (DM) harvest, via urea (46% N) applications. Nutrient application was designed so that soil fertility would be non-limiting in this experiment. The mean soil bulk density of the pots at the commencement of the experiment was 1.46 g/cm^3.

On March 4, 2021, the pots were planted with either PL cv. *Agritonic* or RG cv. *Maxsyn* by planting several seeds in five locations within each plot. On April 14 seedlings of both species were thinned, to leave five remaining plants per pot, a density of ~107 $plants/m^2$. Plants in all pots were cut by hand to 50mm above the soil surface and removed on May 5, May 26, and June 7 before the commencement of the stress period on June 8.

3.3.2 Experimental design and treatments

Treatment combinations consisted of the two species; PL and RG, and three levels of waterlogging: normal watering (control), wet, and waterlogged, as described below. Treatments were applied to the pots in a complete randomised block design, with 10 blocks serving as replicates of each treatment (6 pots per block; Figure 3-1). At the end of the stress period, 5 of these blocks were selected to be destructively harvested. These blocks were chosen according to their position within the glasshouse, which ensured that all subsections of the glasshouse were represented equally in the harvest, and subsequent regrowth period for the remaining 5 blocks.

Water treatments were defined by soil volumetric water content (VWC), calculated by dividing the volume of water in the pot (measured by weight) by the volume of the pot. Field capacity was determined to be 31% soil VWC by saturating six representative non-experimental pots and weighing them immediately following the cessation of drainage.

Pots in the control and wet treatments were topped up to 23% and 31.5% soil VWC, respectively, every two days throughout the stress period. The wet treatment was designed to mimic soils which are free draining but are kept in a wet state (greater than field capacity) because of frequent water supply. These conditions have the potential to limit optimal oxygen supply to roots, because some of the macropores are partially filled with water (McLaren & Cameron 1996).

Pots in the waterlogged treatment were placed into large tubs, where the water level was maintained at 50mm below the soil surface in the pot, and holes in the bottom of the pots facilitated the waterlogging of soil within the pots. During the stress period, waterlogged pots were kept in the large tubs for five days, before two days of drainage, and then the commencement of a further five days of waterlogging. The waterlogging treatment was carried out this way to avoid killing the plants through too long a period of waterlogging.

The water treatments were imposed for 39 days, before a 27-day recovery period. This allowed for two 3-week regrowth periods while plants were under the water treatments (to capture the onset of stress, and then the regrowth of stressed plants following defoliation), and one regrowth to act as a recovery period. During the recovery period, pots from all treatments were watered in accordance with the control watering criteria. This meant that over time, the pots either drained or lost water through evapotranspiration until they reached a soil VWC close to 23%.

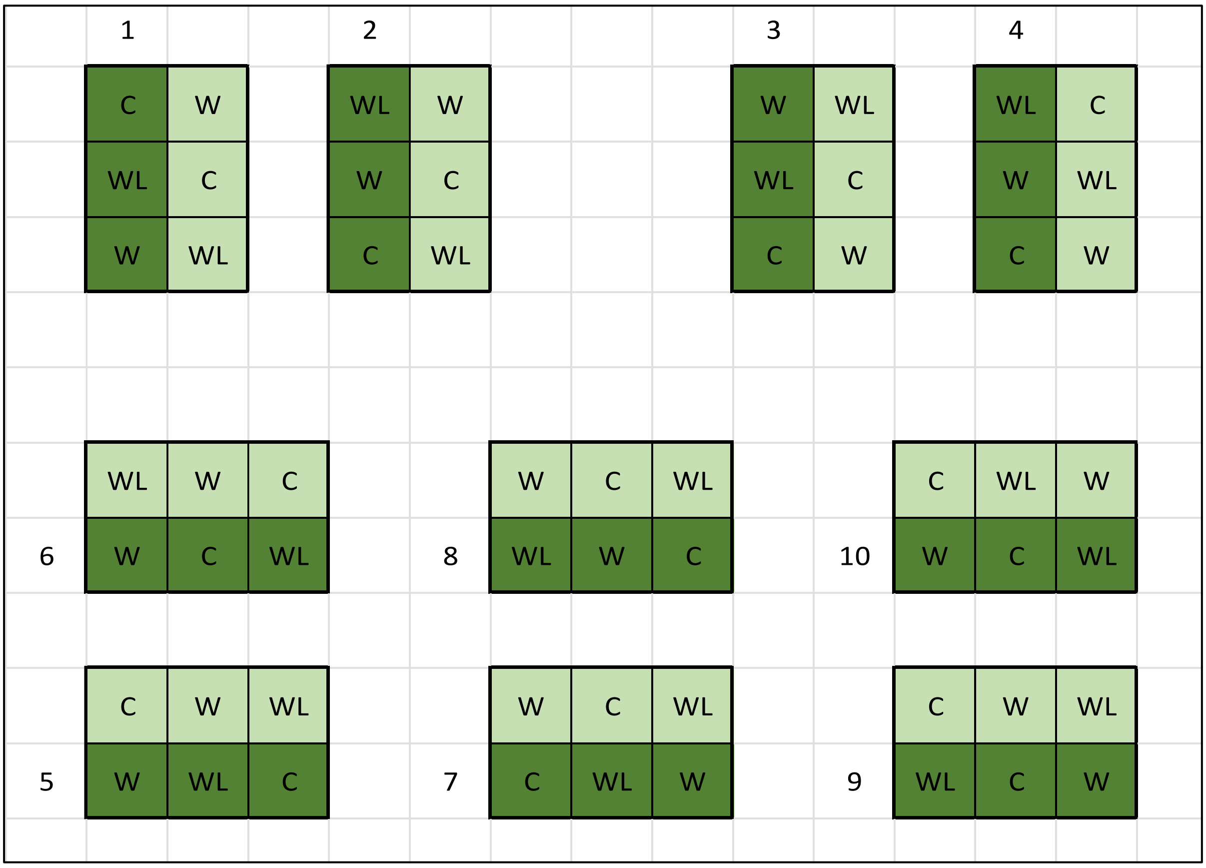


Figure 3-1. Experiment design. Each block contained pots with plantain (dark green) or perennial ryegrass plants (light green), subjected to three water treatments; control (C; 23% soil VWC), wet (W; 31.5% soil VWC) or waterlogged (WL; 37% soil VWC; dashed line).

3.3.3 Experiment timeline

Date	**Event description**
4 March 2021	PL and RG seeds planted
14 April 2021	Plants thinned to five plants per pot
5 May 2021	Plants cut to 50mm height
26 May 2021	Plants cut to 50mm height
7 June 2021	Plants cut to 50mm height
8 June 2021	Commencement of water treatments, day 1 of experiment
30 June 2021	Leaf DM harvest, day 22 of experiment
17 July 2021	Destructive harvest on five of the ten blocks. Day 39 of experiment.
18 July 2021	All treatments commenced control watering. Day 40 of experiment.
13 August 2021	Destructive harvest on remaining five blocks. Day 66 of experiment.

Figure 3-2. Experiment one, prior to the commencement of water treatments on 8th June 2021.

3.3.4 Measurements

Leaf DM above 50mm height was harvested from all pots 22 and 39 days after the commencement of water treatments, and 27 days after the removal of water treatments, for the pots that remained after the first destructive harvest. At each destructive harvest, the stubble DM (including PL petiole and RG pseudostem) below 50mm height was harvested and recorded. Above-ground DM was calculated as the sum of leaf and stubble DM. After collection, all above ground biomass samples were dried in a forced-air oven at 60°C for 48 hours before being weighed.

The number of PL plants per pot, shoots per plant and leaves per shoot were determined no earlier than 24 hours before the shoot biomass harvest.

Specific leaf area (SLA) was determined no earlier than 24 hours before the shoot biomass harvest. Two leaves were removed from each pot (actively growing leaves in PL; youngest fully expanded leaves in RG) for leaf area determination using a LICOR-3100 leaf area meter. Leaves were then dried in a forced-air oven at 60°C for 48 hours to determine DM. The formula for SLA calculation was as follows: leaf area (cm^2)/dry weight (g).

Leaf elongation rate (LER) was measured in PL by selecting two growing leaves per plant from two plants per pot within three days following each harvest. The leaf length of PL leaves was measured as the distance from the base of the petiole to the tip of the leaf, removing any ambiguity concerning the exact definition of the base of the leaf. The LER of RG was measured on two tillers from two different plants per plot. Perennial ryegrass leaf length was measured as the length of the lamina from the ligule of a previously fully expanded leaf to the tip of the leaf, for expanding leaves. The length of fully expanded leaves was measured as the length of the lamina from the ligule to the leaf tip. At least two RG leaves were measured at the beginning of regrowth periods, and new leaves were included as they emerged, so that by the end of the regrowth period, up to 5 RG leaves per tiller were being routinely measured. Leaf length measurements were conducted every two to three days on average from the onset of water treatments to the end of the recovery period. The LER was calculated as the mean increase in leaf length between measurement dates (across all measured leaves within an experimental unit, i.e. two leaves per plant, on two plants per pot for PL), divided by the number of days between measurement dates.

Plant physiological parameters were measured with the use of a LICOR-6800 plant photosynthesis system and its on-board fluorometer. During the stress period, plants were scanned at least twice per week. During weeks two and three of the recovery period, plants were scanned with the LICOR-6800 once. Two leaves per pot of each species (actively growing leaves for PL; youngest fully expanded leaves for RG) were measured on each of the measurement dates. Photosynthesis rate (μmol $m^{-2}s^{-1}$) was measured by enclosing selected leaves inside the chamber and waiting until leaves had acclimatised to the conditions (1000 μmol photons $m^{-2}s^{-1}$, 440ppm CO_2) inside the chamber, before measurements were made. Chlorophyll fluorescence was recorded simultaneously with gas exchange measurements (light adapted) as well as once at two hours after sunset (dark adapted) near the end of stress period. These measurements allowed the calculation of F_v/F_m (maximum quantum efficiency of photosystem two (PSII) dark-adapted and, ΦPSII (PSII operating efficiency: the quantum efficiency of PSII electron transport in the light, sometimes referred to as f_q'/f_m').

Root biomass was harvested from the control and waterlogged pots following the removal of shoot material. Roots were separated from soil material by hand washing with water. The roots of four plants per pot were immediately placed into a freezer at -20°C before they were freeze-dried in a commercial freeze drier and weighed for DM determination. The roots of one plant per pot were stored in a chiller at 4°C for no longer than two weeks, before being analysed with winRHIZO® software. The roots were cut into three segments, based on length from the plant base: 0-50mm, 50-150mm and 150mm and below. These sections were then scanned at 400 dpi with Regent winRHIZO® software for the determination of root diameter, tap-root diameter, and root volume. The segments were then dried in an oven at 60°C for 48 hours to determine the DM of each segment of root. Root tissue density was calculated by dividing the DM of each segment by the root volume of each segment. The ratio of shallow root DM (within 50mm of the plant base) to deep root DM (beyond 50mm from the plant base) (SR:DR) was also calculated. The taproot diameter of PL was measured as the diameter of the thickest part of the taproot, below the base of the crown.

3.3.5 Statistical analysis

Data were analysed using the PROC MIXED procedure of SAS 9.4 (version 9.4, SAS institute 2018) using a model for a complete randomised block design. Pair-wised t-tests were used for mean comparisons and significance was declared at $P<0.05$. Soil VWC, leaf DM, total shoot and stubble DM, SLA, LER, photosynthesis rate, F_v/F_m, ΦPSII, root DM, SR:DR, root diameter and root tissue density were analysed within measurement dates with species, treatments and their interactions as fixed effects. Block (or replicate) was included as the random effect. Root diameter and root tissue density data were analysed with root distance from the plant base as an additional fixed effect. The number of shoots and leaves per PL plant, and the taproot diameter of PL were analysed within measurement dates with treatment as the fixed effect, and block as the random effect.

Soil VWC, leaf DM, total shoot and stubble DM, SLA, LER, root DM, SR:DR, root diameter and root tissue density were analysed for repeated measures with species, treatment, measurement date and their interactions as fixed effects. Block was included as the random effect. Root diameter and root tissue density data were analysed with root distance from the plant base as an additional fixed effect. Photosynthesis rate, ΦPSII, F_v/F_m and LER were analysed for repeated measures within each species, with treatment, measurement date, and their interactions as the fixed effects, and block as the random effect. The number of shoots and leaves per PL plant, and the taproot diameter of PL were also analysed for repeated measures with treatment, measurement date and their interactions as fixed effects, and block as the random effect.

Non-significant interactions between fixed effects have not been reported in the results.

3.4 Results

3.4.1 Soil volumetric water content

During the stress period there was no effect of species on soil VWC, however during the recovery period, RG pots had a significantly higher mean soil VWC than PL pots (P<0.01) (Figure *3*-*3*).

During the stress period, the soil VWC was significantly different between all treatments (P<0.01), being 36% on average in waterlogged pots, and 18 and 30% in control and wet pots, respectively. Following the removal of water treatments on day 40, the soil VWC of previously wet and waterlogged pots converged to a soil VWC similar to that of control pots, however control pots (20%) had a lower mean soil VWC than both wet (23%) and waterlogged (25%) pots during the recovery period (P<0.01). Previously waterlogged pots also had a greater average soil VWC than previously wet pots during the recovery period (P<0.01).

Mean soil VWC increased gradually throughout the stress period, regardless of species or water treatment (P<0.01), while during the recovery period, mean soil VWC declined gradually regardless of species or water treatment (P<0.01).

During the recovery period, control PL pots had a significantly lower mean soil VWC than previously wet or waterlogged PL pots (P<0.01), and previously waterlogged PL pots had a significantly greater mean soil VWC than control or wet PL pots (P<0.01). Previously waterlogged PL and RG pots a had similar mean soil VWC during the recovery period.

The soil VWC of both PL and RG pots decreased significantly between the start and the end of the recovery period, regardless of water treatment (P<0.01). Soil VWC varied between sampling dates within each water treatment during the stress and recovery periods, with

VWC decreasing over time for wet and waterlogged pots during the recovery period ($P<0.01$).

Soil VWC varied between measurement dates within each species × treatment combination during the recovery period, with control and wet PL pots having particularly low soil VWC values at days 55 and 60 of the experiment.

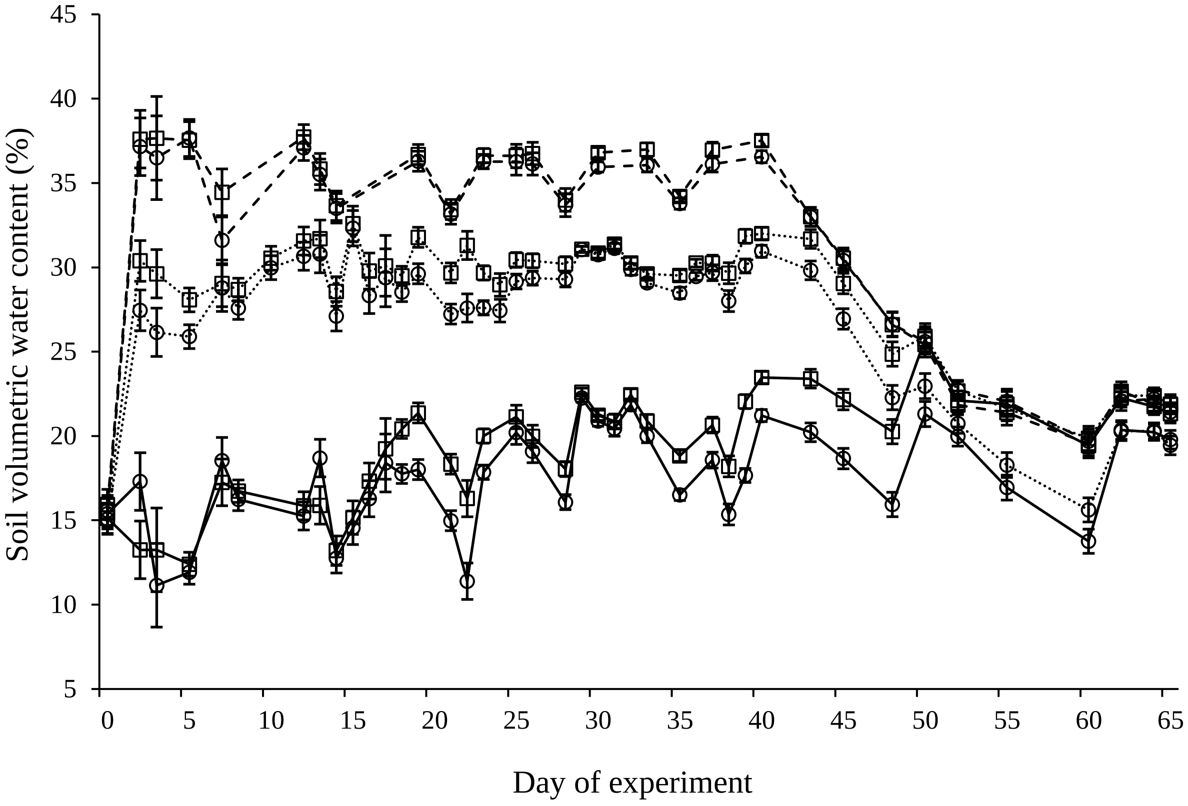


Figure 3-3. Soil volumetric water content (VWC) of plantain (circles) and perennial ryegrass (squares) pots under control (23% soil VWC; solid line), (wet 31.5% soil VWC; dotted line) and waterlogged (37% soil VWC; dashed line) soil conditions for 39 days, followed by 27 days under control soil conditions. Field capacity was 31% soil VWC. Vertical bars show the standard error of the mean.

3.4.2 Leaf dry matter

PL produced a similar amount of leaf DM to RG during the first phase of the stress period (days 1-22), on average over the entire stress period (days 1-39), and during the recovery period (days 39-66) (Table 3-1). However, RG produced more leaf mass than PL during the second phase of the stress period (days 22-39) ($P<0.01$).

Waterlogging significantly reduced leaf DM, regardless of species, at all harvests, in comparison with the control ($P<0.01$). Plants in the wet treatment produced more leaf DM than those under the control treatment during days 1-22 ($P<0.01$) and on average over the first 39 days of the stress period, regardless of species ($P<0.01$). However, during days 22-39, control plants produced a similar amount of leaf DM to wet plants and during the recovery period control plants produced more leaf DM than wet plants ($P<0.01$).

The leaf DM of PL was reduced by the waterlogging treatment by 37% and 38%, respectively, during the stress and recovery periods, in comparison with the control ($P<0.01$). During the first 22 days of the stress period, PL leaf DM was 27% lower under waterlogging than under the control treatment, while during the remainder of the stress period (days 22-39), PL leaf DM was reduced by 51% under waterlogging in comparison with the control ($P<0.01$). Under the wet treatment, PL plants produced 11% more leaf DM than control plants during the stress period ($P<0.01$), but 18% less leaf DM than control plants during the recovery period ($P<0.01$).

In contrast, the leaf DM of RG plants was only reduced by 18% by waterlogging during the stress period in comparison with the control ($P<0.01$) and was not reduced by waterlogging during the recovery period, in comparison with the control. The leaf DM of RG was 17% and 20% lower under waterlogging than under the control treatment during days 1-22 and 22-39, respectively. The leaf DM produced by RG plants under the wet and control treatments was similar during the stress and recovery periods.

Under waterlogging, RG plants produced 28% and 45% more leaf DM than PL plants during the stress and recovery periods, respectively ($P<0.01$). During days 1-22, PL plants produced a similar amount of leaf DM to RG under waterlogging, but during days 22-39, RG plants produced 69% more leaf DM under waterlogging than PL ($P<0.01$).

Table 3-1 Leaf dry matter (DM) (g/pot) of plantain and perennial ryegrass following 22 and 39 days under control (23% soil volumetric water content (VWC)), wet (31.5% soil VWC), and waterlogged (37% soil VWC) soil conditions, and a further 27 days (to day 66) under control soil moisture conditions. Field capacity was 31% soil VWC. Cumulative leaf DM up to day 39 was calculated by adding leaf DM from the day 22 and day 39 harvests together.

Species	**Treatment**	**Leaf DM**			
		22-day harvest	39-day harvest	Total stress period	66-day harvest
		Stress period			Recovery period
Plantain	Control	9.38	6.28a	15.66b	9.60a
	Wet	10.75	6.60a	17.35a	8.17b
	Waterlogged	6.81	3.09c	9.90d	6.00c
Perennial ryegrass	Control	9.07	6.51a	15.58b	8.96ab
	Wet	10.11	6.31a	16.42ab	8.02b
	Waterlogged	7.51	5.21b	12.72c	8.71ab
Standard error of the mean		0.417	0.333	0.651	0.472
Significance	Species	0.789	<0.01	0.197	0.113
	Treatment	<0.01	<0.01	<0.01	<0.01
	Species × Treatment	0.195	<0.01	<0.01	<0.01

Letters indicate means within columns that are significantly different ($P<0.05$).

3.4.3 Total shoot and stubble dry matter

The total shoot DM (stubble DM below 50mm height + leaf DM above 50mm height) was similar between RG and PL following both the stress and recovery periods (Table 3-2). Plants in the waterlogged treatment had a lower shoot DM than plants in the control and wet treatments following the stress period regardless of species (P<0.01). Plants in the wet treatment also had a greater shoot mass than plants in the control treatment, following the stress period (P<0.05), regardless of species.

At the end of the recovery period, waterlogged plants had a significantly lower shoot DM than plants in the control and wet treatments regardless of species (P<0.01). Control and wet plants had a similar total shoot DM following the recovery period.

Following the stress period, waterlogging reduced the total shoot DM of PL plants by 25% and 32% in comparison with the control and wet treatments, respectively (P<0.01). However, the shoot DM of wet PL plants was 11% greater than control PL plants following the stress period (P<0.05). The shoot DM of RG plants was 16% lower under waterlogging than under the wet treatment during the stress period (P<0.01), while wet and control RG plants had a similar shoot DM. The shoot DM of waterlogged RG plants was 17% greater than that of waterlogged PL plants following the stress period (P<0.05).

At the end of the recovery period, waterlogged PL plants had an average total shoot DM that was 20% and 23% lower than that of control and wet PL plants, respectively (P<0.01). The total shoot DM of control and wet PL plants was similar following the recovery period. There was no difference between the shoot DM of RG plants under the different water treatments at the day 66 harvest. The shoot DM of waterlogged RG plants was 28% higher than that of waterlogged PL plants following the recovery period (P<0.01).

At the end of the stress period, PL had a significantly larger stubble DM than RG, regardless of water treatment, however the two species had a similar stubble DM following the recovery period. At the end of the stress period, plants under the wet treatment had a significantly greater stubble DM than plants under the control or waterlogged treatments regardless of species, while control and waterlogged treatments had a similar stubble DM.

Following the recovery period, plants under the waterlogging treatment had a lower stubble DM than plants under the control or wet treatments regardless of species, while wet and control plants had a similar stubble DM.

Table 3-2. Shoot dry matter (DM; = leaf DM + stubble DM) and stubble DM (g/pot) of plantain and perennial ryegrass plants following 39 days under control (23% soil volumetric water content (VWC)), wet (31.5% soil VWC), and waterlogged (37% soil VWC) soil conditions, and a further 27 days (to day 66) under control soil moisture conditions. Field capacity was 31% soil VWC.

Species	**Treatment**	**Shoot DM**	**Stubble DM**	**Shoot DM**	**Stubble DM**
		Day 39 harvest		Day 66 harvest	
Plantain	Control	10.63bc	5.24	17.66a	8.06
	Wet	11.75a	5.95	16.49a	8.32
	Waterlogged	7.95e	4.80	12.42b	6.42
Perennial ryegrass	Control	10.29bcd	4.75	17.48a	8.52
	Wet	11.09ab	5.20	16.07a	8.05
	Waterlogged	9.32d	4.53	15.87a	7.16
Standard error of the mean		0.377	0.231	0.722	0.449
Significance	Species	0.679	0.013	0.122	0.348
	Treatment	<0.01	<0.01	<0.01	<0.01
	Species × Treatment	0.030	0.577	0.024	0.430

Letters indicate means within columns that are significantly different (P<0.05).

3.4.4 *Number of shoots and leaves per plant*

The number of leaves or shoots per PL plant was not significantly affected by water treatment during the stress or recovery periods (Table 3-3). The number of leaves per PL shoot was significantly reduced by the waterlogging treatment following the stress period ($P<0.05$), but there was no effect of water treatment on the number of leaves per PL shoot following the recovery period.

Table 3-3 Mean number of leaves and shoots per plant, and number of leaves per shoot for plantain plants following 39 days under control (23% soil volumetric water content (VWC)), wet (31.5% soil VWC), and waterlogged (37% soil VWC) soil conditions, and a further 27 days (to day 66) under control soil moisture conditions. Field capacity was 31% soil VWC.

Treatment	**Leaves** per plant	**Shoots** per plant	**Leaves** per shoot	**Leaves** per plant	**Shoots** per plant	**Leaves** per shoot
	39-day harvest			**66-day harvest**		
Control	22	4	5a	31	5	14
Wet	24	4	6a	33	4	16
Waterlogged	20	5	4b	28	5	13
Standard error of the mean	1.46	0.24	0.29	2.14	0.42	1.34
Significance						
Treatment	0.198	0.146	0.033	0.246	0.677	0.153

Letters indicate means within columns that are significantly different ($P<0.05$).

3.4.5 Specific leaf area

The SLA of PL was similar to that of RG following 39 days under the watering regime (Figure 3-4). Following the 27-day recovery period, RG had a significantly larger ($P<0.01$) SLA than PL. When averaged across species, plants from different water treatments had a similar SLA following both the stress and recovery periods. During the stress period, waterlogging tended to reduce the SLA of PL leaves in comparison with the control ($P=0.09$), while in RG, SLA was not significantly different between the treatments.

The SLA of waterlogged PL leaves was significantly lower than that of waterlogged RG leaves after 39 days under the water treatments ($P<0.05$).

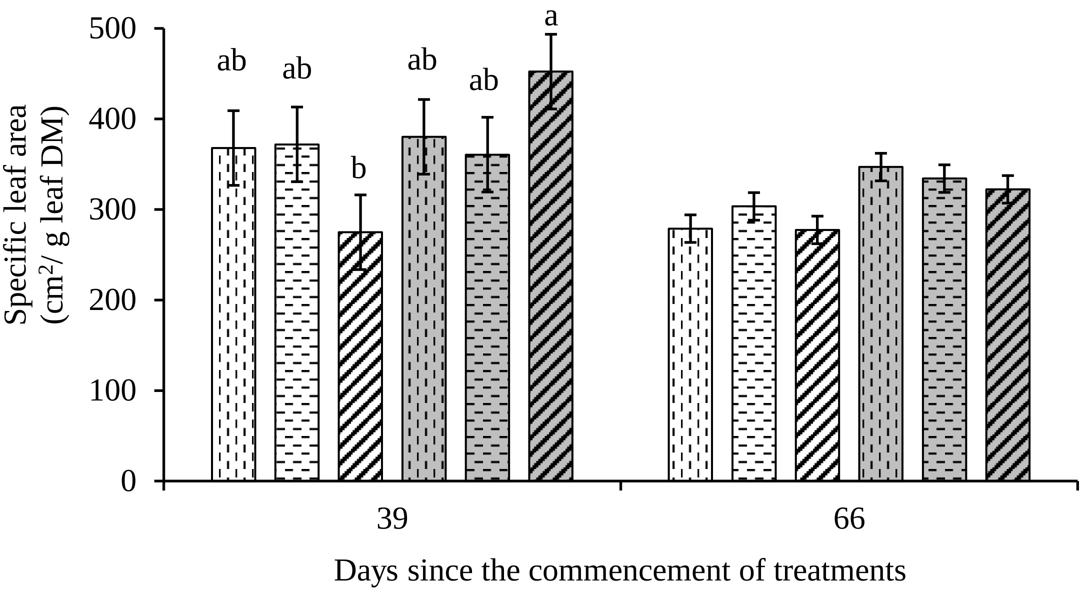


Figure 3-4. Specific leaf area (cm^2/ g leaf DM) of plantain (white bars) and perennial ryegrass (grey bars) following 39 days under control (23% soil volumetric water content (VWC); vertical dashes), wet (31.5% soil VWC; horizontal dashes), and waterlogged (37% soil VWC; diagonal lines) soil conditions, and a further 27 days (to day 66) under control soil moisture conditions. Field capacity was 31% soil VWC. Means with different letters are significantly different (P<0.05). Vertical bars show the standard error of the mean.

3.4.6 Leaf elongation rate

The LER of PL leaves in all treatments varied throughout the stress and recovery periods, ranging from 3 mm day^{-1} to 19 mm day^{-1} (Figure 3-5). On average the LER of waterlogged plants was 37% lower than control plants during the stress period ($P<0.01$). However, during the recovery period, the mean LER of waterlogged plants was not significantly different from control plants. There was no significant difference between the mean LER of PL plants under the control and wet treatments during the stress or recovery periods. The date of measurement had a significant effect on the LER of PL plants during the stress and recovery periods, regardless of treatment ($P<0.01$), with a noticeably higher LER occurring prior to days 8 and 35 of the stress period.

The LER of RG leaves ranged between 8 mm day^{-1} to 25 mm day^{-1} and varied considerably throughout the stress and recovery periods (Figure 3-6). Water treatment had no significant effect on the LER of RG plants during the stress or recovery periods. The date of measurement had a significant effect on the LER of RG plants during the stress period, with the greatest LER occurring prior to days 8 and 15 ($P<0.01$).

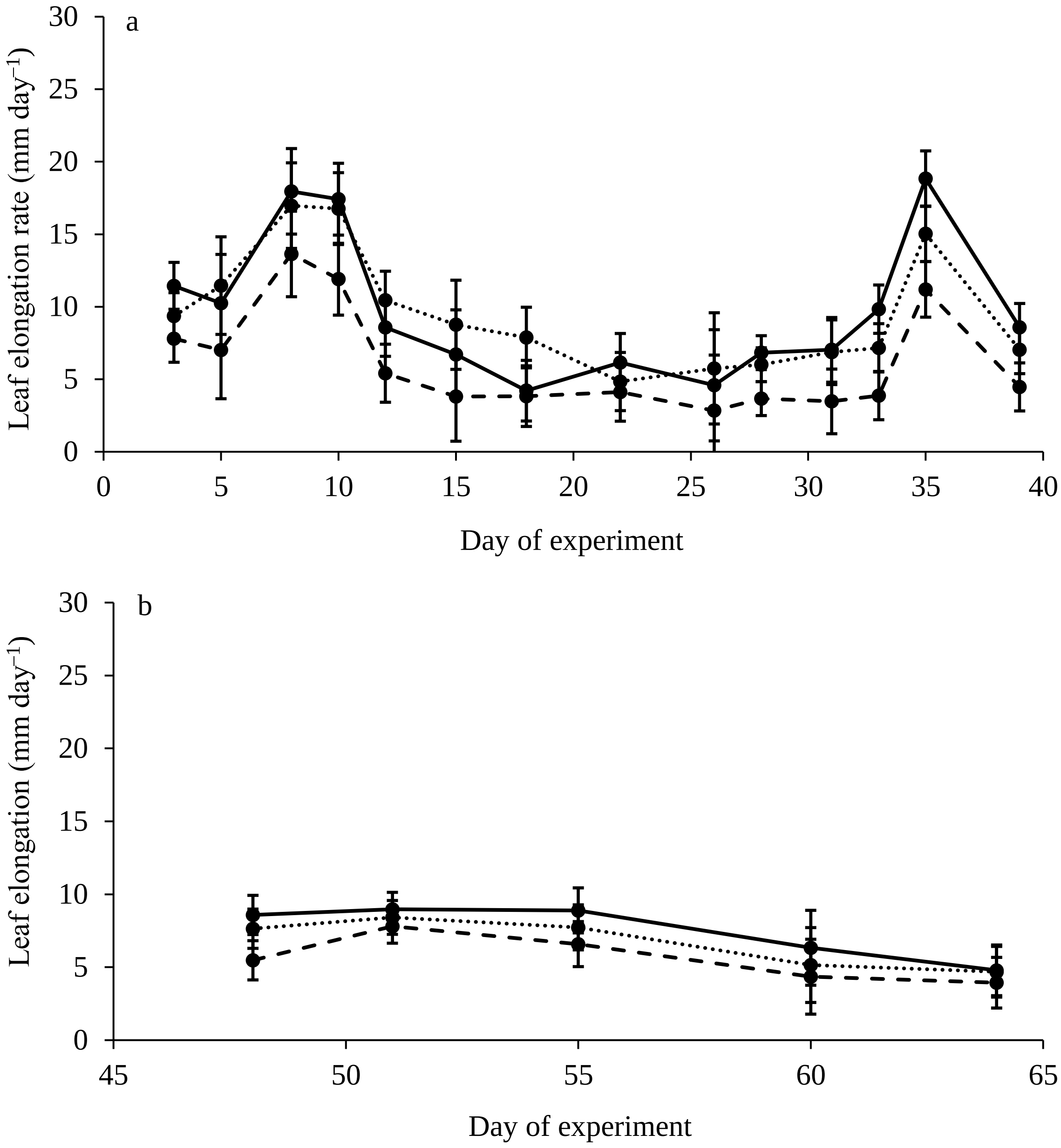


Figure 3-5. Leaf elongation rate (mm leaf $^{-1}$day^{-1}) of plantain plants under control (23% soil volumetric water content (VWC); solid lines), wet (31.5% soil VWC; dotted lines), and waterlogged (37% soil VWC; dashed lines) soil conditions for (a) 39 days, and (b) 27 days under control soil moisture conditions. Field capacity was 31% soil VWC. Vertical bars show the standard error of the mean.

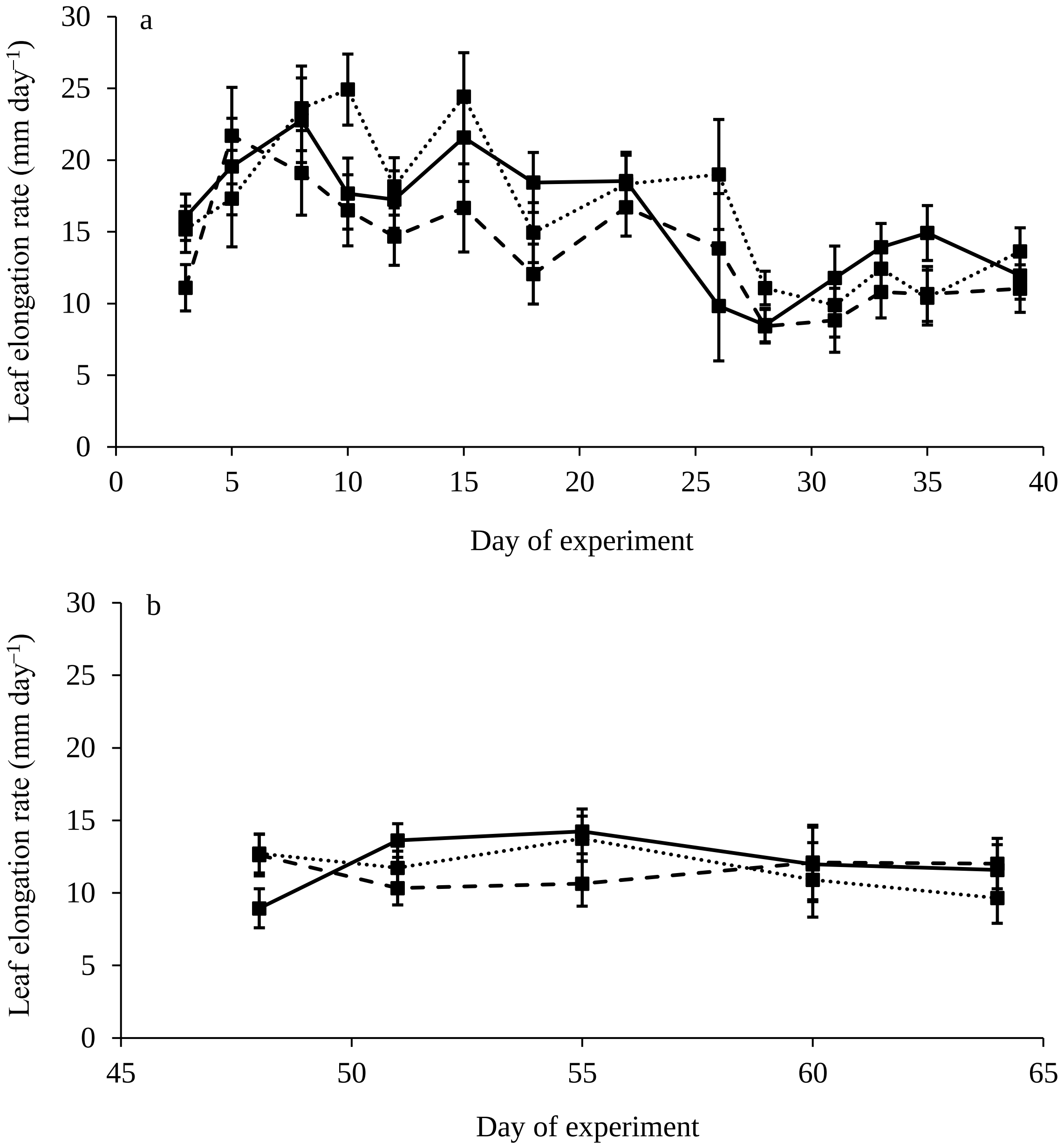


Figure 3-6. Leaf elongation rate (mm leaf^{-1} day^{-1}) of perennial ryegrass plants under control (23% soil volumetric water content (VWC); solid lines), wet (31.5% soil VWC; dotted lines), and waterlogged (37% soil VWC; dashed lines) soil conditions for (a) 39 days, and (b) 27 days under control soil moisture conditions. Field capacity was 31% soil VWC. Vertical bars show the standard error of the mean.

3.4.7 Photosynthesis

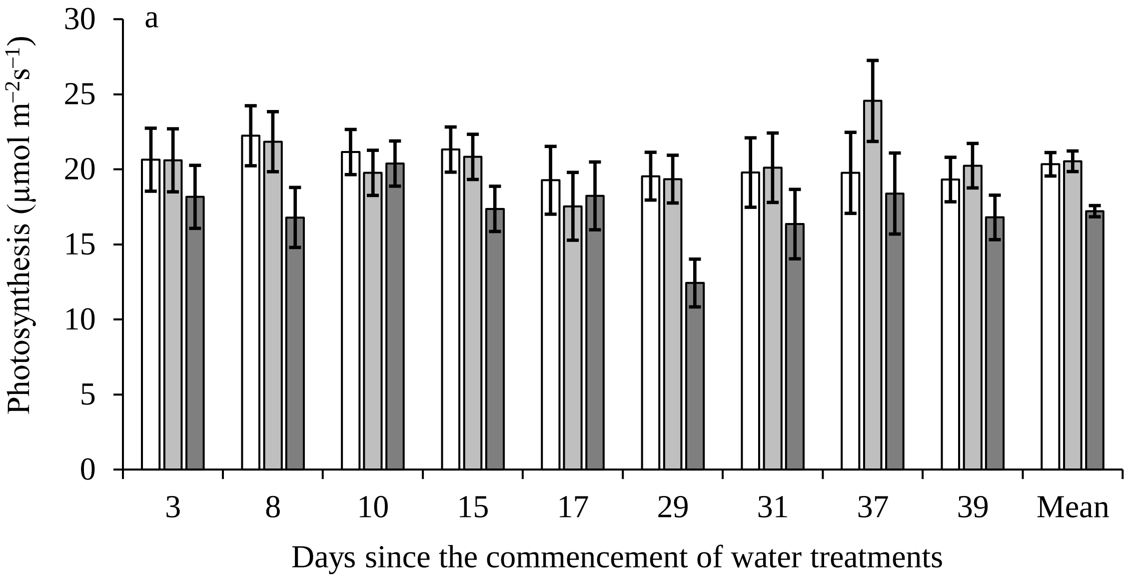
3.4.7.1 Photosynthesis rate

The photosynthesis rate in PL leaves was similar to that of RG leaves during the stress period but was significantly higher than that of RG leaves during the recovery period, regardless of water treatment ($P<0.01$). When averaged across plant species and sampling dates, water treatment had no significant effect on photosynthesis rate during the experiment.

During the stress period, sampling date had a significant effect on the photosynthesis rate of plants regardless of species or water treatment, with the greatest photosynthesis rates occurring on day 37 ($P<0.05$). Sampling date had no significant effect on photosynthesis rate during the recovery period.

The rate of photosynthesis in PL leaves ranged between 12 µmol $m^{-2}s^{-1}$ to 25 µmol $m^{-2}s^{-1}$ during the experiment (Figure 3-7). Waterlogging reduced the rate of photosynthesis in PL leaves by 15% on average in comparison with control watering during the stress period ($P<0.05$), however waterlogged and control plants had a similar mean photosynthesis rate during the recovery period. The photosynthesis rate of PL plants in the control and wet treatments was similar throughout the experiment and was 20 µmol $m^{-2}s^{-1}$ and 21 µmol $m^{-2}s^{-1}$ on average during the stress and recovery periods respectively.

The rate of photosynthesis in RG leaves ranged between 11 µmol $m^{-2}s^{-1}$ to 20 µmol $m^{-2}s^{-1}$ during the experiment (Figure 3-8). There was no significant effect of water treatment on the rate of photosynthesis of RG leaves during the stress or recovery periods. Photosynthesis rate differed significantly between sampling dates for RG leaves, with particularly high photosynthesis rates occurring on days 17 and 37 of the stress period ($P<0.01$).



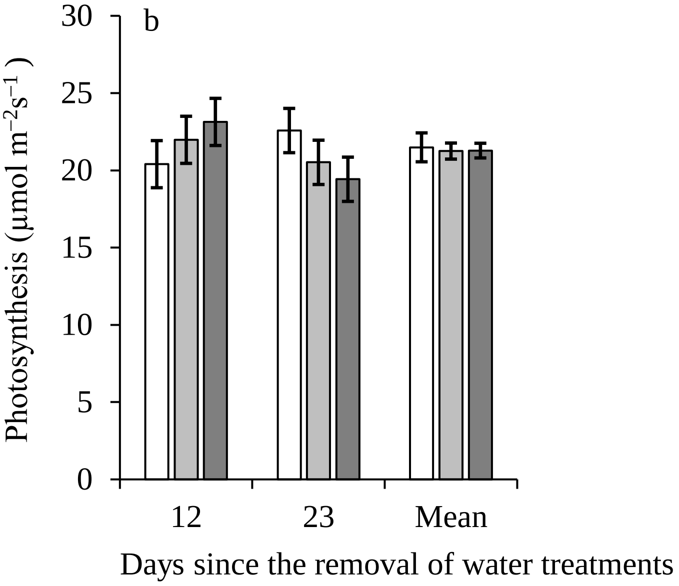
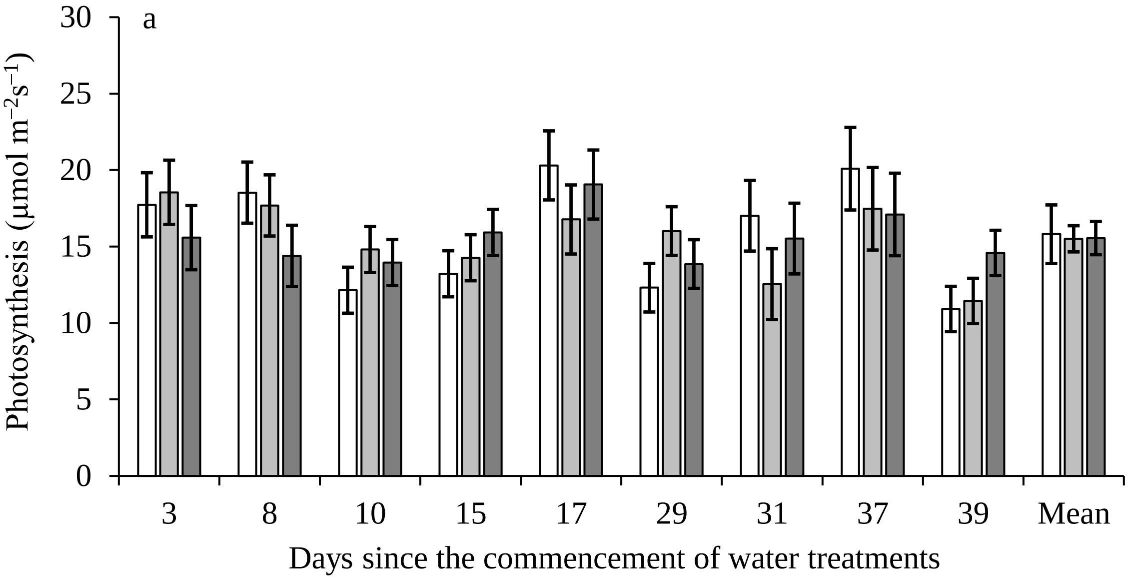


Figure 3-7. Photosynthesis rate (CO_2 uptake = mmol/(m^2/second) of plantain under control (23% soil volumetric water content (VWC); white bars), wet (31.5% soil VWC; light grey), and waterlogged (37% soil VWC; dark grey) soil conditions for (a) 39 days, and (b) 27 days under control soil moisture conditions. Field capacity was 31% soil VWC. Vertical bars show the standard error of the mean.



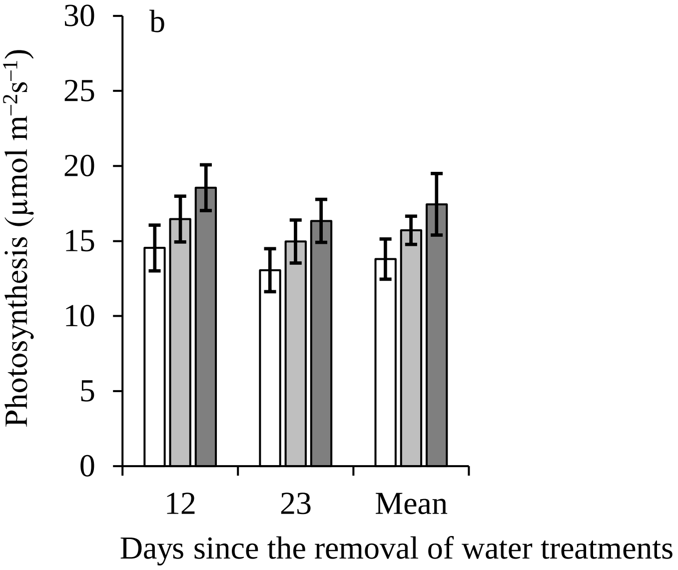


Figure 3-8. Photosynthesis rate (CO_2 uptake = mmol/(m^2/second) of perennial ryegrass under control (23% soil volumetric water content (VWC); white bars), wet (31.5% soil VWC; light grey), and waterlogged (37% soil VWC; dark grey) soil conditions for 39 days (a), and 27 days under control soil moisture conditions (b). Field capacity was 31% soil VWC. Vertical bars show the standard error of the mean.

3.4.7.2 Dark-adapted chlorophyll fluorescence

Water treatment did not significantly affect the mean fluorescence of PL or RG leaves at the conclusion of the water stress period (Figure 3-9).

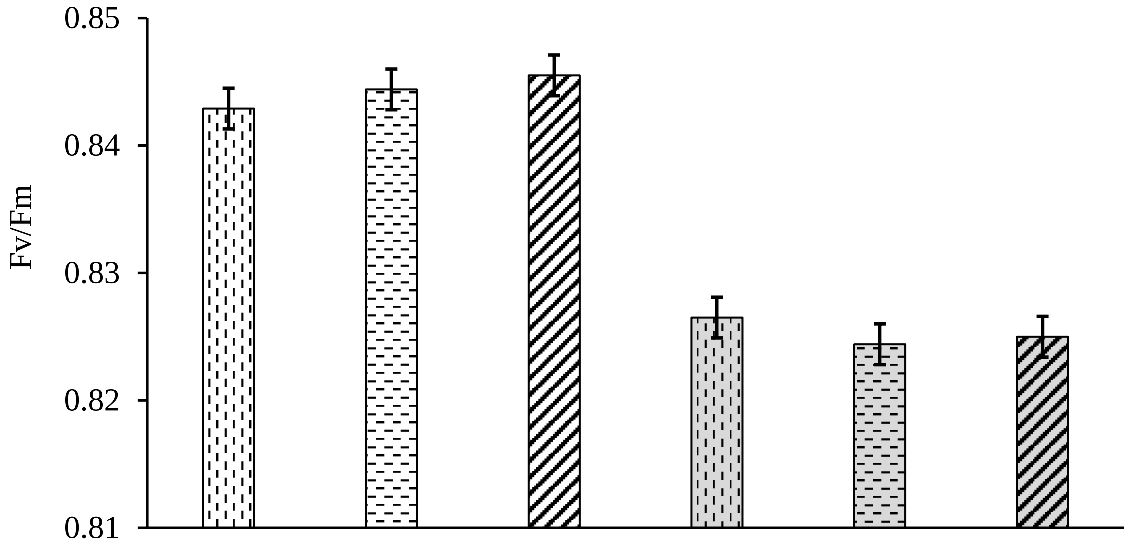
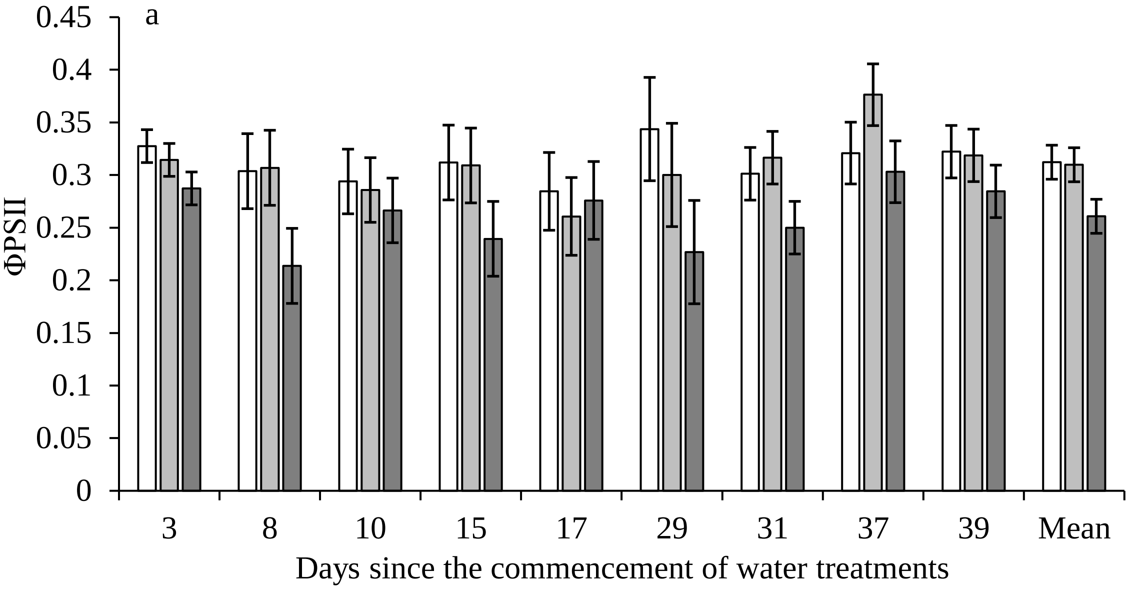


Figure 3-9. Fluorescence (Fv/Fm) of plantain (white bars) and perennial ryegrass (grey bars) after 39 days under control (23% soil volumetric water content (VWC); vertical dashes), wet (31.5% soil VWC; horizontal dashes), and waterlogged (37% soil VWC; diagonal lines) soil conditions. Field capacity was 31% soil VWC. Vertical bars show the standard error of the mean.

3.4.7.3 Operating efficiency of photosystem II

Plantain leaves under waterlogged conditions had a significantly lower photosystem two operating efficiency (ΦPSII) on average over the duration of the stress period, than PL leaves under control or wet conditions ($P<0.01$) (Figure 3-10). On average, ΦPSII was reduced by 17% in waterlogged plants in comparison with control plants. Plantain leaves under the control or wet treatment had a similar mean ΦPSII during the stress period.

Water treatment did not significantly affect mean ΦPSII in RG leaves during the stress period, although it varied between sampling dates ($P<0.05$). The ΦPSII of RG was lower at sampling events on days 8, 10 and 15 than the sampling event on day 3 ($P<0.05$). However, ΦPSII recovered to day 3 levels by the day 17 sampling event.



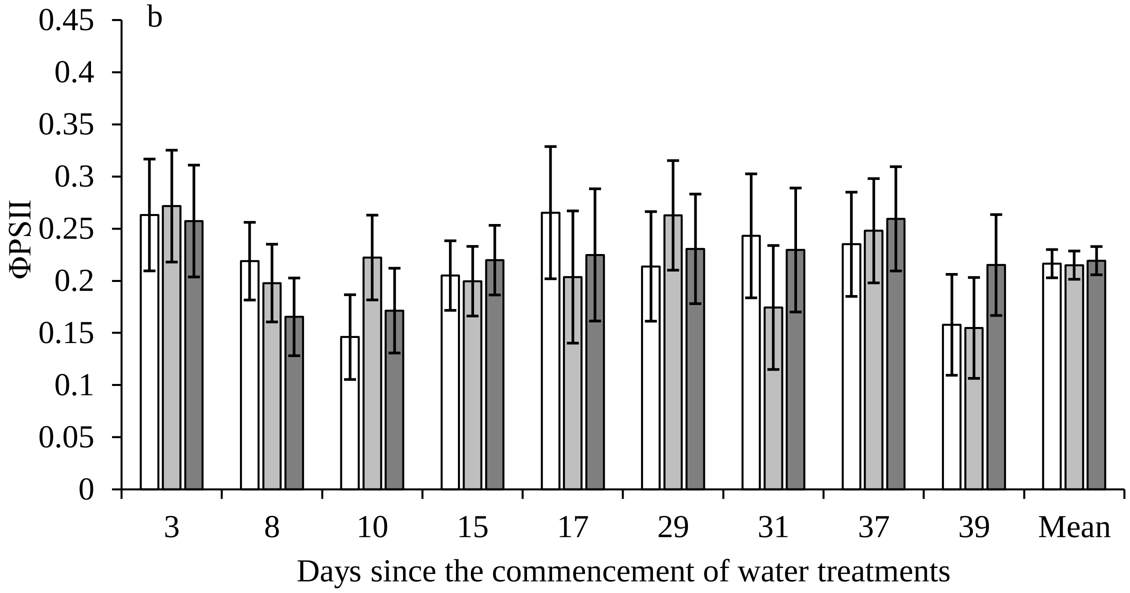


Figure 3-10 Operating efficiency of photosystem II (ΦPSII) of plantain (a) and perennial ryegrass (b) leaves over 39 days under control (23% soil volumetric water content (VWC); white bars), wet (31.5% soil VWC; light grey), and waterlogged (37% soil VWC; dark grey) soil conditions. Vertical bars show the standard error of the mean.

3.4.8 Root dry matter

The root DM was similar between species following the 39-day stress period, but RG plants had 120% more root DM than PL plants following the recovery period ($P<0.01$) (Table 3-4). Root DM increased by 17% under the waterlogging treatment in comparison with the control regardless of species during the stress period, but water treatment had no effect on root DM during the recovery period, when averaged across the species.

The root DM of PL was not significantly different between water treatments following the stress or recovery periods. The root DM of RG tended to be higher under waterlogging in comparison with the control following the stress period ($P=0.09$), but not different than the control following the recovery period.

The mean root to shoot DM ratio (R:S) of RG was 34% and 98% higher than that of PL plants during the stress and recovery periods, respectively. The R:S significantly increased under waterlogging regardless of species during the stress period, although this effect disappeared over the recovery period.

Table 3-4. Total root dry matter (DM) (g/pot) and root to shoot DM (leaf DM + stubble DM) ratio of plantain and perennial ryegrass following 39 days under control (23% soil volumetric water content (VWC) and waterlogged (37% soil VWC) soil conditions, and 27 days recovery (to day 66) under control soil moisture conditions. Field capacity was 31% soil VWC. Means with different letters within the same column are significantly different (P<0.05).

Species	**Treatment**	**Root DM**	**Root: Shoot DM**	**Root DM**	**Root: Shoot DM**
		39-day harvest		66-day harvest	
Plantain	Control	6.97b	0.66b	7.04b	0.40c
	Waterlogged	6.85b	0.86b	5.35b	0.43c
Perennial ryegrass	Control	8.45b	0.83b	12.13a	0.70b
	Waterlogged	11.23a	1.20a	15.09a	0.94a
Standard error of the mean		0.920	0.100	1.350	0.080
Significance	Species	0.117	0.013	<0.01	<0.01
	Treatment	<0.01	<0.01	0.581	0.077
	Species × Treatment	0.091	0.364	0.061	0.158

3.4.9 Root morphology

3.4.9.1 Ratio of shallow to deep roots

The SR:DR was similar between species following 39 days under the water treatments (Figure *3-11*). Waterlogging significantly increased the SR:DR in comparison with the control, regardless of species ($P<0.01$). Waterlogged PL plants had a 120% greater SR:DR than control PL plants and a 72% greater SR:DR than waterlogged RG plants following the stress period ($P<0.01$).

Following the recovery period, PL and RG plants had a similar SR:DR, and waterlogged plants had a significantly greater SR:DR, than control plants, regardless of species ($P<0.05$).

There was a strong, negative correlation ($R^2 = -0.48$) between the DM of deep roots and the SR:DR ($P<0.01$; data not shown) of PL and RG, regardless of species or water treatment following the treatment and recovery periods.

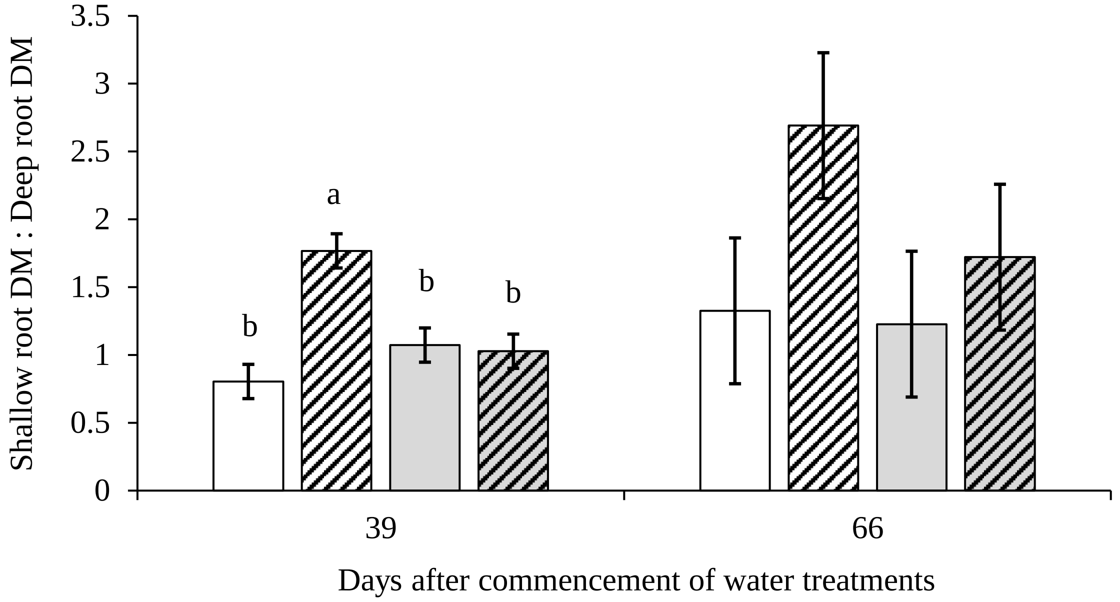


Figure 3-11. Ratio of shallow root dry matter (DM; within 50mm of the plant base) to deep root DM (beyond 50mm from the plant base) of plantain (white bars) and perennial ryegrass (grey bars) following 39 days under control (23% soil volumetric water content (VWC); solid fill), and waterlogged (37% soil VWC; diagonal lines) soil conditions, and 27 days recovery (to day 66) under control soil moisture conditions. Vertical bars show the standard error of the mean. Means with different letters are significantly different ($P<0.05$).

3.4.9.2 Root diameter

PL had a significantly higher mean root diameter than RG regardless of treatment or distance from plant base ($P<0.05$), after 39 days under the water treatments (Figure 3-12). Water treatment did not have any significant effect on the root diameter at the day 39 harvest, when averaged across plant species and distance from plant base.

Following the recovery period, PL plants had a significantly larger root diameter than RG regardless of treatment or distance from plant base ($P<0.01$). There was no effect of water treatment on root diameter at this harvest. At day 66, the diameter of roots within 50mm of the plant base was significantly larger than that of roots 50-150mm from the plant base or beyond 150mm from the plant base, regardless of species or water treatment ($P<0.01$). The root diameter of PL roots within 50-150mm of the plant base and beyond 150mm from the plant base was significantly larger than those of RG roots ($P<0.05$) following the recovery period.

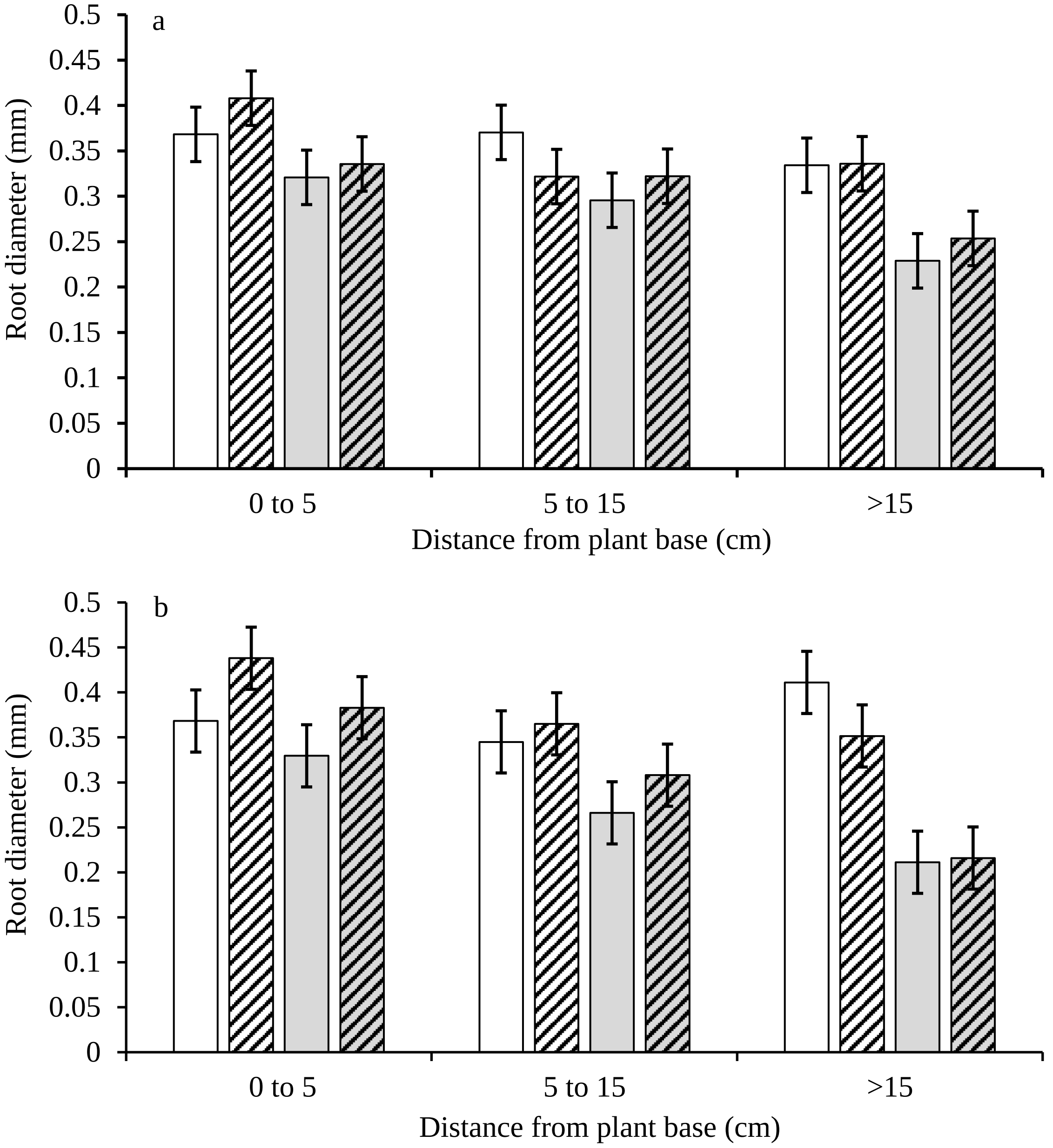


Figure 3-12. Mean root diameter of plantain (white bars) and perennial ryegrass (grey bars) roots within 0-50mm and 50-150mm, and beyond 150mm from the plant base, following (a) 39 days under control (23% soil volumetric water content (VWC); solid fill), and waterlogged (37% soil VWC; diagonal lines) soil conditions, and (b) 27 days recovery under control soil moisture conditions. Vertical bars show the standard error of the mean.

3.4.9.3 Root tissue density

The density of PL roots was greater than that of RG roots following the stress period, regardless of water treatment or distance from the base of the plant ($P<0.01$) (Figure 3-13). Plants under the control treatment had a significantly higher root tissue density than plants under the waterlogging treatment at the day 39 harvest, regardless of species ($P<0.01$). Root tissue density decreased significantly with an increase in the distance from the base of the plant, regardless of species or water treatment ($P<0.01$) following the stress period. At the day 39 harvest, PL roots had a higher density in the 0-50mm and 50-150mm segments than RG roots ($P<0.01$) regardless of water treatment, but the species had a similar root tissue density in the beyond 150mm segment.

Following the recovery period, PL roots had a greater density than RG roots regardless of water treatment or distance from the plant base ($P<0.01$). There was no effect of water treatment on root tissue density at the day 66 harvest. Root tissue density decreased significantly with an increase in the distance from the base of the plant, regardless of species or water treatment ($P<0.01$) following the recovery period. At the day 66 harvest, PL roots had a higher density in the 0-50mm segment than RG roots regardless of water treatment ($P<0.01$), however the root tissue density was similar between the species for the 50-150mm and beyond 150mm segments.

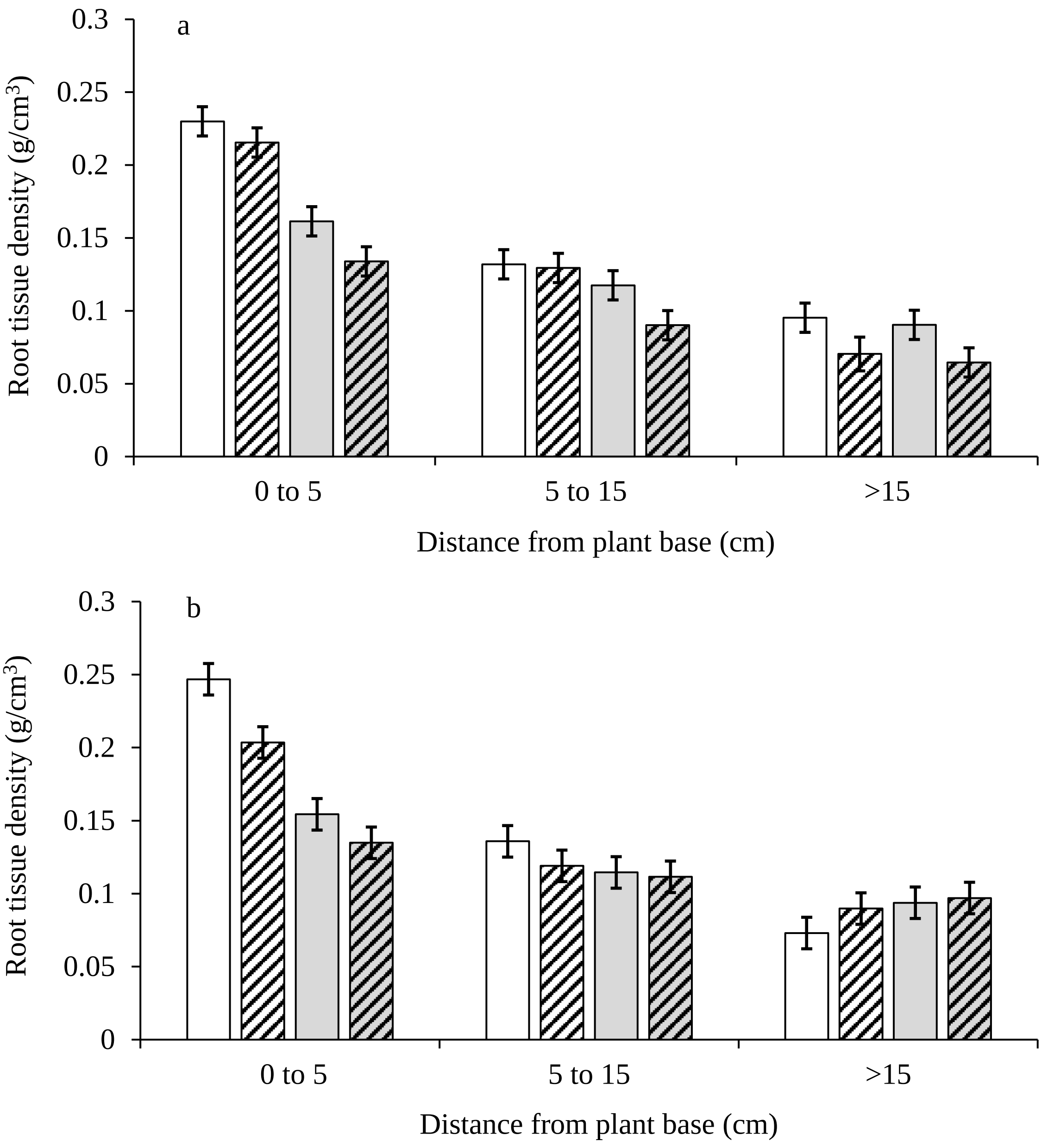


Figure 3-13. Mean root tissue density of plantain (white bars) and perennial ryegrass (grey bars) roots within 0-50mm and 50-150mm, and beyond 150mm from the plant base, following (a) 39 days under control (23% soil volumetric water content (VWC); solid fill), and waterlogged (37% soil VWC; diagonal lines) soil conditions, and (b) 27 days recovery under control soil moisture conditions. Vertical bars show the standard error of the mean.

3.4.9.4 Taproot diameter

When averaged across the harvest dates, PL taproot diameter was similar between plants under different water treatments (Figure 3-14). The mean taproot diameter of PL plants was not significantly affected by water treatment at either of the harvest dates, although waterlogged PL plants tended to have a lower taproot diameter than control plants at the day 39 harvest (P=0.07). The PL taproot diameter was not significantly different between harvest dates.

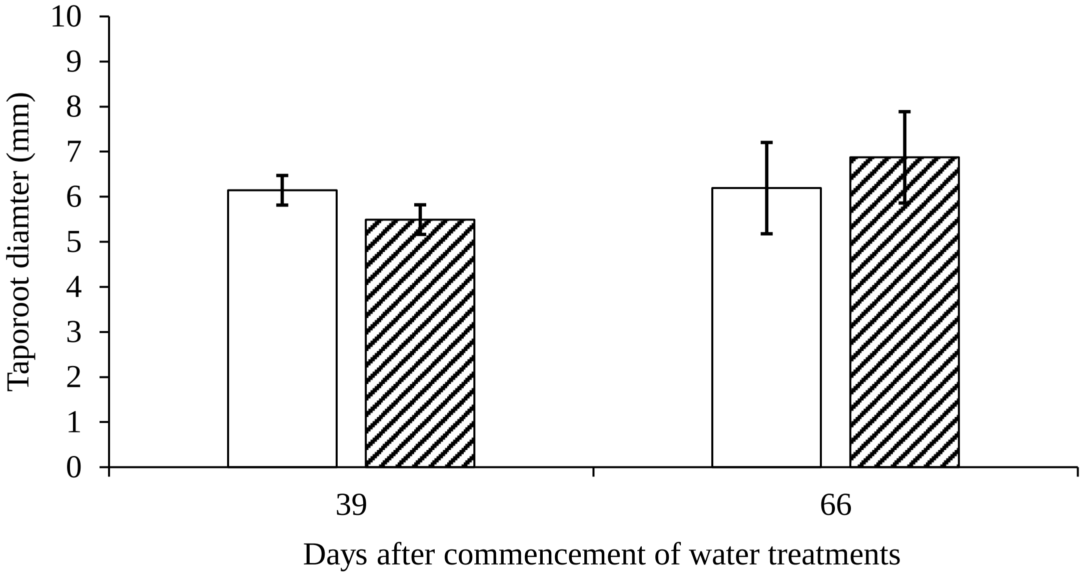


Figure 3-14. Mean taproot diameter of plantain plants following 39 days under control (23% soil volumetric water content (VWC); solid fill), and waterlogged (37% soil VWC; diagonal lines) soil conditions, and 27 days recovery (to day 66) under control soil moisture conditions. Vertical bars show the standard error of the mean.

3.5 Discussion

3.5.1 Water treatments

Although there was a significant difference in soil VWC between the three waterlogging treatments, during the stress period, the intended soil VWC was not achieved for the wet and control treatments, evidenced by the lower-than-expected mean soil VWC for those treatments. This was likely due to the rapid loss of water from pots, both through drainage immediately following watering, and through evapotranspiration. Due to the nature of soil moisture measurement in this study (manual weighing of 60 11kg pots), it was decided that measurements would be made no less frequent than once every two days, and where possible, every day. The results suggest that this frequency of soil moisture measurement and maintenance was not sufficient to maintain precise soil VWC's in these pots. Future studies that use similar pot sizes and soil mixes could consider measuring and maintaining soil moisture two times per day or incorporate automated soil moisture monitoring equipment for more effective treatment implementation.

However, it was not believed that these limitations affected the validity of the results in this experiment since the soil VWC of the three treatments was significantly different during the stress period.

3.5.2 Plantain and perennial ryegrass shoot growth and morphology under waterlogging

The reduction in PL leaf DM under the waterlogging stress had lasting effects, evidenced by the slower recovery of waterlogged PL in comparison with waterlogged RG following the cessation of water treatments. The reduction in PL leaf DM yield under waterlogging was probably caused by a combination of a reduced LER and photosynthetic rate. Similarly, in work by Grimoldi et al. (2005), above ground biomass was reduced by 65% in PL plants subjected to above ground waterlogging for 60 days (water level 6cm above soil surface but not above leaves), while in barley plants (*Hordeum vulgare* L.) subjected to surface waterlogging for 21 days (water level at soil surface), shoot biomass was reduced by 65-80% in sensitive cultivars (Pang et al. 2004). The leaf DM of RG recovered relatively quickly from the waterlogging stress in comparison with PL, suggesting that RG may have been in a less-stressed state at the cessation of the water treatments, due to employing water tolerance mechanisms (Liu & Jiang 2015), and/or being able to rapidly restore shoot growth processes following the end of the waterlogging stress, through compensatory growth mechanisms (Korte & Chu 1983; Nicholas et al. 2004).

Leaf elongation is highly sensitive to changes in plant water status and has been identified as an early indicator of plant sensitivity to waterlogging stress (Dias-Filho & De Carvalho 2000). The reduction in the LER of PL under waterlogging stress was similar to that of Brachiaria grass (*Urochloa brizantha*) subjected to flooding for 28 days (Beloni et al. 2017), and indicates that PL is sensitive to waterlogging (Dias-Filho & De Carvalho 2000). However, the waterlogging stress did not cause a total cessation of growth in PL leaves, suggesting that PL may possess waterlogging tolerance features. In the pasture grasses cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.), the restoration of oxygen supply to waterlogged tissues through morphological changes was a major waterlogging tolerance mechanism which allowed for the continuation of above ground growth (Mui et al. 2021). In the current study, waterlogged PL plants may have developed additional adventitious roots close to the oxygenated soil surface or drawn oxygen from above the soil surface through lysigenous aerenchyma cells (Grimoldi et al. 2005; Mui et al. 2021). Additionally, the similarity in the LER of control and waterlogged PL plants during the recovery period suggests that plants recovered quickly from waterlogging, possibly due to the maintenance of important growth functions while under stress.

While the waterlogging stress did not lead to the death of PL plants in this experiment, a longer stress period, or the combination of stresses that would normally occur in a grazed pasture, could have a more significant impact on PL survival in the field. The reduction in the LER of a PL plant would limit its ability to generate leaf area, and thus capture light for use in photosynthesis (Teramura et al. 1981). This could be particularly critical for PL, as it has been suggested that PL has a limited capacity for energy storage in its crown and roots (Ayala et al. 2011b), and thus PL may be more reliant on residual leaf area and current photosynthesis for leaf regrowth following defoliation. Therefore, the decrease in the LER of PL under waterlogging could lead to further reductions in light interception, energy production and growth following subsequent defoliation. This effect might be exacerbated if PL were to co-exist in a grazed pasture with RG, which this study and others (Di Bella et al. 2022), have shown to be productive and therefore competitive, in waterlogged soil. Additionally, the improved ability of RG to recovery quickly from abiotic stresses (Barker et al. 1985) could prolong the effect, leading to the continual shading of PL plants, even after the end of waterlogging stress.

While the effect of waterlogging on the stubble DM of PL and RG plants appeared to be small following the stress period, waterlogging had reduced the stubble DM of both species following the recovery period. This reduction could indicate that the two species exhibited a similar response to the cessation of waterlogging, in which plants allocated DM to other organs before increasing stubble DM. Chicory and PL plants have been shown to rapidly increase leaf growth during recovery from drought stress (Cave et al. 2013), which could indicate that resources were allocated preferentially to leaf growth during recovery from water stress. However, the leaf DM production of previously waterlogged PL plants was lower than that of control and wet plants during the recovery period, possibly indicating that recovering PL plants had limited remaining resources to allocate to leaf regrowth, due to using those resources to support survival mechanisms during the stress period. In contrast, RG plants produced a similar quantity of leaf DM in previously waterlogged plants to that of control and wet plants during the recovery period, possibly indicating a preference for the allocation of resources to leaf growth before stubble growth, following the end of the stress period.

On first assessment, the absence of any water treatment effect on the number of leaves and shoots per PL plant suggests that the reduction in total above-ground shoot DM in waterlogged plants during the stress period was more related to changes in leaf or stubble growth under waterlogging. However, the reduction in the number of leaves per PL shoot under the waterlogging stress, likely contributed to the reduction in leaf DM of PL plants during the stress period. The decrease in the number of leaves per PL shoot suggests that the waterlogging stress might have negatively affected leaf appearance, an effect that has been previously observed in water-stressed PL plants (Guisande-Collazo et al. 2022) and RG plants (Rawnsley et al. 2010). Furthermore, a reduction in the potential leaf area produced by an individual PL shoot could potentially affect its survivability in longer-term waterlogging events, due to a reduced ability to intercept light.

The contrasting morphological response of PL and RG plants to waterlogging could suggest a difference in the waterlogging tolerance strategy of the two species. The relatively lower SLA of PL plants under waterlogging may have been related to reduced leaf expansion (Guisande-

Collazo et al. 2022), and/or an accumulation of photosynthates in leaves, due to a reduction in their transport to other storage sites, as was observed in waterlogged Brachiaria grass (*Brachiaria* spp.) (Dias-Filho & De Carvalho 2000). For PL, a reduction in SLA under waterlogging might further impact its ability to compete for light while under stress, due to the likely reduction in photosynthetic surface area. In contrast, the relatively higher SLA of waterlogged RG plants at the end of the stress period could have been caused by an increase in the translocation of photosynthates away from the leaf to support the increased root growth, which appeared to occur during that time. However, it was also possible that the relatively higher SLA of RG plants under waterlogging may have occurred simply because the waterlogging treatment encouraged leaf elongation through an unlimited water supply.

3.5.3 Plantain and perennial ryegrass root growth and morphology under waterlogging

The increase in the mean root DM of waterlogged plants during the stress period was likely driven by the trend for increased root growth in waterlogged RG plants. The maintenance of root growth under waterlogging has been associated with waterlogging tolerance in RG (McFarlane et al. 2003; Di Bella et al. 2022). While increased root growth did not appear to be a waterlogging tolerance strategy of PL, root DM may have remained constant under waterlogging, through the replacement of dead primary roots with new adventitious roots in oxygenated areas close to the soil surface, which was evidenced by the increase in SR:DR in PL plants, and has also been observed in waterlogged wheat (*Triticum aestivum* L.) (Malik et al. 2001). However, a closer examination of root morphology revealed a strong, negative relationship between the quantity of root DM in deeper, less-oxygenated regions of soil, and SR:DR. Combining this negative relationship with a poor to non-existent relationship between shallow root DM and SR:DR makes clear that the increased mean SR:DR of plants under waterlogging during the stress period was likely caused by a reduction in deep root DM, rather than an increase in shallow root DM. Thus, it is unlikely that any maintenance of root DM in PL plants under waterlogging resulted from increased adventitious root growth in shallow, oxygenated soil.

The increase in mean R:S under waterlogging during the stress period was likely caused by a combination of a reduction in leaf DM production of plants under waterlogging, along with the preservation of root DM growth in those plants. The higher R:S of RG plants in comparison with PL plants throughout the experiment may partly explain the improved recovery of the former once the stress had subsided. Previous research showed that a reduction in the leaf growth of RG in response to abiotic stress led to an accumulation of soluble sugars in leaves (Barker et al. 1985), and resulted in compensatory growth following the removal of the stress (Korte & Chu 1983; Barker et al. 1985). While the R:S of RG increased under waterlogging during the stress period in the present study, this effect diminished during the recovery period as RG plants likely allocated more resources to leaf production.

The roots of PL plants had a greater mean diameter and root tissue density than RG plants throughout the experiment, highlighting an important morphological difference between the root systems of the two species, which could have important implications for plant growth and survival under stress conditions. The lower tissue density of the fibrous roots of RG plants might have enabled a rapid response to waterlogging, as low-density tissue enables a fast growth rate and allows for a rapid acquisition of resources with a low DM investment (Ryser & Lambers 1995). This could explain the apparent increase in the root growth of RG plants under the waterlogging treatment, as roots searched for favourable environments within flooded pots. In contrast, it is possible that the denser, branched-taproot system of PL probably reduced the speed at which root growth processes could adjust to the waterlogged soil, with PL plants opting to respond to stress by preserving existing tissues (Ryser & Lambers 1995) and altering internal root structures instead. One such alteration of internal root structure and important waterlogging avoidance strategy that has been previously observed in PL (Grimoldi et al. 2005) is the formation of aerenchyma in root tissue. One typical indicator of aerenchyma formation is an increase in root diameter (Striker et al. 2007). Although it appeared that both species' roots were thicker in the 0-50mm segment under waterlogging in comparison with the control during the experiment, this was not significant. However, because aerenchyma formation has been shown to occur in PL without an increase in root diameter (Grimoldi et al. 2005), the formation of aerenchyma in PL roots under waterlogging in this experiment cannot be ruled out.

Tap-rooted species store carbohydrates and nitrogenous compounds in their taproot to enable the recovery of shoot regrowth following defoliation (Fankhauser et al. 1989), and taproot diameter has been used as an indicator of these energy reserves in tap-rooted species (Li et al. 1997c). Since leaf DM and photosynthesis rates, and likely carbohydrate synthesis, were reduced in PL under waterlogging in the current experiment, a larger reduction in PL taproot diameter was expected than the observed trend for thinner taproots in waterlogged plants. However, this result was in line with that of Cranston et al. (2016), who showed that PL taproot diameter was not significantly different between plants under drought stress and well-watered conditions. Taken together, these results might suggest that PL taproot diameter is insensitive to water stress and may not be a reliable indicator of shoot regrowth potential following release from stress.

3.5.4 Plantain and perennial ryegrass photosynthesis under waterlogging

The reduction in photosynthesis and ΦPSII in PL leaves under the waterlogging stress suggest that PL is sensitive to waterlogging at a physiological level. Although the observed reduction in the photosynthesis of PL leaves under waterlogging (-15%) was significant, it was a considerably smaller response than the 82% reduction observed in waterlogged wheat (Malik et al. 2001), and the 80% reduction observed in waterlogged cocksfoot (Ploschuk et al. 2017). This suggests that PL might cope better under waterlogging than some other common temperate grass species, although it is possible that the contrast between the photosynthesis rates of waterlogged plants in the current experiment and those in other studies could be due to differences in the application of waterlogging treatments. In contrast with PL, the rate of photosynthesis and ΦPSII in RG leaves was not significantly affected by the waterlogging stress, and this suggests that RG might possess a relatively higher tolerance to waterlogging than PL at a physiological level. However, it has been shown previously that photosynthesis rates were reduced by 30-40% in RG leaves after plants were waterlogged for 28 days (McFarlane et al. 2003); thus the absence of a water treatment effect on the photosynthesis rate of RG leaves in the current experiment was unexpected.

Waterlogging stress may result in the production of reactive oxygen species (ROS) that can lead to the destruction of photosynthetic machinery (Zhang et al. 2019). In lucerne (*Medicago sativa* L.) seedlings subjected to 10 days of waterlogging, a reduction in net photosynthesis and photochemical efficiency occurred concurrently with an increase in cell lipid peroxidation, suggesting that ROS had caused damage in the chloroplasts (Zhang et al. 2019). While the rate of photosynthesis was reduced in PL leaves under waterlogging stress in the current study, the rapid recovery of photosynthesis rate following the removal of the stress could suggest that there was no permanent damage to photosynthetic apparatuses.

Leaf photosynthetic capacity can also be assessed through chlorophyll fluorescence (F_v/F_m) measurements (Murchie & Lawson 2013). Smethurst and Shabala (2003) found that waterlogged lucerne plants had a reduced F_v/F_m in comparison with non-waterlogged plants following 7 days of waterlogging. Lower fluorescence values can indicate a loss of photosynthetic capacity due to the injury of photosynthetic apparatus by ROS (Zhang et al. 2019). However, in the current study, there were no significant differences in the chlorophyll fluorescence of PL or RG leaves under different water treatments, providing further confirmation that the photosynthetic machinery, including PSII and thylakoid membranes, of waterlogged PL and RG leaves was not significantly damaged by the waterlogging. This could have been due to adaptive processes such as an increase in antioxidant activity, which has been shown to be a potential waterlogging tolerance mechanism in RG (Liu & Jiang 2015). These results suggest that the reduction in photosynthesis in PL leaves was likely related to something less permanent, such as a reduction in stomatal conductance (McFarlane et al. 2003).

3.6 Conclusions

The waterlogging stress caused a reduction in PL leaf DM growth, number of leaves per shoot, leaf elongation, photosynthesis rate and ΦPSII, and a non-significant trend for a reduction in specific leaf area, suggesting that PL growth functions were particularly sensitive to waterlogging during the current experiment. However, the survival of PL under the waterlogging stress and the recovery of leaf growth and photosynthesis following the removal of stress suggest that important physiological functions remained intact under waterlogging, possibly due to tolerance mechanisms. In contrast, RG proved to have an enhanced ability for coping under waterlogging stress, in comparison with PL.

These findings suggest that while waterlogging may cause limitations to PL growth, there is no evidence to suggest that it alone could cause irreversible damage to plants and thus prevent their recovery. Rather, waterlogging stress could undermine the ability of PL to compete with the more waterlogging-tolerant RG under such conditions, should they co-exist in a mixed sward.

DRC 16

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Chapter 4 Plantain (*Plantago lanceolata* L.) content within plantain + perennial ryegrass (*Lolium perenne* L.) pastures may be temporarily reduced by livestock treading damage.

Chapter 3 showed that although waterlogging is detrimental to PL growth, it is unlikely to be a principal cause of PL plant death in mixes dairy pastures. The obvious next step would be to investigate the impact of waterlogging on the growth and survival of PL within a grazed mixed pasture under field conditions. However, since waterlogging is difficult to simulate under field conditions it was not possible to conduct an experiment of this nature within a PhD cost budget and timeline. But, since an investigation of the impact of abiotic stresses on PL within grazed mixed pastures was the core aim of this thesis, it was vital that the next phase of experimentation be under field conditions.

Since there is a high chance that waterlogging and treading damage by grazing livestock may occur together, the following field experiments focused on the impact of treading damage on PL but had also incorporated measurements that allowed for the speculation of the presence and impact of any waterlogging stress on PL plants. Chapter 4 details two field studies in which the impact of treading damage on PL growth and survival within grazed mixed pastures was investigated.

This chapter was published in the Journal of New Zealand Grasslands vol 85.

Wilson S, Donaghy D, Horne D, Navarrete S, Kemp P. 2023. Plantain (*Plantago lanceolata* L.) content within plantain/perennial ryegrass (*Lolium perenne* L.) pastures may be temporarily reduced by livestock treading damage. *Journal of New Zealand Grasslands 85*: 177-186.

4.1 Abstract

There is evidence that plantain (*Plantago lanceolata* L.) persistence within perennial ryegrass (*Lolium perenne* L.) dominant dairy pastures is usually poor. One factor potentially limiting plantain content and persistence is treading damage, caused by cows grazing pasture while the soil moisture content is high. The impact of treading damage on plantain regrowth and survival was investigated in two field experiments at Massey University, Palmerston North. In a small-plot study, cow treading damage was simulated on separate plantain and perennial ryegrass pastures using a novel treading device. Subsequently, a larger-scale experiment investigated the impact of treading damage by grazing dairy cows on plantain regrowth and survival within a mixed plantain + perennial ryegrass pasture. Damage by the treading device, in late spring, immediately reduced the growth rate of both plantain and perennial ryegrass pastures by 30%, however neither plantain content nor density were reduced. In cow treading experiment, treading damage reduced the growth rate of the mixed pasture by 50% in early spring, but it recovered during late spring. Plantain content and density were both initially reduced by the treading damage treatment but recovered to pre-treading levels by autumn. These results suggest that treading damage from cows may be an important cause of reduced plantain content in dairy pastures during spring, however there was no evidence that treading damage reduced plantain persistence.

4.2 Introduction

Plantain (*Plantago lanceolata* L., PL) is becoming an increasingly important component of New Zealand dairy pastures because of its ability to mitigate nitrogen (N) losses. When included in dairy pastures, PL can reduce N loading in urine patches (Navarrete et al. 2022) and suppress soil nitrification (Judson et al. 2019), leading to a reduction in N losses from the pastoral system (Nguyen et al. 2022a). These attributes, along with a high nutritive value, have made PL a suitable, low-cost option for reducing N losses from highly productive, perennial ryegrass (*Lolium perenne* L. - RG) based dairy farm systems.

However, there is growing anecdotal and scientific evidence that PL has poor persistence in RG-based pastures (Dodd et al. 2019) under conventional management (Ayala et al. 2011b). Data from New Zealand farms suggest that PL contents greater than 30% on a dry matter (DM) basis are only attainable in the first two years following establishment as a mixed PL-grass pasture (Dodd et al. 2019). This poses a problem, as at least 30% PL is required in a cow's diet to enable meaningful reductions in urine N concentration and excretion (Minnée et al. 2020). Competition from pasture and weed species (Stewart 1996; Sanderson et al. 2003), and livestock treading damage and associated soil compaction (Blom 1979) are potential causes of this decline in PL content. Herb species are reportedly particularly sensitive to livestock treading in wet soil (Jones & Haggar 1994), however literature detailing the effects of livestock treading damage on PL-inclusive pastures or PL plants, is limited. Blom (1979) showed that PL shoot and root growth was highly sensitive to treading, which agrees with observations by Chappell et al. (1971), who found denser populations of PL in lightly trampled areas, than in well-worn areas. In contrast, RG is one of the most treading-tolerant pasture species (Edmond 1964), although significant growth reductions are possible following severe treading damage (Nie et al. 2001). Menneer et al. (2005) showed that pasture botanical composition can be altered following treading damage, whereby the content of species with a good tolerance of treading increases, while the content of species sensitive to treading decreases.

Treading damage, also known as pugging, is regarded as inevitable on many New Zealand farms (Drewry et al. 2000), where high stock densities are managed in confined winter and spring rotations. The extent of damage to any given soil during winter and spring is largely governed by the soil moisture content (SMC) during that period (Howes 2019). Livestock treading damage is inherently random and can result in a large variability of treading pressure, density, and spatial pattern (Di et al. 2001). Therefore, the use of livestock for implementing treading damage is impractical in small-plot or high-precision experiments where consistent treatment replication is required. In these situations, a mechanical device capable of producing repeatable, realistic treading damage is desirable. There have been attempts to mechanically simulate the treading impact of cattle (Scholefield & Hall 1986; Di et al. 2001; Hu et al. 2018), and while these machines were able to deliver treading precisely, they often failed to simulate the lateral soil damage effect of cattle hooves or had poor manoeuvrability. In the current study, we created and assessed the effectiveness of a novel technique for simulating cattle treading damage on pasture.

The current studies aimed to 1. investigate the impact of treading damage on the productivity and PL plant populations of PL dominant vs RG dominant pastures; 2. investigate the impact of treading damage on PL content and persistence within a PL/RG pasture; and 3. consider the effectiveness of a novel treading device for simulating pasture treading damage.

4.3 Materials and Methods

4.3.1 Experimental sites and layout

Simulated treading experiment – Massey No. 4 Dairy Farm, November 2021 to April 2022

This experimental site included two paddocks with different pasture compositions: 1. PL cv. *Agritonic*/ white clover (*Trifolium repens* L.) cv. *Tribute*/ RG cv. *One*^{50}; and 2. RG cv. *One*^{50}/ white clover cv. *Tribute*. Both pastures were drilled in April 2019 into a Tokomaru silt loam. Nutrients were non-limiting in this experiment. There was 534 mm of rainfall between November 2021 and April 2022. Daily maximum temperatures ranged between 21°C for November to 25°C in January and daily minimum temperatures ranged between 12°C for November to 13°C for January. Meteorological data was sourced from the NIWA weather station, Palmerston North, approximately 1km from the site. Experiment site GPS coordinates 40°23'31.5"S 175°36'50.5"E.

Cow treading experiment – Massey Pasture and Crop Research Unit (PCRU), August 2022 to April 2023

This experimental site was established in autumn 2022. PL cv. *Agritonic* and RG cv. *Maxsyn* were direct drilled at 10 kg and 5 kg per hectare, respectively, on April 8 with the seeding rate designed to achieve an equal proportion (on a DM basis) of PL and RG at the start of the experiment. The soil at the site is a Manawatu fine sandy loam. Nutrients were non-limiting in this experiment. There was 759 mm of rainfall between August 17, 2022, and April 28, 2023. Daily maximum temperatures ranged between 15°C in August to 24°C in January and daily minimum temperatures ranged between 7°C for August to 15°C for January. Meteorological data was sourced from the NIWA weather station, Palmerston North, approximately 200m from the site. Experiment site GPS coordinates 40°22'56.6"S 175°36'27.55"E.

4.3.2 Treatments

Simulated treading experiment

The treatments consisted of the PL and RG pastures subjected to three treading damage severities: undamaged (UD), light damage (LD) and heavy damage (HD). Within each pasture type there were 15 plots (three treading treatments, five replicates) of 4 m^2, laid out in a randomised complete block design. Herbage was cut and removed with a Honda push mower to a height of 5 cm prior to treading and during subsequent harvests, which occurred whenever mean estimated pasture DM across the experiment exceeded 3000 kg DM/ha.

The severity of treading damage was governed by the SMC at the time of treading. All plots were watered prior to treading with the intent to reach saturation, which was equivalent to 44% gravimetric water content (GWC), (Howes 2019). The cutting of HD and UD plots and treading of the HD plots was carried out on 1 November. Treading was implemented with a novel device, named the ‘puggo-stick’. It consisted of a fence-post laden with 20 kg of cast-iron weights, steel footrests and handles, and cost ~$150. The base area of the device was 78.5 cm^2 and with an operator, delivered a pressure on the soil surface of approximately 167 kPa, which is comparable to that of one hoof, exerted by a stationary dairy cow (132.5 kPa) (Scholefield & Hall 1986).

Soil resistance to treading was monitored in the following days by measuring the depth of pug marks produced by the puggo-stick in areas adjacent to LD plots. When the mean depth of pug marks was less than half of those produced on 1 November, it was determined that the risk of damage had fallen sufficiently. On 4 November, the LD plots were cut (as previously described), and treading implemented.

Cow treading experiment

The cow treading experiment consisted of two treading treatments, damaged (DD) and undamaged (ND) with four replicates of each treatment, for a total of eight plots, in a randomised complete block design. Each plot was 13 m by 14 m (182 m^2). Dairy cows were used to implement treading damage, which was based on a difference in SMC at the time of grazing. Procedures were approved by the Massey University Animal Ethics Committee (AEC 22/34).

Based on previous work (Howes 2019), it was decided that grazing at a soil water deficit of 5 - 10 mm, or 37% - 33% soil volumetric water content (VWC), would ensure that ND plots were not damaged by the cows. On August 17, ND plots were grazed. Each plot was grazed with 6 pregnant dairy cows at a density equivalent to 330 cows/ha. In the 48 hours following this initial grazing there was 25 mm of rainfall, which greatly increased soil VWC and so DD plots were grazed (and damaged) on August 19. At each grazing, plots were grazed for 2.5 hours. Subsequent grazings were carried out with lactating dairy cows whenever mean estimated pasture mass across the site reached approximately 2800 kg DM/ha. The target residual was 1500 kg DM/ha and care was taken to avoid further treading damage to the plots by cows.

4.3.3 Measurements

Simulated treading experiment

Soil measurements were taken to assess treading damage severity. Pug mark depth (cm) was determined by averaging the three deepest and three shallowest pug marks in three 0.25m^2 quadrats per plot with a ruler. Pug mark density (pugs/m^2) was determined by counting the number of pug marks within the same quadrat. Soil GWC was measured by collecting four soil cores to 10 cm depth per plot, which were then weighed and oven-dried at 105°C until a constant weight was achieved. Soil GWC data for the UD plots in the RG pasture were missed, so were assumed to be comparable to that of the HD plots in that pasture.

Pasture DM yield was measured before each lawnmower cut by hand-clipping three random 0.1m^2 quadrat cuts per plot to 5 cm height, then drying the herbage at 60°C for 48 hours. Harvests were made on December 1 (H1), January 11 (H2), February 21 (H3), and March 29 (H4; PL only). The RG pasture could not be harvested at H4 due to unforeseen circumstances. Herbage accumulation rate was calculated by dividing DM yield by the number of days in the regrowth period and then fitting those rates to specific months.

Botanical composition was visually evaluated in the PL pasture by scoring the proportion of ground cover occupied by PL, RG, white clover, and dock (*Rumex obtusifolius* L.) in each plot prior to each DM harvest (Lynch 1960).

At H1, one yield sample per plot was used to measure leaf area index (LAI) using a LI-COR 3100 leaf area meter. Plant density was determined in the PL pasture by counting the number of PL plants within three 0.25m^2 quadrats per plot.

Cow treading experiment

Estimations of treading damage severity followed the same method as in the simulated treading experiment but included five quadrats per plot. Soil VWC was measured with a time-domain reflectometer to 15cm depth in 10 locations per plot.

Pasture DM yield pre- and post-grazing was measured by hand-clipping herbage to ground level in three 0.1m^2 quadrats per plot and drying the herbage at 60°C for 48 hours. Quadrat locations were selected for the purpose of obtaining a representative pasture sample from each plot. Where necessary, soil was removed from samples by washing. Pasture growth rate was calculated by subtracting the current pre-grazing DM from the post-grazing DM of the previous grazing and dividing by the number of days in the regrowth period.

Light interception measurements were made using a Spectrosense2$^{+®}$ device 14 days after each grazing event by measuring light intensity above and below the canopy in three fixed locations in each plot.

Herbage was cut from a 10×50 cm strip adjacent to each pre-grazing DM quadrat and separated into PL, RG, annual poa (*Poa annua* L.), dead matter, broadleaf weeds, PL seed head and volunteer white clover. Samples were dried at 60°C for 48 hours to determine pasture botanical composition on a DM basis.

The density of PL and RG was measured by counting the number of shoots (PL) or tillers (PR) in four fixed 0.1m^2 quadrats per plot (tillers were counted in one half of the quadrat, i.e., 0.05 m^2).

4.3.4 Statistical analyses

Statistical analyses were conducted using the MIXED procedure is SAS (version 9.4, SAS institute 2018). Pair-wised t-tests were used for mean comparisons and significance was declared at $P<0.05$. In the simulated treading experiment botanical composition and PL plant density were analysed at each sampling date with treatment as the fixed effect. All other results from simulated treading experiment were analysed at each sampling with pasture, treatment, and pasture×treatment as fixed effects. All data were also analysed for repeated measures, with month, pasture×month, treatment×month and pasture×treatment×month as fixed effects. In the cow treading experiment all results were analysed within each sampling date with treatment as the fixed effect. All data were also analysed for repeated measures, with season and treatment×season as fixed effects. Non-significant interactions between fixed effects have not been reported in the results.

Figure 4-1. Simulated treading damage experiment, Massey University No.4 Dairy Farm.

4.4 Results

4.4.1 Simulated treading experiment

4.4.1.1 Treading damage

Soil GWC, pug depth and pug density were significantly higher in the PL pasture, in comparison with the RG pasture (Table 4-1). Soil GWC was significantly higher ($P<0.01$) during the treading of the HD plots, than the LD plots, regardless of pasture type. Pug marks were deeper in HD plots in comparison with LD plots ($P<0.01$), but LD plots had a significantly higher pug density than HD plots ($P<0.05$).

4.4.1.2 Pasture production and leaf area index

The RG pasture had a higher herbage accumulation rate than the PL pasture ($P<0.01$) throughout the experiment (Table 4-2). Herbage accumulation rate was significantly lower in HD plots in comparison with LD and UD plots, regardless of pasture type, during November, January, and February ($P<0.01$). The DM production for PL and RG pastures was 31% and 32% lower respectively in HD plots than in UD plots during November. The PL herbage accumulation rate was highest during December and March and was lowest during January and February ($P<0.01$), while RG Herbage accumulation rate was highest in November and December and decreased over January and February ($P<0.01$). In the PL pasture the LAI of HD plots was 43% lower than that of UD plots at the end of the first regrowth following the treading damage, although the treatment effect was non-significant.

4.4.1.3 Botanical composition

The mean PL content decreased from 18% at H1, to 9% at the end of the experiment ($P<0.01$) (Table 4-3). The mean white clover content was constant over the experiment, while the RG content increased. There was more white clover in HD plots than UD plots at H1 ($P<0.05$). The mean dock content of plots declined from 22% at H1 to 8% at the end of the experiment

(P<0.01). Treading treatment appeared to have had little effect on the botanical composition of this pasture.

Table 4-1. Mean soil gravimetric water content (%) at the time of treading damage in November 2021, and the mean pug mark density (pugs/m^2) and pug depth (mm) resulting from plantain and perennial ryegrass-based pastures that were subjected to no damage (UD) light damage (LD) or heavy damage (HD).

Pasture	**Treatment**	**Soil water content**^{1} (%)	**Pug depth** (mm)	**Pug density** (pugs/m^2)
Plantain	UD	48	-	-
	LD	42	13	95
	HD	51	35	92
Perennial ryegrass	UD	-	-	-
	LD	36	11	91
	HD	45	18	78
SEM		2.62	1.67	2.83
ANOVA p-values				
Pasture		<0.01	<0.01	<0.01
Treatment		<0.01	<0.01	0.010
Pasture*Treatment		0.899	<0.01	0.127

Table 4-2. Herbage accumulation rate (kg DM/ha/day) and leaf area index (LAI; cm^2 leaf/ cm^2 ground) of three-year-old plantain and perennial ryegrass-based pastures following no treading damage (UD), light treading damage (LD) or heavy treading damage (HD) in November 2021. Sown April 2019.

		Herbage accumulation rate (kg DM/ha/day)						**LAI (cm^2 leaf/ cm^2 ground)**
		2021		**2022**				
Pasture	**Treatment**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Mean**	**02 Dec-21**
Plantain	UD	96	110	77	69	103	91	3.73
	LD	85	114	85	77	104	93	3.05
	HD	67	107	75	69	111	86	2.12
Perennial ryegrass	UD	143	138	109	94	*	121	-
	LD	147	128	116	110	-	125	-
	HD	100	124	100	86	-	103	-
SEM		8.82	7.31	4.12	3.62	3.28	4.39	0.39
ANOVA p-values								
Pasture		<0.01	<0.01	<0.01	<0.01	-	<0.01	
Treatment		<0.01	0.549	0.017	<0.01	0.220	<0.01	0.052
Month							<0.01	
Pasture*Month							<0.01	
Treatment*Month							<0.01	

* Perennial ryegrass treatment ended before the March harvest.

Table 4-3. Botanical composition (% ground cover) of a three-year-old plantain pasture following no treading (UD), light treading damage (LD) and heavy treading damage (HD) over the summer of 2021-2022. Sown April 2019.

		Treatment			
Date	**Species**	**UD**	**LD**	**HD**	**SEM**
1 Dec 2021	Plantain	17.6	21.0	16.6	2.99
	White clover	51.8^{ab}	50.2^{b}	69.8^{a}	5.16
	Perennial ryegrass	2.6	3.8	1.2	1.74
	Dock	28.0	25.0	12.4	5.80
22 Mar 2022	Plantain	8.0	8.8	9.8	1.80
	White clover	67.0	61.2	67.0	3.99
	Perennial ryegrass	15.4	20.4	19.0	4.96
	Dock	9.6	9.6	4.2	2.39

Letters indicate means within rows that are significantly different (P<0.05).

4.4.1.4 Plantain plant density

The mean PL plant density across the experiment was 38 plants/m^2 and 29 plants/m^2 in November and December respectively, but there was no difference between treatments (Figure 4-2). In January, HD plots had a PL density that was 60% and 48% higher than UD and LD plots, respectively (P<0.05). In March, the PL plant density in HD plots was 144% and 78% greater than that of UD and LD plots, respectively (P<0.01). In April, PL density was not significantly different between treatments. The mean PL density decreased significantly between November and January but was constant between January and April (P<0.01). During the experiment, PL density decreased by 51% on average across the site (P<0.01).

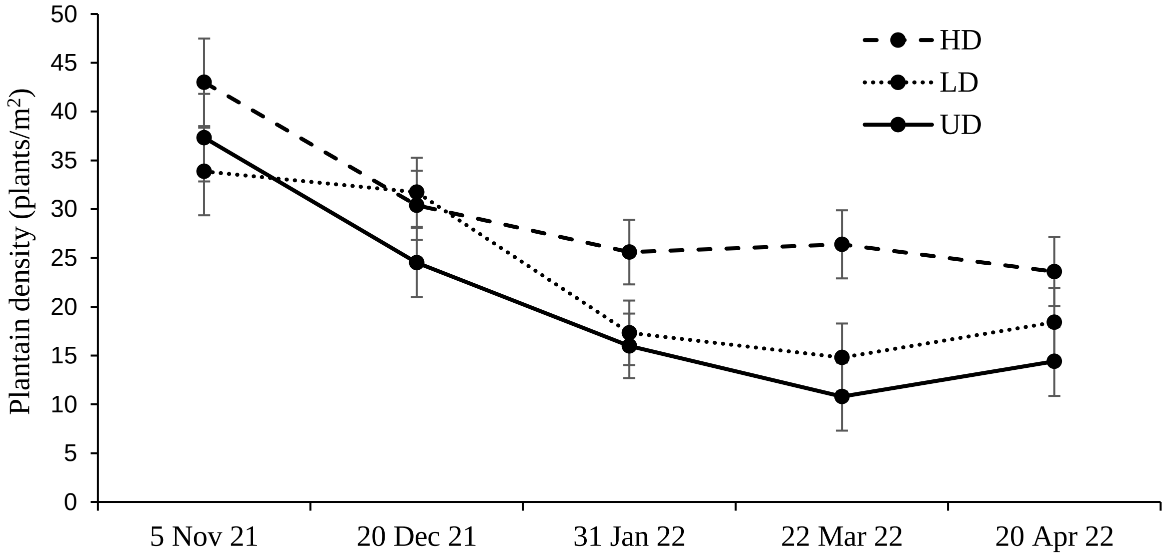

Figure 4-2. Density of plantain plants (plants/m^2) in a three-year-old plantain-dominant pasture following no treading (UD – solid line), light damage (LD – dotted line) and heavy damage (HD – dashed line). Bars show standard error of the mean. Sown April 2019.

4.4.2 Cow treading experiment

4.4.2.1 Treading damage

Soil volumetric water content was significantly higher ($P>0.01$) on the day that DD plots were treaded than when the ND plots were treaded, two days earlier (Table 4-4). The mean pug depth ($P<0.01$) and pug density ($P<0.01$) were significantly greater in DD plots than ND plots following the treading treatment.

Table 4-4. Mean soil volumetric water content (%) during treading by dairy cows in August 2022, and resulting mean pug mark density (pugs/m^2) and depth (mm).

Treatment	**Soil volumetric water content** (%)	**Pug depth** (mm)	**Pug density** (pugs/m^2)
Damaged	43.0	34.3	45.3
Undamaged	35.2	13.8	37.3
SEM	0.8	1.7	2.2
ANOVA p-values	<0.01	<0.01	<0.01

4.4.2.2 Pasture production and canopy light interception

During early spring, pasture growth was 50% lower in DD plots than in ND plots ($P<0.05$) but was not statistically significantly different between treading treatments from late spring onwards (Table 4-5). Average pasture growth rate increased from 36 kg DM/ha/day in early spring to 82 kg DM/ha/day during late spring, but then decreased to 46 kg DM/ha/day during early summer ($P<0.01$). Mean pasture growth was similar between early and late summer, but then decreased during autumn, to 31 kg DM/ ha/day. The total mean pasture growth rate was not significantly different between treading treatments. The mean canopy light interception of DD plots was significantly lower than that of ND plots 14 days after the treading damage in early spring ($P<0.01$) but was not significantly affected by treading treatment in any other season. The mean light interception at 14 days after grazing, across the experiment, was greater from early summer to autumn than in early or late spring (P<0.01).

Table 4-5. Pasture growth rate (kg DM/ha/day) and light interception (%) of a plantain + perennial ryegrass pasture sown in April 2022, following treading damage (DD) or no treading damage (ND) from dairy cows during August 2022.

	Pasture growth rate (kg DM/ha/day)			**Light interception[1] (%)**		
Season	DD	ND	SEM	DD	ND	SEM
Early spring (17/08-13/09)	24^{b}	48^{a}	8.85	84^{b}	95^{a}	1.22
Late spring (14/09-24/11)	82	83	12.57	88	90	1.02
Early summer (25/11-12/01)	52	41	6.02	91	93	1.14
Late summer (13/01-03/03)	38	47	5.49	93	94	0.95
Autumn (04/03-28/04)	26	36	3.95	93	92	0.90
Mean	44	51	3.92	90	93	0.79
ANOVA p-values						
Treatment	0.307			0.069		
Season	<0.01			<0.01		
Treatment×Season	0.252			<0.01		

[1] Mean canopy light interception measured 14 days after each grazing event.

Letters indicate means within rows that are significantly different (P<0.05).

4.4.2.3 Botanical composition

Prior to the experiment, PL content was on average 41% across the site, while RG (46%) made up much of the remaining DM (Figure 4-3). During early spring, PL content was 19% lower in DD plots than in ND plots, though this difference was not significant. In contrast, the mean RG content was 28% higher in DD plots than in ND plots over the same period (P<0.05). The effect of treading damage was evident in DD plots during late spring, where PL content was 26% lower than in ND plots (P<0.05). The content of RG was significantly higher in DD plots than ND plots during late spring (P< 0.05). The effects of treading damage treatment on PL and RG content diminished after late spring. The PL content varied in both treatments between the seasons and after declining to 20% on average during late spring recovered throughout summer to reach 44% on average across the site in autumn (P<0.05). In contrast, the RG content increased in both treatments during spring and then decreased over summer to 40% on average across the site in autumn. When averaged over the whole experimental period, PL content tended (P=0.07) to be higher in ND plots (35%) than in DD plots (31%). Other notable changes to pasture composition, were the reduction in annual poa content between spring and summer (P<0.01), and the increase in dead material from spring to summer (P<0.01).

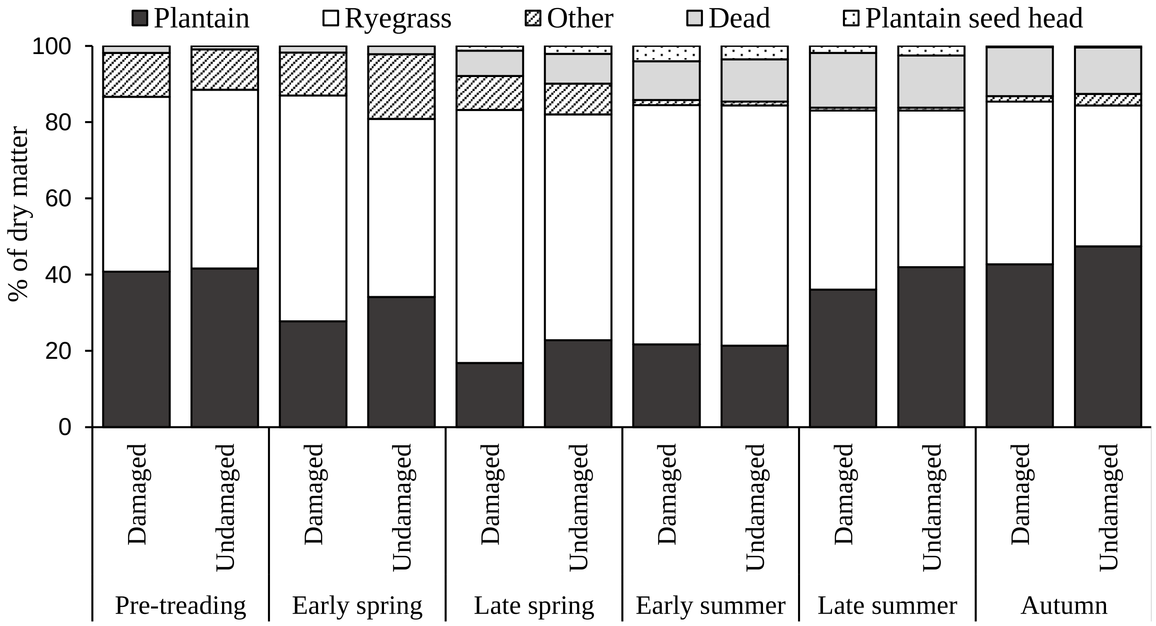

Figure 4-3. Botanical composition (% of dry matter) of a plantain + perennial ryegrass pasture sown in April 2022, following treading damage by dairy cows in August 2022. ‘Other’ comprised of annual poa, weeds and white clover.

4.4.2.4 Plantain shoot density and perennial ryegrass tiller density

Prior to the implementation of the treading treatments, PL shoot density was 309 shoots/m^2 on average (Figure 4-4). The PL shoot density was 21% and 29% lower in DD plots than in ND plots during early and late spring respectively ($P<0.05$). In late summer PL shoot density was 20% lower in DD plots than in ND plots ($P<0.05$), but there was no difference between treatments during autumn. When averaged over the whole season, DD plots (255 shoots/m^2) appeared to have a lower PL shoot density than ND plots (311 shoots/m^2) although this effect was not significant. Following the first grazing (and treading) in August, mean PL shoot density across the site decreased ($P<0.01$) to 225 shoots/m^2. During late spring, this increased to 271 shoots/m^2 ($P<0.01$) and then remained steady into early summer. Mean PL shoot density increased further during late summer to 311 shoots/m^2 ($P<0.01$) but there was no further change from late summer to autumn. The mean RG tiller density across the site was 2755 tillers/m^2 prior to the treading damage from cows. Treading treatment had no significant effect on RG tiller density during the experiment. The mean RG tiller density didn’t differ significantly between seasons.

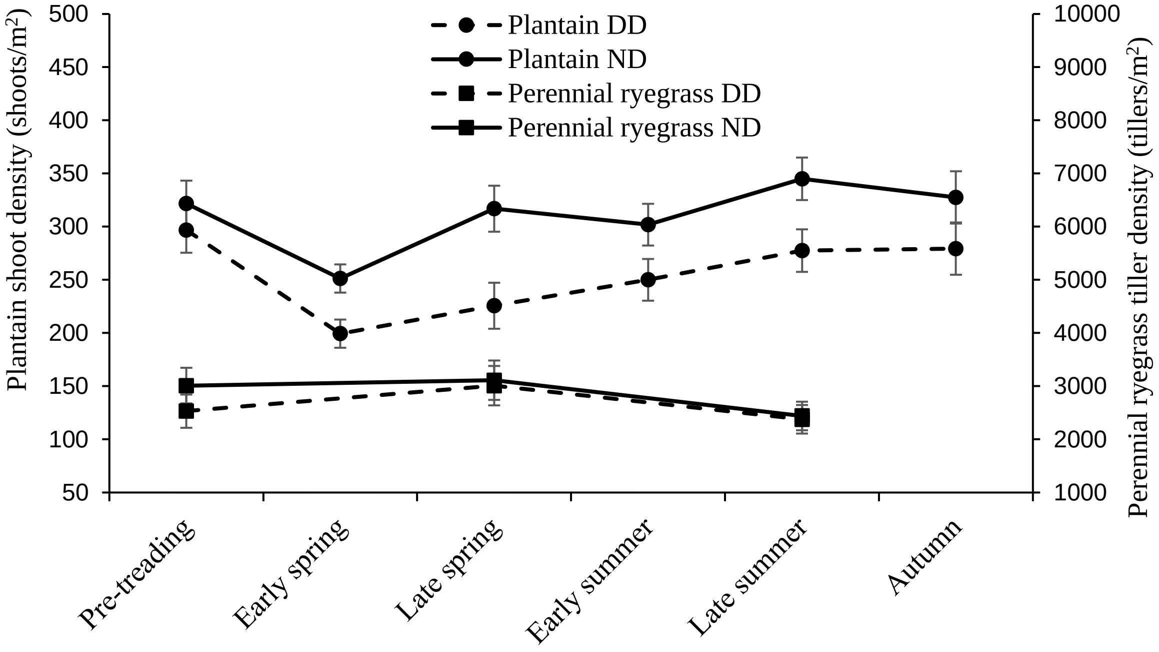

Figure 4-4. Density (number/m^2) of plantain shoots (circles) and perennial ryegrass tillers (squares) in a first-year plantain + perennial ryegrass pasture sown in autumn, following treading by dairy cows in early spring. Damaged by treading (DD – dashed lines), not damaged by treading (ND – solid lines). Bars show standard error of the mean.

4.5 Discussion

4.5.1 Treading damage

In both experiments, pug depth and thus treading damage severity, increased with an increase in SMC at the time of grazing, which agrees with previous studies (Climo & Richardson 1984), and confirms that the treading treatments were implemented as planned. In the simulated treading experiment, the greater treading damage severity observed in the PL pasture appeared be a consequence of the higher SMC in those plots during treading, in comparison with the RG pasture, with the difference most likely due to site differences in soil water holding capacity. However, it is difficult to rule out any effect that different species, composition, and plant density might have had on the resistance of soil to treading. The treading damage caused by the puggo-stick is comparable to damage caused by cattle in other studies. For example, Betteridge et al. (2003) reported pug depths greater than 30mm in a saturated silt loam, Nie et al. (2001) reported larger pug depths of 43mm in a wet clay loam and in the current work a mean pug depth of 34mm was recorded in a wet silt loam in the cow treading experiment. The lateral soil damage effects of the puggo-stick were not evaluated.

4.5.2 Plantain + perennial ryegrass pasture DM production following treading damage

The effects of treading damage on herbage production in the current experiments agree with previously established reductions of between 30-50% (Pande et al. 2000; Nie et al. 2001) which provides further confirmation that the treading treatments were implemented appropriately by the puggo-stick and cows. One important finding was that the loss of pasture production caused by the puggo-stick damage was similar for both the PL and RG pasture types. This potentially contradicts earlier statements (Stewart 1996) that PL is more sensitive to treading damage than RG. However, since the PL content in the PL pasture was low, it is not possible to claim that PL was as tolerant of treading damage as RG. In the cow treading experiment the pasture growth rate of DD plots appeared to recover as soon as the second regrowth period after treading, suggesting that any negative impacts of the damage had subsided. However, in the simulated treading experiment, HD plots still had a reduced herbage accumulation rate in January and February, suggesting that the damage may have caused a longer-term impact such as a deterioration of soil physical quality (Hu et al. 2018). The influence of temperature on pasture growth could explain why pasture growth appeared to be limited less by damage in the simulated treading experiment than in the cow treading experiment i.e., November vs August pugging events. Temperature is a major driver of pasture growth rate (Baars & Waller 1979). Daily temperatures were closer to optimal for temperate species (20-25°C) following treading in the simulated treading experiment, than in the cow treading experiment, likely enabling a faster pasture regrowth in the former, regardless of pasture type or treading treatment. If an interaction between temperature and treading damage exists, it might be expected that the impact of treading damage on pasture regrowth would be more severe in cooler seasons, therefore future studies might consider implementing treading in autumn or winter. It was noted that herbage accumulation rates during the simulated treading experiment were very large and care should be taken when comparing these with other studies. The high growth rates were potentially due to the nature of the experiment site including the optimal soil moisture and nutrient inputs, and the absence of typical grazing detriments including further treading damage and over-grazing.

The immediate effect of the damage on pasture growth appears to have been driven by a reduction in the light intercepting capacity of the pasture canopy, potentially caused by the burial of plants and a reduction in leaf elongation on injured shoots. This agrees with work by Pande et al. (2000), who found that the LAI of a RG pasture was reduced by cattle treading damage, which was strongly correlated with reduced pasture growth. In the current study, an apparent reduction in LAI and reduced pasture growth were observed in HD plots in the simulated treading experiment. Although canopy light interception, and subsequently pasture growth, were initially reduced in the DD pasture in the cow treading experiment, these recovered quickly, probably due to the increased growth of RG in those plots during spring.

4.5.3 Plantain content and plantain persistence following treading damage

The effects of the simulated treading damage on PL content in the simulated treading experiment were not obvious, probably having been confounded by the low PL content and PL density in the sward prior to the experiment, which are common features of a three-year-old sward (Dodd et al. 2019). An interesting finding was the apparent enhancement of PL plant density by the HD treatment and consequent diversion from a decline during summer, which is typical of older PL swards (Ayala et al. 2011b). It is plausible that new PL plants emerged in HD plots, in areas of bare soil which commonly occur following treading damage (Elliott et al. 2002) and which provide an environment ideal for seedling establishment (Bakker 1985). Alternatively, cattle treading has been shown to speed up clonal fragmentation of prostrate-growing pasture plants (Edmond 1964; Menneer et al. 2005), which could also be responsible for increased PL plant density under heavy treading. Lastly, it is possible that improved light availability in HD plots, due to the low dock content following treading, enhanced the survival of existing PL plants. In contrast, the high dock content of UD plots may have shaded PL plants and contributed to PL density decline. Weed invasions were shown to coincide with PL density decline by Sanderson et al. (2003).

In the cow treading experiment, treading damage reduced PL content during early and late spring, likely due in part to the reduction in PL shoot density in DD plots during that time. In contrast, RG tiller density appeared to be largely unaffected by the treading damage, probably allowing for a quicker recovery of leaf production and growth following the damage, confirming previous findings that RG is one of the most treading-tolerant pasture species (Edmond 1964) . Menneer et al. (2005) previously showed that following treading damage, white clover, a prostrate and treading-sensitive species, also had a significantly lower content in a RG pasture. The reduction in PL content in DD plots in the cow treading experiment also likely enhanced the increase in RG growth in the sward from early spring to early summer, which is its major growing season (Kemp et al. 1999). Additionally, low temperatures following treading damage could have further limited PL regrowth (Teramura et al. 1981) and encouraged the shift in pasture composition from PL to RG in all plots during spring. The PL and RG content in the pasture appeared to be negatively correlated, which was particularly evident when comparing pasture composition for late spring and late summer. More work is required to gain a full understanding of what factors drove the shift from RG dominance in spring to PL dominance in late summer.

It is difficult to determine from the cow treading experiment, whether the treading damage had any permanent effect on PL persistence. The reduction in PL shoot density in both treatments following the first grazing event, suggests that the PL population was sensitive to the early-spring grazing, regardless of grazing management. Although the PL shoot density of DD plots was lower than ND plots during spring, it recovered to within a similar range of ND plots by autumn. It is plausible that the PL shoot density recovered in DD plots because of new shoot production from individual plants, which could compensate for a loss of plants in the short-term, such as has been observed in chicory (Li et al. 1997b). However, it is unknown how these new shoots would respond to repeat treading damage during subsequent years. Since the PL shoot density in DD plots eventually recovered to near pre-experiment levels, it would be theoretically capable of sustaining the pasture PL content achieved during this experiment, into the future. Therefore, there is no evidence that treading damage had any negative effect on PL persistence.

The general trend for the recovery of PL shoot density over the course of the year in the cow treading experiment was in stark contrast with the continual decrease in the PL density of the

3-year-old PL sward over summer in the simulated treading experiment. The difference between these trends suggests that PL population age may have important implications for the recovery of PL density following damaging events and might suggest that PL populations are more tolerant of density loss within the first year following sowing due to their improved capacity for recovery.

The current study highlighted the necessity for continued research on other factors affecting PL content and persistence, including the long-term effects of treading damage, the effect of repeat treading damage, the interaction of PL age with treading damage, suitable companion species, and the interaction of treading damage with other abiotic stresses such as low temperature.

4.6 Conclusions

The reductions in the DM production of the PL pasture following simulated treading damage and the PL + RG pasture following cattle treading damage were comparable to reductions previously observed for PR-dominant pastures. The results indicate that treading damage may be an important cause of reduced PL content in PR-based dairy pastures during spring and highlight the need for careful management of pastures that are prone to treading damage, to ensure sufficient PL content. It was not clear from the current experiments that treading damage reduced the ability of PL to persist. On first assessment, the puggo-stick appeared to be an effective method for simulating cattle treading damage, although further work is required to fully validate the method.

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Wilson. S, Donaghy. D, Horne. D, Navarrete. S, Kemp. P, 2024. An inexpensive and effective method for simulating cattle treading damage on pasture. In: Opportunities for improved farm and catchment outcomes. (Eds C.L Christensen, D.J. Horne, and R. Singh). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 36. Farmed Landscapes

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Chapter 5 An inexpensive and effective method for simulating cattle treading damage on pasture.

This chapter provides an opportunity for a more in-depth discussion of the effectiveness of the tool developed for simulating cow treading damage on pasture. While chapter 4 is concerned with the effect of this device on PL and RG pastures, this chapter discusses the usefulness of the device for simulating treading damage on pasture, and lists limitations which aim to assist future researchers in the use or manufacture of this or similar devices.

This chapter was published in the occasional report of the Farmed Landscapes Research Centre, 2024.

Wilson. S, Donaghy. D, Horne. D, Navarrete. S, Kemp. P, 2024. An inexpensive and effective method for simulating cattle treading damage on pasture. In: *Opportunities for improved farm and catchment outcomes*. (Eds C.L Christensen, D.J. Horne, and R. Singh). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 36. Farmed Landscapes Research Centre, Massey University, Palmerston North, New Zealand. 9.

5.1 Abstract

It may seem obvious to use livestock to study the effect of treading damage on pasture, however live animals limit the likelihood of achieving spatially uniform damage, and therefore are unsuitable for the study of treading damage in small plot experiments, or where studies necessitate the identical treading of replicate plants. In these situations, a mechanical device capable of generating repeatable and realistic treading damage is desirable. There have been multiple attempts at creating such a device, however these are typically manufactured at substantial cost and are often complicated and/or poorly maneuverable in the field. Thus, the challenge was to create a simple, low-cost, novel treading device that would provide a realistic simulation of cattle treading on pasture plants. The device created consisted of a wooden post laden with cast-iron weights with steel handles and foot pegs so that a single operator could shift the device into position before stepping onto it to generate the pug mark. The device was designed to produce a pressure equivalent to the hoof of a 520-560 kg cow. Due to its resemblance to a pogo stick, the device was aptly named the puggo-stick. In an experiment at Massey University's No.4 Dairy farm, the effects of treading damage at varying severities by the puggo-stick were assessed on plantain (*Plantago lanceolata* L.) and perennial ryegrass (*Lolium perenne* L.) dominant pastures. The treading severities imposed on each pasture type were facilitated by differences in soil moisture content at the time of treading and included heavy damage (high soil moisture), light damage (medium soil moisture) and no treading. Measurements included pug mark depth and density, and the herbage regrowth of each pasture type following damage. The treading damage caused by the puggo-stick was comparable to damage caused by cattle in previous studies, with severity (pug depth) increasing with an increase in soil moisture content. The resulting reduction in the herbage accumulation of both pasture types following damage by the puggo-stick was comparable to the reduction in herbage growth observed by earlier researchers for pastures

subjected to cattle treading damage. These results suggest that the puggo-stick generated treading damage comparable to that of live cattle, and so appears to be an inexpensive and effective method for simulating cattle treading damage in pastures and could be considered for use in future experiments.

5.2 Introduction

Treading damage by grazing livestock is regarded as inevitable on most New Zealand pastoral farms in any given year (Howes 2019). Treading damage typically occurs between late-autumn and early spring, when soil moisture content (SMC) is elevated due to increased rainfall and low evapotranspiration, and when livestock are managed in confined rotations. The extent to which a pasture is damaged during this period is largely governed by SMC, because at a high SMC, soil is generally susceptible to treading damage regardless of its texture or structure (Howes 2019). The treading damage caused by livestock is typically spatially random, and can result in a large variability of treading pressure (Di et al. 2001), both of which reflect the randomness of livestock movement and behavior within an allotted area of pasture. Thus, the use of grazing livestock for implementing treading damage in small-plot experiments, or in experiments that require uniform treading damage is impractical. In such experiments, a mechanical device capable of producing repeatable and life-like treading damage is desired.

There have been several attempts at manufacturing such a device, however they appear to have come at a high cost for investigators and/or have required proper technical aptitude (Scholefield & Hall 1986; Abdel-Magid et al. 1987; Di et al. 2001; Hu et al. 2018). Given that PhD researchers, such as the current author, are often lacking somewhat in one or both of these areas, and are usually confined by time limits, experiments often require a certain amount of ingenuity. On this occasion, that manifested in the development of a novel treading device. The current work set out to create and assess an uncomplicated and low-cost alternative device. The newly created puggo-stick was evaluated based on its ability to produce pug depths similar to those of dairy cows and cause pasture damage similar to that documented in previous cattle treading studies.

5.3 The puggo-stick

The treading in this trial was simulated using a newly fabricated treading device, known as the ‘puggo-stick’ (Figure 5-1). The instrument comprised of a weighted fencepost with a 100mm diameter base. It also had handles and footrests, so it was possible to stand on the device in a manner similar to a pogo-stick (hence its name). The instrument (25.5kg fully loaded) was pressed into the ground with the weight of a human operator (109kg). The hoof had a surface area of 78.54cm^2 and so the impact pressure of the device on the soil surface was approximately 168kpa. This was relatively close to the pressure of a single hoof exerted by a stationary, standing dairy cow (Scholefield & Hall 1986). The operator stepped onto the device in a controlled manner and, attempted to keep the device as vertical as possible while the hoof sank into the soil surface. The puggo-stick cost around $150 (NZ) to construct and was assembled in around two hours, thus satisfying the key criteria of inexpensive and quick to manufacture.

Figure 5-1. The puggo-stick, with 20kg of weights attached to it.

5.4 Materials and methods

5.4.1 Experimental site

The experimental site at Massey University's No.4 Dairy farm included two paddocks with different pasture compositions: 1. Plantain cv. Agritonic/ White clover (*Trifolium repens* L.) cv. Tribute/ Perennial ryegrass cv. One50; and 2. Perennial ryegrass cv. One50/ White clover cv. Tribute. Both pastures were three years old. The experiment was conducted in November 2021.

5.4.2 Treatments

The treatments consisted of the plantain and perennial ryegrass pastures subjected to three treading damage severities: undamaged (UD), light damage (LD) and heavy damage (HD). Within each pasture type there were 15 plots (three treading treatments, five replicates) of 4m^2, laid out in a randomised complete block design. The severity of treading damage was governed by the SMC at the time of treading. All plots were watered prior to treading with the intent to reach saturation, which was equivalent to 44% gravimetric water content (GWC), (Howes 2019). The cutting of HD and UD plots and treading of the HD plots was carried out on 1 November. Herbage was cut and removed with a Honda push mower to a height of 5 cm prior to treading. Before treading with the puggo-stick, two square posts were laid down along opposing sides of each plot and a plank was laid across them which extended over the whole plot (Figure 5-1). This made it possible for the operator to stay off the soil surface and provided a steady base from which the puggo-stick could be stepped onto. The intention was to produce pug marks directly adjacent to one another so that 100% of the soil surface could be treaded. Soil resistance to treading was monitored in the days following the treading of HD plots by measuring the depth of pug marks produced by the puggo-stick in areas adjacent to LD plots. When the mean depth of these pug marks was less than half of those produced in the HD plots, it was determined that the risk of damage had fallen sufficiently. On 4 November, the LD plots were cut (as previously described), and treading implemented.

5.4.3 Measurements

Soil measurements were taken to assess treading damage severity. Pug mark depth (mm) was determined by averaging the three deepest and three shallowest pug marks in three 0.25m^2 quadrats per plot with a ruler. Pug mark density (pugs/m^2) was determined by counting the number of pug marks within the same quadrat. Soil GWC was measured by collecting four soil cores to 10 cm depth per plot, which were then weighed and oven-dried at 105°C until a constant weight was achieved. Soil GWC data for the UD plots in the perennial ryegrass pasture were missed, so were assumed to be comparable to that of the HD plots in that pasture. Pasture DM yield was measured on December 1 by hand-clipping three random 0.1m^2 quadrat cuts per plot to 5 cm height, then drying the herbage at 60°C for 48 hours.

5.4.4 Statistical analysis

Statistical analyses were conducted using the MIXED procedure is SAS (version 9.4, SAS institute 2018). Pair-wise t-tests were used for mean comparisons and significance was declared at $P<0.05$. Soil GWC, pug depth and pug density were analysed with pasture and treatment as fixed effects, while DM yield was analysed with treatment as the fixed effect.

5.5 Results

5.5.1.1 Treading damage

Soil GWC, pug depth and pug density were significantly higher in the plantain pasture, in comparison with the perennial ryegrass pasture (Table 5-1). Soil GWC was significantly higher (P<0.01) during the treading of the HD plots, than the LD plots, regardless of pasture type. Pug marks were deeper (P<0.01) in HD plots in comparison with LD plots, but LD plots had a significantly higher (P<0.05) pug density than HD plots.

Table 5-1 Mean soil gravimetric water content (%) at the time of treading damage, and the mean pug depth (mm) and pug mark density (pugs/m^2) in plantain and perennial ryegrass-based pastures that were subjected to light damage (LD) or heavy damage (HD) with the puggo-stick.

Pasture	**Treatment**	**Soil water content** (%)	**Pug depth** (mm)	**Pug density** (pugs/m^2)
Plantain	UD	48	-	-
	LD	42	13	95
	HD	51	35	92
Perennial ryegrass	UD	-	-	-
	LD	36	11	91
	HD	45	18	78
SEM		2.62	1.67	2.83
ANOVA p-values				
Pasture		<0.01	<0.01	<0.01
Treatment		<0.01	<0.01	0.010

5.5.1.2 Pasture production

Herbage accumulation was significantly lower (P<0.01) in HD plots in comparison with LD and UD plots, regardless of pasture type (Table 5-2). The DM production for plantain and perennial ryegrass pastures was 30% and 32% lower respectively in HD plots than in UD plots.

Table 5-2 Herbage accumulation relative to undamaged plots (UD) of three-year-old plantain and perennial ryegrass-based pastures following light treading damage (LD) or heavy treading damage (HD) with the puggo-stick in November 2021. Sown April 2019.

Pasture	**Treatment**	**Herbage accumulation relative to UD**
Plantain	UD	100
	LD	89
	HD	70
Perennial ryegrass	UD	100
	LD	103
	HD	68
ANOVA p-values		
Treatment		<0.01

Figure 5-2.: Treading damage produced by the puggo-stick in this experiment.

5.6 Discussion

Pug depth and thus treading damage severity, increased with an increase in SMC at the time of grazing, which agrees with previous work (Climo & Richardson 1984), and confirms that the treading treatments were implemented as planned. The greater damage severity observed in the plantain pasture was likely a consequence of the higher SMC in those plots during treading, in comparison with the perennial ryegrass pasture, with the difference most likely due to site differences. However, it is difficult to rule out any effect that botanical composition and plant density might have had on the resistance of soil to treading. The likely lower growing point density and higher proportion of bare soil in the plantain pasture relative to the perennial ryegrass pasture may have increased the susceptibility of the former to greater treading damage. The lower pug density recorded for the perennial ryegrass pasture than the plantain pasture may have come as a result of pug marks being less identifiable in the former, since the pug marks were often concealed by plant material. Similarly, pug density may have appeared lower in HD plots due to greater soil displacement causing pug marks to overlap each other, and thus make them difficult to count individually.

The treading damage caused by the puggo-stick was comparable to damage caused by cattle in other studies. For example, Betteridge et al. (2003) reported pug depths greater than 30mm in a saturated silt loam and Nie et al. (2001) reported pug depths of 43mm in a wet clay loam. Furthermore, the effects of puggo-stick damage on herbage production agreed with previously established reductions of between 30-50% following cattle treading (Pande et al. 2000; Nie et al. 2001), which provided further confirmation that the puggo-stick method provided a reasonable simulation of cattle treading. The effect of puggo-stick stick treading on herbage accumulation was also worsened with an increase in SMC. Treading at a ‘safe’ SMC appeared to marginally increase herbage accumulation in the perennial ryegrass pasture, but drastically reduce it at an elevated SMC.

There were however, some limitations associated with the puggo-stick; 1. The method required a considerable physical effort on the part of the operator due to its weight and the necessity to move the device for every new pug mark, 2. It was difficult to ensure that every pug mark resulted from an identical exertion of force since that pressure was driven by the operator's physical actions, 3. Being a flat post-end, the force was generally only directed vertically, so any lateral cutting or soil displacement was probably minimal in comparison to that achieved under a walking cow's hoof. However, when considering the likeness of the soil and pasture damage produced by the puggo-stick and cattle treading damage, these limitations appeared to be minor.

5.7 Conclusions

The treading damage caused by the puggo-stick was comparable to damage caused by cattle in previous studies, with severity (pug depth) increasing with an increase in SMC. The resulting reduction in the herbage accumulation of both pasture types following damage by the puggo-stick was comparable to the reduction in herbage growth observed by earlier researchers for pastures subjected to cattle treading damage. These results suggest that the puggo-stick generated treading damage comparable to that of live cattle, and so appears to be an inexpensive and effective method for simulating cattle treading damage in pastures and could be considered for use in future experiments.

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Name/title of Primary Supervisor:	Danny Donaghy

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Chapter 6 Investigating the impact of treading damage on the plantain (*Plantago lanceolata* L.) content and performance of a plantain + perennial ryegrass (*Lolium perenne* L.) pasture over two years.

Chapter 4 showed that treading damage by cows may be an important cause of reductions in pasture PL content during spring. However, since the pasture PL content and PL shoot density of damaged plots were similar to those of undamaged plots by autumn, there was no evidence to suggest that there was any permanent negative impact of treading damage on PL. This result reflects previous literature which has shown PL contents to peak around 15 months, before decreasing. It is unknown if previously damaged PL populations could recover from further damage events in the second year after sowing. Therefore, the study of PL populations within mixed pastures, which are damaged in the second year after sowing is warranted. Additionally, a two-year study of a PL population within a grazed, mixed pasture is interesting since it might elucidate factors responsible for reductions in PL content and persistence, which often occur in the second year following sowing. Chapter 6 is a continuation of chapter 4 and measured the response of a PL population subjected to treading damage in early spring in its first year, to a repeat damage event during early spring in its second year. This chapter allowed for conclusions to be made about the impact of treading damage on PL within mixed pastures and provided an opportunity to speculate on possible causes of reduced PL contents within mixed pastures in the second year following sowing.

This chapter was under review for the Journal of New Zealand Grasslands vol 86.

Wilson S, Donaghy D, Horne D, Navarrete S, Kemp P. 2024. Investigating the impact of treading damage on the plantain (*Plantago lanceolata* L.) content and performance of a plantain + perennial ryegrass (*Lolium perenne* L.) pasture over two years. *Journal of New Zealand Grasslands 86*.

6.1 Abstract

There is industry concern over the difficulty of maintaining a satisfactory plantain (*Plantago lanceolata* L.) content within plantain + perennial ryegrass (*Lolium perenne* L.) dairy mixed pastures. One cause of the sharp decline in plantain content may be treading damage from grazing dairy cows during early spring. In an experiment at Massey University, Palmerston North, the impact of cow treading damage on the plantain content of a plantain + perennial ryegrass pasture was investigated over two production years. Measurements of pasture yield, canopy development, botanical composition and growing point density captured the response of the pasture to the treading damage. Early spring treading damage reduced pasture growth by 50% and 75% during the early spring periods of year one (2022) and two (2023), respectively ($P<0.05$). Plantain content and shoot density tended to be lower in damaged plots during early spring, before recovering throughout summer, although this effect was more pronounced during year one. These results re-iterate the importance of avoiding treading damage to avoid reductions in pasture production and plantain content throughout spring. The reduction in plantain content in all plots during the second year of the experiment reflected industry observations, suggesting that treading damage likely provides only a part of the explanation for declines in plantain content observed on-farm. An alternative cause of the decline in plantain content in year two may have been shading from perennial ryegrass during late spring. Future research should consider the effect of grazing management and other companion species on plantain content.

6.2 Introduction

The incorporation of plantain (*Plantago lanceolata* L., PL) into high-performing perennial ryegrass (*Lolium perenne* L., RG) based pastures is one potential low-cost tool for reducing nitrogen (N) losses from New Zealand dairy farm systems (Doole et al. 2021). However, there is growing evidence that PL content within these pastures is variable (Nguyen et al. 2022b) and persistence is usually poor (Dodd et al. 2019). One study on New Zealand farms has shown that PL contents rarely exceed more than 30% of the sward dry matter (DM) in grass-based pastures after two years following sowing (Dodd et al. 2019), while another study of a dairy pasture showed that the PL content peaked at 47% during the first 15 months after sowing, then declined to 20% after 23 months (Nguyen et al. 2022b). This decline is a significant issue as the efficacy of PL for reducing N losses from pastures is strongly associated with the proportion of PL in the diet of cows (Minnée et al. 2020; Navarrete et al. 2022).

While PL has been labelled a 2 to 3 year forage species in grazed pastures (Kuiper & Bos 1992), it has been described by ecologists as having potential life-span greater than 12 years (Grime et al. 1989). This implies that grazed pastures introduce additional stresses for PL, suggesting some potential for improving PL persistence in pastures by alleviating these stresses. Potential causes of the shortened lifespan and corresponding rapid decline in PL content in RG-based pastures include competition from other pasture species (Bryant et al. 2019), insect pressure (Kelly & Foley 2019; Dodd et al. 2024), sub-optimal grazing management (Ayala et al. 2011b; Dodd et al. 2019), waterlogging (Van Groenendael 1985), and treading damage and compaction (Blom 1979).

Chapter 4 showed that cow treading damage during early spring caused an immediate reduction in the PL content within a four month old PL + RG pasture. However, in that study PL content recovered to pre-damage levels by autumn, and it appeared that there was no permanent effect of the damage on PL persistence. However, it was unknown how the recovered PL population would perform following damage in the second year. It is possible that treading damage events might have a larger impact in the second year after sowing than in year one, which could account for the typically observed rapid decline in PL content in year two (Nguyen et al. 2022b). One potential reason for this could be the lasting effects from the damage in year one, such as plant injury and burial (Brown & Evans 1973), and soil compaction (Blom 1979) and their perpetuation following any further treading damage events in year two. Further to this, a reduced ability for growth following successive treading damage events might adversely impact the ability of PL to compete with companion species for light, specifically the somewhat treading-tolerant RG (Brown & Evans 1973). Dense RG-based pastures may also inhibit the emergence of new PL seedlings (Bryant et al. 2019), and thus limit the recovery of the PL population through seed. Additionally, given that declines in PL content normally occur during the second year after sowing, older PL populations might also have a reduced capacity for recovery via asexual shoot propagation. A better understanding of these dynamics could lead to improved management strategies for maintaining high PL contents in RG-based dairy pastures at risk of repeated treading damage.

By definition, the PL content of a pasture (on a DM basis) is derived from the ratio of PL DM to all other pasture DM, and thus is driven by: 1. the DM yield of PL, and 2. the DM yield of all other pasture species. Since the benefits of PL for reducing environmental impacts are driven by the proportion of PL in a sward or cows' diet (Minnée et al. 2020; Navarrete et al. 2022), studies have concentrated on PL content when discussing the fitness of PL in a particular climate + soil type + system combination (Dodd et al. 2019; Nguyen et al. 2022b). However, this approach fails to account for the important contribution of companion species DM in the determination of PL content. The current work attempted to separate PL and companion DM growth to explain the effects of treading damage stress on PL independently.

The current study is an extension of an earlier experiment (chapter 4) and aimed to: 1. investigate the impact of treading damage, in two successive years, on the productivity of a PL + RG pasture; and 2. investigate the impact of treading damage, in two successive years, on the content and density of PL within a PL + RG pasture.

6.3 Materials and Methods

6.3.1 Experimental site and climate

The experiment site was established in autumn 2022 on a Manawatu fine sandy loam. Plantain cv. Agritonic and perennial ryegrass cv. Maxsyn were direct drilled at 10 kg/ha and 5 kg/ha, respectively, on April 8 with the seeding rate designed to achieve an equal proportion of PL and RG in the pasture at the start of the experiment. Nutrients were non-limiting in this experiment. In year one of the experiment (August 2022 - July 2023) there was 958 mm of rainfall, and during the measurement period of year two (August 2023 - February 2024) there was 470 mm of rainfall. The long-term average annual rainfall (1991 - 2020) for the site is 984 mm. Mean daily temperature ranged between 8℃ in August 2023 to 20 ℃ in January 2024. During the treatment period in August 2022, total thermal time was 182.2℃ d, while during the treatment period in August 2023 total thermal time was 98℃ d. The thermal time base temperature was 5℃. Meteorological data were sourced from a NIWA weather station, Palmerston North, 200m from the study site. Experiment site GPS coordinates 40°22'56.6"S 175°36'27.55"E.

6.3.2 Experimental layout and treatments

The experiment consisted of two treading treatments, damaged (DD) and undamaged (ND) with four replicates of each treatment, in a randomised complete block design. Each plot was 182 m^2. The soil at the site is a Manawatu fine sandy loam which is described as a well-drained, fluvial recent soil with a high soil water holding capacity and high structural vulnerability (Landcare-Research 2019). Dairy cows were used to implement treading damage, which was based on a difference in soil volumetric water content (VWC) between treatments at the time of grazing. Procedures were approved by the Massey University Animal Ethics Committee (AEC 22/34). Based on previous studies, it was decided that grazing at a soil water deficit of 5 - 10 mm, or 37% - 33% soil VWC, would ensure that ND plots were not damaged by the cows.

On 17th August 2022, ND plots were grazed with 6 pregnant dairy cows (representing a stocking density of 330 cows/ha). In the 48 hours following this grazing there was 25 mm of rainfall, which increased soil VWC sufficiently and so DD plots were grazed (and damaged) on August 19th with the same cows.

Subsequent grazings on both treatments were carried out with lactating dairy cows for the remainder of the production year, and once with dry cows during winter. All grazing events were carried out with 6 cows per plot. Grazings occurred whenever the estimated mean pasture mass across the site reached approximately 2800 kg DM/ha, and the target residual was 1500 kg DM/ha. However, soil conditions were closely monitored throughout the year, and plots were only grazed when soil VWC was less than 37% to ensure that plots were not damaged further by cows.

On 7th August 2023, ND plots were grazed with 6 lactating dairy cows per plot. On 8th August, 15 mm of irrigation water was applied to the site, which sufficiently increased soil VWC and so DD plots were grazed and damaged on 9th August with the same cows. Later grazing events in the second year were then carried out as described above, until the last grazing on 21st February, 2024. All grazing events during the experiment lasted an average of 2.5 hours.

6.3.3 Measurements

Soil measurements for treading damage severity included pug mark depth, which was measured with a ruler, and pug mark density, which was determined by counting the number of pug marks within a defined area. Soil VWC was measured with a portable TDR system (Time Domain Reflectometry – HandiTRASE® TDR, Soilmoisture Equipment Corp, USA) in each plot during each treading event. Soil measurements are detailed further in chapter 4.

Pasture DM yield pre- and post-grazing was measured by hand-clipping herbage to ground level in three 0.1 m^2 quadrats per plot and drying the herbage at 60°C for 48 hours. Pasture growth rate was calculated by subtracting the pre-grazing DM yield from the post-grazing DM yield of the previous grazing and dividing by the number of days in the regrowth period. Herbage was cut from a 10×50 cm strip adjacent to each pre-grazing DM quadrat and separated into PL leaf, PL seed head, RG, dead matter and 'other' which comprised annual poa (*Poa annua* L.), broadleaf weeds (e.g., broadleaf dock, *Rumex obtusifolius* L. and volunteer chicory, *Cichorium intybus* L.), and white clover (*Trifolium repens* L.). Samples were dried at 60°C for 48 hours to determine pasture botanical composition on a DM basis. The growth rates of PL and RG were calculated by multiplying the pasture DM growth rate during a given season by the proportion of PL and RG in the pasture during that same period.

Light interception (LI) measurements were made using a Spectrosense2$^{+®}$ device by measuring photosynthetically active radiation (PAR) above and below the canopy three times in three fixed quadrats within each plot. Leaf area index (LAI) was measured using a LICOR LAI-2000® portable light meter by scanning once above the canopy and three times below the canopy at three fixed quadrats within each plot. Measurements of normalised difference vegetation index (NDVI) were also made with a RapidScan$^®$ CS-45 canopy analyser by scanning a pre-determined and consistent path through each third of every plot. Measurements of LI, LAI and NDVI were consistently conducted 14 days after each grazing event so that measurements could be made before canopy closure. Due to unforeseen circumstances, these measurements were made 21 days after the treading event in year two. The relationship between LI and LAI between late spring and early summer in year two was explored using the scatter plot function in Microsoft Excel.

The density of PL growing points was measured by counting the number of shoots (PL) in four fixed 0.1 m^2 quadrats per plot. The density of RG growing points was measured by counting the number of tillers (PR) in four fixed 0.05 m^2 quadrats per plot.

6.3.4 Statistical analysis

Statistical analyses were conducted using the MIXED procedure for an ANOVA in SAS (version 9.4, SAS institute 2018). Pair-wise t-tests were used for mean comparisons and significance was declared at P<0.05. All results were analysed within each sampling date with treatment as the fixed effect. All data were also analysed for repeated measures. Results for pasture growth rate, botanical composition, and PL and RG density were analysed for repeated measures with treatment, climatic season, and their interactions as fixed effects. Results for soil VWC and treading damage, were analysed for repeated measures with treatment, study year and their interactions as fixed effects. Results for pasture growth rate, botanical composition, and PL and RG density from the period between early spring to late summer, were also analysed for repeated measures in a separate analysis with treatment, study year and their interactions as fixed effects. Results for LI, LAI and NDVI were analysed for repeated measures with treatment, season, and their interaction as fixed effects. Results for PL and RG growth rates were analysed for repeated measures with species, treatment, season and their interactions as fixed effects. Interactions between fixed effects were presented only when they were statistically significant.

6.4 Results

6.4.1 Treading damage

During the treading events in both years, soil VWC was significantly higher ($P<0.01$) on the day that DD plots were treaded than when the ND plots were treaded two days earlier (Table 6-1). Both the mean pug depth ($P<0.01$) and pug density ($P<0.01$) were significantly greater in DD plots than ND plots following the treading treatments in both years. The soil VWC in ND plots at the time of treading tended to be higher in year two than in year one ($P=0.06$).

6.4.2 Pasture growth rate, Light Interception, Leaf Area Index and Normalised Difference Vegetation Index

Pasture growth was reduced by 50% and 75% following treading damage in the early spring periods of year one and two respectively ($P<0.05$; Table 6-2). However, treading treatment did not significantly affect pasture growth during any other seasons. When averaged over each year and over the entirety of the experiment, pasture growth rate was not significantly different between treading treatments.

Pasture growth varied significantly between climatic seasons ($P<0.01$). In year one, the mean pasture growth rate across treatments increased from 36 kg DM/ha/day in early spring to a peak of 82 kg DM/ha/day during late spring before decreasing over summer to 31 kg DM/ha/day in autumn and winter. Mean pasture growth decreased significantly in the early spring of year two, to 17 kg DM/ha/day, before increasing to 76 kg DM/ha/day in late spring ($P<0.01$). The mean pasture growth rate was similar in early summer, before decreasing to 42 kg DM/ha/day in late summer ($P<0.01$). The mean pasture growth rate between early spring and late summer was similar between the two years of the experiment.

Canopy light interception was significantly lower in DD plots than ND plots during early spring in both years ($P<0.05$). However, it was not significantly affected by treatment during any other season (Table 6-3). Mean canopy LI was greatest during the summer of year one, and lowest during late summer in year two (season effect P<0.01).

In year two, the mean LAI of the DD pasture was 50% lower than the LAI of ND pasture during early spring (P<0.05) but was not significantly different between treading treatments during any other season. The mean LAI was greatest during late spring and early summer and lowest during early spring (P<0.01). There was a strong logarithmic regression relationship between canopy LI and LAI between late spring and early summer (R2 = 0.66), with LI reaching 95% at an LAI of 4.9 (Figure 6-1).

During early spring in year one of the experiment, NDVI was significantly lower in DD plots than ND plots 14 days after the treading damage (P<0.05). There was no significant difference between the NDVI of treading treatments during any other season. Mean NDVI was greatest during late spring in both years and was lowest during late summer in year two (P<0.01).

Table 6-1. Mean soil volumetric water content (VWC) during treading by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2) and the resulting mean pug mark density and depth in a plantain + perennial ryegrass pasture.

Year	**Treatment**	**Soil VWC (%)**	**Pug depth (mm)**	**Pug density (pugs/m^2)**
1	Damaged	43	34	46
	Undamaged	35	13	37
2	Damaged	42	31	48
	Undamaged	38	18	39
SEM		0.5	1.8	2.2
ANOVA p-values				
Treatment		<0.01	<0.01	0.047
Year		0.246	0.602	0.202

SEM = Standard error of the mean

Table 6-2. Pasture growth rate (kg DM/ha/day) of a plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (DD) or no treading damage (ND) from dairy cows during early spring 2022 (year 1) and early spring 2023 (year 2).

		Pasture growth rate (kg DM/ha/day)		
Year	Season	DD	ND	SEM
1	Early spring	24[b]	48[a]	8.85
	Late spring	82	83	12.57
	Early summer	52	41	6.02
	Late summer	38	47	5.49
	Autumn	26	36	3.95
	Winter	31	33	2.88
2	Early spring	7[b]	27[a]	5.53
	Late spring	75	77	5.27
	Early summer	86	76	10.10
	Late summer	48	36	6.09
	Mean	47	50	2.49
ANOVA p-values				
Treatment		0.371		
Season		<0.01		
Year		0.679		

Means within rows with different letters are significantly different (P<0.05)

SEM = Standard error of the mean

Table 6-3. Pasture light interception (LI), leaf area index (LAI) and normalised difference vegetation index (NDVI), measured 14 days after each grazing event, in a plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (DD) or no treading damage (ND) from dairy cows during early spring 2022 (year 1) and early spring 2023 (year 2).

		LI (%)		**LAI (cm^2 leaf/ cm^2 ground)**		**NDVI**	
Year	Season	DD	ND	DD	ND	DD	ND
1	Early spring	84b	95a	-	-	0.79b	0.83a
	Late spring	88	90	-	-	0.79	0.79
	Early summer	91	93	-	-	0.69	0.68
	Late summer	93	94	-	-	0.70	0.71
	Autumn	93	92	-	-	0.71	0.72
2	Early spring*	83b	89a	0.66b	1.33a	0.67	0.70
	Late spring	86	87	2.66	2.91	0.83	0.84
	Early summer	88	89	3.19	3.29	0.76	0.76
	Late summer	79	75	1.70	1.53	0.51	0.50
	Mean	89	87	2.05	2.27	0.72	0.72
ANOVA p-values							
Treatment		0.188		0.342		0.591	
Season		<0.01		<0.01		<0.01	

Means within rows with different letters are significantly different ($P<0.05$)

*Early spring measurement taken 21 days after the treading damage event.

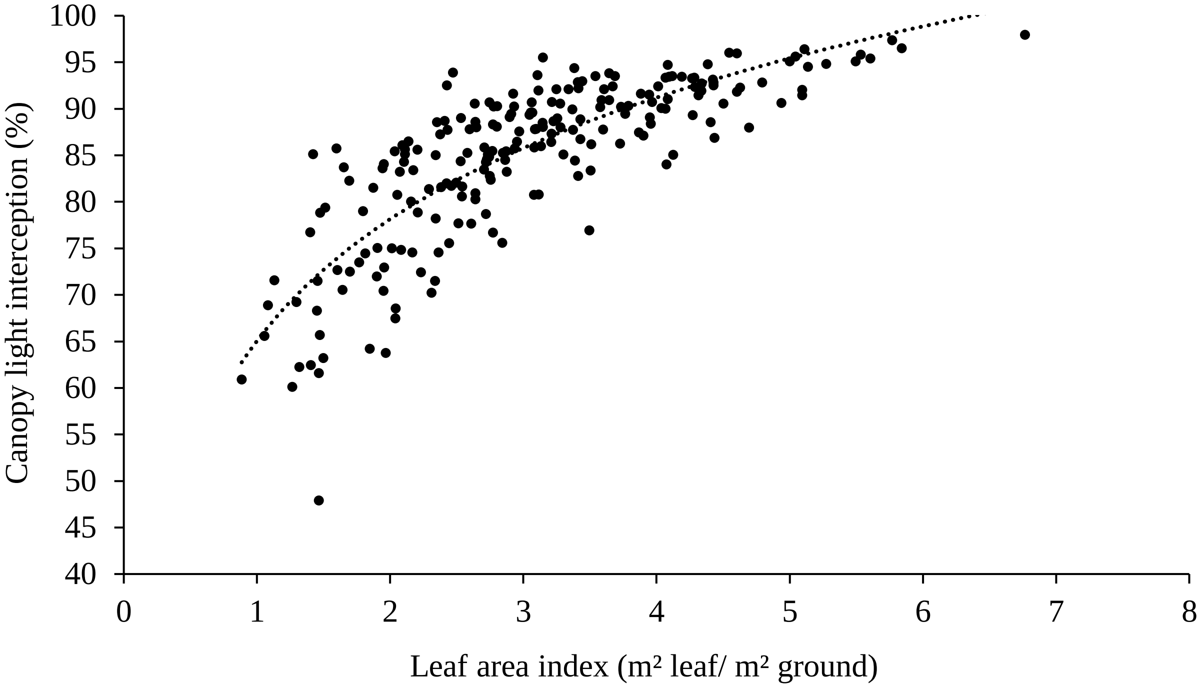


Figure 6-1. Relationship between leaf area index and light interception during several regrowth cycles between late spring and early summer for a two-year-old plantain + perennial ryegrass pasture. Equation $y = 18.866\ln(x) + 65.051$.

6.4.3 Pasture botanical composition

During early spring in year one, following the treading event, PL content tended to be lower (P=0.097) in DD plots than in ND plots while RG content was 28% higher in DD plots than in ND plots during the same period (P<0.05, Figure 6-2). During late spring, PL content was 26% lower in DD plots than in ND plots (P<0.05), while RG content was significantly greater in DD plots than in ND plots (P< 0.05). There was no significant effect of treading treatment on PL and RG content during the remainder of year one. During early spring in year two, following treading damage, PL content appeared to be lower in DD plots than in ND plots (P=0.19), but RG content was similar in both DD and ND plots. There was no significant effect of treatment on pasture PL or RG content during the remainder of the study. When averaged over the entire experimental period, treading treatment had no significant effect on the PL content (DD = 27%, ND = 29%) or RG content (DD = 53%, ND = 51%) of plots.

The mean pasture PL and RG content varied significantly between seasons (P<0.01) In general, PL content peaked during autumn and winter, where it exceeded 40% of DM, and was lowest during late spring and early summer, where it made up ~20% of sward DM in year one and ~10% of sward DM in year two. In year one, RG content was greatest during early spring and late summer (63%) and lowest in autumn (40%), while in year two, RG content was greatest in late spring (70%) and lowest in late summer (42%). The mean pasture PL content decreased from 35% in year one to 17% in year two (P<0.01), while the mean RG content was similar between years.

The portion of pasture DM not comprising RG or PL was largely made up of annual poa, which appeared throughout spring in both years, and dead matter, which comprised 28% and 34% of sward DM during early and late summer in year two, respectively. Additionally, PL seed head content was significantly higher during the second year of the experiment, than the first year ($P<0.05$), while the broadleaf weed content increased gradually over the course of the experiment.

6.4.4 Plantain and perennial ryegrass growth rates

The growth rate of RG was significantly higher than that of PL during late spring and early summer in year one ($P<0.01$), and during late spring, early and late summer in year two ($P<0.01$) but similar to that of PL in all other seasons (Figure 6-3). There was a tendency for a higher growth rate for RG than for PL during early spring in both year one ($P=0.09$) and year two ($P=0.07$). The mean RG growth rate (26 kg DM/ha/day) was 138% greater than that of PL (11 kg DM/ha/day) on average over the entire experiment ($P<0.01$).

The mean growth rate across PL and RG was reduced by treading damage during early spring in both year one ($P<0.05$) and year two ($P<0.01$), and during autumn in year one ($P<0.05$). When averaged over the entire experiment, the mean growth rate across PL and RG was not significantly different between treading treatments.

The growth rate of both PL and RG varied significantly between seasons, regardless of treatment ($P<0.01$). In early spring of year one, the PL growth rate was 12kg DM/ ha/ day. The mean PL growth rate then peaked during late spring and late summer (17kg DM/ha/day) and was similar throughout autumn and winter. In early spring of year two, the mean PL growth rate decreased to 5kg DM/ha/day, before increasing again in late spring and then trending down for the remainder of year two. In early spring of year one, the growth rate for RG was 18kg DM/ha/day. This increased during late spring to 51kg DM/ha/day, but then decreased from early summer onward and was around 18kg DM/ha/day throughout autumn and winter. The mean RG growth rate was not significantly different between winter and the early spring of year two but increased to 53kg DM/ha/day during late spring. The growth rate of RG then decreased gradually between early and late summer.

6.4.5 Plantain shoot density and perennial ryegrass tiller density

In year one, the PL shoot density in DD plots was 21% and 29% lower than in ND plots during early and late spring, respectively ($P<0.05$) (Figure 6-4). During late summer, PL shoot density was 20% lower in DD plots than in ND plots, but from autumn until late summer in year two, PL shoot density was similar between treatments. When averaged over the whole experimental period, PL shoot density was not significantly different between treading treatments.

Treading damage had no significant effect on RG tiller density during any season in the study.

Following the first grazing (and treading) in August 2022, mean PL shoot density across the site decreased from 309 to 225 shoots/m^2 ($P<0.01$). The mean PL shoot density then increased gradually over year one, to reach 311 shoots/m^2 in autumn. Mean PL shoot density decreased from autumn into winter ($P<0.05$). In year two, following the treading event, PL shoot density remained steady across the site and was 252 shoots/m^2 in early spring, before decreasing by 37% between early and late spring ($P<0.01$) to 160 PL shoots/m^2, and then remained constant into late summer. The mean PL shoot density decreased by 53% on average over the experiment and was significantly lower in year two than in year one ($P<0.01$).

There was also an effect of a significant interaction between treading treatment and climatic season on PL shoot density ($P<0.05$). After decreasing significantly following the treading event in year one, PL shoot density in DD plots recovered steadily and was similar to pre-experiment levels by late summer. In contrast, following a reduction in early spring, the PL shoot density of ND plots had recovered to pre-experiment levels by late spring, and then increased gradually until autumn. Between autumn in year one and the end of the experiment in year two, the shoot density of DD plots and ND plots followed a similar downward trend, with the largest reduction occurring in late spring in year two.

In year one, the mean RG tiller density was similar between the pre-experimental measurement and the late spring measurement, although it decreased significantly between late spring and late summer ($P<0.05$). The mean RG tiller density was similar between late summer and winter (1869 tillers/m^2). The mean RG tiller density increased significantly during early spring in year two, to 3971 tillers/m^2 ($P<0.01$) and then remained constant into late summer. In contrast to PL, the mean RG tiller density was significantly greater in year two than in year one ($P<0.01$).

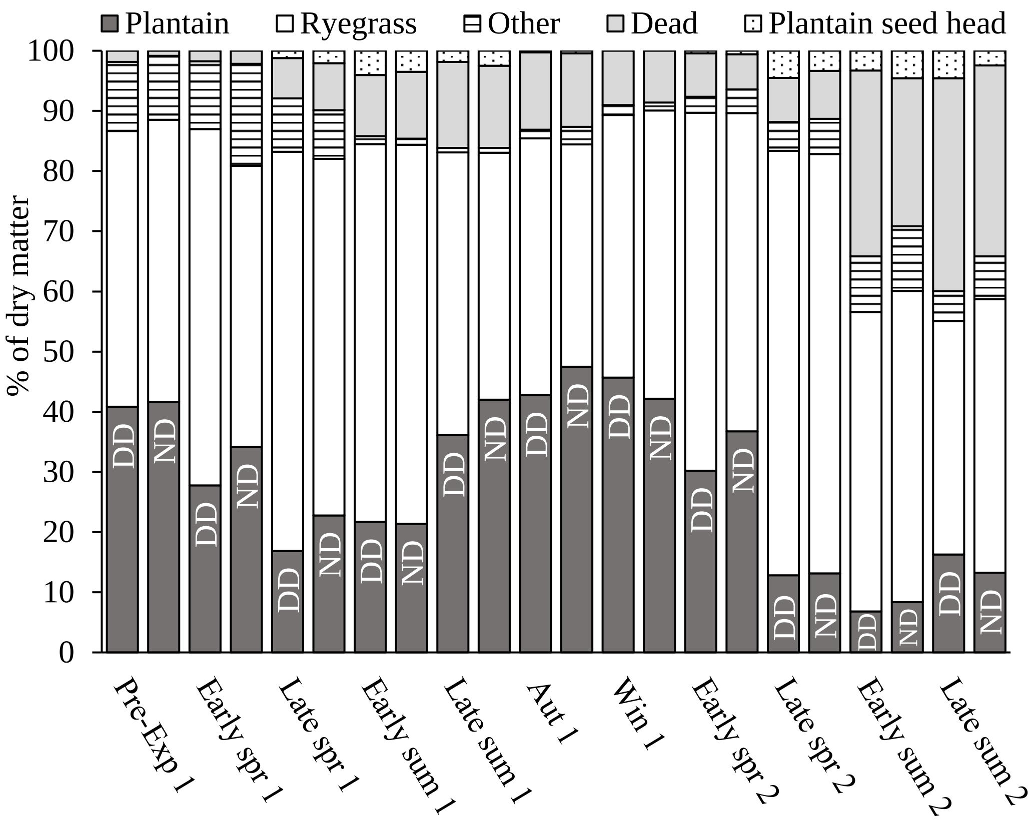


Figure 6-2. Botanical composition of a plantain + perennial ryegrass pasture sown in autumn 2022 over two years, following treading by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). DD= Damaged, ND= Undamaged.

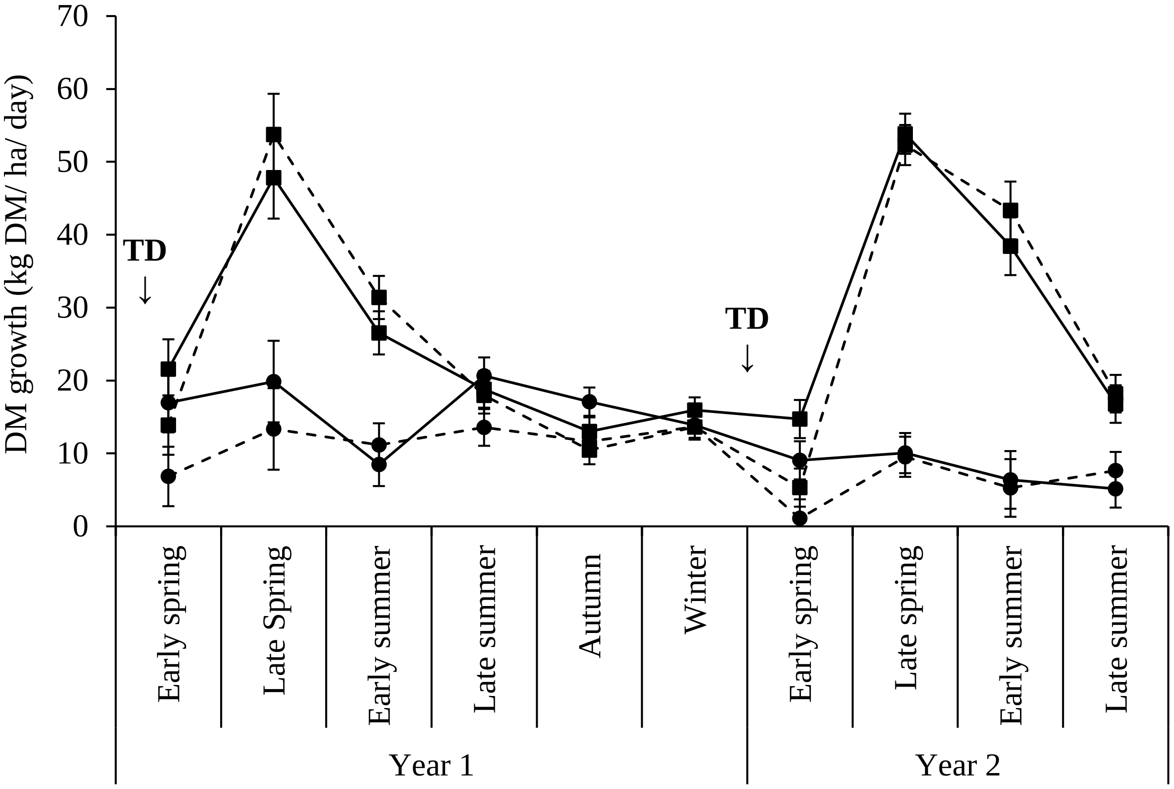


Figure 6-3. Daily growth rates (kg DM/ ha/ day) of the plantain (circles) and perennial ryegrass (squares) components of a plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (dashed lines) or no treading damage (solid lines) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean.

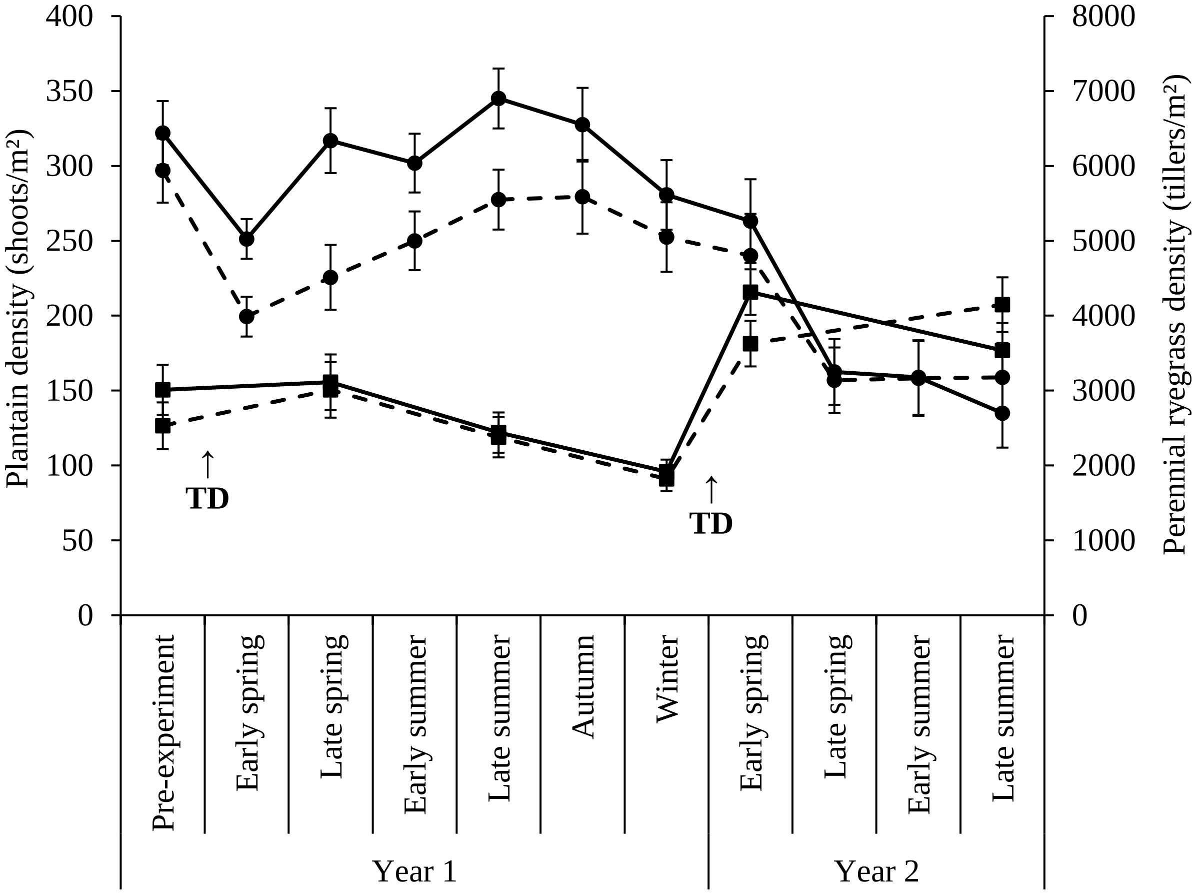


Figure 6-4. Density (number/m^2) of plantain shoots (circles) and perennial ryegrass tillers (squares) in a second-year plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (dashed lines) or no treading damage (solid lines) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean.

6.5 Discussion

6.5.1 Treading damage

As expected during both years, mean pug depth increased with an increase in soil VWC at the time of grazing (Climo & Richardson 1984), and led to the significantly greater damage severity observed in DD plots than in ND plots after each treading event. The mean pug depths observed in DD plots across the two years are comparable to those previously observed by Betteridge et al. (2003) in a cattle treading experiment on a silt loam soil. The greater mean pug density in DD plots than ND plots was unexpected and may have resulted from deep pug marks being more easily identified than shallow pug marks, rather than an effect of animal behaviour.

6.5.2 Pasture growth rate, Light Interception, Leaf Area Index and Normalised Difference Vegetation Index

In agreement with previous pasture treading studies (Brown & Evans 1973; Pande et al. 2000), treading damage had an immediate, negative impact on the growth of the PL + RG pasture, with a reduction in pasture growth rate of 50% and 75% in the first regrowth period following treading in years one and two respectively. Additionally, since the reduction in growth rate following treading damage was similar to that observed for RG-dominant pastures in other studies (Pande et al. 2000), it didn’t appear that inclusion of PL increased sensitivity of the mixed pasture to treading damage in the current study. Pasture growth rates recovered in DD plots during late spring in both years, suggesting that the damage caused no permanent limitations to pasture growth. Pasture growth rates followed a typical trend for RG-based swards, peaking between late spring and early summer, and declining during winter (Kemp et al. 1999). The low pasture growth rates and larger reduction in growth following treading damage in the early spring of year two than in year one probably reflected cooler temperatures during that time (Baars & Waller 1979).

Pasture growth rate is strongly associated with a pasture's ability to capture incident light through leaves (Smetham 1973). Treading damage has been shown to reduce pasture LAI and thus growth rate in the initial regrowth period following the damage event (Pande et al. 2000). In the current study, treading damage initially reduced canopy LI in both years and LAI in year two, most likely driving the reduction in growth rate during that period (Smetham 1973).

The use of NDVI for capturing the negative effects of treading damage on canopy growth proved useful in early spring in year one of the study, where DD plots had a lower NDVI value than ND plots, reflecting a reduction in green leaf material following damage. However, following the treading event in year two, NDVI measurement failed to capture the severity of treading damage on pasture in DD plots. This may have been a result of the NDVI scanner receiving reflectance from dying residual herbage trampled into the soil surface in DD plots, below the height at which LI and LAI measurements are made. Therefore, the measurement of NDVI was useful for detecting damaged pasture, particularly where there was a high proportion of bare soil, although more work needs to be done to determine the limitations of its use.

6.5.3 Plantain content and density

Treading damage by dairy cows caused a notable reduction in PL content within the PL + RG pasture in early spring in year one, likely via the destruction and burial of PL growing points (Brown & Evans 1973), along with the impairment of leaf regrowth. However, the effects of the treading damage on PL content in year two were less obvious. In contrast with PL, treading damage appeared to cause an increase in RG content in both years, reaffirming its status as a more treading-tolerant pasture species (Brown & Evans 1973). Pasture PL content was lower than expected during the experiment, given that similar sowing rates of both PL and RG resulted in a peak PL content of 64% in another study in the same region (Nguyen et al. 2022b). Low emergence rates, along with the rapid decline in PL content during year two, represent some of the challenges frequently observed following the incorporation of PL with RG (Bryant et al. 2019; Nguyen et al. 2022b).

The pasture PL and RG contents appeared to be negatively correlated throughout the current study. As anticipated, RG growth rates peaked during late spring in both years (Kemp et al. 1999) and made up a large proportion of sward DM. However, despite also reaching a peak growth rate in late spring in both years, PL content was generally very low during this period. In contrast, during late summer and autumn in year one of the study, there was a peak in PL content, coinciding with significantly lower RG growth rates. Taken together, these results suggest that PL content was largely driven by RG growth and illustrate the impact that companion species growth has on the determination of PL content.

Pasture PL content and DM yield are also likely to be driven somewhat by PL shoot density (Ayala et al. 2011b). The reduction in PL shoot density in DD plots following treading in year one appeared to account for the reduction in PL content in the pasture during that time. However, in year two, treading damage didn’t appear to have any major effect on PL shoot density, which might have been due to larger, better established PL plants (chapter 7; Powell et al. 2007) providing greater protection to individual shoots from the treading damage imposed.

For PL to persist, it is necessary for PL populations to replace dead shoots with new shoots, either asexually through secondary shoot development, or via seedling recruitment (Grime et al. 1989). While the PL shoot population proved to be sensitive to treading in early spring in year one (Ayala et al. 2011b), in year two the population largely survived this early treading, possibly also due to shoots being bunched together on larger PL plants. However, the continual reduction in PL shoot density during late spring and summer in year two is in stark contrast with the gradual increase in PL shoot density during those seasons in year one. That could indicate that older PL populations have a reduced capacity for recovery via new shoot production or seedling recruitment.

In year two, the largest reduction in PL shoot density, regardless of treading treatment, occurred between early and late spring, at a time when both the tiller density and growth rate of RG increased considerably. This result might suggest that a significant driver of reduced PL shoot density was increased competition from RG, which agrees with previous findings (Bryant et al. 2019; Dodd et al. 2019), and, in the context of PL persistence, could signify that RG is an unsuitable companion for PL. In a study by Mook et al. (1989), PL mortality in dense vegetation was concentrated in the late spring period, coinciding with a period of low light transmission through the canopy. Studies have suggested that PL growth and development are particularly sensitive to shading (van der Toorn & Pons 1988; Kuiper & Bos 1992), which has been shown to reduce PL leaf photosynthesis rates (Kuiper & Bos 1992), and is associated with suppressed secondary shoot development (Van Tienderen & van der Toorn 1991). Furthermore, low-light conditions in dense grass-based swards may inhibit the successful establishment of new PL seedlings (van der Toorn & Pons 1988; Bryant et al. 2019), thus limiting the potential for PL population maintenance or increase via seedling recruitment.

If the main driver of PL shoot survival through late spring and early summer is light captured by shoots, one potential mitigation could be to defoliate the pasture at an earlier regrowth stage than what is recommended for a RG-based pasture. Since RG pastures usually consist of thin, erect leaves, canopies may reach 95% LI, and thus their maximum growth rate (critical LAI), at a LAI of 7 in summer (Brougham 1957). However, it appears that the PL + RG pasture in the current study achieved 95% LI at a LAI of 4.9, which is closer to the critical LAI for pastures containing flat-leaved plants such as white clover (Brougham 1957).

Since pasture growth rate declines gradually after a pasture reaches its critical LAI (Smetham 1973), it may be in the best interest of both PL shoot survival and pasture growth to defoliate PL + RG pastures at this lower LAI. However, more work is required to determine how an earlier defoliation would affect RG productivity, the allocation of pasture to cows, and influence feed budgeting on farm.

Figure 6-5. Cow treading experiment site during a late spring grazing event in year two.

6.6 Conclusions

While results from the current study suggest that livestock treading damage might provide only part of the explanation for low PL contents in dairy pastures, it remains important to avoid treading damage to avoid reductions in PL content and pasture growth throughout spring.

The decline in PL content within the PL + RG pasture over the two-year experiment seemed to be largely driven by high DM yields of RG in late spring and early summer in both years, and the reduction in PL shoot density during the second year of the study. These results suggest that PL shoot survival is negatively impacted by shading in RG-based swards. By grazing PL + RG pastures at an earlier regrowth stage during late spring, it may be possible to optimise pasture productivity as well as PL shoot survival and thus improve PL persistence.

Chapter 7 Investigating the impact of treading damage on the growth and survival of plantain (*Plantago lanceolata* L.) plants within a grazed dairy pasture

Chapter 6 showed that although livestock treading damage might provide only part of the explanation for low PL contents in dairy pastures, treading damage caused negative implications for the PL component of the pasture during early spring, especially in year one. In contrast, the effects of cow treading damage on PL were much smaller in year two of the study. It is possible that younger, smaller PL shoots and plants were more susceptible to treading damage events than older, larger PL shoots and plants. Additionally, since PL shoot density continued to decrease following damage in year 2, it is possible that older PL populations had a limited capacity for recovery, and/or another stress impacted the PL population during that time. However, it is not possible to make any sound conclusions on these matters from the results of chapter 6 alone.

Since chapter 6 is a sward-level study of PL within pastures, if fails to account for any plant-level impacts of treading damage on the PL component of the pasture. Given that plant-level functions like the number of shoots per plant and shoot DM yield are ultimately what underpin the performance and persistence of a PL population over time, a study of the plant-level effects of treading damage on PL was warranted. Chapter 7 measured the plant-level responses of PL to treading damage, as well as other stresses that occur within grazed pastures, to explain changes in the PL population over two years.

7.1 Abstract

The New Zealand dairy industry has identified plantain (*Plantago lanceolata* L.) as a tool to mitigate nitrogen losses from pasture-based systems, however, reports of poor plantain persistence within dairy pastures have led to industry concern over its viability. One cause of reduced plantain persistence is treading damage caused by cows grazing pastures when soil moisture contents are high. In an experiment at Massey University, Palmerston North, the impact of cow treading damage on the growth and survival of plantain within a plantain + perennial ryegrass (*Lolium perenne* L.) pasture was investigated over two production years. Measurements of plantain growth and population density captured the response of plantain plants to treading damage in a grazed pasture. Early spring treading damage caused an immediate reduction in plantain leaf and shoot (leaf + petiole) mass in year one, which was likely underpinned by a reduction in leaf elongation rate. Treading damage also caused a reduction in plantain shoot density, likely reflecting the death of growing points. However, the negative effects of treading damage on plantain were short-lived, as growth of damaged plantain plants and the density of plantain shoots in damaged plots, recovered throughout the first year of the experiment. One-year-old plantain plants appeared to be more susceptible to treading damage than two-year-old plantain plants, although the one-year-old population probably had a greater capacity for recovery through vegetative shoot production. An observed reduction in plantain shoot density in late spring in year two coincided with a reduction in petiole energy reserves (water soluble carbohydrates and nitrogen), possibly caused by shading from perennial ryegrass. Treading damage has the potential to exacerbate this effect since it might induce a reduction in the expansion of plantain leaves, and thus reduce potential photosynthate production in plantain plants. Future studies should consider the effects of grazing management and companion species selection on shading during late spring as a means of improving plantain performance within grazed mixed pastures.

7.2 Introduction

The identification of plantain (*Plantago lanceolata* L., PL) as a low-cost option for New Zealand dairy farms to reduce the loss of nitrogen (N) from their system to the environment (Doole et al. 2021) has led to PL becoming an increasingly important component of perennial ryegrass (*Lolium perenne* L., RG) based dairy pastures. However, suggestions of PL's poor persistence within RG-based pastures have gained prominence (Dodd et al. 2019), leading to concern over its potential as a sustainable N-loss mitigation tool. Several studies have shown that the PL content of PL + RG pastures usually peaks within two years of the sowing date and declines steeply thereafter (Dodd et al. 2019; Navarrete et al. 2022; Nguyen et al. 2022b). This is a significant issue given that the level of N-loss mitigation provided by PL is driven by the PL content in a pasture and/or in a cow's diet (Minnée et al. 2020). While pasture PL content at any given moment is driven by both the performance of PL (PL biomass yield) and the biomass yield of other pasture species, declines in PL content over time are most likely caused by factors affecting the PL population independently. The performance of a PL population within a pasture over time is underpinned by the growth (biomass accumulation) and survival (density per unit area) of individual PL plants and shoots. Given the importance that the New Zealand industry has placed upon PL for mitigating N-losses from dairy pastures, an investigation of factors affecting the performance of PL populations within dairy pastures is essential.

While PL is generally considered a two-to-three-year forage species in grazed pastures (Kuiper & Bos 1992), there is evidence that PL plants may persist for longer than 12 years in natural environments (Grime et al. 1989). While that implies that PL plants are sensitive to stresses associated with grazed pasture systems, it also suggests that there is an opportunity to extend the lifespan of PL plants, and thus PL persistence within pastures, through the removal of such stresses. There may be several potential causes of reduced PL lifespan in grazed pastures, which could include competition from other pasture species (Bryant et al. 2019), insect pressure (Kelly & Foley 2019; Dodd et al. 2024), sub-optimal grazing management (Ayala et al. 2011b; Dodd et al. 2019), and waterlogging (Van Groenendael 1985). Another potential cause of PL population decline within grazed pastures could be treading damage and compaction from livestock (Blom 1979). Previous researchers suggested that herb species are vulnerable to physical damage from livestock treading on wet soil (Jones & Haggar 1994). Additionally, Blom (1979) showed that PL shoot and root growth was highly sensitive to treading, which concurred with observations by (Chappell et al. 1971), who found denser populations of PL in lightly trampled areas, than in well-worn areas. However, it is likely that PL possesses some tolerance to treading disturbance, given that PL is usually a prominent species along the margins of well-worn tracks and pathways (Sagar & Harper 1964). That could suggest that treading only becomes significant for PL where plants are routinely grazed, however studies on the impact of treading damage on grazed PL populations are scarce.

Treading damage occurs when elevated soil moisture contents are combined with the management of livestock in confined rotations, situations which occur during late winter and early spring on many New Zealand dairy farms (Drewry et al. 2000). The damage itself is characterised by hoof-sized soil impressions (pugging), torn plant material and mud covering the plant and soil surfaces (Brown & Evans 1973). Treading damage may reduce PL leaf growth through the burial and destruction of growing points (Brown & Evans 1973) and the injury of remaining leaves (Sun & Liddle 1993). Reduced leaf growth would then limit the ability of PL shoots and plants to develop sufficient leaf area to maximise the capture of photosynthetically active radiation (PAR). This could be critical for PL, as PL may be reliant on energy produced in photosynthesis for regrowth following defoliation, due to its supposed limited capacity for energy storage in the crown and root (Ayala et al. 2011b). This effect could then be exacerbated by the shading of PL by other pasture species, notably RG, which is largely treading tolerant (Brown & Evans 1973) and so is likely to have a competitive advantage over PL following treading damage events.

Photosynthesis may also be negatively affected by the impact of treading damage on internal leaf structures. The trampling of PL under glasshouse conditions has been shown to reduce leaf photosynthesis (Mills et al. 1996), potentially due to direct effects on photosystem II and/or reduced stomatal conductance. Additionally, treading damage may reduce the photosynthetic efficiency of PL leaves by inducing a reduction in specific leaf area (SLA). Dijkstra and Lambers (1989) suggested that while a lower SLA was associated with improved trampling tolerance in broadleaf plantain (*Plantago major* L.), this may have resulted in internal leaf shading due to the high concentration of cell components per unit area of leaf. The combination of reduced leaf area and reduced photosynthetic capacity could lead to a decrease in water soluble carbohydrate (WSC) production in PL leaves, and a subsequent reduction in carbohydrate translocation to storage organs. The failure of PL plants to replenish carbohydrate reserves between defoliation events would then ultimately lead to a decline in plant dry matter (DM) accumulation (Lee et al. 2015b). In the long term, a consistent failure of plants to replenish carbohydrate stores between defoliation events, could lead to a loss of shoot and plant persistence, as has been observed for RG tillers (Fulkerson & Donaghy 2001).

The negative effects of treading damage, including soil compaction (Blom 1979) and creation of areas of bare ground (Elliott et al. 2002), may also be exacerbated by repeated treading damage events on the same PL population. The study of repeat damage events is of particular interest, as PL pastures are likely to encounter more than one damaging event in their lifetime, and each successive damage event could be potentially worsened by the previous event. For example, areas of bare ground resulting from the death of plants following damage are more susceptible to further treading damage than those areas with pasture cover (Betteridge et al. 1999), which could increase the severity of damage for remaining plants. Experiments that have investigated the effect of repeated damage treatments on the same pasture for more than a year are rare (Howes 2019), so the current study also provides some novelty by measuring the responses of pasture to treading damage over two production seasons.

Given the importance that the New Zealand dairy industry has placed on PL for mitigating N losses from dairy farm systems, an investigation of factors affecting the performance of PL within dairy pastures is of high interest. The current study aimed to investigate the impact of treading damage on the growth and survival of PL populations within PL + RG pasture. This study is an extension of the experiment carried out in chapter 6 but includes a greater focus on the plant-level effects of treading damage.

7.3 Materials and Methods

7.3.1 Experimental site

The experiment site was established in autumn 2022 on a Manawatu fine sandy loam. Plantain cv. *Agritonic* and RG cv. *Maxsyn* were direct drilled at 10 kg and 5 kg/ha, respectively, on April 8 with the seeding rate designed to achieve an equal proportion of PL and RG in the pasture at the start of the experiment. Nutrients were non-limiting in this experiment. In year one of the experiment (August 2022 - July 2023) there was 958mm of rainfall, and in year two (August 2023 - February 2024) there was 470mm of rainfall (Figure 7-1). As a comparison, the long-term average annual rainfall (1991 - 2020) for the site is 984mm. Mean daily temperature ranged between 8℃ in August 2023 to 20℃ in January 2024. During the treatment period in August 2022, total thermal time was 182℃ d, while during the treatment period in August 2023 total thermal time was 98 ℃ d. The thermal time base temperature was 5℃. Meteorological data were sourced from a National Institute of Water and Atmospheric (NIWA) weather station, Palmerston North, 200m from the study site. Experiment site GPS coordinates 40°22'56.6"S 175°36'27.55"E.

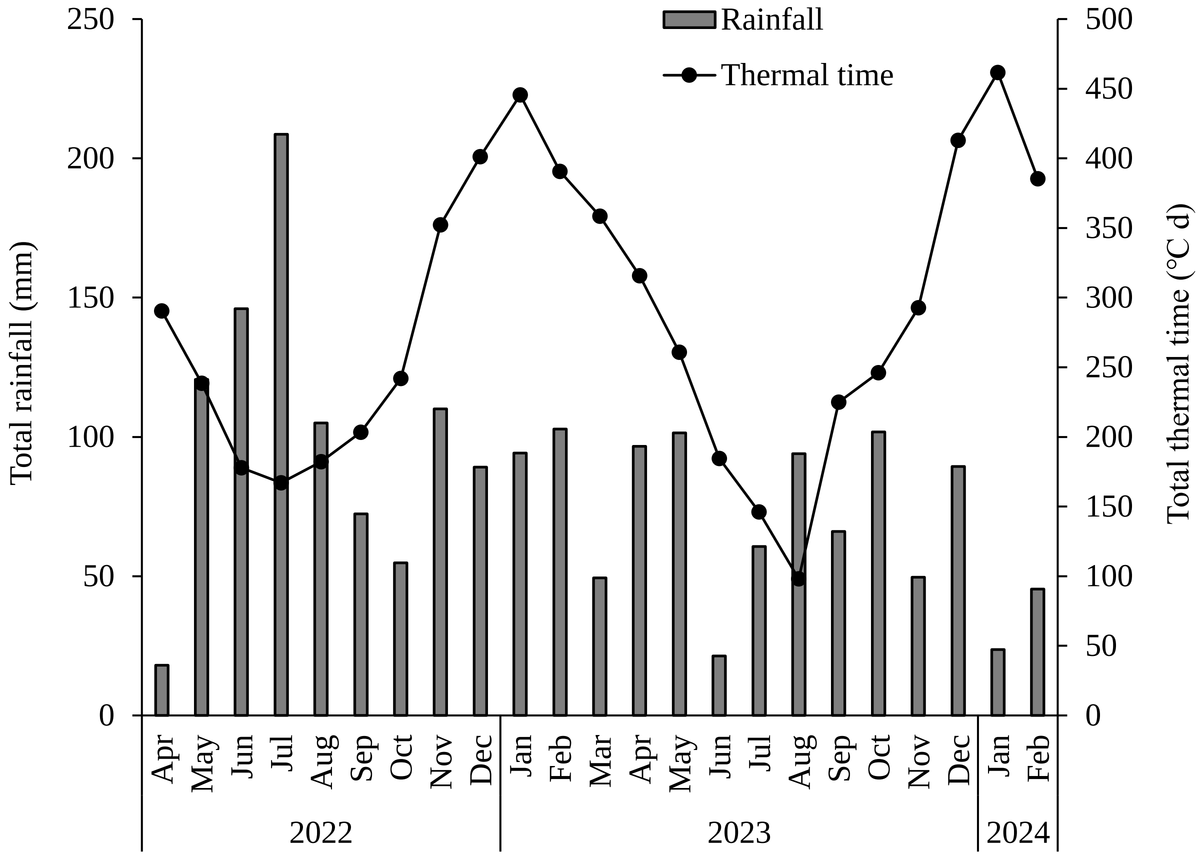


Figure 7-1. Total monthly rainfall (mm) and thermal time (°C d) at Palmerston North, between April 2022 and February 2024. The thermal time base temperature was set at 5°C.

7.3.2 Experimental layout and treatments

The experiment consisted of two treading treatments, damaged (DD) and undamaged (ND) with four replicates of each treatment, in a randomised complete block design. Each plot was 182 m^2. The soil at the site is a Manawatu fine sandy loam which is described as a well-drained, fluvial recent soil with a high soil water holding capacity and high structural vulnerability (Landcare-Research 2019). Dairy cows were used to implement treading damage, which was based on a difference in soil volumetric water content (VWC) between treatments at the time of grazing. Procedures were approved by the Massey University Animal Ethics Committee (AEC 22/34). Based on previous studies, it was decided that grazing at a soil water deficit of 5 - 10 mm, or 37% - 33% soil VWC, would ensure that ND plots were not damaged by the cows.

On 17th August 2022, ND plots were grazed with 6 pregnant dairy cows (representing a stocking density of 330 cows/ha). In the 48 hours following this grazing there was 25 mm of rainfall, which increased soil VWC sufficiently and so DD plots were grazed (and damaged) on August 19th with the same cows.

Subsequent grazings on both treatments were carried out with lactating dairy cows for the remainder of the production year, and once with dry cows during winter. All grazing events were carried out with 6 cows per plot. Grazings occurred whenever the estimated mean pasture mass across the site reached approximately 2800 kg DM/ha, and the target residual was 1500 kg DM/ha. However, soil conditions were closely monitored throughout the year, and plots were only grazed when soil VWC was less than 37% to ensure that plots were not damaged further by cows.

On 7th August 2023, ND plots were grazed with 6 lactating dairy cows per plot. On 8th August, 15 mm of irrigation water was applied to the site, which sufficiently increased soil

VWC and so DD plots were grazed and damaged on 9th August with the same cows. Later grazing events in the second year were then carried out as described above, until the last grazing on 21st February, 2024. All grazing events during the experiment lasted an average of 2.5 hours.

7.3.3 Measurements

Soil measurements for treading damage severity included pug mark depth, which was measured with a ruler, and pug mark density, which was determined by counting the number of pug marks within a defined area. Soil VWC was measured with a portable TDR system (Time Domain Reflectometry – HandiTRASE® TDR, Soilmoisture Equipment Corp, USA) in each plot during each treading event. Soil measurements are detailed further in chapter 4. Treading damage was also measured with a visual scoring method developed by Howes (2019). One treading damage score was recorded for each plot which ranged from 1 (no surface disruption) to 5 (intense surface disruption).

Individual PL plants were sampled from each plot at the conclusion of selected regrowth periods throughout the experiment. Approximately 20 individual PL plants per plot were harvested manually with a trowel, between 0700 and 0900 hours, to avoid the diurnal changes in the plant WSC profile that occur during the day. Following each harvest, individual plants were washed manually with cool water, before being placed into a chiller at 5°C until they could be processed. During processing, the plants were separated into two categories: primary and secondary shoots (refer to Figure 2-1). Within each category, shoots were dissected into leaf, petiole, reproductive stems, and dead material for DM measurement. The initial shoot coming from the plant crown (usually having emerged from seed) was defined as the primary shoot, and lateral shoots coming from the crown were defined as secondary shoots. The main plant caudex (thickened organ from which the crown is formed) was included in the primary petiole category. Other morphological features were then recorded, including the number of leaves, shoots, and reproductive stems within each category. Before dissection, the crown width (combined width of all primary and secondary shoots at the base of rosettes on a single plant) and tap-root thickness (thickest part of the tap root, near the soil surface) were also measured with digital callipers. Roots that remained attached to plants following the harvesting process (most fine roots were broken off and lost during digging) were also kept for chemical analysis. Immediately following the dissection of plants, samples were placed into a freezer at -20°C until the samples could be freeze dried, to prevent the loss of WSC.

Plant samples were dried in a commercial freeze drier no later than 30 days after sampling, and later ground through a 1mm sieve for chemical analysis. Petioles from harvested primary PL shoots were analysed for WSC using an 80:20 ethanol: water extraction and colorimetric determination (DuBois et al. 1956) and total N via the Dumas combustion method. Primary petioles were also analysed for starch; however, concentrations were often less than the 0.5% detection limit and so are not reported on in the current study.

Leaf area was measured for harvested leaves with a LI-3100C® (LICOR) leaf area meter, and with the later incorporation of leaf DM, specific leaf area (SLA) was determined using the following equation: SLA= leaf area/ leaf DM. Due to a processing error, primary leaf DM for winter and early and late spring in year two was not able to be measured directly. During these seasons primary leaf DM was estimated by dividing the leaf area which was measured, by the mean SLA of primary leaves measured during the corresponding season in year one. Mean primary leaf SLA from early spring in year one was used to estimate both winter and early spring leaf DM in year two.

Leaf elongation rate (LER) was determined for five pre-selected PL plants, located randomly within fixed quadrats in each plot. Following each grazing event, the two youngest leaves on each plant were marked with wire hoops and measured during the subsequent regrowth at 2-

to 4-day intervals with a ruler. LER was determined as the difference in leaf length between two measurements, divided by the number of days between measurements.

Leaf photosynthetic parameters were measured with a LI-6800® (LICOR) portable photosynthesis system on several occasions following treading damage. The photosynthesis rate (μmol $m^{-2}s^{-1}$) and operating efficiency of photosystem two (ΦPSII) were measured on the youngest leaf that was big enough to fill the LICOR head window ($6cm^2$) on five PL plants per plot.

The density of PL growing points was measured by counting the number of shoots in four fixed $0.1m^2$ quadrats per plot.

7.3.4 Statistical analysis

Statistical analyses were conducted using the MIXED procedure in SAS (version 9.4, SAS institute 2018). Pair-wised t-tests were used for mean comparisons and significance was declared at $P<0.05$. All results were analysed within each measurement date with treatment (TMT) as the fixed effect. All data were also analysed for repeated measures. Soil VWC, pug depth, pug density and treading damage visual score were analysed with TMT, year (YR) and TMT×YR as fixed effects. Photosynthesis rate and ΦPSII were analysed with TMT, measurement date (DATE) and TMT×DATE as fixed effects. LER was analysed with TMT, regrowth (RG) and TMT×RG as fixed effects. All other measurements of PL growth and density were analysed with TMT, climatic season (SN) and TMT×SN as fixed effects. Non-significant interactions between fixed effects have not been reported in the results.

7.4 Results

7.4.1 Treading damage

During the treading events in both years, soil VWC was significantly higher ($P>0.01$) on the day that DD plots were treaded than when the ND plots were treaded two days earlier (Table 7-1). The mean pug depth ($P<0.01$), pug density ($P<0.01$) and visual damage score ($P<0.01$) were significantly greater in DD plots than ND plots following the treading treatments in both years.

There was no significant difference between the mean soil VWC, pug depth, pug density, nor visual score between years one and two, regardless of treading treatment. The soil VWC in ND plots at the time of treading tended to be higher in year two than in year one ($P=0.06$).

Table 7-1. Mean soil volumetric water content (VWC) during treading by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2) and the visual damage assessment, and pug depth and pug density measured following each treading event in a plantain + perennial ryegrass pasture.

Year	**Treatment**	**Soil VWC (%)**	**Pug depth (mm)**	**Pug density (pugs/m^2)**	**Visual damage score**
1	Damaged	43	34	46	3.0
	Undamaged	35	13	37	1.1
2	Damaged	42	31	48	3.0
	Undamaged	38	18	39	1.1
SEM		0.5	1.8	2.2	0.2
ANOVA p-values					
Treatment		<0.01	<0.01	0.047	<0.01
Year		0.246	0.602	0.202	1.000
Treatment×Year		0.061	0.128	1.000	1.000

SEM = standard error of the mean

7.4.2 Plantain dry matter growth

7.4.2.1 Total above-ground plant yield and yield of plant components

Prior to the implementation of treading treatments in year 1, total plant DM as well as the DM yield of all plant components were similar between the treatments (Figure 7-2). Treading treatment did not have any significant effect on total plant DM or the DM yield of any plant component during any climatic season. However, there was a trend for lower total above-ground DM in DD plants than ND plants during early summer (P=0.095) and late summer in year one (P=0.06), and early summer in year two (P=0.08). There was also a trend for lower primary petiole DM for DD plants than ND plants in early summer in year one (P=0.09). When averaged over the entire experiment, neither total above-ground plant DM nor the DM yield of any plant component was significantly affected by treading treatment.

Climatic season had a significant effect on total above-ground plant DM and the yield of plant components within PL plants (P<0.01). Mean primary leaf DM decreased by 34% between the pre-experiment and early spring measurements in year one and then gradually increased until late summer, before remaining steady until early spring in year two. Mean primary leaf DM decreased by 23% during late spring and decreased further by 42% during early summer, before increasing during late summer. Mean primary leaf DM was greater in late summer in year one than in all other seasons except for autumn, winter and early spring in year two. Mean secondary leaf DM decreased between the pre-experiment and early spring measurements in year one by 75%, and then gradually increased until winter. Between winter and early spring in year two, mean secondary leaf DM increased by 68%, before decreasing in late spring and then remaining constant into late summer. Mean secondary leaf DM was greater in early spring in year two than in all other seasons except for pre-experiment and late summer in year two.

Between the pre-experiment and early spring measurements in year one, primary petiole DM decreased by 44%, before remaining steady until late summer. Between late summer and autumn, primary petiole DM increased by 92% and then remained steady into winter. During early spring in year two, mean primary petiole DM increased by 25% and then remained constant into late spring, before decreasing in early summer and then increasing again during late summer. Mean primary petiole DM was greater during early spring in year two than in all other climatic seasons except for late spring and late summer in year two. Mean secondary petiole DM decreased between the pre-experiment and early spring measurements in year one and then gradually increased throughout year one until winter. Between winter and early spring in year two, mean secondary petiole DM increased by 83% and then remained steady until late summer. Mean secondary petiole DM was greater in early and late spring in year two, than in early and late spring in year one.

There was no primary stem DM prior to late spring in year one. Mean primary stem DM increased between late spring and early summer and then remained steady during late summer. There was no primary stem DM during autumn and winter. Primary stem DM appeared during early spring in year two and increased during late spring, before increasing further during early summer. Mean primary stem DM was similar between early and late summer in year two. Mean secondary stem DM followed a similar pattern to primary stem DM, although it decreased significantly between early and late summer in year two. Mean primary and secondary stem yield was greatest during early and late summer in both years, although primary stem yield was greater during late summer in year two than in late summer in year one.

Mean primary dead DM increased gradually during both years of the study and was greatest during autumn in year one and late summer in year two. Mean secondary dead DM followed a similar pattern to primary dead DM, gradually increasing throughout both years of the experiment.

Mean total above-ground plant DM decreased by 51% between the pre-experiment and early spring measurements in year one, then increased gradually to early summer. Total above-ground plant DM increased by 29% between early and late summer and then remained steady until winter. During early spring in year two, total above-ground plant DM increased by 25%, before decreasing in late spring. Total above-ground plant DM was similar between late spring and early summer, before increasing during late summer. Total above-ground plant DM was greatest during early spring and late summer in year two and lowest during early spring in year one.

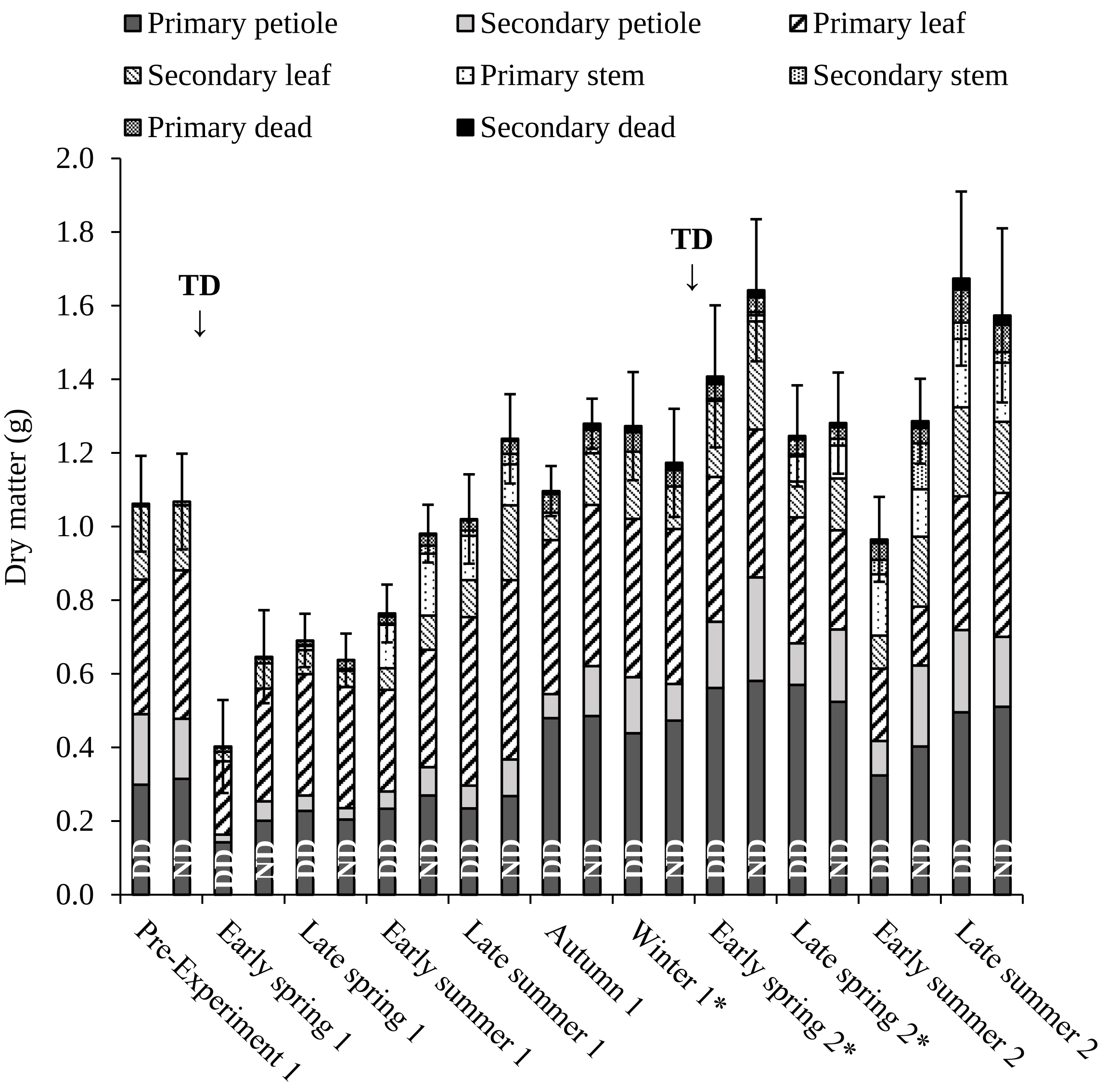


Figure 7-2. Mean total above-ground dry matter (g) (whole bars) and mean primary and secondary leaf, petiole, reproductive stem, and dead dry matter components (g) for plantain plants within in a plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (DD) or no treading damage (ND) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean for total above-ground plant dry matter. *Primary and secondary leaf dry matter for winter, and early and late spring in year two were calculated by incorporating leaf area from those harvests, with specific leaf area from the corresponding seasons in year one.

7.4.2.2 Shoot dry matter yield

Prior to the experiment, total shoot DM (mean of all shoots including both primary and secondary shoots) was similar between the treading treatments (Figure 7-3). Treading damage reduced shoot DM by 44% during early spring in year one ($P<0.05$) but did not have any significant effect on shoot DM during any other climatic season. On average, over the entirety of the experiment, PL shoot DM was not significantly different between DD and ND plots.

The mean shoot DM across the treatments differed significantly between climatic seasons ($P<0.01$). Mean shoot DM decreased by 54% between the pre-experiment and early spring measurements in year one. The mean shoot DM then increased between early and late spring, and then was constant into early summer. The mean shoot DM increased by 22% in late summer and then remained constant until late spring in year two. The mean shoot DM tended to decrease in early summer ($P=0.05$) and then remained constant into late summer. The mean shoot DM was greater at the pre-experiment measurement than during any climatic season except for late summer in year one, or between winter and late spring in year two. The mean shoot DM was lowest during early spring in year one.

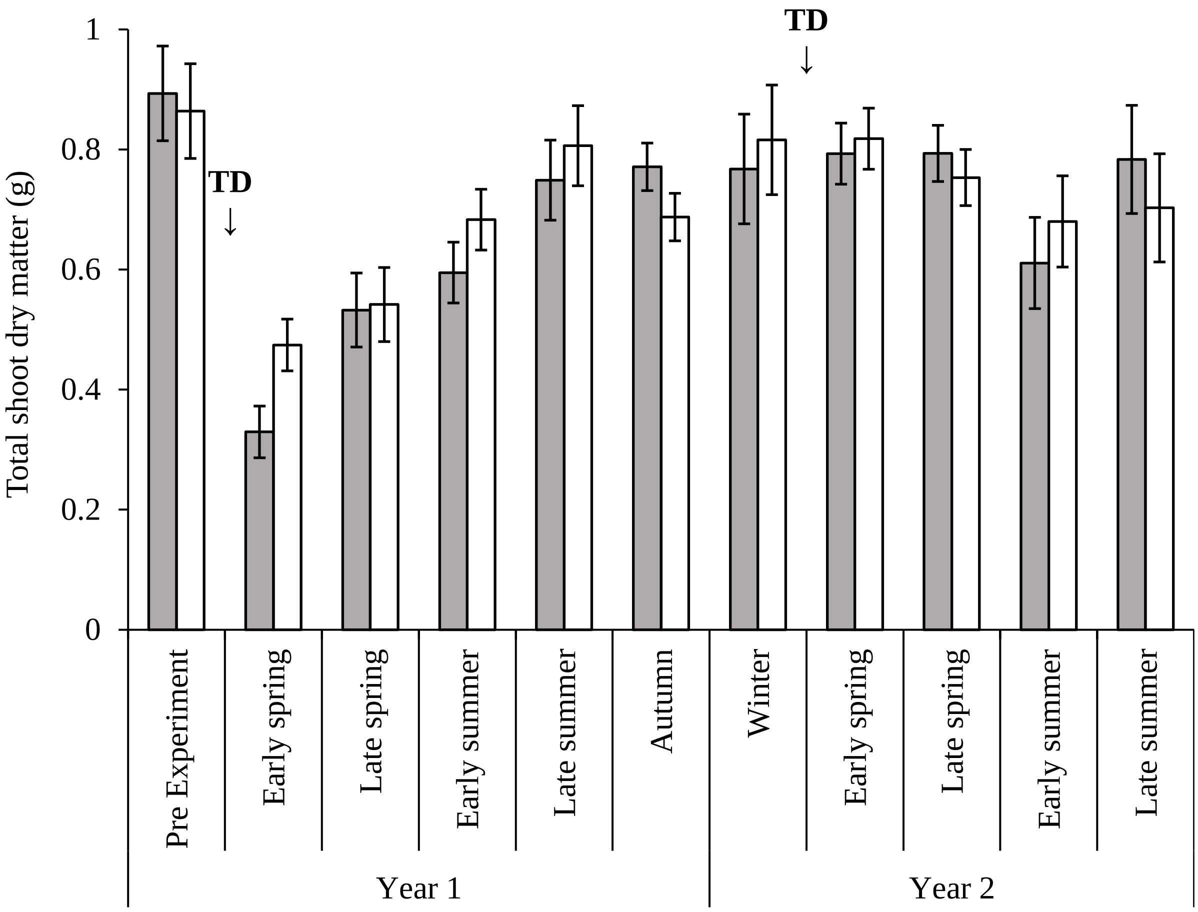


Figure 7-3. Mean shoot dry matter yield (g) for plantain shoots within in a plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (grey bars) or no treading damage (white bars) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean.

7.4.2.3 Leaf elongation rate

Leaf elongation rate (LER) was 41% lower on average for PL plants in DD plots than for PL plants in ND plots during the first regrowth (early spring) following the treading event in year one ($P<0.01$) (Figure 7-4). The LER was 24% lower on average in DD plants than in ND plants during the second regrowth (late spring) following the treading event ($P<0.01$). There was a trend for reduced LER in DD plants in comparison with ND plants during the third regrowth (also late spring) following the treading event in year one ($P=0.07$). In the first two regrowth periods (early spring and late spring) following the treading event in year two, there was no significant difference in the LER of DD and ND plants. When averaged over the early and late spring periods of both years, there was a trend for a lower LER in PL plants in DD plots than in ND plots ($P=0.06$).

The mean LER of PL plants different significantly between regrowth periods, regardless of treading treatment ($P<0.01$). The mean LER of PL plants increased by 56% between the first and second regrowth periods following treading damage in year one. There was a trend for an increase in mean LER between the second and third regrowth periods ($P=0.07$). In year two, the mean LER of PL plants was greater in the second regrowth period following the treading event, than during the first regrowth period immediately following the treading event. The mean LER was lower during the first regrowth period in year two, than during any other regrowth period, and was 41% lower than during the first regrowth period in year one.

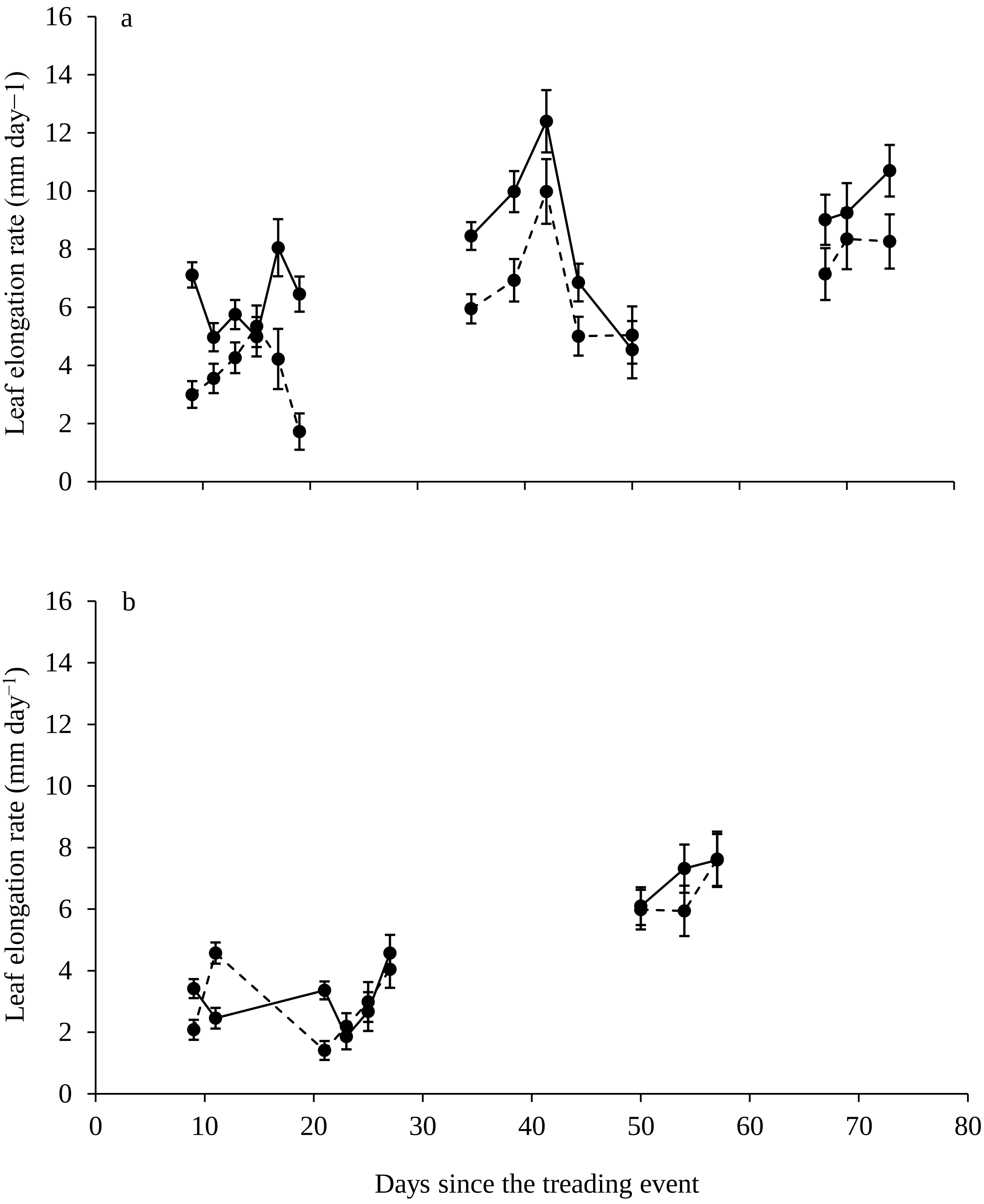


Figure 7-4. Mean leaf elongation rate (mm day^{-1}) of plantain plants within in a plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (dashed line) or no treading damage (solid line) by dairy cows in early spring 2022 (year 1 - a) and early spring 2023 (year 2 - b). Vertical bars show the standard error of the mean.

7.4.2.4 Leaf area per plant and specific leaf area

There was no significant effect of treading treatment on the primary or secondary leaf area per plant during early and late spring in year one, but there was a trend for lower primary leaf area per plant in DD plots than in ND plots during early summer (P=0.06) (Figure 7-5). The primary and secondary leaf area per plant was similar between treatments during all seasons except for late spring in year two, where plants in DD plots had a significantly higher primary leaf area than plants in ND plots (P<0.01). When averaged over the entirety of the experiment, primary and secondary leaf area per plant were not significantly affected by treading treatment. The mean primary and secondary leaf area of plants differed significantly between climatic seasons, regardless of treading treatment (P<0.01). Mean primary leaf area was greatest in late summer and autumn in year one, and lowest in early spring and early summer in year one, and in early summer in year two. The mean secondary leaf area of plants peaked in late summer in year one.

There was no significant effect of treading treatment on the SLA of primary or secondary shoots during any climatic season in the experiment. However, there was trend for lower SLA on primary shoots in DD plots than in ND plots during early spring in year one (P=0.06) and during early summer in year two (P=0.06). When averaged over the entirety of the experiment, the SLA of primary and secondary shoots was not significantly affected by treading treatment. The mean SLA of primary and secondary shoots differed significantly between climatic seasons, regardless of treading treatment (P<0.01). Mean primary SLA was greatest in late summer and autumn in year one, and in early summer in year two. Mean primary SLA was lowest in late summer in year two. Following a significant increase between early and late spring in year one, the mean SLA of secondary shoots followed a similar seasonal pattern to mean SLA of primary shoots. Mean secondary SLA was lowest in early spring in year one.

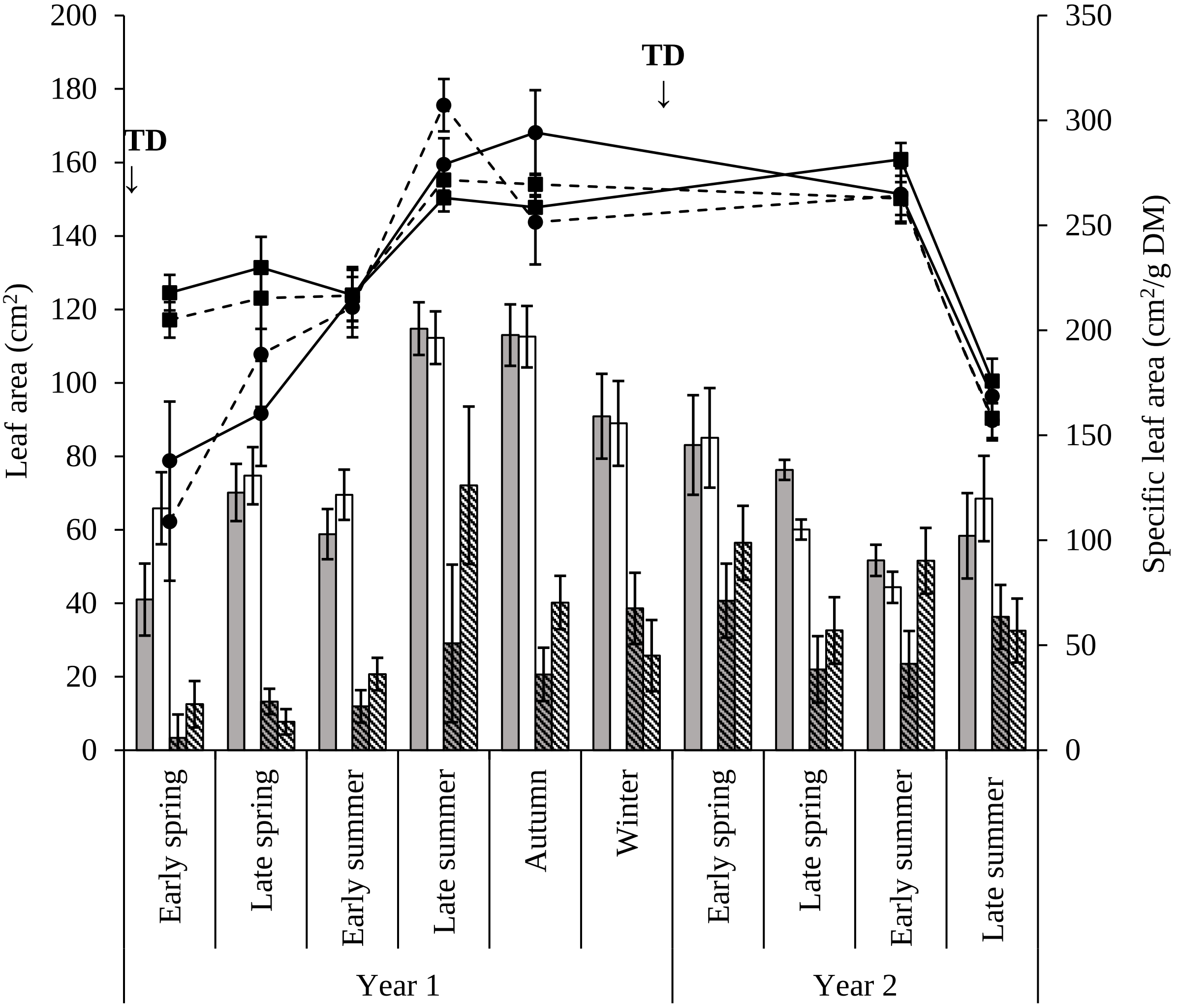


Figure 7-5. Mean primary (solid bars and squares) and secondary (striped bars and circles) leaf area (cm^2 - bars) and specific leaf area (cm^2/g DM - lines) per plantain plant within in a plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (grey bars and dashed lines) or no treading damage (white bars and solid lines) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean.

7.4.2.5 Photosynthesis rate and operating efficiency of Photosystem II

Photosynthesis rate was 33% lower in PL leaves in DD plots than in PL leaves in ND plots 10 days after the treading event in year one (P<0.01) (Figure 7-6). There was no difference in photosynthesis rate between PL plants in DD plots and ND plants 20 days after the treading event, nor during the second regrowth period following the treading event. When averaged over all measurements during the first and second regrowth period following the treading event in year one, the photosynthesis rate was significantly lower in PL leaves in DD plots, than in PL leaves in ND plots (P<0.05).

The mean photosynthesis rate differed significantly across measurement dates with the first and second regrowth period following the treading event, regardless of treading treatment (P<0.01). The mean photosynthesis rate of PL leaves peaked at 14 days after grazing during the second regrowth period following the treading event and was lowest 10 days after the treading event. There was also a significant interaction between treading treatment and climatic season on photosynthesis rate (P<0.01). The photosynthesis rate of PL leaves in DD plots was lowest at the 10 days after treading measurement and similar between the other measurements. The photosynthesis rate of PL leaves in ND plots was lower at the 20 days after treading measurement in regrowth one than at all other measurement dates except for the 21 days after grazing measurement in regrowth two.

Following the treading event in year one, PL leaves in DD plots had a significantly lower ΦPSII than PL leaves in ND plots at the 10 days after treading measurement (P<0.05). At the 20 days after treading measurement during the first regrowth period following the treading event, PL leaves in DD plots had a greater ΦPSII than PL leaves in ND plots. Treading treatment did not have a significant effect on ΦPSII at either measurement during the second regrowth period following the treading event. When averaged over all measurements during the first and second regrowth period following the treading event, treading treatment had no significant effect on ΦPSII.

Mean ΦPSII differed significantly across measurement dates within the first and second regrowth period following the treading event, regardless of treading treatment ($P<0.01$). Mean ΦPSII was greatest at the 14 days after grazing measurement during the second regrowth period following the treading event and was lowest 10 days after the treading event. There was also an effect of a significant interaction between treading treatment and climatic season on ΦPSII ($P<0.01$). The mean ΦPSII of DD leaves was greatest at the 14 days after grazing measurement in regrowth two and lowest at the 10 days after treading measurement in regrowth one. The mean ΦPSII of ND leaves was greater during the measurements in regrowth two than during the measurements in regrowth one.

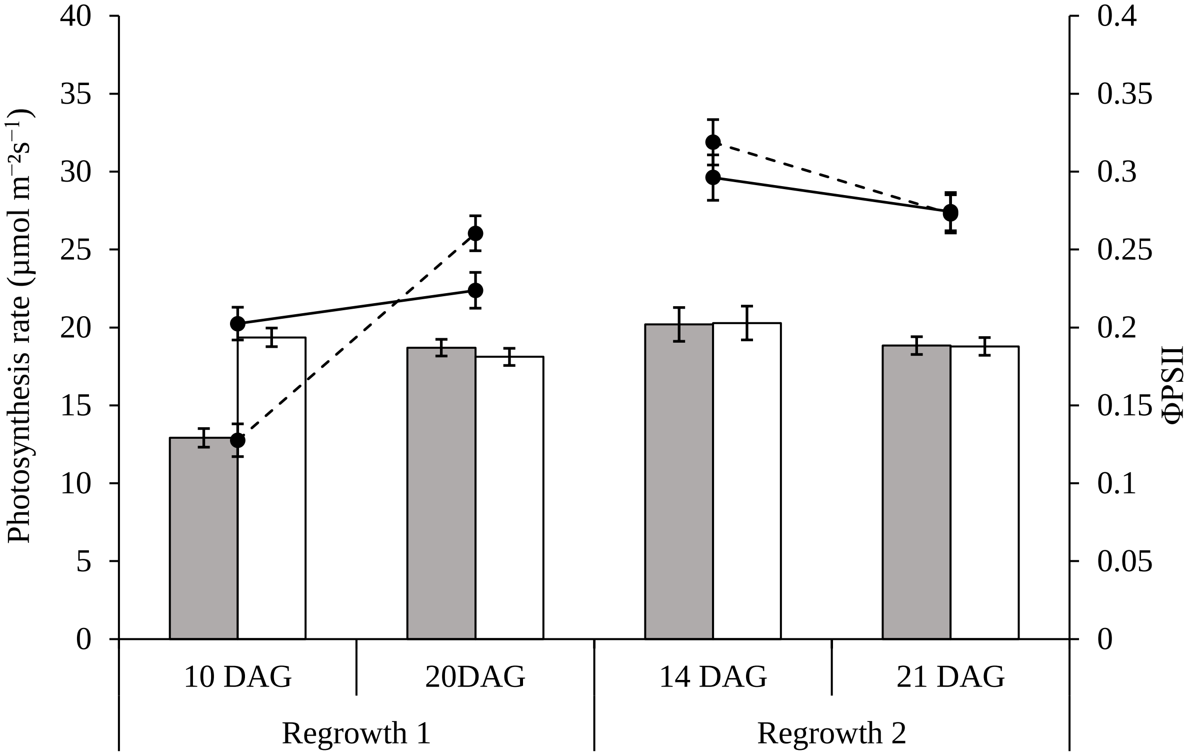


Figure 7-6. Photosynthesis rate (µmol $m^{-2}s^{-1}$ - bars) and operating efficiency of photosystem two (ΦPSII - lines) of plantain leaves within in a plantain + perennial ryegrass pasture during the first and second regrowth periods following treading damage (grey bars and dashed lines) or no treading damage (white bars and solid lines) by dairy cows in early spring 2022. Pasture was sown in autumn 2022. DAG = Days after grazing. Vertical bars show the standard error of the mean.

7.4.2.6 Number of leaves per shoot

Treading treatment had no significant effect on the number of leaves on primary or secondary PL shoots during the experiment. The number of leaves on primary shoots (L/PS) and secondary shoots (L/SS) varied significantly between climatic seasons ($P<0.01$) (Figure 7-7). The L/PS decreased from 5 to 3.7 leaves between the pre-experiment measurement and early spring in year one. Primary shoots had a greater number of leaves during autumn (7 leaves) than during any other season except for late summer in both years. Primary shoots had the lowest number of leaves between early spring and early summer in year one (4 leaves). Prior to the experiment, secondary shoots had 3 leaves on average. There was no significant change in mean L/SS between the pre-experiment measurement and early spring. The mean L/SS was greatest during late summer in year one (5 leaves) and lowest during early spring in year one.

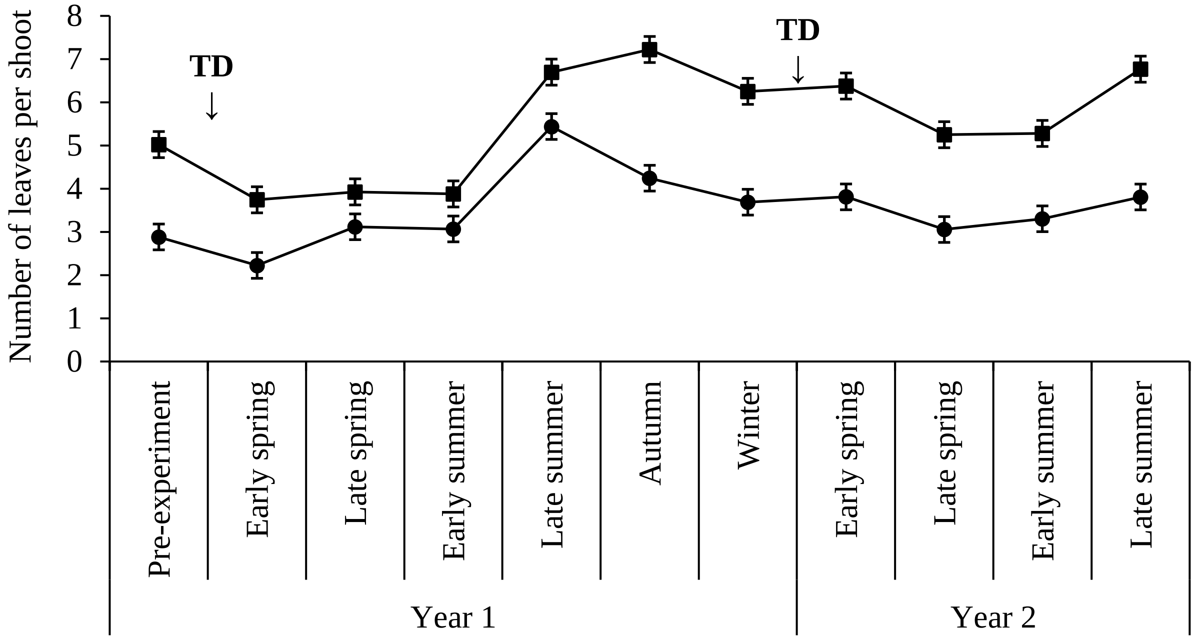


Figure 7-7. Number of leaves on primary (squares) and secondary (circles) shoots of plantain plants within in a plantain + perennial ryegrass dairy pasture over the 2022-2023 (year 1) and 2023-2024 (year 2) production years. Pasture sown in autumn 2022. TD = Treading damage event. Vertical bars show the standard error of the mean.

7.4.2.7 Crown and taproot diameter

There was no significant difference between the crown diameter (CD) of PL plants in DD and that of PL plants in ND plots during any climatic season (Figure 7-8). However, there was a trend for a lower CD on plants in DD plots than plants in ND plots during late summer in year one (P=0.07). When averaged over the entirety of the experiment, the CD of PL was not significantly affected by treading treatment.

The mean CD of PL plants differed significantly between climatic seasons, regardless of treading treatment (P<0.01). Mean CD was greater between early spring and early summer in year two, than at any time prior to winter in year one, and was smaller prior to the experiment than during any other season than early or late spring in year one.

Following the first regrowth period after the treading event in year one, there was a trend for a lower taproot diameter (TPD) for PL plants in DD plots than for PL plants in ND plots (P=0.08). Following the first regrowth period after the treading event in year two, PL plants in DD plots had a TPD than was on average 15% lower than that of PL plants in ND plots (P<0.05). Treading treatment had no significant effect on PL TPD during any other season in the experiment. When averaged over the entirety of the experiment, the TPD of PL was not significantly affected by treading treatment.

The mean TPD of PL plants differed significantly between climatic seasons, regardless of treading treatment (P<0.01). Mean TPD was greater during early summer in year two than in all other seasons except for early summer and autumn in year one. Mean TPD was lowest prior to the start of the experiment.

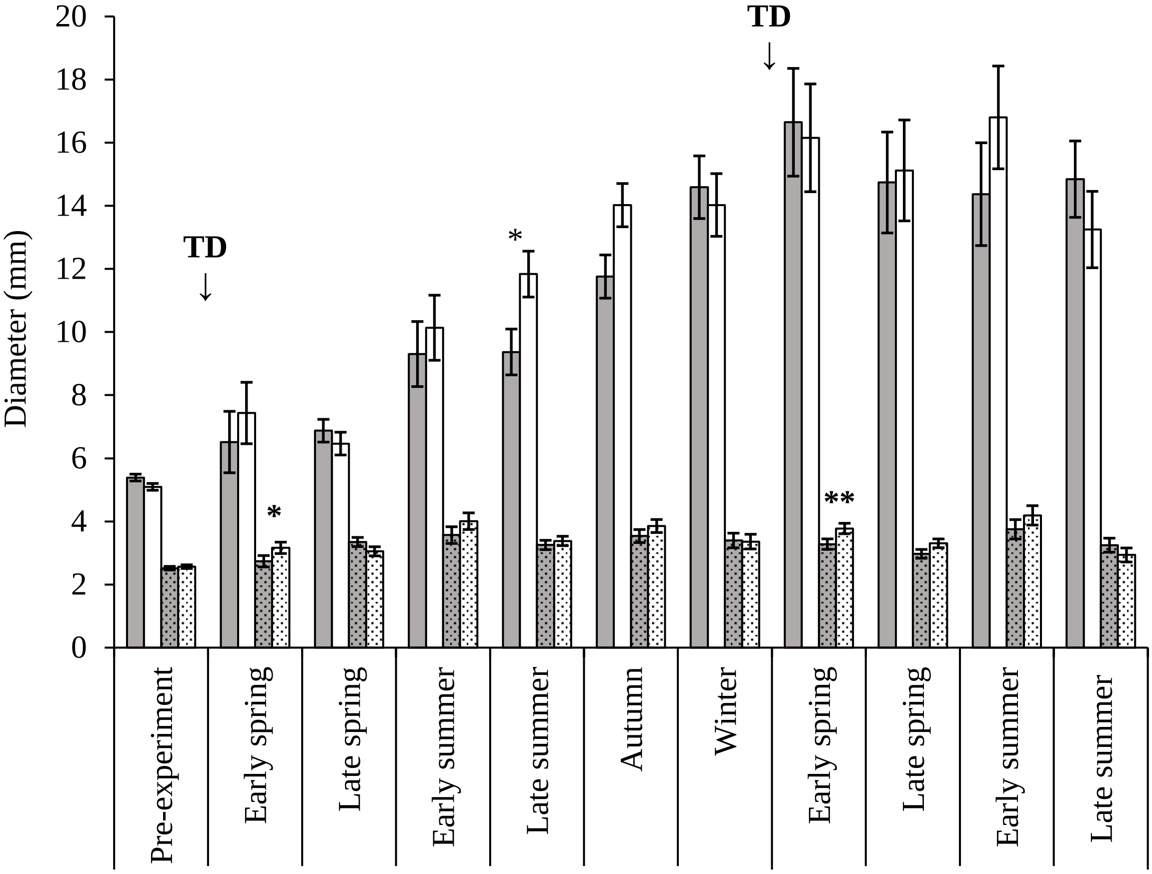


Figure 7-8. Crown (solid bars) and taproot (dotted bars) diameter (mm) of plantain plants within in a plantain + perennial ryegrass pasture sown in autumn 2022 following treading damage (grey bars) or no treading damage (white bars) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean. Asterisk's show significant treatment × time interactions. One asterisk denotes a trend (P<0.1) while two asterisks denotes a significant difference (P<0.05).

7.4.2.8 Water soluble carbohydrate and nitrogenous reserves

Treading treatment had no significant effect on the total WSC reserves or WSC concentration in PL petiole DM during any season in the experiment (Figure 7-9). However, during early spring (P=0.097) and early summer (P=0.09) in year one, there was a trend for lower total WSC reserves in DD petioles than in ND petioles. When averaged over the entirety of the experiment, neither total WSC reserves nor WSC concentration within PL petiole DM were significantly affected by treading treatment.

The mean total WSC reserves and WSC concentration in the petiole DM of PL plants differed significantly between climatic seasons, regardless of treading treatment (P<0.01). The mean total petiole WSC decreased by 46% between the pre-experiment measurement and early spring but was constant between early spring and late summer. Between late summer and autumn, total petiole WSC increased by 114%, but did not change between autumn and winter. Mean total petiole WSC increased during early spring in year two, before decreasing in late spring. Mean total petiole WSC was greatest during early spring in year two and lowest between early spring and early summer in year one. The mean petiole WSC concentration was similar between the pre-experiment measurement and late spring, before decreasing by 30% in early summer. Mean petiole WSC concentration was constant between winter and early spring in year two, before it decreased by 26% in late spring. Mean petiole WSC concentration was greater prior to the experiment than during all other seasons except for between early and late spring, and autumn in year one. Mean petiole WSC concentration was lowest in early summer in year one and in late spring in year two.

Treading treatment had no significant effect on the total N reserves or N concentration in PL petiole DM during any season in the experiment (Figure 7-10). Although there was a trend for lower total N reserves in the petiole DM of DD plants than in the petiole DM of ND plants during winter in year one (P=0.08). When averaged over the entirety of the experiment, neither total N reserves nor N concentration within PL petiole DM were significantly affected by treading treatment.

The mean total N reserves and N concentration in the petiole DM of PL plants differed significantly between climatic seasons, regardless of treading treatment ($P<0.01$). Mean total petiole N decreased by 56% between the pre-experiment measurement and early spring in year one. The mean petiole N was similar in winter and early spring in year two, before it decreased in late spring. Mean total petiole N was greater in early spring in year two than in all other seasons except for in winter. Mean total petiole N was lower during early spring in year one than all other seasons except for late spring and early and late summer in year one. Between the pre-experiment measurement and early spring in year one, the mean petiole N concentration decreased by 21%. The mean petiole N concentration peaked in winter in year one, then decreased during both early and late spring in year two. Mean petiole N concentration was greatest during winter in year one and was lower during early spring in year one than in all other seasons except for early summer in year one.

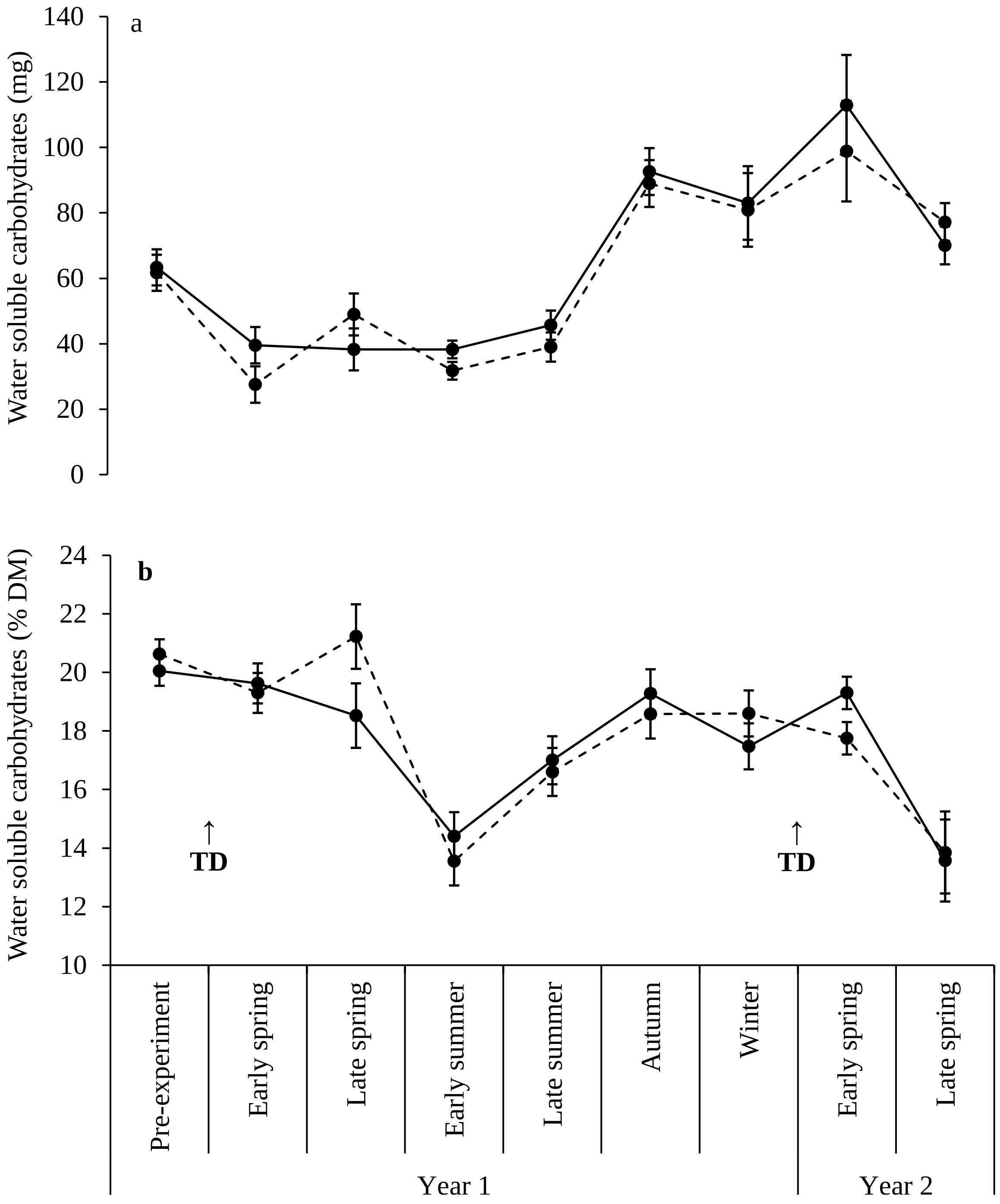


Figure 7-9. Mean total primary petiole water-soluble carbohydrate content (mg/plant - a) and primary petiole water-soluble carbohydrate concentration (% of dry matter - b) of plantain plants within in a plantain + perennial ryegrass pasture sown in autumn 2022 following treading damage (dashed lines) or no treading damage (solid lines) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean.

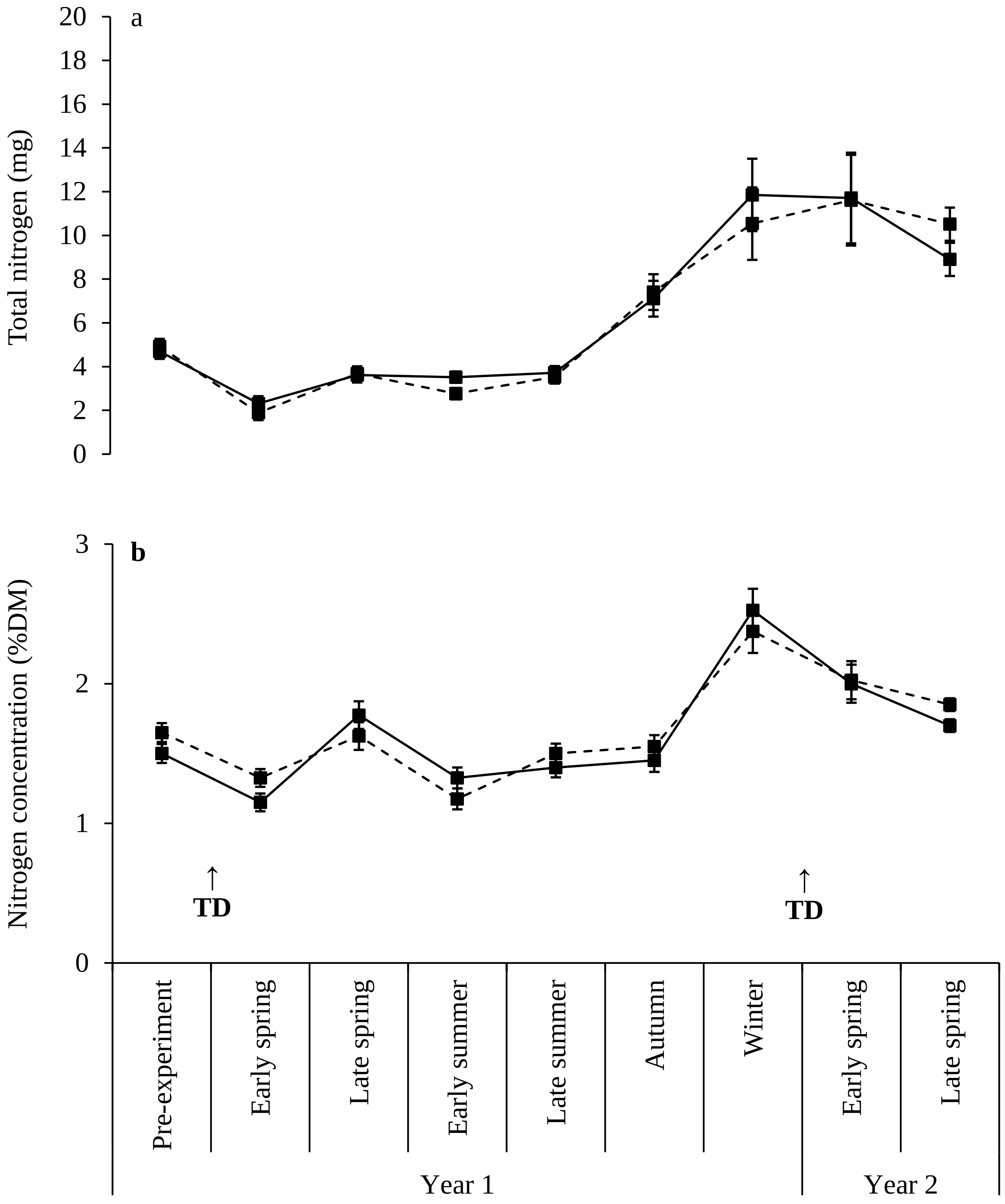
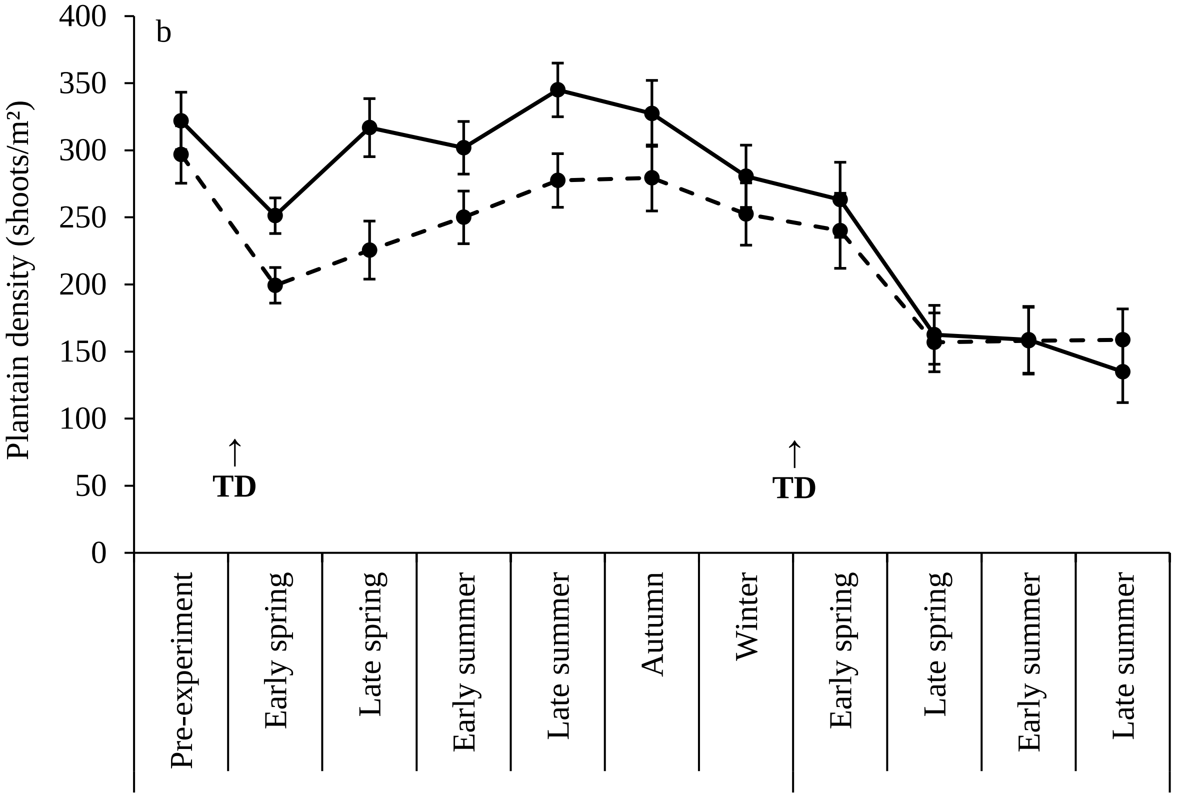


Figure 7-10. Mean total primary petiole nitrogen content (mg/plant - a) and primary petiole nitrogen concentration (% of dry matter - b) of plantain plants within in a plantain + perennial ryegrass pasture sown in autumn 2022 following treading damage (dashed lines) or no treading damage (solid lines) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean.

7.4.3 Plantain shoot density

7.4.3.1 Number of shoots per plant

There was no significant difference in the number of shoots/plant between PL plants in DD or ND plots during any season except for during early summer in year one, when plants in DD plots had fewer shoots than plants in ND plots (P<0.05) (Figure 7-11). Additionally, during autumn, there was a trend for a lower number of shoots/plant for PL plants in DD plots, than for plants in ND plots (P=0.097). When averaged over the entirety of the experiment, the number of shoots/plant was not significantly affected by treading treatment.



The mean number of shoots/plant differed significantly between climatic seasons, regardless of treading treatment (P<0.01). The number shoots/plant increased gradually throughout year one and was 30% greater in winter, than at the pre-experiment measurement. Between winter and early spring in year two, the number of shoots/plant increased by 22%, before it tended to decrease during late spring (P=0.05). The number of shoots/plant was similar between late spring and early summer, then it increased by 24% during late summer. Over the course of the experiment, the mean number of shoots/plant increased from 1.2 to 2.2 (an 83% increase).

7.4.3.2 Shoot density

In year one, the PL shoot density in DD plots was 21% and 29% lower than in ND plots during early and late spring, respectively ($P<0.05$) (Figure 7-11). During late summer, PL shoot density was 20% lower in DD plots than in ND plots, but from autumn until late summer in year two, PL shoot density was similar between treatments. When averaged over the whole experimental period, PL shoot density was not significantly different between treading treatments.

The mean PL shoot density differed significantly between climatic seasons, regardless of treading treatment ($P<0.01$). Following the first grazing (and treading) in August 2022, mean PL shoot density across the site decreased from 309 to 225 shoots/m^2 ($P<0.01$). The mean PL shoot density then increased gradually over year one, to reach 311 shoots/m^2 in autumn. Mean PL shoot density decreased from autumn into winter ($P<0.05$). In year two, following the treading event, PL shoot density remained steady across the site and was 252 shoots/m^2 in early spring, before decreasing by 37% between early and late spring ($P<0.01$) to 160 PL shoots/m^2, and then remained constant into late summer. The mean PL shoot density decreased by 53% on average over the experiment ($P<0.01$).

There was also an effect of a significant interaction between treading treatment and climatic season on PL shoot density ($P<0.05$). The mean shoot density of DD plots decreased by 33% between the pre-experiment measurement and early spring. The shoot density of DD plots then increased gradually to late summer before tending to decrease gradually to early spring in year two ($P=0.06$). During late spring in year two, the PL shoot density of DD plots decreased by 35% and then remained constant until late summer. There was no difference in the shoot density of DD plots between the pre-experiment measurement and the measurements in late summer and autumn in year one. The PL shoot density in DD plots was lowest between late spring and late summer in year two. The mean shoot density of ND plots decreased by 22% between the pre-experiment measurement and early spring in year one. Between early and late spring, the shoot density of ND plots increased by 26%, but was constant into early summer. During late summer, the mean shoot density of ND plots increased further and then remained steady into autumn. Between autumn and winter, the shoot density of ND plots decreased by 14% before remaining steady into early spring in year two. The shoot density of ND plots then decreased by 38% between early and late spring and then was constant from late spring to late summer. There was no difference in the shoot density of ND plots between the pre-experiment measurement, and all of the measurements between late spring and autumn in year one. The shoot density of ND plots was lowest between late spring and late summer in year two.

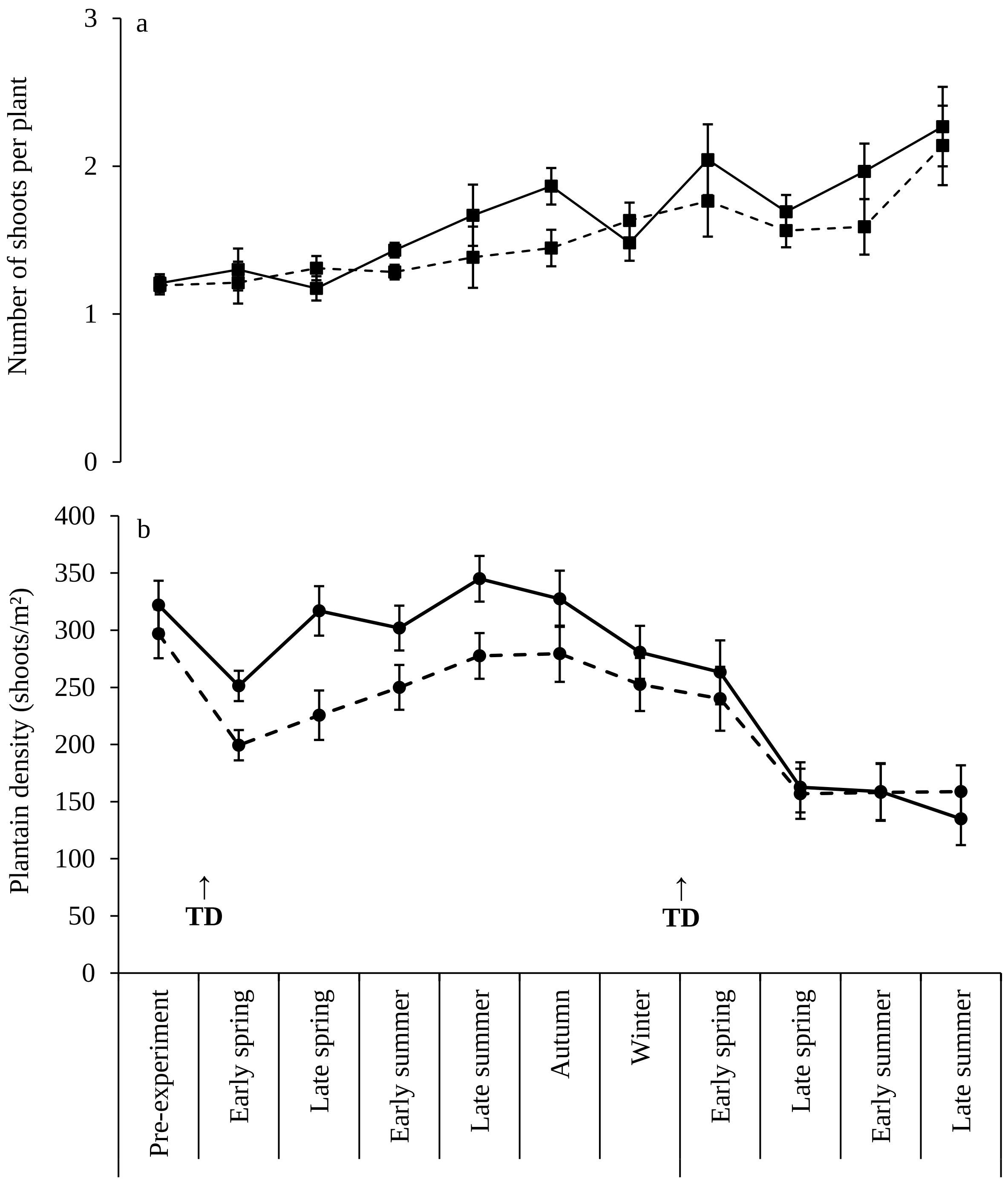


Figure 7-11. Number of shoots per plantain plant (a) and density (number/m^2) of plantain shoots within a plantain + perennial ryegrass pasture sown in autumn 2022, following treading damage (dashed lines) or no treading damage (solid lines) by dairy cows in early spring 2022 (year 1) and early spring 2023 (year 2). TD = Treading damage event. Vertical bars show the standard error of the mean.

7.5 Discussion

7.5.1 Treading damage

During both years, mean pug depth increased with an increase in soil VWC at the time of grazing (Climo & Richardson 1984), and led to the significantly greater visual damage scores recorded for DD plots than ND plots after each treading event. The mean pug depths observed in DD plots across the two years were comparable to those previously observed for cattle treading damage on a silt loam soil (Betteridge et al. 2003) and marginally shallower than those observed on a clay loam soil (Nie et al. 2001). Although pug density was consistently higher in DD plots than ND plots, it was hypothesised that this likely resulted from deeper pug marks being more easily identified, rather than an effect of animal behaviour or the treading treatments. The visual damage scoring method consistently showed DD plots to be more severely damaged than ND plots, however the technique may have been even more informative if it was conducted on a number of pseudo-replicates within each plot, for example in each quarter of the plot.

Figure 7-12. Damaged and undamaged plots following the treading event in year two.

7.5.2 Plantain growth in a successively damaged, grazed mixed pasture

The primary effects of treading damage on RG DM yield, at both the individual plant and monoculture level, have been reported on extensively (Brown & Evans 1973; Nie et al. 2001; Betteridge et al. 2003; Howes 2019), with reductions of 30-50% reported during the regrowth period immediately following the treading event. Among other effects, the physical damage to growing points and the covering of leaves with films of mud that cause these reductions in RG growth (Brown & Evans 1973) were also observed for PL plants in DD plots in the current study.

Treading damage by cows caused an immediate reduction in PL shoot DM yield and LER during early spring in year one. Similar observations were made for a related species by Ikeda and Okutomi (1995), who found that the leaf length and plant DM yield of asiatic plantain (*Plantago asiatica* L.) were both reduced under heavy trampling treatments. It is possible that herb species such as PL may be particularly susceptible to treading damage because of their relatively fragile leaf structures (Sun & Liddle 1993). High leaf strength, associated with an elevated cellulose content and greater proportion of cell wall, was found to be one important factor for the improved treading tolerance of RG varieties, as it provides a protective cushion to growing points (Evans 1967). Compared with RG, PL leaves have a lower proportion of cell wall and less cellulose (Stewart 1996; Navarrete et al. 2022). Sun and Liddle (1993) also suggested that erect herbaceous plants with broad leaves might by less tolerant of trampling than grass species with long, narrow leaves, since a greater proportion of leaves were broken in the former under trampling damage. Taken together with results from the current study, it is evident that PL leaves are not as well suited as RG leaves to withstanding the physical pressures exerted by cattle treading. The susceptibility of PL leaves to treading damage is most likely what drove the reduction in LER and shoot DM yield in DD plants during early spring in year one.

One response of PL plants to the treading damage was a reduction in SLA, possibly as a tolerance strategy in anticipation of future treading events. In broadleaf plantain, a reduction in SLA resulting from an increase in cell wall and protein per unit area leaf, was thought to have inferred greater tramping tolerance (Van Dijk 1985). The reduction in the SLA of PL following treading damage could have simultaneously improved the physical resistance of PL leaves (Dijkstra & Lambers 1989) and reduced the probability of them being impacted in future events, through having a smaller surface area. Furthermore, a smaller leaf size could have enhanced the conservation of energy and water following damage to leaves and roots (Xu et al. 2009). One potential disadvantage of a reduction in SLA is a loss of photosynthetic capacity per unit area of leaf, due to internal leaf shading (Terashima & Saeki 1983; Dijkstra & Lambers 1989). However, in contrast with trampled broadleaf plantain (Van Dijk 1985; Dijkstra & Lambers 1989), it was not obvious that a reduction in photosynthesis rate per unit leaf area was related to the lower SLA in damaged PL leaves in the present study, since a suppression of photosynthesis was found only in the initial stages of recovery from damage.

While the notable reduction in SLA didn’t appear to reduce the photosynthetic efficiency of leaves, it occurred concurrently with a reduction in LER. This likely reduced the potential for individual leaves to develop a long and wide lamina for maximum light capture which probably contributed to the reduced shoot DM yield during early spring. The potential reduction in light capture by PL plants in DD plots may also explain the lower taproot diameter of those plants following damage, since additional energy reserves required for shoot recovery may have been drawn from the taproot to compensate for a loss in photosynthate production by leaves. Although the SLA, LER and DM yield of DD shoots recovered as early as late spring, it is possible that a further damage event during the next grazing might have caused a low SLA and LER to be maintained for longer and thus resulted in a low shoot DM yield throughout spring.

The reduction in photosynthesis in damaged PL leaves following the treading event in year one appeared to be short lived, suggesting no permanent impairment to photosynthetic capacity. Mills et al. (1996) observed a 62% reduction in photosynthesis of in trampled PL leaves 24 hours after trampling. Measurement of thylakoid activity in those trampled PL leaves revealed that approximately half of the reduction in photosynthesis was caused by an inhibition of primary photosynthetic apparatus, which includes photosystem II (PSII). The author concluded that inhibition of photosynthesis in PL leaves is complex but may contain a large contribution from increased stomatal or mesophyll resistance (Mills et al. 1996). In the current study, the closure of stomata could have served as a temporary water conservation strategy for PL following damage to the root system during treading, and subsequent restriction in water supply (Wong et al. 1979). Given that ΦPSII in DD leaves had recovered fully 20 days after the treading damage, the reduction in ΦPSII in DD leaves 10 days after the damage was probably caused by a reduction in CO_2 supply suppressing processes downstream of PSII following stomal closure (Baker 2008), as opposed to any permanent damage in PSII. It is possible that more severe treading damage, or the combination of treading damage with other abiotic stresses that prompt stomatal closure, might therefore prolong the effects of treading damage on PL photosynthesis.

From late spring onward in year one, and through the remainder of the experiment, there was no apparent effect of the treading treatments on the above-ground growth of PL plants. During this time, PL plant DM yields appeared to be driven largely by leaf and petiole yields, which were likely underpinned by the number of leaves and leaf area per shoot, and the number of shoots per plant. Between early spring and early summer in year one, and during late spring and early summer in year two, the number of leaves and total leaf area per PL shoot prior to grazing events was relatively low. This may have been a consequence of sub-optimal temperatures reducing the LER of PL (Teramura et al. 1981), or the more frequent defoliations during that period, which were necessary to control RG growth and prevent shading. The number of leaves and leaf area per PL shoot were generally high during late summer in both years and during autumn in year one, probably due to warmer conditions driving a greater LER (Teramura et al. 1981), and less frequent defoliations following a decline in leaf growth rate and a shift to reproductive stem production in RG (Langer 1973). Additionally, the increased openness of the pasture, and greater irradiance during this time, potentially encouraged an increase LER and thus leaf area (Teramura 1983).

The petiole DM of PL plants appeared to be a function of the leaf area on PL shoots during and prior to the climatic season in which it was measured, which was probably a reflection of leaf area driving photosynthate production (McKenzie et al. 1999) and subsequent DM accumulation in petioles. Of note was the large increase in petiole DM between late summer and autumn in year one, whilst both leaf DM and leaf area were constant over that time. That indicates that the proportion of DM allocated to storage increased during autumn and could also suggest that petioles are an important energy store for PL. The accumulation of energy reserves in autumn has been shown to be important for the survival of chicory plants through winter, when leaf growth becomes limited (Li et al. 1997a). While PL is a tap-rooted species (Cranston et al. 2015a), it is not clear from previous experiments that the taproot is the primary storage organ of PL. Previous studies comparing PL and chicory (which possesses a much larger taproot than PL) have shown that while the taproot diameter of chicory was reduced under frequent or hard cutting, PL taproot diameter remained unaffected (Yu et al. 2008; Cranston et al. 2016). These findings agree with the results of the current study which showed that while petiole DM increased following large increases in leaf area and presumably photosynthate production, the diameter of PL taproots remained largely constant. Taken together, these results could suggest that petioles are an important energy storage organ for PL and that the measurement of energy reserves in petiole DM could provide valuable insight into the energy status of PL plants.

Between early and late summer in both years, and during late spring in year two, PL plant DM yield was also boosted by reproductive stems. There appeared to be a trade-off between reproductive stem and petiole for the allocation of DM, which was evidenced by the sharp increase in petiole DM following the cessation of stem production in autumn. In chicory, the removal of primary stems by grazing caused a reduction in stem production from primary shoots, and following the release from apical control, encouraged the growth and development of secondary shoots during summer and autumn (Li 1997). Although strategies for the allocation of photosynthates in chicory are different from that of PL (Cranston et al. 2015a), any increase in the photosynthetic surface area of plants is likely to increase the amount of photosynthate available for growth or storage (McKenzie et al. 1999). If petiole DM is indeed an important energy source for PL growth and survival, the control of reproductive stem production might increase the amount of energy that is allocated to petiole DM for vegetative growth or stress tolerance strategies and therefore improve the growth and survival of the PL population.

7.5.3 Plantain survival in a successively damaged, grazed mixed pasture

The reduction in PL shoot density following the treading damage in year one appeared to be the most obvious and prolonged effect that treading damage had on the PL population. The large reduction in PL shoot density between the pre-experiment measurement and early spring in year one indicates that shoots died as a direct result of the treading damage, probably due to them being physically severed by cow hooves or being buried deep in mud. However, shoot density recovered in DD plots throughout year one and was similar to that of ND plots by autumn, indicating that the population had recovered. The treading damage in year two did not impact PL shoot density to the same extent as it did in year one and appeared to become less important throughout year two. These results could suggest that first-year PL shoot populations may be more susceptible to treading damage but have an enhanced ability for recovery following damage events, and that second-year PL populations are more tolerant of treading damage.

Young PL plants are vulnerable to the effects of livestock grazing during establishment, especially before plants (or shoots given that young PL plants usually comprise of one shoot) attain at least 6 leaves (Powell et al. 2007). Based on this principle, Powell et al. (2007) suggested that PL plants should not be grazed until 725°C.day after sowing, to ensure PL density losses are less than 10%. Although the pasture was sown in early April in the current study, dry conditions (soil water deficit of 101mm) delayed the germination of seedlings until late April, which meant that 725°C.day was achieved one day before the grazing of ND plots. However, given that drier than normal conditions prevailed until mid-May, DM accumulation in PL plants was likely reduced, and plants may not have reached an ideal size or mass at their first grazing. This could explain the contrast in PL plant DM after 725°C.day between plants in the work by Powell et al. (2007) (1.4g) and plants in the current study (1.07g), even with both experiments being conducted at the same site. Since the PL plants in the current experiment had around 8 leaves at the first grazing, the 22% reduction in PL shoot density in ND plots after the initial grazing was higher than that expected, based on the work by Powell et al. (2007). This result may therefore suggest that plant DM is also an important determinant of PL survival following early grazing events. However, it is possible that the grazing activities of cows contributed to the larger loss of PL density observed in the current study, than the previous study which involved sheep. The combined effects of this potentially premature grazing and the physical damage and burial of growing points are probably what led to the 33% reduction in PL shoot density in DD plots after the initial grazing. This result might then also suggest that PL shoot losses in damaged plots could have been significantly reduced if the first grazing, and treading damage event, were delayed.

When comparing PL shoots prior to treading damage between years one and two, first-year PL shoots had a lower petiole DM, a thinner crown and taproot, and less WSC and N reserves in the petiole than second-year PL shoots. Taken together, these limitations probably impaired the ability of first-year shoots to repair tissue damage and re-emerge from burial. In contrast, second-year PL shoots were more likely to be paired with and partially protected by another shoot on the same plant and likely had access to a greater amount of stored WSC and N reserves through the greater petiole, crown, and taproot size. Together, these factors probably inferred greater treading damage tolerance and led to the relatively smaller effect of damage in year two on PL shoot density. These results imply that under conditions of elevated soil VWC that are expected to result in the partial damage of some paddocks, established pastures should be grazed in preference to new pastures, as the damage to PL plants and subsequent reduction in PL density would likely be lower.

The first-year PL population probably had a greater capacity for recovery from damage and early grazing due to its ability to increase via asexual shoot propagation as well as seedling recruitment throughout the year. Some PL plants in the current study had developed secondary shoots prior to the first grazing, although much of the new shoot emergence occurred from late summer onward in year one. Secondary shoot development may have been promoted during late summer and autumn, following a decline in RG growth, which allowed for a greater penetration of light into the base of the pasture. Van Tienderen and van der Toorn (1991) found that PL plants that grew in a grazed, open pasture, characterised by high light transmission at soil level (inverse of canopy light interception), developed more secondary shoots than plants in a dense hayfield, characterised by low light transmission at soil level. Improved light supply and quality (greater red/far-red ratio) at soil level, and warmer soil temperatures also possibly encouraged PL seedling recruitment during late summer and autumn, since both factors influence PL germination in field situations (Pons & van der Toorn 1988). Furthermore, the establishment of germinated PL seedlings may be more successful during periods when the pasture is more open, as their long, thin cotyledons are generally not well suited to low-light conditions which occur beneath dense grass canopies (van der Toorn & Pons 1988).

Two-year-old PL populations probably have a lower capacity for recovery via secondary shoot development, since the potential number of new shoots able to be produced is dependent on the number of existing PL plants. Li et al. (1997b) observed a similar phenomenon in chicory (*Cichorium intybus* L.), whereby chicory populations that had a density of less than 150 shoots/m^2 could not compensate for plant losses through asexual shoot propagation, and therefore renewal was required. In the current study, this effect was evidenced by the contrasting trends in PL shoot density between the early spring and late summer periods of years one and two. Between early spring and late summer in year one, PL shoot density increased steadily, whereas in year two, PL density decreased dramatically during late spring and then remained steady until late summer. While the specific shoot density below which PL populations cannot recover is unknown, the results from the current study indicate that two-year-old PL populations may have a reduced capacity for recovery via asexual means, which would explain the rapid losses of PL density commonly observed on-farm from the second year after sowing onwards (Navarrete et al. 2022; Nguyen et al. 2022b).

The dramatic reduction in PL density during late spring in year two may have been driven by shading from RG (as indicated in chapter 6), and the responses of PL to low-light conditions. This agrees with findings by Mook et al. (1989) who showed that for small PL plants (less than 4 leaves) in particular, mortality in dense swards was almost entirely concentrated in late spring, coinciding with the period of lowest light transmission. However, in larger plants (more than 4 leaves), light transmission to the base of the sward was not as important for survival through spring and summer, which agrees with findings of van der Toorn and Pons (1988), who found that larger plants were more competitive, especially in shaded canopies. That could suggest that the reduction in shoot density observed in late spring in the current study, under increased shading, was caused by the death of small plants, rather than the death of shoots on plants with multiple rosettes, which would probably have access to greater amounts of resources from the primary petiole. However, the current investigation did not differentiate between asexually propagated shoots, new seedlings which had germinated during the study, or asexually propagated shoots that had separated from parent plants to form new ramets (Grime et al. 1989). Therefore, the type of shoots that died during the late spring period in year two was not known, and requires further research.

The significant reduction in petiole WSC concentration and total plant WSC reserves during late spring in year two might indicate that PL plants continually failed to replenish energy reserves between defoliations through that period. Combined with a reduction in leaf DM during that period, it would appear that the reduction in energy reserves during late spring in year two were due to low amounts of photosynthate production, rather than an increase in energy use for growth. The coincidence of the reduction in petiole WSC reserves with the considerable reduction in shoot density, could suggest that the death of PL shoots may have been partly caused by the exhaustion of energy reserves. The trend for a reduction in the number of shoots per plant during the same period might suggest that newly formed secondary shoots were sacrificed on PL plants, in an attempt to conserve energy. The depletion of WSC reserves in RG plants has been shown to negatively impact tillering, leading to a reduction in tiller density in the long-term (Fulkerson & Donaghy 2001). The cause of depleted WSC reserves during late spring was most likely a reduction in the light interception and thus photosynthate production of PL shoots due to shading from large RG leaves, which dominated the sward canopy during that time (chapter 6).

Due to its influence on LER and SLA, treading damage may exacerbate this effect, particularly where treading damage is severe or repeated within the same season. Since a reduction in LER or SLA might lead to a reduction in the photosynthetic leaf area and/or efficiency per unit of leaf area, treading damage would be expected to further reduce the capacity of PL plants to capture light or produce photosynthates. Furthermore, in situations of little or no shading, the strategy of PL plants to reduce SLA following treading damage might be an effective survival strategy (Ikeda & Okutomi 1995), however in denser pasture canopies, particularly those with RG, which is largely treading tolerant (Brown & Evans 1973) as the major companion species, the reduction in SLA might exacerbate the initial effects of treading damage by reducing the ability of PL to compete with RG for light. Under the treading treatments in the current study, SLA of PL plants recovered quickly following treading damage in year one. Unfortunately, as SLA was not measured during the initial stages of recovery from treading damage in year two, future studies designed to measure this will need to be undertaken.

7.6 Conclusions

Early spring treading damage caused an immediate reduction in PL leaf and shoot DM in year one, which was likely underpinned by a reduction in LER and a brief decrease in photosynthesis rate. Treading damage also caused an immediate reduction in PL shoot density, probably reflecting the death and burial of growing points. However, the negative effects of treading damage on PL growth were short-lived, and damaged PL plants, as well as the density of PL shoots in damaged plots, recovered throughout the first year of the experiment. One-year-old PL plants appeared to be more susceptible to treading damage than two-year-old PL plants, although the one-year-old population probably had a greater capacity for recovery through vegetative shoot production. The reduction in PL density in late spring in year two coincided with a reduction in plant energy reserves and was possibly caused by increased shading from RG. Treading damage may have the potential to exacerbate this effect since it appeared to encourage a reduction in the SLA and LER of PL leaves, thereby further reducing light capture and subsequent energy production in PL plants. Future studies should consider the effects of grazing management and companion species selection on shading during late spring as a means of improving PL performance within grazed mixed pastures.

Chapter 8 General discussion and conclusions

8.1 Introduction

The pollution of freshwater resources with N lost from grazed pastoral systems is of major societal concern in New Zealand (Cameron et al. 2013; Joy 2015; Schullehner et al. 2018; Richards et al. 2022). One low-cost, N-loss mitigation strategy for high-producing dairy systems is the inclusion of PL within RG-based pastures (Navarrete et al. 2018; Simon et al. 2019; Doole et al. 2021; Nguyen et al. 2022a). However, there is industry concern over the poor persistence of PL in RG-based pastures, with several studies showing that pasture PL content decreases rapidly from the second year after sowing (Dodd et al. 2019; Nguyen et al. 2022b). This is a significant issue, as the efficacy of PL for reducing N losses from pastures is strongly associated with the proportion of PL in the diet of cows (Minnée et al. 2020; Navarrete et al. 2022).

There may be some potential for improving PL persistence in dairy pastures by alleviating stresses which are introduced to PL within grazed pastures. There is some evidence that PL does not cope well in wet soil conditions which may introduce additional stresses to PL in the form of waterlogging or treading damage (Blom 1979; Van Groenendael 1985; Mook et al. 1989). However, literature detailing the effects of wet soil conditions on PL in an agricultural setting are limited. Furthermore, studies which have investigated the impact of wet soil conditions on PL within grazed RG-based pastures are extremely scarce. Given the importance the industry is placing on PL for reducing N losses from dairy farm systems, an investigation of the impact of wet soil conditions on the growth and survival of PL within grazed RG-based pastures is warranted.

The primary objectives of this research were to: 1. Investigate the impact of waterlogging on the growth and survival of PL; 2. Investigate the impact of treading damage on the growth and survival of PL; and 3. Investigate the impact of interactions between the effects of these stresses and other stresses which occur within RG-based pastures, on PL growth and survival.

A series of glasshouse and field experiments investigated the impact of waterlogging and treading damage on PL growth and survival within grazed RG-based pastures. The results of these experiments are discussed in detail in chapters 3-7. This section sets out to address the research objectives and highlight the implications of key findings for pasture management strategies. The morphological and physiological responses of PL and RG to waterlogging and treading damage observed in this thesis have been summarised in a visual representation (Figure 8-1).

8.2 Waterlogging

The first experimental chapter of this thesis investigated the impact of waterlogging on PL and RG growth and survival in a glasshouse. Waterlogging proved to be highly detrimental to PL growth, with large reductions in leaf DM, LER and photosynthesis measured (Figure 8-1). However, since all PL plants survived under waterlogging, and recovered quickly following the removal of waterlogging stress, it appeared that PL displayed a degree of tolerance to waterlogging, possibly through morphological changes such as the development of aerenchyma (Grimoldi et al. 2005), or through physiological changes such as an upregulation of antioxidant activity, as occurs in RG (Liu & Jiang 2015). These results suggest that PL may survive for a prolonged period in waterlogged soils and that waterlogging stress is unlikely to be a solitary cause of PL plant mortality within grazed mixed pastures.

In contrast with PL, the growth and physiology of RG plants didn’t appear to be significantly affected by waterlogging stress in this experiment, except for a slight reduction in leaf growth while under waterlogging. The recovery of RG plants following the removal of waterlogging stress was rapid, which agrees with other research that showed that RG has an improved ability for recovering following periods under abiotic stress (Barker et al. 1985). The growth of RG was superior to that of PL under waterlogging, and this continued during the recovery period following removal of waterlogging stress, suggesting that the former had a greater capacity for coping with, and recovering from, waterlogging.

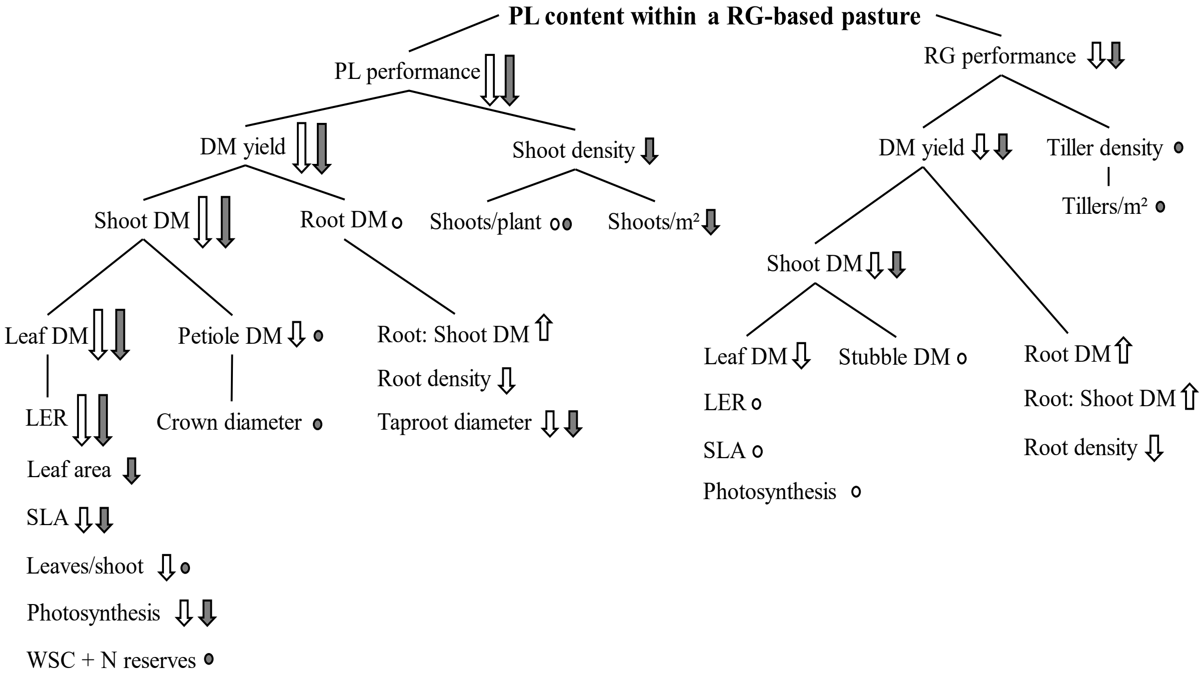


Figure 8-1. Schema of the measured impacts of waterlogging and treading damage on the content and performance of plantain (PL) within a PL + perennial ryegrass (RG) pasture. The direction of arrows show how each measured variable is affected by waterlogging (white) or treading damage (grey) during this study. The size of the arrows signifies the severity of impact. Circles show where measured variables were not significantly affected by waterlogging or treading damage treatments. The measurements included above and below ground dry matter (DM) yield, leaf elongation rate (LER), leaf area per plant, specific leaf area (SLA), number of leaves per shoot, number of shoots per plant, photosynthesis rate, crown and taproot diameter, internal root tissue density and water-soluble carbohydrate (WSC) and nitrogenous (N) reserves in PL petiole.

While all PL plants survived under the waterlogging stress in this experiment, the reduced leaf growth of PL under waterlogging would likely have negative long-term consequences for PL survival in a mixed species pasture, especially when RG is the main companion species. The reduction in the leaf growth of a PL plant would limits its ability to generate leaf area, and thus capture light for use in photosynthesis (Teramura et al. 1981). This could be particularly critical for PL, since PL plants may have a limited capacity for energy storage in the crown and roots (Ayala et al. 2011b), and thus may be more reliant on energy produced in photosynthesis for leaf re-growth following defoliation. Therefore, the decrease in the leaf growth of PL under waterlogging could lead to further reductions in light interception, energy production and regrowth following any subsequent defoliation. This effect might be exacerbated if it were to co-exist in a grazed pasture with RG, which the current study has shown to be more productive than PL under waterlogged conditions. Additionally, the improved ability of RG to recovery quickly from abiotic stresses (Barker et al. 1985) could prolong this effect, leading to the continual shading of PL plants, even after the end of waterlogging stress periods. Future studies should therefore consider the impact of waterlogging on PL growth and survival within PL + RG pastures, to identify interactions between waterlogging and competition for light, on the survival of grazed PL plants.

These findings suggest that while waterlogging may cause limitations to PL growth, there is no evidence to suggest that it alone could cause irreversible damage to plants and thus prevent their recovery. Rather, waterlogging stress could undermine the ability of PL to compete with the more waterlogging-tolerant RG under such conditions, should they co-exist in a mixed sward.

8.3 Treading damage

Two field experiments investigated the impact of treading damage on PL growth and survival. In chapters 4 and 5, the impact of simulated treading damage on PL, within a PL-dominant sward, was investigated in a small plot mowing study, while in chapters 4, 6 and 7, the impact of treading damage on PL within a PL + RG sward, by grazing cows was investigated in a larger plot study. An investigation of the impacts of treading damage on multiple sward types including PL-dominant and RG-dominant pastures, in addition to the mixed PL + RG pasture, was enabled by the development of an artificial treading device (chapter 5). The puggo-stick allowed treading damage to be applied and quantified in small-plot experiments, which enabled a direct comparison of responses to treading damage by PL-dominant and RG-dominant swards (chapter 4). Additionally, given that the PL-dominant pasture subjected to simulated treading damage was three years old, this study also allowed a comparison of the responses to treading damage by older PL populations with those of newly established PL populations.

Treading damage had an immediate and severe impact on PL morphology and physiology when imposed on a newly established population during early spring (chapters 4, 6 and 7); Figure 8-1). Treading damage reduced the LER and photosynthesis rate of PL during the initial regrowth period following the damage, which likely contributed to a temporary reduction in shoot DM and taproot diameter in damaged plants. This reduction in PL growth and the concurrent maintenance of growth in RG, following damage, likely contributed to a reduction in PL content and a concomitant increase in RG content of damaged pasture during that period. Similarly, the growth rate of the PL-dominant pasture was reduced by heavy simulated treading damage in the initial regrowth period following the damage event (chapter 4). These findings agreed with previous studies, which suggested that plants with broad leaves and a relatively low cellulose content, such as PL (Stewart 1996; Navarrete et al.

2022), were more susceptible to leaf injury caused by treading damage (Sun & Liddle 1993). The relatively limited capacity of PL leaves to protect growing points from damage likely also contributed to the significant reduction in PL shoot density following treading damage (Evans 1967), although a large number of PL shoots were also likely buried in wet soil, as is commonly observed in damage pastures (Brown & Evans 1973).

The effects of cow treading damage on PL plants didn’t appear to be long-lived, with a reduction in LER being the only noticeable effect in damaged plants during the second and third regrowth periods after the year one cow treading event (chapters 4, 6 and 7). Furthermore, the PL shoot density of damaged plots recovered throughout subsequent regrowth periods after treading events. Combined, the increase in PL shoot density and DM growth throughout summer months likely contributed to the recovery of PL content in damaged plots in the newly established sward. Since pasture PL content was similar between damaged and undamaged plots in autumn after year one, it appeared that the treading damage had no permanent negative effect on PL survival. The recovery of PL density in the months following treading damage was observed for PL populations within both the PL-dominant sward (simulated treading) and in the PL + RG pasture (cow treading). The recovery of PL population density within damaged plots throughout summer and autumn was possibly due to secondary shoot production as well as seedling recruitment.

Following early spring treading damage by cows in the PL + RG sward, PL shoot density recovered to 94% of the pre-damage density by autumn during year one, and to 63% of the pre-damage density by late summer during year two, while following simulated spring treading damage, PL plant density within the 3-year-old PL-dominant sward recovered to 55% of the pre-damage density by the following autumn. That suggests that younger PL populations might have a greater capacity for recovery following treading damage, than older PL populations. The recovery of shoot density per m^2 in the younger PL population coincided with an increase in the number of shoots per plant, which could imply that the younger population recovered primarily via asexual means. In contrast, the upward trend for PL plant density per m^2 within the damaged PL-dominant sward during late summer could suggest that PL density in the sward had recovered via the emergence of new PL plants from seed, in areas of bare-ground created by treading damage (Elliott et al. 2002). Given that open spaces within pasture which have improved light availability are crucial for the establishment of PL seedlings (van der Toorn & Pons 1988), recovery via seedling recruitment may not be feasible for PL populations within dense pastures, and so populations may be reliant on asexual reproduction to recover in those situations.

In the cow treading experiment, there were no obvious differences between damaged and undamaged PL populations during the second production year, despite the treading damage event being re-implemented in early spring, to the same level of damage as year one. That suggested that PL plant populations might have an improved capacity for coping with treading damage in their second year, possibly because plants were larger (Powell et al. 2007), had more shoots and access to a greater amount of stored energy (chapter 7), than the PL plants did in year one. The perceived improvement in the tolerance of PL to treading damage by cows in year two may have also simply been due to a sparser population of plants having a smaller chance of being physically impacted by cow hooves, than in year one, where plants were closely situated along drill-rows. Regardless, the mean PL shoot density across both treatments continued to decrease throughout year two, possibly due to a reduced capacity for asexual reproduction in older PL populations, in comparison with newly established PL populations.

The response of RG to treading damage, varied between experiments, with a similar reduction in DM growth to the PL pasture following simulated treading damage, but a smaller reduction in DM growth than PL following damage by dairy cows. However, it should be noted that the comparison between PL and RG pasture growth following simulated treading damage is probably not a direct comparison between species, since the PL content of the PL pasture was only around 20%. The results from the cow treading experiment are aligned with previous research that reported RG to be one of the most treading-tolerant pasture species (Brown & Evans 1973).

The reduction in the SLA of PL leaves following treading damage could also undermine the ability of PL to compete with RG for light during spring. While a reduction in SLA may result in an improved tolerance of leaves to treading damage (Van Dijk 1985; Dijkstra & Lambers 1989), it would also likely reduce the ability of PL shoots to develop sufficient leaf area for maximal light capture and could affect the light use efficiency of affected leaves (Terashima & Saeki 1983; Dijkstra & Lambers 1989). Within relatively fast-growing mixed pastures, and when paired with the largely treading-tolerant RG (Brown & Evans 1973), a reduction in light capture and subsequent reduction in photosynthate storage in PL shoots prior to defoliation would likely result in a depletion of plant energy reserves, which would negatively impact the regrowth of leaves following defoliations (Fulkerson & Donaghy 2001). This effect could be more pronounced during late spring and early summer when high RG growth rates may lead to increased shading within pastures. While the SLA of PL leaves appeared to recover quickly following treading damage in the current study, it is possible that more severe or repeated treading events, especially those close to the original event, may prolong or accentuate this effect. Therefore, future research should consider the effect of treading damage and other abiotic stress on the SLA of PL, since it could impact the performance of PL within grazed pasture mixtures.

Taken together, these results suggest that early spring treading damage is unlikely to be a primary cause of PL content decline within grazed pastures, although it might contribute to temporary reductions in PL content during spring, particularly in newly established swards.

However, given that treading damage caused a reduction in processes which drive leaf growth, it is possible that treading damage could reduce the ability of PL to compete with treading-tolerant species such as RG following treading damage events in grazed mixed pastures.

8.4 Interactions between waterlogging and treading damage on plantain

Given that waterlogging and treading damage stress both result from excess soil moisture, there is the potential for them to occur concurrently. While the interaction between waterlogging and treading damage on PL was not directly tested in this study, the differences in soil VWC between years one and two in the cow treading experiment allowed for some speculation. Rainfall was greater prior to the treatment period in year one than in year two, which is reflected in the slightly higher soil VWC observed during the treading of DD plots in year one. Although the monitoring of soil VWC only covered the treading events themselves, the rainfall data suggest that the soil VWC at the experimental site likely exceeded field capacity several times in the months before the implementation of treading treatments in year one.

As alluded to earlier, one response of PL to excess soil moisture, or reduced soil oxygen, is the development of lysigenous aerenchyma to support the flow of oxygen from oxygenated areas of plants, to roots within hypoxic zones of soil (Grimoldi et al. 2005). However, the development of lysigenous aerenchyma weakens PL root structure since it requires cell lysis within root tissue to create air channels (Striker et al. 2007). This effect may have been evidenced by a reduction in root tissue density in PL under waterlogging in chapter 3. This could suggest that due to the wet soil conditions prior to treading in year one, PL plants had developed lysigenous aerenchyma, and thus had a weaker root structure at the time of treading. This would provide some additional explanation for the larger reduction in PL shoot density observed following treading damage during year one, than during year two, when the volume of rainfall before the treading event was lower.

Furthermore, the development of aerenchyma likely reduced the ability of PL roots to expand in compacted soil following treading damage (Blom 1979; Engelaar et al. 1993; Striker et al. 2007). Treading damage may therefore have reduced the ability of surviving PL plants to acquire water and nutrients from soil, which could explain the relatively slow recovery of LER in those plants, which is strongly associated with the uptake of water (Hay & Porter 2006) and N (MacAdam et al. 1989). Given that there is a high probability that a period of waterlogging may precede treading damage on farms, future studies might consider investigating the impact of an interaction between waterlogging and treading damage on PL, to identify better strategies for the effective management of periodically waterlogged pastures containing PL.

8.5 Other factors that impact plantain performance within grazed mixed pastures

The performance (DM yield) of a PL population within a pasture is underpinned by the number of PL shoots in the pasture and, also by the size (DM yield) of those shoots. Therefore, factors which affect the density or growth of PL shoots will affect the performance of the PL population. In addition to abiotic stresses, these may include agronomic factors such as grazing management or companion species selection.

The largest reduction in PL shoot density observed in the cow treading experiment was in late spring in the second year of the experiment. One obvious cause of this reduction was shading from RG, which had high growth rates during that time, probably due in part to the onset of reproductive stem production and a significant increase in tiller density during early spring. Similarly, in the simulated treading experiment, PL plant density declined significantly between late spring and early summer within the PL-dominant sward. This decline in PL plant density also coincided with high pasture growth rates (>100 kg DM/ha/day), which would have inevitably led to increased shading with the sward.

Results from this thesis agree with those of previous studies, which showed that shading during late spring led to high mortality rates in a population of PL shoots (Mook et al. 1989), which are known to have some sensitivity to shading (van der Toorn & Pons 1988; Kuiper & Bos 1992). While the exact cause of this late spring sensitivity to shading is unknown, it may have been related to a reduction in WSC reserves within PL petioles, resulting from reduced light interception.

Since RG typically exhibits high growth rates which lead to increased shading during late spring, and since PL appears to be sensitive to shading during this period, RG may therefore not be an ideal companion species for PL in grazed pastures. Future research should consider the effect of alternative companion species, with different seasonal DM growth distributions to RG, as a means of reducing late spring shading and thus improving PL persistence.

It may be possible to alleviate the negative effects of RG shading by grazing the pasture at an earlier stage of regrowth, than is normally recommended for RG-based pastures. As discussed in chapter 6, the PL + RG pasture achieved 95% light interception at an earlier stage than a RG monoculture, likely due to the larger proportion of flat leaves in the former pasture (Brougham 1957). Since pasture growth rates begin to decline a short time after 95% light interception is achieved (Smetham 1973), it may therefore be in the best interest of both PL shoot survival and overall pasture growth to defoliate PL + RG pastures at an earlier regrowth stage than what is recommended for RG-based pastures. However, further research is required to determine the impact of earlier defoliations on PL + RG pastures on PL shoot survival, RG productivity and the allocation of pasture to cows, particularly during late spring and early summer.

8.6 Main conclusions

1. It does not appear that waterlogging alone causes a major decline in PL density within grazed mixed pastures.
2. However, waterlogging stress may undermine the ability of PL to compete with the more waterlogging-tolerant RG under such conditions, should they co-exist in a mixed sward.
3. Early spring treading damage is also unlikely to be the primary cause of PL content decline within grazed mixed pastures, although it is likely to contribute to temporary reductions in PL content during spring, particularly in newly established swards.
4. As with waterlogging, treading damage may reduce the ability of PL to compete with treading-tolerant species such as RG following treading damage events in mixed pastures. Such a reduction in competitive ability may lead to declines in PL density in the longer term.
5. Increased shading during late spring, resulting from high pasture growth rates, may lead to a reduction in PL density within both PL-dominant pastures and mixed PL + RG pastures.

8.7 Recommendations for farmers

1. Prioritise paddocks with more freely draining soils to establish PL + RG pastures, to reduce the likelihood of waterlogging and treading damage potentially impacting PL performance.
2. Avoid grazing PL + RG pastures during periods of high soil moisture content (<5 mm soil water deficit for a Manawatu fine sandy loam) during winter and early spring, to ensure optimal PL contents throughout spring.
3. If circumstances necessitate the grazing of PL + RG pastures at a high soil moisture content, graze older pastures before younger pastures, as the negative impacts on PL content and persistence are likely to be lower.
4. Ensure that PL + RG pastures are not under-grazed during late spring and that rotations are sufficiently short to reduce pasture shading during this period.

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