THE EFFECTS OF INTENSIVE GRAZING ON NITROGEN FIXATION BY WHITE CLOVER

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JOHN CHRISTOPHER MENNEER

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Abstract

The effects of the grazing animal (treading, defoliation, and excreta) on N_2 fixation were investigated in an intensively managed legume-based dairy pasture in the Waikato region, North Island, New Zealand. Two major 12-month field experiments were conducted in a long-term (>30 years) permanent mixed pasture of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) to determine the full effect of grazing animals on legume growth and N_2 fixation. Each study was commenced in spring (1998 and 1999), when pasture was taken out of grazing and the relevant treatments applied in a randomised block design. Subsequently, pasture was defoliated by mowing at regular intervals and the herbage dry matter yield and pasture species composition determined. N_2 fixation was estimated using the ^{15}N isotope dilution technique to give an integrated estimate of the proportion of clover N derived from atmospheric N_2 (%Ndfa) at each harvest.

The first experiment (initiated in October 1998) investigated the effects of urine additions on N_2 fixation in pasture managed with light and moderately-severe cutting treatments. Urine application (equivalent to 746 kg N ha^{-1}) increased average annual production by 85%, but did not significantly impact on clover production, except in the light cutting treatment at days 50-100. Urine application reduced %Ndfa to 25% of the control (as measured by ^{15}N methods), with recovery taking almost a year, thus reducing total fixed N in clover herbage from 232 to 145 kg N ha^{-1} yr^{-1}. Four methods of estimating %Ndfa from ^{15}N data were used in the urine treated plots. The use of ^{15}N-labelled urine gave estimates of %Ndfa that were 20-30% below values calculated using conventional ^{15}N-labelling during the first 161 days. These differences were probably due to differences in the rooting depth between ryegrass and white clover in conjunction with treatment differences in ^{15}N distribution with depth. The ^{15}N natural abundance technique gave highly variable estimates of %Ndfa (-56 to 24%) during the first 23 days after urine application, but thereafter estimates of %Ndfa were similar to those using ^{15}N-labelling methods. Defoliation severity had no immediate effect on N_2 fixation, but during summer and autumn %Ndfa in moderately-severe plots was consistently higher than under light defoliation. The greater abundance and productivity of vigorous growing weed and summer-grass species recorded in moderately-severe plots during summer and autumn indicate that these species were probably regulating N_2 fixation by changing soil N availability. Overall, total N fixed increased by 36% under moderately-severe defoliation compared to light defoliation. Defoliation severity had no significant effect on clover production.

The second major experiment (initiated in September 1999), examined the effects of pugging intensity on pasture growth and N_2 fixation. A single pugging event at stocking rates of 4.5 cows 100 m^{-2} for 1.5 or 2.5 hours on a wet silt loam soil initiated the experiment, and was equivalent to a moderate or severe pugging event in spring. Fixed N in clover herbage decreased significantly from 76 kg N ha^{-1} yr^{-1} in the non-pugged control, to 66 kg N ha^{-1} yr^{-1} and 36 kg N ha^{-1} yr^{-1} under moderate and severe pugging, respectively. The decrease in fixed N was mainly due to treading damage to clover plants and an associated reduction in clover production which persisted for up to 259 days under severe pugging. Detailed clover morphology measurements revealed an increase in stolon fragmentation and a decrease in plant density, which heightened the vulnerability of plants to dry soil conditions following pugging. A medium-term reduction in grass growth under pugging probably led to increased soil inorganic N and would account for an observed decline in %Ndfa that occurred during the first 3-months of the study. Although pugging caused a major disturbance and rearrangement of the soil surface layers this only had a marginal short-term effect on soil physical properties. Soil aeration was reduced for approximately 21 days and did not measurably affect N_2 fixation. However, an increase in denitrification during the first 21 days after pugging suggested biological process were affected by the reduction in soil aeration.

A conceptual model is proposed to describe the principal processes that affect N_2 fixation by grazing animals in intensively managed clover-grass pastures. The use of a whole farm dairying model of N cycling to further investigate animal impacts in legume-based pastures highlighted the need to consider pasture management strategies and interactions between treading, defoliation and excreta. In the future, more complex models specifically dealing with legume-based pasture ecosystems will be needed to integrate detailed animal-plant-soil components and thus assist in identifying how N_2 fixing efficiency can be improved.

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General Introduction

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General Introduction

I. Introduction

In agricultural systems globally, biological fixation of atmospheric dinitrogen (N_2 fixation) by forage legumes makes a key contribution to the nitrogen (N) economy of grassland. Notably, forage legume species of the genera *Trifolium* (clovers), *Medicago* (lucerne), *Melilotus* (lotus), and *Vicia* (vetches), hold a prominent position in legume-based farm systems in many temperate climates. Global estimates of the total annual inputs of fixed N by legume-based pastures are about 50 Tg N yr^{-1}, and represent about 30-40% of the total fixed N input for terrestrial ecosystems (Burns and Hardy, 1975; Hauck, 1988; Mosier, 2002). While N_2 fixation in legume-based pastures does provide a significant replenishment of soil N, many agricultural systems are still heavily reliant on fertiliser N. At current consumption rates, annual fertiliser N use in grassland systems is about 60 Tg N y^{-1}, and is expected to exceed 80 Tg N yr^{-1} by 2020 as agricultural production increases to meet world food demands (Mosier, 2002). Presently, various concerns exist regarding the environmental consequences associated with the continued intensive use of fertiliser N, including, loss of non-renewable resources, nitrate leaching into groundwater and the contamination of aquatic systems (Mosier, 2002; Vitousek et al., 1997). In comparison to the fossil-energy expenditure in an N-fertilised grass system, a legume-based system reportedly expends only 5% of the fossil energy when equivalent inputs of N are considered

(Wood, 1996). Due to the apparent sustainability of legume-based systems many researchers have indicated that increased N_2 fixation inputs into agricultural systems *per se* are necessary to reduce the proportion of fertiliser N used (e.g. Peoples et al., 1995; Ledgard, 2001).

Several other factors have also led to a resurgence of interest in legume-based pasture systems. Firstly, in many countries (e.g. Ireland, United Kingdom, and United States of America) a desire to decrease farm costs has resulted in renewed interest in extending the outdoor grazing season and reducing the housing of animals (Ledgard, 2001; Leep et al., 2002). Some recent research has begun to investigate the utility of various grass-legume mixtures for such systems (e.g. Leep et al., 2002). Secondly, an overall desire by farmers globally for greater productivity and profitability is leading to increased intensification of farming. In these high producing systems legumes are often used, but it is uncertain how legume performance is affected by intensification, or how efficiently N_2 fixation is operating. In particular, there are growing concerns about the response of N_2 fixation to increased grazing animal impacts (e.g. treading, defoliation, and excreta), and this has yet to be resolved.

In New Zealand, grassland-based grazing systems are heavily reliant on N_2 fixation by white clover (*Trifolium repens*) to achieve desired levels of pasture productivity. Estimated total annual N_2 fixation by white clover in New Zealand grasslands is comparatively large, at 1.57 Tg N, when compared against the meagre amounts of N fixed in some other countries with similar areas of farmed grassland (e.g. 0.8 Tg N in the United Kingdom). During the past decade New Zealand farming has not been exempt from the global trend of greater intensification (e.g. dairy farming). Due to New Zealand's successful use of legumes in undeveloped and less intensive farming systems (e.g. extensive sheep farming), many high producing dairy systems presently operate on grass-white clover pastures. Although white clover has the potential to contribute significant amounts of fixed N (up to 210 kg N ha^{-1} yr^{-1}; Ledgard et al., 2001) in these intensive systems, the full effects of grazing animal impacts on clover growth and N_2 fixation are not well understood. Major issues relate to direct effects on N_2 fixation (e.g. high excreta N inputs), as well as indirect effects via legume growth and persistence (e.g. treading and compaction). Therefore, the research presented in this thesis was undertaken to differentiate the effects of treading, defoliation, and excreta on clover growth and N_2 fixation in permanent (>30 years) clover-grass swards entirely dependent on N_2 fixation.

More specifically, the objectives of the research reported in this thesis were:

1. To investigate the direct impact of treading on clover and grass growth and indirect impacts on clover N_2 fixation through effects on soil physical properties.
2. To investigate the impacts of defoliation severity on clover and grass growth and clover N_2 fixation.
3. To establish the effect of urine N additions on clover and grass growth and clover N_2 fixation.
4. To compare estimates of N_2 fixation using ^{15}N natural abundance and three different ^{15}N isotope dilution techniques.

The research was carried out on a dairy farm located in one of the most intensive dairy farming regions in the world, the Waikato region, North Island, New Zealand.

II. Layout of this thesis

The thesis is structured so that 5 chapters, which consist of the literature review (Chapter 2) and the main research findings (Chapters 3, 4, 5 and 6), are manuscripts which have either been accepted or submitted for publication in internationally recognised, peer reviewed journals. Details of the chapters submitted for publication and presented as manuscripts are as follows:

Chapter 2: The impact of grazing animals on N_2 fixation in legume-based pastures and management options for improvement. *Advances in Agronomy* 83, 181-241 (*In press*).

Chapter 3: The effect of a single application of cow urine on annual N_2 fixation under varying simulated grazing intensity, as measured by four ^{15}N isotope techniques. *Plant and Soil* 254, 469-480.

Chapter 4: The effects of treading by dairy cows on white clover productivity, growth and morphology in a mixed grass/clover pasture. *Grass and Forage Science* (Submitted on the 30th November 2003).

Chapter 5: Animal treading reduces N_2 fixation in mixed grass-clover pasture. *Plant and Soil* (Submitted on the 30th November 2003).

Chapter 6: Animal treading stimulates denitrification in soil under pasture. *Soil Biology and Biochemistry* (Submitted on the 30th November 2003).

In the final chapter (Chapter 7), the results of the study are summarised and discussed to provide a synthesis of the information on the effects of grazing animals on clover N_2 fixation. A dairying model of N cycling was used to further assess the impact of grazing animals on pasture growth, N_2 fixation, and farm productivity.

All the manuscripts are authored by J.C. Menneer, and co-authored by S.F. Ledgard, C.D.A McLay, and W.B. Silvester who jointly supervised the research. The manuscripts were initially written by, and all diagrams, tables, and graphs drafted by J.C. Menneer. Except for minor formatting changes to conform to thesis presentation requirements, each chapter is presented as it was submitted for publication. Due to the presentation of chapters as 'stand alone' manuscripts, some repetition exists between the chapters (e.g. parts of the introduction and methodology). In addition, relevant research findings presented in this thesis have also been summarised in the literature review (Chapter 2) to provide an up-to-date manuscript for publication.

In this thesis, each chapter contains a separate reference list, therefore, no master reference list is given at the end of the thesis.

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2

Literature Review: Grazing Animal Impacts on N_2 Fixation and Options for Improvement

2

The impact of grazing animals on N_2 fixation in legume-based pastures and management options for improvement[1]

Keywords: Treading, defoliation, excretion, N_2 fixation, legume, grass-white clover, pasture management, farm management

I. Introduction

Grazing animals have profound effects on individual plants and plant communities in several interrelated ways (Balph and Makchek, 1985; Vallentine, 2001), including: (1) physical impacts on soil and plant material through treading, (2) plant defoliation, and (3) nutrient removal by grazing and redistribution through excreta. These effects are common to all grazed pastoral systems, but in legume-based pastures they play a major role in regulating the efficiency of N_2 fixation by pasture legumes. The effects that are relevant to legume-based pastures are depicted schematically in Figure 1. At the individual plant level, grazing animal effects are manifest by changes in legume morphology and physiology, and at the community level they act through modifying the balance of competition between plants in the legume-grass association (Schwinning and Parsons, 1996a). When either of these plant or community related processes cause

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[1] Menneer J C, Ledgard S F, McLay C D A and Silvester W B 2003 The impact of grazing animals on N_2 fixation in legume-based pastures and management options for improvement. Advances in Agronomy 83, 181-241 (*In press*).

the legume to be disadvantaged through the influence of the grazing animal then legume performance can be adversely affected (e.g. Brock et al., 1988; Cluzeau et al., 1992; Menneer et al., 2001; 2003). In grazing systems this is reflected by decreasing legume production, persistence and/or a diminishing legume content in the sward, especially if management ignores the legume component. Competition between the grass and legume component in legume-grass swards can also be influenced by their differing susceptibilities to various other factors such as nutrient deficiencies, pests and diseases, and climatic stresses, and these aspects have been discussed in various reviews (e.g. Ledgard and Steele, 1992; Woodfield and Caradus, 1996).

The magnitude of legume response to treading, defoliation and excretion is largely determined by the intensity of animal grazing. As grazing intensity increases, it amplifies the negative impacts of the grazing activities (e.g. Curll and Wilkins, 1983; Greenwood and McKenzie, 2001; Menneer et al., 2001; 2003). Other farm system attributes that have been reported to contribute to the adverse effects of grazing on the legume component and N_2 fixation include: animal type, pasture management, grazing regime (e.g. continuous versus rotational), and soil properties (e.g. Hay and Baxter, 1984; Murphy et al., 1995a, b; Fothergill et al., 2000; Nolan et al., 2001).

Future farming systems are likely to become increasingly intensive due to the limited availability of prime agricultural land and a need to meet greater world food demands. Under this scenario a greater reliance on efficient N_2 fixation to meet the N requirements of high-yielding pastures is desirable to reduce the economic and environmental costs of N fertiliser use (Mosier, 2002). In addition, current farming trends of increased dependence on grazing-based systems and a move away from the housing of animals to reduce costs and labour requirements has also led to a greater reliance on legume-based pastures for meeting plant N requirements (Leep et al., 2002). If future legume-based pasture systems are to derive a consistent and significant contribution from N_2 fixation, and operate at a high-level of efficiency, then an understanding of the full effects of grazing animals and the underlying processes involved is necessary. For example, it has been reported that intensive grazing of pasture in winter by cattle is often associated with very high stocking rates to ration feed at a time of low pasture growth, and this greatly increases the possible negative impacts on N_2 fixation from treading damage and high rates of excreta return (Ledgard et al., 1996a).

This review reports the effects of grazing animals in legume-grass pasture systems. Most emphasis is on white clover (*Trifolium repens*)-grass associations, and reference to other legume-based agricultural systems is for comparative purposes only. The impacts of treading, defoliation, and excreta (Sec. II, III, and IV, respectively) on legume growth and efficiency of N_2 fixation and the underlying processes involved are described. The role of key management practices to reduce grazing animal impacts and optimise legume production and N_2 fixation will be discussed (Sec. V and VI).

II. Animal treading

Animal treading of pasture can affect plants directly by plant injury, death and/or burial, and indirectly through soil compaction and puddling resulting from hoof penetration (e.g. pugging) in wet soil and treading on dry soil (Greenwood and McKenzie, 2001). Evidence from limited research with pasture legumes and more extensively with crop legumes in compacted soils (Voorhees et al., 1976; Asady and Smucker 1989; Henderson, 1991; Cook et al., 1996; Grath and Arvidsson, 1997; Mapfumo et al., 1998) indicates that increased soil bulk density is likely to have two component effects on legume growth, productivity and N_2 fixation in grazing systems. Firstly, it can cause an increase in mechanical impedance to root penetration, and secondly, a reduction in aeration and/or an increase in waterlogging of soil. Although numerous studies in grazing systems have shown large negative effects of treading on grass and legume production (Table 1), most have failed to adequately separate plant damage effects from the soil physical effects and few have described the underlying processes involved or related them to effects on N_2 fixation (Figure 1).

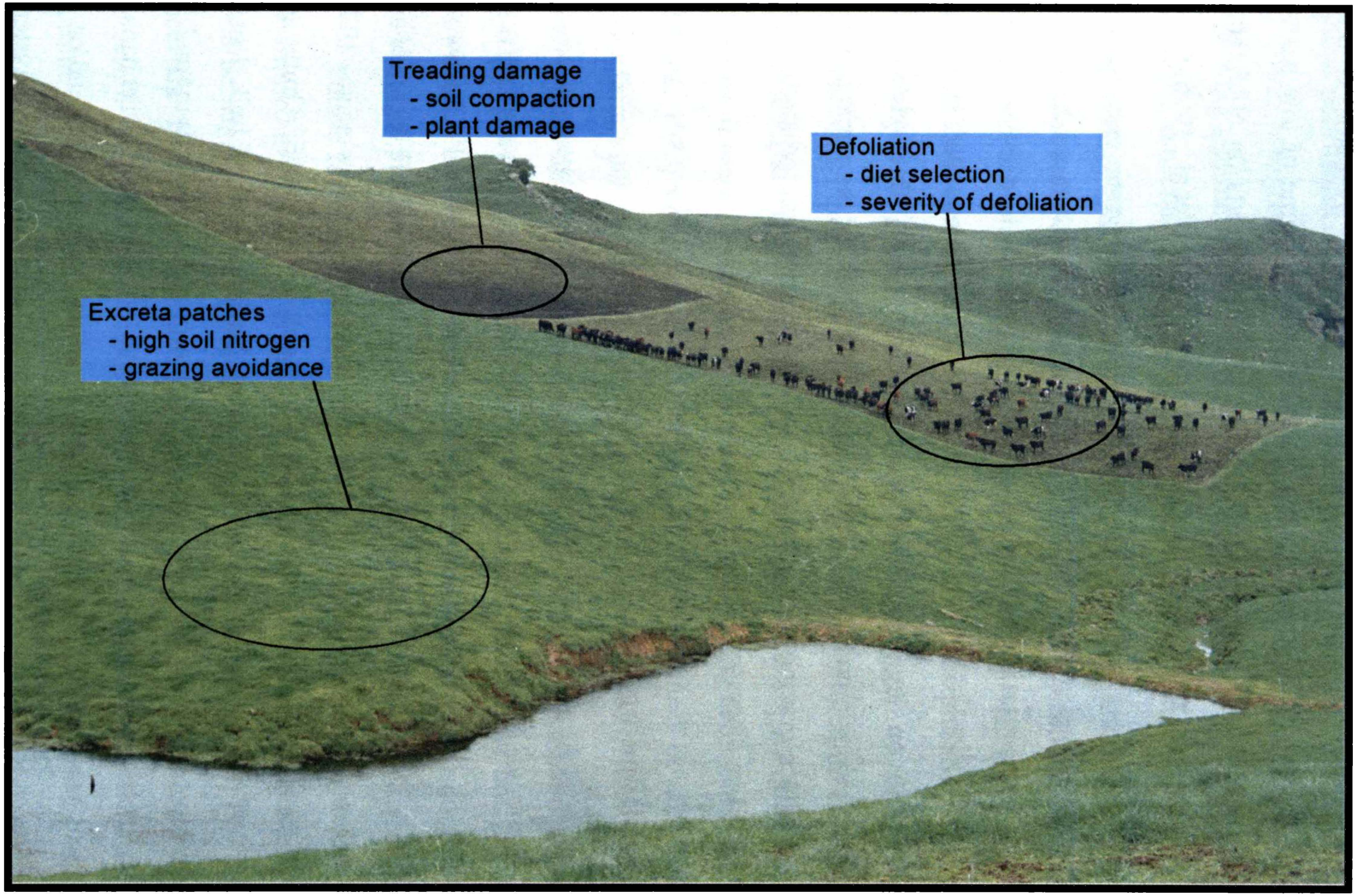

Plate 1. Photo showing the main factors associated with grazing animals that affect N_2 fixation in intensively grazed legume-based pastures.

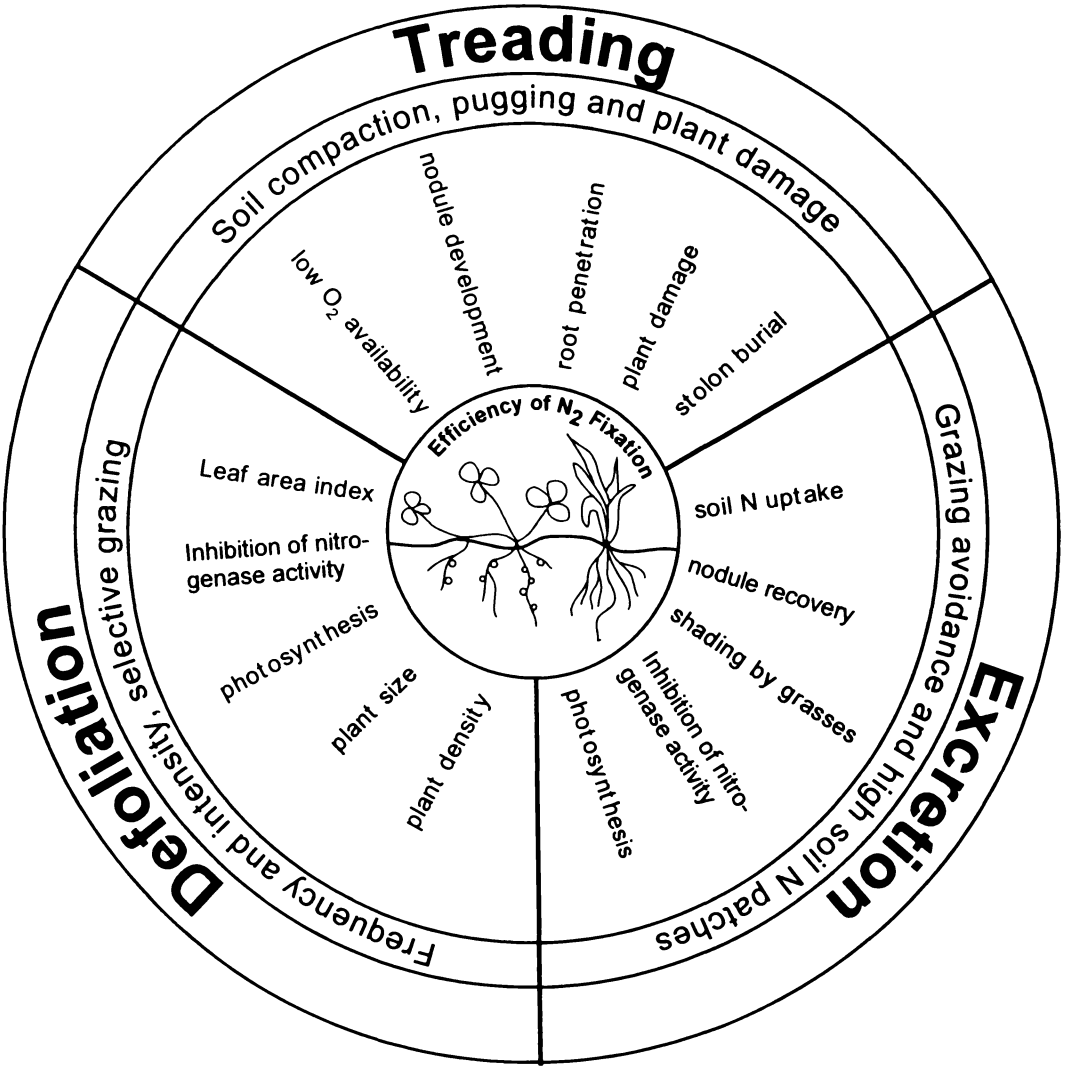

Figure 1. Schematic representation of how grazing animals may affect N_2 fixation in legume-based pasture systems.

Table 1. Summary of treading effects on grass, clover and total pasture production in mixed grass/white clover pastures (not all components measured in some studies)

Animal type	Stocking rate (animals ha^{-1} day^{-1})	Species DM as % difference from non-trodden control:			Reference
		Ryegrass	White clover	Grass/ clover mix	
Cattle					
	2.5			+1	Edmond (1970)
	20	-	-	-24	Edmond (1970)
	2.7	-	-81	-70	Cluzeau (1992)
	67 ha^{-1} 7 h	-	-	-7	Nie et al. (2001)
	133 ha^{-1} 7 h	-	-	-40	Nie et al. (2001)
Sheep					
	15	ns	+7[ab]		Brown (1968b)
	29	ns	-74[ab]	-	Brown (1968b)
	29	+13[a]	-16[a]	+12[a]	Brown (1968a)
	118	-56[a]	-90[a]	-69[a]	Brown (1968a)
	10	-17	-26	-18	Edmond (1958a)
	49	-64	-95	-66	Edmond (1958a)
	15	-57	-8	-18[b]	Edmond (1962)
	29	-68	-30	-40[b]	Edmond (1962)
	10	-42	-5	-	Edmond (1964)
	78	-50[c]	-59[c]	-	Edmond (1964)
	25	-	-	-7	Edmond (1970)
	100	-	-	-29	Edmond (1970)

[a] Varied with season.

[b] Varied with soil moisture.

[c] Varied with type of associated grass species in sward.

A. Plant damage and burial by hoof action

Studies in mixed legume-grass pastures under treading have shown large effects of treading on grass and white clover production with little difference between sheep and cattle (Table 1). In the majority of these studies it is difficult to determine the relative contribution of soil compaction versus direct damage to plants, but it is likely that plant damage is the key factor responsible for reduced pasture yield in these shorter-term (less than 1 year) investigations. For example, in the numerous studies of animal treading by Edmond (1958a, 1958b, 1962, 1963, 1964) that ranged in duration from 3 to 10 months, plant yield reductions following treading were mainly caused by direct damage to plants by hoof action rather than changes to soil physical properties. In one of these studies using white and red clover (*Trifolium pratense*) grown in mixture with grasses, Edmond (1962) measured white clover yield reductions of 22% on dry soil, 23% on moist soil, and 30% on saturated soil when treading of 24 sheep equivalents per hectare was compared to no treading over 3 months. White clover had a greater tolerance to treading than red clover, with the latter showing a two-fold greater reduction in yield. Compared to white clover, red clover plants (and lucerne, *Medicago sativa*) grow from a central crown containing basal buds that are more sensitive to treading damage (Frame et al., 1998; Lodge, 1991).

In general, the sensitivity of individual pastures species to treading depends on the intensity of grazing and the plant species (e.g. Table 2). Notwithstanding, white clover is more susceptible to treading compared to several of its common-companion species (e.g. ryegrass or poa). In recent studies (e.g. Cluzeau et al., 1992; Menneer et al., 2001), white clover further proved its greater susceptibility to treading damage compared to ryegrass by producing much lower yields than its sward associate after treading for up to 4 months. Treading has also been shown to affect clover content with early research on temperate pastures in Europe and the United Kingdom (Bates, 1935; Davies, 1938) ranking *Lolium perenne*, *Poa annua*, *Poa pratensis* and *Trifolium repens* as the most resistant to treading damage. Recently, Menneer (2003) measured white clover content in a mixed

Table 2. Pasture species tolerance to treading as measured by percent reduction of pasture yield from sheep treading at two stocking rates[ab]

Species	Yield reduction (%) at :	
	20 sheep ha^{-1} day^{-1}	80 sheep ha^{-1} day^{-1}
Perennial ryegrass (*Lolium perenne*)	5	23
Kentucky bluegrass (*Poa pratensis*)	6	31
Roughstalk bluegrass (*Poa trivialis*)	0	50
Short-rotation ryegrass (*Lolium perenne*)	9	56
White clover (*Trifolium repens*)	10	60
Browntop (*Agrostis tenius*)	24	60
Timothy (*Phleum pratense*)	22	62
Cocksfoot (*Dactylis glomerata*)	26	80
Red clover (*Trifolium pratense*)	37	87
Yorkshire fog (*Holcus lanatus*)	57	91

[a] Data from Edmond (1964).

[b] Relative to nil grazing; Data is based on mean DM yield over 11 months and 9 separate treading events.

Table 3. Pasture production and N_2 fixation over 48 days after pugging. Yield data are the sum of two harvests. Treatments followed by a different letter for each plant measurement are significantly ($P<0.01$) different[†].

	Pugging Severity			
	Nil	Moderate	Severe	SED
Total pasture yield (DM kg ha^{-1})	2219a	1319b	527c	240
Clover yield (DM kg ha^{-1})	244a	94b	12b	50
Clover % of total pasture yield	12a	6b	2b	2.9
%N derived from N_2 fixation	88a	79a	47b	8.2
Total N fixed (kg ha^{-1} 48 $days^{-1}$)	10.8a	3.8b	0.4b	2.3

[†] Data from Menneer et al. (2000).

clover-grass sward in the medium-term after a single treading event, and recorded a clover content of 10% under severe treading compared to 40% in the nil-treading control soil.

In intensively grazed clover-grass systems, differences in grass and clover tolerance to treading could infer a general growth advantage to associated grasses over clover. Other research has shown that competitive interactions between grass and clover are important in governing clover performance and content in mixed pastures, e.g. self-regulation by clover-grass swards of soil inorganic N concentration (Chapman et al. 1996; Schwinning and Parsons, 1996a, b). Consideration, therefore, should be given to the choice of companion species with clover in mixed swards and their potential to interact through treading.

Compared to the upright tufting growth habit of ryegrass and poa, white clover with its prostrate growth form appears to be more prone to burial and stolon fragmentation. In two recent studies using white clover the aerial biomass of stolon decreased by 50-60% (Cluzeau et al., 1992; Menneer et al., 2001), in the short-term (first 48 days) after severe treading. Burial of stolon tissue in mixed clover-grass grazing systems is not unusual though, with workers in New Zealand (Hay and Chapman, 1984; Hay et al., 1987; Harris, 1994) and Scotland (Gooding and Frame, 1997; Marriott and Smith, 1992) demonstrating a seasonal cycle of stolon burial in winter (up to 80-90%), stolon fragmentation and re-emergence of growing points in spring, followed by surface stolon development over summer-autumn (up to 40% stolon burial). Along with worm castings, animal treading is an important factor controlling stolon burial (Hay et al., 1987; Marriott and Smith, 1992). In high rainfall areas or on poorly drained soils where treading causes excessive burial of stolon material the balance between stolon growth and decomposition may be such that the amount of white clover in the sward decreases (Marriott and Smith, 1992). In addition, the smaller plant units resulting from spring fragmentation of parent plants may be more vulnerable to direct treading effects thereby reducing white clover content and yield.

Decreased white clover yield due to treading damage or burial is not the only contributor to losses of fixed N in pasture as direct effects on N_2 fixation can also occur. For example, Menneer (2003) measured a small proportion of clover N derived from atmospheric N_2 (%Ndfa) in mixed clover-grass pasture during the first 48 days after a severe pugging event by dairy cows (Table 3). This short-term decrease in %Ndfa in combination with a measured decrease in annual white clover yield culminated in a significant reduction in total N fixed, under severe pugging. This study, highlighted the potential for direct negative effects of treading on N_2 fixation, as well as losses of white clover yield via plant damage, and indicates that other indirect processes due to treading (e.g. mechanical impedance and/or reduced aeration) may also operate where severe treading occurs.

B. Soil compaction: Mechanical impedance effects on legumes

In grazing systems, there are no reported effects of increased mechanical impedance due to compaction by animal treading on legume growth and N_2 fixation. This is in spite of many studies reporting increased soil bulk density in grazed pastures due to treading (Table 4).

Therefore, in this section, pot experiments and field studies using wheeled agricultural machinery are reviewed with respect to soil compaction and the associated effects of increased mechanical impedance on legume growth and N_2 fixation (e.g. Frame, 1985; Cook et al., 1996; Grath and Arvidsson, 1997).

In general, the effects of increased mechanical impedance on plant growth and function are largely caused by restricted root growth and reduced soil water availability, and their influence on decreasing water and nutrient uptake (Bennie, 1991; Henderson, 1991; Cook et al., 1996). Some studies (e.g. Cook et al., 1996; Passioura, 1991) suggest hormonal signalling from the impeded roots may be involved in slowing the growth of shoots.

Table 4. Effect of treading by livestock on bulk density of soils in grazed pasture systems[a]. Studies included are both short-term (months) and long-term (years).

Animal type	Stocking rate/treatment	Depth (mm)	Bulk density[a] (g cm^3)	Reference
Sheep	0-50 ha^{-1} day^{-1}	60	1.08→1.28	Edmond (1958b)
	25, 50 ha^{-1} day^{-1}	0-50	1.12→1.42[b]	Curll and Wilkins (1983)
	25, 50 ha^{-1} day^{-1}	0-50	1.10→1.26[c]	Curll and Wilkins (1983)
	0-22 ha^{-1} day^{-1}	0-60	0.89→1.05	Willatt and Pullar (1983)
	2.5-37 ha^{-1} day^{-1}	0-38	1.15→1.43	Langlands and Bennett (1973)
	7.4-22 ha^{-1} day^{-1}	0-50	1.27→1.57	Carter (1977)
	Light vs. intensive	0-50	0.83→1.06	Greenwood and McNamara (1992)
	3 x above control	0-60	1.16→1.28	Russell (1960)
	0-20 ha^{-1} day^{-1}	0-80	1.17→1.26	Greenwood et al. (1997)
Cattle	0-40 ha^{-1} day^{-1}	0-51	1.34→1.61[d]	Stephenson and Veigel (1987)
	0-50 ha^{-1} day^{-1}	0-50	1.04→1.30[e]	Daniel et al. (2002)
	0-1.06 ha^{-1} $year^{-1}$	0-80	1.00→1.29	Taboada and Lavado (1988)
	350 ha^{-1} for 8 h	20-84	1.42→1.50	Kelley (1985)
	0-0.9 AUM ha^{-1}	0-75	1.02→1.07	Naeth et al. (1990)
	0-4.4 AUM ha^{-1}	0-75	0.89→1.07	Naeth et al. (1990)
	0-4.8 AUM ha^{-1}	0-75	0.75→0.90	Naeth et al. (1990)
	400 ha^{-1} for 12 h	0-50	0.52→0.76	Singleton and Addison (1999)
	Sheep vs. cattle	0-50	1.12→1.37	Murphy et al. (1995a)
	80 ha^{-1} for 24 h	50-100	0.96→1.06	Drewry and Paton (2000)

[a] Modified from Greenwood and McKenzie (2001).

[b] Without excretal return.

[c] With excretal return.

[d] Mean of 5 sampling dates.

[e] After 10 years of treatment.

AU = animal unit, a measure of grazing pressure equivalent to a dry cow weighing 450 kg.

AUM = animal unit per hectare for 1 month.

Table 5. Summary of effects of increasing soil bulk density under controlled conditions on shoot and root growth of selected pasture legumes

		Bulk density ($g\ cm^{-3}$)		Percent decrease in:			
Species	Soil type	Base level	Treatment level	Shoot Biomass	Root Biomass	Root length	Root diameter
White clover[a] (*Trifolium repens* L.)	Sand culture	1.50	1.70	38	45	37	-
			1.80	37	35	44	-
Subterraneum clover[b] (*Trifolium subterraneum* L.)	Silt loam	1.10	1.2	nil	-	ns	ns
			1.4	38	-	55	41
			1.6	52	-	82	62
Lucerne[c,d] (*Medicago sativa* L.)	Clay loam	1.15	1.27	18	nil	-	-
			1.38	40	nil	-	-
			1.50	78	31	-	-
	Sandy loam	1.20	1.38	38	nil	-	-
			1.56	53	nil	-	-
			1.74	84	57	-	-

Data from [a]Cook et al. (1996); [b]Nadian et al. (1996); [c]Mapfumo et al. (1998).
[d]May have also been limited by reduced aeration.

1. Legume shoot and root growth

Research with pasture legumes has shown reduced root elongation and diameter, and a decrease in shoot dry weight as bulk density increases (Table 5). For example, the adverse effects of mechanical impedance (under controlled conditions without the effects on soil aeration) has been measured for white clover, with Cook et al. (1996) reporting marked reductions in root length, and root and shoot dry weight, when plants potted in sand were subjected to an increase in bulk density at 20 mm depth from 1.50 to 1.80 g cm^{-3} (Table 4). In the same study, comparisons of white clover with several grass species (*Lolium perenne* and *Agrostis capillaris*) revealed that the effect of increased bulk density on root length appeared to be less with white clover. This may be due to the greater ability of dicotyledonous species (which have a thick seminal taproot) to penetrate compacted soil than monocotyledonous plants (with thinner roots) (Materechera et al., 1991), and could modify the competitive interaction of clover in legume-grass based pastures. Other research (Nadian et al., 1996) with subterranean clover (*Trifolium subterraneum*) examined root length and diameter across an increasing continuum of bulk densities (1.10 to 1.60 g cm^{-3}). Plant growth parameters were not affected until bulk density exceeded 1.20 g cm^{-3} and then shoot growth was reduced by up to 52%. Similarly, in lucerne (*Medicago sativa*) grown in pot culture, increased bulk density reduced shoot and root growth (by up to 78% and 57%, respectively; Mapefumo et al., 1998).

The pot experiments reported above were carried out using seedling plants, and extending this work to pastures that are dependent on the vegetative propagation of legumes could be problematical. For example, in established clover-grass pastures (>2 years), white clover seedling recruitment is usually rare and plants typically spread by vegetative growth (Brock et al., 2000; Gustine and Sanderson, 2001). Compared to clover seedlings which have a seminal taproot, stolon fragments have shallow, weakly-taprooted nodal roots and could differ in their response to soil compaction. This could be important in spring when clover-grass pastures rely on successful root initiation and the establishment of small fragmented stolon units to maintain the sward clover population. In one report that used white clover stolon cuttings in cores of compacted field soil, shoot and root growth were reduced, but differentiation of root resistance effects from reduced aeration was not possible (Blaikie and Mason, 1993).

Currently, insufficient information exists to rank grass and legume species according to their tolerance of compaction, and potential use in compacted pasture soils. Recent work with lucerne (Mapefumo et al., 1998) has shown that plant response to compaction is greatly influenced by soil texture, and the component of plant response measured (e.g. leaf, roots, or branches). Thus, future research and comparisons between pasture species grown in compacted soils will need to relate observed changes in growth to the response factor measured and the soil characteristics. In addition, pot experiments and field studies are required to establish compaction effects across a broader lower-end-range of bulk density values (e.g. 0.75 to 1.0 g cm^{-3}) to include coarser textured soils that comprise some temperate pasture systems (e.g. New Zealand).

Field studies in pasture soils compacted by wheeled agricultural machinery (e.g. silage making operations) generally confirm the work of pot experiments, and show that soil compaction can reduce legume productivity (e.g. red clover and lucerne; Davies and Hughes, 1980; Frame, 1985; Rechel et al., 1987; Henderson, 1991). For example, in mixed swards of grass/red clover, compaction by wheel traffic reduced red clover yield by 17-25% and was mainly linked to an increase in bulk density (from 1.24 to 1.40 g cm^{-3}) (Frame, 1985). Similarly, fieldwork in Australia by Henderson (1991) with subterranean clover and medic (*M. littoralis*) showed that restricted root growth and function led to reduced shoot growth of 30% when bulk density increased from 1.32 to 1.50 g cm^{-3} in the top 0-50 mm soil depth after wheel traffic.

In these field studies, plant growth limitations were probably not only due to mechanical impedance, but also to the effects of reduced soil aeration (discussed in Sec. II.C). Nonetheless, the evidence reviewed here strongly suggests that the magnitude of bulk density increases seen in grazed pasture due to animal treading

Table 6. Summary of effects of increasing bulk density on nodulation and N_2 fixation by crop legumes

Species	Soil bulk density ($g\ cm^{-3}$)		Percent decrease in:			References
	Base level	Compacted	Nodule number	Nodule weight	Specific nitrogenase activity	
Soybean	na[a]	na	30	36	-	Voorhees et al. (1976)
	1.16	1.28	19	26	ns[b]	Lindemann (1982)
	1.20	1.40	23	17	10	Tu and Buttery (1988)
		1.60	46	24	46	Tu and Buttery (1988)
White bean	1.20	1.40	15	11	26	Tu and Buttery (1988)
		1.60	30	30	48	Tu and Buttery (1988)
Field pea	na	na	60	-	-	Grath and Hakansson (1992)

[a] na = not available.

[b] ns = not significant.

NB: data significant unless otherwise stated.

could potentially have a negative effect on legume root and shoot yield through increased mechanical impedance to roots. In legume-based pastures, fixed N in roots can potentially be 30-60% of the fixed N in leaves (e.g. white clover, Jorgensen and Ledgard, 1997; subterranean clover, McNeill et al., 1997). Thus, any reduction in root biomass due to increased root resistance will have serious implications for total N_2 fixation and the cycling of N in pasture systems, as well as the causative effect of reduced above ground biomass.

2. N_2 Fixation

There appears to be no published studies on the effects of soil compaction *per se* resulting from animal treading on nodulation and N_2 fixation in pasture legumes. Hence, the discussion here relies on studies using crop legumes grown in arable soils compacted by wheeled agricultural machinery. Generally, these studies have not determined the relative importance of mechanical impedance and soil aeration status specifically.

Studies with crop legumes have related increases in soil compaction to decreases in nodulation and N_2 fixation (Table 6). Under areas of compaction in field grown soybean (*Glycine max*), Voorhees et al. (1976) found decreases of about 20-30% in nodule numbers and a 36% smaller total nodule mass. Similarly, soybean and common bean (*Phaseolus vulgaris*) grown in pots at bulk densities from 1.2 to 1.6 g cm^{-3} showed a 30 – 50% reduction in nodule number and a 25 – 30% reduction in nodule fresh weight per plant (Tu and Buttery, 1988). Nitrogenase specific activity (using the acetylene reduction assay) was reduced by about 50% (Tu and Buttery, 1988). Such a reduction in nitrogenase specific activity suggests that while impedance may impact dramatically on root biomass and nodulation, nodule functioning may also be affected by other factors, in particular poor soil aeration.

C. Soil compaction: Aeration and/or waterlogging effects on legumes

The adverse effect of poor aeration and/or waterlogging on growth and N_2 fixation of pasture legumes has been reported by many workers and is caused by a

Table 7. Summary of shoot and root dry matter after waterlogging, or reduced aeration (if stated) of the major pasture legume species under controlled conditions

Species	Days of water-logging or reduced O_2	Percent difference from control in:		References
		Shoot DM	Root DM	
White clover				
Ladino type	10^a	-23, -30, -16	-11, -17, -25	Finn et al. (1961)
Ladino type	30^a	-36, -29, +11	-33, -30, -9	Finn et al. (1961)
Intermediate type	3, 6, 10^b	-21, -43, -62	-40, ns, ns^c	Hoveland and Mikkelsen (1967)
Ladino type	3, 6, 10^b	-20, -23, -30	-29, ns, ns	Hoveland and Mikkelsen (1967)
White clover	20	-70	-80	Heinrichs (1970)
White clover	60	+70	ns	Pugh et al. (1995)
Miscellaneous clovers				
Strawberry clover (*T. fragiferum*)	3, 6, 10^b	-5, -24, -18	ns	Hoveland and Mikkelsen (1967)
Persian clover (*T. resupinatum*)	3, 6, 10^b	0, -18, -15	ns	Hoveland and Mikkelsen (1967)
Red clover (*T. pratense*)	20	-60	-50	Heinrichs (1970)
Strawberry clover (*T. fragiferum*)	20	-50	-40	Heinrichs (1970)
Alsike clover (*T. hybridum*)	20	-60	-60	Heinrichs (1970)
Subterranean clover				
cv. *subterraneum*	21	-26	-45	Francis and Devitt (1969)
cv. *yanninicum*	21	ns	-26	Francis and Devitt (1969)
cv. *brachycalcycinum*	21	-44	-63	Francis and Devitt (1969)

Lucerne				
M. sativa	10[a]	-81, -45, -30	-90, -42, -29	Finn et al. (1961)
M. sativa	30[a]	-90, -69, -58	-87, -79, -54	Finn et al. (1961)
M. sativa	60 at 1% pO_2	ns	-50	Arrese-Igor et al. (1993)
M. sativa	14	-72[d]	-	Shiferaw et al. (1992)
Lotus				
Lotus corniculatus	10[a]	+11, -6, +11	-27, -10, -26	Finn et al. (1961)
Lotus corniculatus	30[a]	-24, -16, +11	-34, -29, -2	Finn et al. (1961)
Lotus corniculatus	14	-50[d]	-	Shiferaw et al. (1992)
Lotus pendunculatus	14	-45[d]	-	Shiferaw et al. (1992)
Lotus uliginosus	60[d]	+40	ns	James and Crawford (1998)

[a] Plants subjected to 0, 25 and 20 cm moisture tensions, representing air-filled porosities of 0, 14, and 20%, respectively; waterlogged for 10 or 30 days out of 60 days.

[b] 3, 6 and 10 days waterlogging out of 10 days for 4 months

[c] ns = not significant.

[d] Total plant weight reported only (roots+shoots).

NB: data significant unless otherwise stated.

lack of O_2 for root metabolism (affecting nutrient and water uptake) and N_2 fixation (Finn et al., 1961; Hoveland and Mikkelson 1967; Arrese-Igor et al., 1993) (Table 7). In addition, increased accumulation of toxic products (e.g. Mn, Fe, and ethylene) under poor aeration/waterlogging have also been shown to negatively affect the growth of both legumes and non-legumes (e.g. Hoveland and Mikkelson, 1967; Ponnamperuma, 1984; Pezeshki, 1994). These negative effects on legume growth and N_2 fixation are quite variable and can differ with the extent of exposure and species tolerance to conditions of poor aeration and/or waterlogging.

1. Legume shoot and root growth

In general, reduced aeration or waterlogging has a large negative effect on legume shoot and root growth (Table 7). For example, Finn et al. (1961)

measured root and shoot yield decreases for lucerne, white clover, and lotus across a range of different soil aeration (0, 14, and 20% air-filled porosity), with similar relative decreases in root yield. Lucerne was most sensitive to reduced aeration. In subsequent work by Hoveland and Mikkelson (1967) the tolerance to waterlogging in 3 different species of clover was reported to be in the order of Persian (*Trifolium resupinatum*)≥strawberry (*Trifolium fragiferum*)>Ladino white (*Trifolium repens* L. regal)>intermediate white clover (*Trifolium repens* L. S1), when plants were either intermittently flooded (3 and 6 days out of every 10 for 4 months) or continuously flooded. The white clover cultivars were most sensitive to flooding and experienced a decrease in shoot dry matter yield of up to 62% under the most extreme conditions of flooding treatment. Differences in response between white clover cultivars were also evident with the larger-leafed ladino cultivar appearing to be far more tolerant of flooding than the smaller-leafed intermediate cultivar (Table 7). The reduced clover yields in this study were due to a combination of root decay and reduced root growth, decreased N uptake, and possibly Mn toxicity.

In contrast, there have been reports of increased legume growth in waterlogged soil or under low O_2 conditions. For example, Pugh et al. (1995) reported that white clover subjected to a period of prolonged waterlogging (9 weeks) resulted in an 81% increase in shoot yield and no change in root yield compared to a normally watered regime. Other workers too (e.g. lucerne, Arrese-Igor et al., 1993; lotus, James and Crawford, 1998) have measured similar legume growth enhancement or maintenance when plants have been exposed to waterlogging and/or reduced aeration for extended periods, especially when other anaerobic stresses are avoided (e.g. toxic levels of Mn, Fe, and ethylene). In these cases and others with crop legumes (cowpeas, Dakora and Atkins, 1990a, 1991; soybean, Parsons and Day, 1990) important structural adaptations of enhanced production of lenticels and/or aerenchyma have been cited as key features that enable the increased supply of O_2 through tissue air spaces from shoot to root in low O_2 environments.

Table 8. Summary of nodulation and N_2 fixation under conditions of waterlogging or reduced aeration (if stated) in selected pasture and cropping legumes

Species	Days of waterlogging or reduced O_2	Percent difference from control in:		References
		Nodule weight	Specific nitrogenase activity	
Pasture legumes		-		
White clover	60	-	+34	Pugh et al. (1995)
White clover	7	-	-96	Pugh et al. (1995)
Kenya white clover (*Trifolium semipilosum*)	14	ns	-	Shiferaw et al. (1992)
Lucerne (*M. sativa*)	14	-100	-	Shiferaw et al. (1992)
Lucerne	60 at 1% pO_2	-50	ns	Arrese-Igor et al. (1993)
Lotus (*Lotus corniculatus*)	14	-80	-	Shiferaw et al. (1992)
Lotus (*Lotus penduncculatus*)	14	-68	-	Shiferaw et al. (1992)
Lotus (*Lotus uliginosus*)	60[c]	+35	ns[d]	James and Crawford (1998)

Crop legumes				
Cowpea (*Vigna unguiculata*)	69 at 1% pO_2	-63	-70[a]	Dakora and Atkins (1990)
Cowpea (*Vigna unguiculata*)	4 - 32	-27 to -59	-18 to -46[b]	Minchin and Summerfield (1976)
Fababean (*Vicia faba*)	21 – 50	-	+10 to +60[b]	Gallacher and Sprent (1978)
Garden pea (*Pisum sativum*)	16	-56	-62	Minchin and Pate (1974)

[a] ns = not significant.

[b] Comparison of two waterlogging treatments with and without aeration of O_2 (i.e. pO_2 0.241 versus 0.094).

[c] Measured on 55th day of 69 day study.

[d] Depended on nodule/plant adaptations.

NB: data significant unless otherwise stated.

2. N_2 *fixation*

Studies investigating the effects of soil aeration and waterlogging on the N_2 fixing performance of various crop and pasture legumes have shown nodulation and N_2 fixation can decrease for up to 30 days, but in many longer-term examples (c. 60 days) effects can actually be positive (Table 8). In the medium-term decreased nitrogenase activity in legumes under reduced aeration and/or waterlogging primarily occurs as a result of reduced oxygen supply to the nodule (Arrese-Igor et al., 1993; Pugh et al., 1995). Early studies (Minchin and Pate, 1975; Minchin and Summerfield, 1976) using seedling pea species (cowpea and field pea) with well-formed nodules showed nodule tissue production decreased by up to 60% and nitrogenase activity per plant by 70% after 16 days of waterlogging. Later, Pugh et al. (1995) observed similar effects when normally watered white clover plants were subjected to waterlogging for 7 days causing a dramatic reduction in N_2 fixation (96%).

Over the long-term legumes can apparently adjust to waterlogging and experience enhanced N_2 fixation. For example, white clover when subjected to constant waterlogging for 9 weeks increased N_2 fixation substantially, compared to normal watering (Pugh et al., 1995). Supporting these findings using a controlled O_2 environment to avoid anaerobic stresses, Arrese-Igor et al. (1993) measured no difference in nitrogenase specific activity in lucerne at ambient and sub-ambient pO_2. This suggests that lucerne is capable of adapting to low aeration, and developing a more efficient N_2 fixation system. As in other studies with crop and non-crop legumes (e.g. Dakora and Atkins, 1990; Parsons and Day, 1990; James et al., 1991) this work has highlighted the importance of various structural adaptations in low oxygen environments, and the influence this has on nodulation and nitrogenase activity. In addition to the previously mentioned production of lenticels and/or aerenchyma, are modifications of cells within the nodule cortex and the infected zone to increase gas diffusion to bacteriods carrying out N_2 fixation (Dakora and Atkins, 1990a, b, 1991; Minchin, 1997). These cellular modifications include increases in the ratio of uninfected cells to microbial-infected cells, and increases in the size of intercellular spaces in both the cortex and the infected region (Atkins et al., 1993). Pugh et al. (1995) also noted that vacuole enlargement within infected cells could help increase O_2 availability to bacteriods. Adaptive cellular mechanisms such as these are thought to operate in conjunction with the 'variable diffusion barrier' that surrounds the site of N_2 fixation and regulates O_2 diffusion into the nodule (Dakora and Atkins, 1990a, b, 1991; Minchin, 1997).

The benefits of these adaptations in low O_2 environments (e.g. 'puddled' pasture soils) is not known, but it is likely that the duration of waterlogging, and the degree of plant response to other factors associated with waterlogging (e.g. Mn, Fe, and ethylene accumulating in the root zone) will also have important effects. In reality, waterlogging in pasture soils is usually short-term/intermittent (e.g. puddling after pugging) so longer-term adaptive mechanisms for enhancing O_2 supply may not counteract the negative plant response to anaerobosis. On the other hand, if compaction via treading is causing a gradual but cumulative decline in soil aeration, or in soils with poor drainage, the development of legume varieties which form adaptations for O_2 transport may be advantageous.

D. Significance of plant and soil factors, and limits of pasture tolerance

The relative significance of plant and soil effects resulting from treading processes on legume growth and N_2 fixation in legume-based pastures is difficult to determine. This is mainly because of the large influence that farm management has on the magnitude of treading processes, and also from differences in soil properties between farm systems. Although damage to plants appears to be the main effect under treading (especially at high stocking rates), systematic studies are required to determine the relative contribution of direct effects of plant damage versus indirect effects of soil compaction. In particular, future work with intensive farming systems should recognise that compaction of pasture soils is likely to be a long-term and cumulative process of soil degradation involving changes in soil strength and aeration (e.g. Daniel et al., 2002; Table 4 and 5). To this end, studies may need to have a much longer term focus (years rather than months) to establish if increasing compaction is causing a slow but important winding-down of legume productivity and N_2 fixation. To achieve this, further insight is required regarding the application of suitable soil physical indices along with modelling of legume growth response and N_2 fixation under field conditions to determine impacts at the farm-scale.

Table 9. Estimated threshold bulk densities to reduce relative yield (%) of shoot and root dry matter for lucerne and brome grass grown in clay loam and sandy clay loam soils with subsurface compaction[a]

		Threshold bulk density			
		Clay loam[b]		Sandy loam[c]	
Parameter	Relative yield	Brome	Lucerne	Brome	Lucerne
	(%)	g cm^{-3}			
Shoot biomass	75	1.28	1.28	1.36	1.35
	50	1.40	1.40	1.55	1.55
	25	1.52	1.52	1.74	1.74
Root biomass	75	1.31	1.55	1.38	1.45
	50	1.45	1.79	1.59	1.69
	25	1.58	2.02	1.81	1.93

[a] Data from Mapfumo et al. (1998).
[b] Base level soil bulk density was 1.15 g cm^{-3}
[c] Base level soil bulk density was 1.20 g cm^{-3}

Establishing critical limits of bulk density that affect plant growth in compacted soils is difficult and large variations exist in reported values due to the influences of soil water content (Eavis, 1972), soil texture (Jones, 1983), and plant species tolerance (Materechera et al., 1991). Additionally, some studies have revealed that even slight increases in bulk density can have adverse affects on the growth of some key pasture species (e.g. ryegrass, Houlbrooke et al., 1997). Therefore, it may be of more use to establish guideline values of bulk density for individual soil types (or properties) and plant species that represent a continuum of incremental reductions in key growth factors rather than defining critical upper limits. This approach was adopted by Mapfumo et al. (1998), using lucerne and a brome grass in two different soil types (a clay loam and sandy loam), which were compacted to a range of bulk densities and growth changes recorded, after which linear regression equations were used to estimate the threshold bulk densities that reduced yields by 25, 50, and 75% of the control (Table 9). Threshold values for decreased shoot dry weight were the same for both species but different for the two soils, and threshold values for root dry weight reductions were different for both soil type and species. This work clearly shows the necessity to consider differences in soil texture, species tolerance, and the response factor measured when establishing 'critical' levels of bulk density causing mechanical impedance problems in pasture soils.

Air-filled porosity is a frequently used indicator for assessing soil aeration status in grazed pasture (e.g. Greenwood and McNamara 1992; Singleton and Addison 1999). As would be expected, increases in bulk density of compacted pasture soils are paralleled with a decrease in air-filled porosity. However, plant function and growth is not normally affected unless the air-filled porosity falls below about 10% (Grable, 1971). It is generally assumed that values of air-filled porosity between 10 and 25% provide adequate aeration but with some limitations to O_2 diffusion under certain conditions, and above 25% air-filled porosity provides good aeration (Stepniewski et al., 1994). As with bulk density, critical values of air-filled porosity vary with plant type and soil texture, because of differences in air-filled pore geometry and stability, and subsequent effects on O_2 diffusion. For example, Bakken et al. (1987) found that O_2 diffusion was reduced to zero when air-filled porosity was 10% for a clay soil, but in a sandy soil zero O_2 diffusion did not occur until the air-filled porosity was 2%.

Despite many authors reporting low air-filled porosity in grazed pasture, only recently has work began to investigate the relationship between yield response and air-filled porosity with ryegrass (Drewry et al., 2001). No similar work has yet been done for white clover. Under simulated treading to reduce plant damage effects, Drewry et al. (2001) produced a pasture response curve that indicated that the optimum air-filled porosity for ryegrass yield was about 16-17%, and a critical air-filled porosity (for >10% yield reduction) was approximately 10-11% (Figure 2). Differences in soil texture and seasonal weather patterns (wet or dry periods) meant that this curve could undergo a lateral shift in either direction and requires additional calibration. Further work is also needed to see if a similar relationship exists for pasture legumes, especially in legume-grass mixtures where any differences in effect may influence the legume-grass competitive interaction and thus the persistence of legumes in the sward.

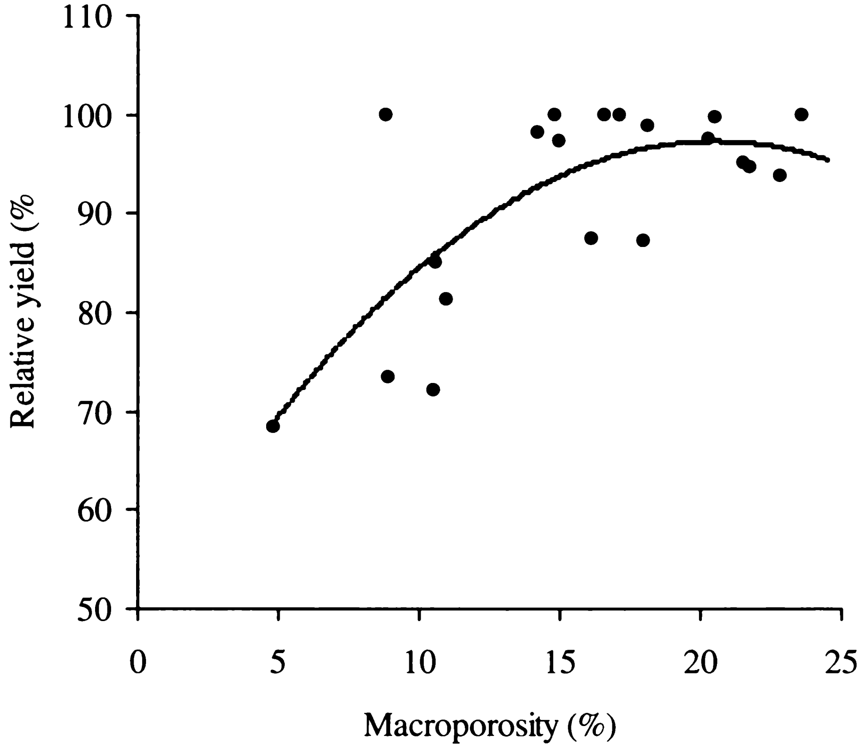

Figure 2. Relationship between relative pasture yield for mid-summer and macroporosity at 0-50 mm soil depth (P=0.001; r^2=0.49) (after Drewry et al., 2001).

III. Animal grazing

Defoliation by grazing animals can affect the size of individual legume plants or plant parts (e.g. leaf size), plant density, and from a physiological standpoint N_2 fixation and photosynthesis (Figure 1). Key components of defoliation that can have either a positive or negative effect on legume productivity and N_2 fixation in pasture relate directly to the foraging behaviour and management of the grazing animal: *viz*, selection by the animal (of certain plants or plant parts) and, frequency and intensity at which plants are defoliated.

A. Diet selection and defoliation effects

1. Diet selection

Pasture species selection by the grazing animal can have a marked influence on the legume content of mixed pastures (e.g. Ledgard and Steele, 1992). Selection for legume varies with the degree of selectivity and the feeding style attributes of the animal type (Table 10). In turn these attributes can lead to differences in legume content of pasture. For example, white clover content of swards grazed by different animals is generally in the order: cattle>goats>red deer≥sheep (Hunt and Hay, 1989; Sheath and Hodgson, 1989; Wright et al., 1992; Semiadi et al., 1995). Because of white clover's lower position in the sward the height at which different animals graze also has a considerable bearing on the amount of white clover ingested (Milne et al., 1982). These differences in grazing selection behaviour between animals can be used advantageously by mixed grazing of different animal types to modify sward composition in favour of white clover (or grass if need be) (Collins, 1989; Curll et al., 1985 a, b; Murphy et al., 1995b; Nolan et al., 1999, 2001). For example, Nolan et al. (2001) highlighted the modifying influence of mixed grazing with sheep and cattle on white clover content in mixed clover-grass swards. After a 3 year period of rotational grazing with mixed cattle and sheep, versus sheep only, white clover contents were significantly different at 13.5 versus 6.1 %, respectively (Nolan et al., 2001). The mixing of goats with cattle or sheep has also been shown to improve white clover content in the sward, and can significantly increase cattle and sheep live weight gains (Osoro et al., 2000; del Pozo et al., 1997). The improved white clover content occurs because of goats' top-down grazing style which results in more grass being ingested than the lower lying white clover foliage (del Pozo et al., 1996).

Table 10. Feeding style attributes of different livestock species[a]

Species	LU[b]	Method	Selectivity	Type	Typical minimum sward height grazed (cm)
Cattle	0.73 – 1.0	Tear with Tongue	Low	Grazer	6
Sheep	0.13 – 0.2	Biting / shearing	High	Grazer / browser	3
Goat	0.1 – 0.2	Biting / shearing	High	Browser / grazer	6
Deer	0.1-0.4	Biting / shearing	High	Browser / grazer	<3

[a] Modified from Bullock and Armstrong (2000).
[b] LU= Livestock Unit (1 dry cow, 600 kg).
Data from Hearn (1995), Crofts and Jefferson (1999), Mayle (1999), Milne et al. (1998), Cosgrove and Hodgson (2002).

2. Defoliation frequency and intensity

While the selective preferences of the grazing animal are of considerable importance for manipulating pasture composition, the frequency, intensity and timing of defoliation has an overriding effect on white clover content in legume based pastures (Curll and Wilkins, 1982; Brock et al., 1988; Parsons et al., 1991a, b; Brock and Hay, 1996; Frame et al., 1998). Defoliation frequency depends solely on stocking rate, whereas the intensity of defoliation depends on stocking rate and the duration of grazing, both of which are features of the chosen grazing management system (e.g. continuous versus rotational) (Lemaire and Chapman, 1996). Through grazing management, defoliation frequency and intensity interact to determine the competitive balance between white clover and grass for light and space and can have a profound effect on white clover productivity.

Early work in mixed swards indicated that white clover is favoured by frequent and intense defoliation with short rest periods (Brougham, 1959; Graham et al., 1961; Ward et al., 1966; Bland, 1967). Brougham (1959), for example, found that white clover content and yields were highest where grazing was most frequent and closest, but with a rest between grazings (i.e. short rotation), and was attributed to the reduced effects of shading from ryegrass under such conditions. In general, these early studies highlighted the importance of rest periods between defoliation events that were not prolonged but still gave adequate time for white clover regrowth without excess shading. Undoubtedly, this early work along with subsequent research (e.g. Widdup and Turner, 1983; Clarke et al., 1984; Newton et al., 1984; Frame and Newbold, 1984, 1986; Brock, 1988; Brock et al., 1988; Hay et al., 1988; Orr et al., 1990; Laws and Newton, 1992; Elgersma et al., 1998; Nolan et al., 2001) have consolidated the commonly-held belief that rotational grazing management with its inherent rest periods tends to enhance white clover content as opposed to continuous grazing management which is often reported to reduce white clover content (but see Sec. V.A.2) and yield by overly frequent and intense defoliation and/or continual selective pressures (Figure 3).

Under rotational grazing, the severe removal of leaf and stolon tissue during defoliation events is usually offset by the generally longer interval between defoliations which allow plants to rebuild photosynthetic area compared to continuous grazing. This allows more leaves per stolon to accumulate, and a high photosynthetic potential and greater plant size. Additionally, competition with grass reduces since grass tiller density and leaf appearance rate decline (Grant and Barthram, 1991), thereby allowing more space for white clover invasion (Brock et al., 1988).

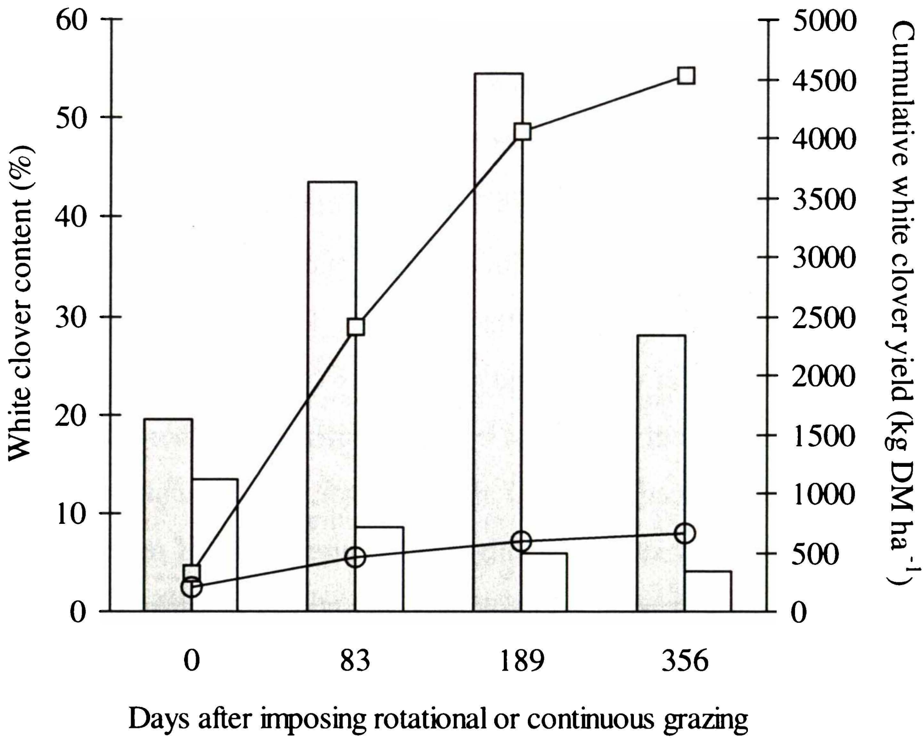

Figure 3. White clover content (bars) and dry matter yield (lines) under rotational grazing (shaded and □) and continuous grazing (open and ○) at a stocking rate of 14 ewes ha^{-1} (derived from Newton et al., 1984).

Larger, more complex structured white clover plants are conducive to white clover survival in the sward, whereas smaller, simple structured plants are more vulnerable to edaphic and animal stresses (Brock et al., 1988; Hay et al., 1989a). The latter have been blamed for white clover decline or 'crashes' that may occur on a 3 to 4 year cycle in swards continuously grazed by sheep (Fothergill et al., 1996). These cycles of declining white clover content under continuous grazing are most evident with sheep because of their high selectivity for clover, though cattle under continuous grazing can also reduce sward white clover contents, albeit gradually (Gibb and Baker, 1989; Laidlaw et al., 1995). Herbage rejection by cattle near dung pats offers patches of white clover a rest from defoliation, and has been suggested to help maintain white clover content compared to sheep grazed swards (Frame et al., 1998).

Although white clover is the most extensively-used legume in mixed legume-grass pasture systems, red clover, and to a lesser extent lucerne, which are generally better suited to conservation, are sometimes used in multispecies mixtures for grazing. With lucerne and red clover, the effects of defoliation frequency are clearer than with white clover. Both species do not persist under frequent and severe defoliation (Frame et al., 1998; Lodge, 1991) and so are better suited to rotational, lenient grazing with sufficient time between grazings to allow for plant recovery. Lucerne regrowth is from crown buds using stored reserves, and the plant needs 4-6 weeks for reserve replenishment (Lodge, 1991). In contrast, red clover relies on terminal meristems for regrowth and needs to maintain adequate residual leaf area for subsequent growth. Persistence of both lucerne and red clover in grazed mixed swards is unstable and appears to be limited to 2 or 3 years for red clover and 4 years for lucerne (Frame et al., 1998; Sprent et al., 1996).

B. Direct effects of defoliation on N_2 fixation

Nitrogen fixation in pasture legumes is greatly reduced or ceases almost immediately after defoliation (Vance et al., 1979; Cralle and Heichel, 1981; Davidson et al., 1990; Ta et al., 1990). In early literature, this decrease in nitrogenase activity resulting from defoliation was attributed to reduced photosynthesis and an associated fall-off in photosynthate supply to the nodule for nodule function and growth (Boller and Heichel, 1983; Macdowall, 1983; Ryle et al., 1986; Ta et al., 1990). An alternate model (Hartwig and Nosberger, 1994; Hartwig and Trommler, 2001) is that defoliation leads to a reduction in shoot demands for nitrogen (reduced N sink strength) and that continuing N_2 fixation leads to an accumulation of N compounds in the nodules. These N compounds trigger an increase in resistance to O_2 diffusion (by an unknown mechanism) resulting in an inhibition of respiration, and thereby decreased nitrogenase activity. Alternatively, N_2 fixation may become a low-priority sink for carbon, which is redirected to regrowth and away from the export of N from nodules.

Table 11. Summary of defoliation effects on nitrogenase activity of selected pasture legumes

Species	Defoliation severity (% shoots removed)	% decrease in N_2 fixation or nitrogenase activity within 48hrs of defoliation	Nº of days for recovery of N_2 fixation (specific activity)	Reference
White clover (*T. repens*)	50	70	5-9[a]	Ryle et al. (1985)
	100	85	10	Chu and Robertson (1974)
	44[b] and 83[c]	nil and 70	nil and 5	Davidson et al. (1990)
	90	62	21	Moustafa et al. (1969)
Lucerne (*M. sativa*)	na	95	24	Kim et al. (1993)
	90	91	12	Macdowall (1983)
	60	80	14	Ta et al. (1990)
	100	78	12	Cralle and Heichel (1981)
	60	88	18	Vance et al. (1979)
Lotus (*L. corniculatus*)	85	70	11	Cralle and Heichel (1981)

[a] Depended on age of plants; N_2 fixation determined from N content.

[b] Successive defoliation's every 10d for 40d.

[c] Single defoliation after 40d.

na = not available.

NB: data significant unless otherwise stated.

In white clover, nitrogenase activity decreases within several hours after defoliation (Moustafa et al., 1969; Chu and Robertson, 1974; Ryle et al., 1985), and recovery takes anywhere from 5-21 days depending on the severity of defoliation. Interestingly, Ryle et al. (1985) observed nodule dry weight decreases of about 30% (as did Chu and Robertson, 1974) in white clover after defoliation, with recovery to original nodule weights after about 6-9 days depending on the photosynthetic ability of the remaining tissue. This indicates that the adverse effect of defoliation on nitrogenase activity is not entirely due to loss of photosynthetic area. Similar, results have been obtained using other pasture legumes with nitrogenase activity decreasing by 60-95% immediately after defoliation, and recovery to control levels taking between 5-24 days, depending on the species, severity of defoliation, and growing conditions (Table 11).

In intensively grazed mixed legume-grass swards the immediate effect of defoliation on nitrogenase activity suggests that N_2 fixation may be operating at suboptimal levels, particularly if insufficient time is allowed between grazing events for return of N_2 fixation to maximum rates. In a rotationally grazed mixed clover-grass sward, Murphy et al. (1986) showed a rapid decline in N_2 fixation occurred at each grazing event, but regrowth was accompanied by a recovery in N_2 fixation shortly (about 20 days) after grazing ceased (Figure 4). Thus, the length of grazing interval needs to be carefully balanced; firstly, to ensure recovery of N_2 fixation, and secondly, to minimise the risk of shading by companion grasses and the potential for further reductions of N_2 fixation (Chu and Robertson, 1974; Halliday and Pate 1976). In the study of Murphy et al. (1986), increased soil N from animal excreta may have also contributed to the lower levels of N_2 fixation that were measured after grazing (e.g. Hoglund and Brock, 1978) and is further discussed in Section IV.

Other more complicated factors also need consideration when pasture is intensely grazed, with recent work (e.g. Menneer et al., 2003) showing defoliation intensity can also affect N_2 fixation (%Ndfa) by regulating soil N availability through manipulation of the botanical composition of the sward. Menneer et al.

(2003) measured increased %Ndfa in a more severe cutting height treatment, but only during the summer months. This was attributed to an increase in drought-tolerant grasses and weeds that had a greater ability to grow and absorb soil N than ryegrass, which led to reduced soil N availability and enhanced the potential for N_2 fixing activity.

IV. Animal excretion

In intensive high-producing pasture systems, dung and urine patches can cover more than a third of grazed pasture in any one year (Haynes and Williams, 1993; Whitehead, 1995). Urine N is in highly mineralisable forms (mainly as 70-90% urea-N) compared to dung N, and within 3-5 days is rapidly converted to plant-available N in soil (e.g. Menneer et al., 2003). This can result in inorganic soil N under urine patches up to 10 times greater than under a dung pat, and more than 30 times greater than areas unaffected by excreta (Afzal and Adams, 1992). Where cattle graze, animal rejection of herbage on or near dung pats is also a problem, and depending on the climate and stocking rate can represent 35-40% of the total sward area and last up to 1 year (Marsh and Campling, 1970).

In mixed clover-grass pasture, high soil N from urine and animal rejection of herbage on or near dung pats adversely affect legume growth and N_2 fixation mainly by altering the legume-grass competitive interaction and/or depressing N_2 fixation (Figure 1). While smothering/burial or scorching of plant tissue by excreta can also affect legume performance, it is far less important than changes caused by the primary processes described above.

A. Increased soil N and grazing avoidance of excreta-affected areas

Typically, increased levels of soil N due to excreta return, particularly urine, have a large negative effect on white clover content and in many cases also white clover yield (Table 12). This commonly measured decrease in white clover content under urine is a dilution effect caused by what is often an impressive increase in grass yield (e.g. up 166%; Menneer et al., 2003) without an

Table 12. Effect of animal excreta on various components of white clover performance and N_2 fixation, measured under cutting unless otherwise stated

Excreta type	Decrease in white clover yield (%)	Change in white clover content (%)	Decrease in total N fixed (%)	Duration of study (days)	Minimum N_2 fixation activity[a]	Days for recovery of N_2 fixation activity to baseline
Urine						
Ball *et al.*, 1979	-	48→12	-	53	5[b]	>53
Curll and Wilkins, 1983						
Grazed at 25 sheep ha^{-1}	13	58→45	-	150	-	-
Grazed at 50 sheep ha^{-1}	nil	58→18	-	150	-	-
Ledgard *et al.*, 1982	27	43→22	72[d]	120	30[b]	>90
Marriott *et al.*, 1987	72[e]	28→5	-	81	10[b]	>80
Menneer *et al.*, 2003	nil	44→33	43	365	25[c]	289
Vinther, 1998	nil	60→30	45	120	28[c]	>120
Dung						
Vinther, 1998[f]	nil	40→30	20	120	75[c]	120

[a] Units are either [b] specific nitrogenase activity or [c] %Ndfa.

[d] Estimate calculated using N_2 fixation value derived by acetylene reduction assay method.

[e] Some severe scorching of herbage by urine also involved.

[f] Measurements within 0-10 cm of dung pat.

accompanying increase in white clover yield. Similarly, the rejection of herbage by grazing animals on or near dung pats, can cause low white clover contents and yield (Vinther, 1998). Death of white clover nodes also occurs directly beneath dung pats shortly after deposition (about 25 days) (MacDiarmid and Watkin, 1979). To some degree this effect is offset by old dung-pat sites providing fresh bare patches for colonization by white clover (Weeda, 1967), and can result in white clover dominance for up to 18 months on such sites.

In mixed clover-grass swards the negative effects of excreta on clover performance are largely due to strong competition from its grass companion for light and space. White clover, because of its relatively low position in the canopy is easily overcome by shading when sward heights are excessive (e.g. when soil N is high or herbage is rejected). High soil N and herbage refusal also increases grass tiller density so reducing the space available for white clover invasion (Davies, 2001). In clover-grass swards, some workers (e.g. Schwinning and Parsons, 1996b) propose that the spatial heterogeneity of soil inorganic N generated by the patchiness of excreta return may be important for desynchronising legume-grass interactions and reducing the risk of large population fluctuations at the field scale. This is the key reason for the typical spatial variability of legume content and N_2 fixation that occurs in intensively grazed pastures.

The extent of competition for light and space between white clover and grass is dependent on defoliation intensity (Curll and Wilkins, 1983; Evans et al., 1992; Menneer et al., 2003). For example, Menneer et al. (2003) measured the effect of a single application of cow urine (746 kg N ha^{-1}) under differing cutting severity, through to complete recovery of N_2 fixation and white clover production. As in some other studies (e.g. Ledgard et al., 1996b; Vinther, 1998) in mixed clover-grass pastures with applied urine or N fertiliser, Menneer et al. (2003) observed that the major effect of urine on N_2 fixation was by the direct effect of increased soil N availability on reducing the N_2 fixing capability of the plant. Appropriate grazing management is therefore important for countering the negative effect of animal excreta on white clover performance, especially in intensively grazed pastures where excretion often covers a large proportion of the grazed area.

B. Direct effects of excreta N on N_2 fixation

Where conditions of high inorganic soil N prevail, N_2 fixing activity in legumes is reduced because of the plant's preference for uptake of N from soil over the more energy dependent process of N_2 fixation (Davidson and Robson, 1985; Phillips et al., 1982). In mixed clover-grass pasture this is reflected in a rapid (within 2 days) decline in N_2 fixation of up to 90% upon deposition of animal urine (Table 12). Reported decreases in N_2 fixation rates near dung affected areas are much less than near urine affected areas, because of the lower availability of dung N, with values falling by only 2-10% within 10 cm of the pat edge (Jorgensen and Jensen, 1997; Vinther, 1998).

Under urine patches, the rate of recovery of N_2 fixation is variable and depends on the time taken for soil inorganic N to return to back-ground levels (e.g. 30-162 days; Ball et al., 1979; Marriott et al., 1987; Vinther, 1998; Menneer, et al., 2003). Recovery of N_2 fixation per unit of legume growth, therefore, may be as quick as 40-50 days (Ball et al., 1979; Marriott et al., 1987) or as long as 120-289 days under urine patches (Menneer, et al., 2003; Vinther, 1998). Only one of these studies (Menneer et al., 2003) measured the full recovery in clover growth and N_2 fixation from urine deposition. In that study, a marked reduction in N_2 fixation due to urine began within 3 days and low N_2 fixation values persisted for up to 289 days even though inorganic N had fallen to back-ground levels in the 0-150 mm soil depth by 160 days (Figure 5). This delayed recovery of N_2 fixation in the absence of high soil N (also measured in the study of Vinther, 1998), may be due to the remobilisation of stored N from roots to shoots, uptake of inorganic N from below the soil sampling depth, and/or the delayed re-establishment of active nodules (Marriott et al., 1987; Munns, 1977). Overall, the prolonged effect of decreased N_2 fixation after urine application resulted in a 38% decrease in total N fixed from 232 to 145 kg N ha yr^{-1} (Menneer et al., 2003).

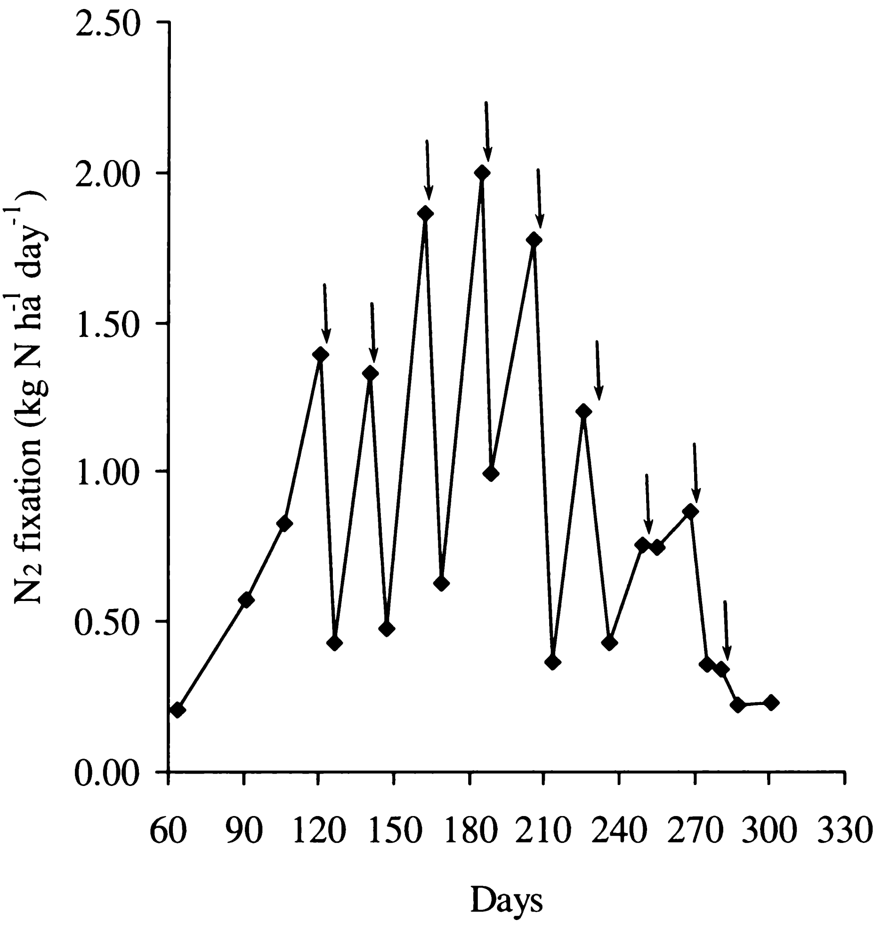

Figure 4. N_2 fixation rate (nitrogenase activity) in a grass-clover sward as influenced by a series of grazings: arrows indicate times of grazing events (redrawn from Murphy et al., 1986).

At the farm scale not all pasture is influenced by urine, with the proportion of affected pasture depending on stocking rates, length of the grazing period, frequency of excretion and area covered by individual urine patches (Haynes and Williams, 1993). On New Zealand dairy farms, the average stocking rate is 2.8 cows ha^{-1} $year^{-1}$ (Livestock Improvement, 2003). Haynes and Williams (1993) reported a typical urination frequency of 10 urinations per day, and the average area covered of 0.30 m^2. Using these reported values and a Poisson distribution to allow for the overlapping of urine patches (Petersen et al., 1956), we calculate that urine would be deposited on 25% of the grazed area each year. However, the area affected by urine usually extends well beyond the wetted urine patch (Whitehead, 1995). Affected areas of 0.5 – 0.7 m^2 have been reported for dairy cows (Lantinga et al., 1987; Richards and Wolton, 1976), and could potentially result in up to 46% of grazed pasture being affected by urine deposition each year. Using a typical estimate of total N_2 fixation with no input of urine of 250 kg N ha^{-1} yr^{-1} (e.g. Ledgard and Steele, 1992; Menneer et al., 2003) we calculate N_2 fixation would decrease by 10% (25 kg N ha^{-1} yr^{-1}) on an annual basis, using the area covered by urine of 25% and the 38% percent decrease of total N fixed reported by Menneer et al. (2003). This reduction due to urine could be as high as 40% by accounting for the total area affected by urine compared with pasture receiving no urine.

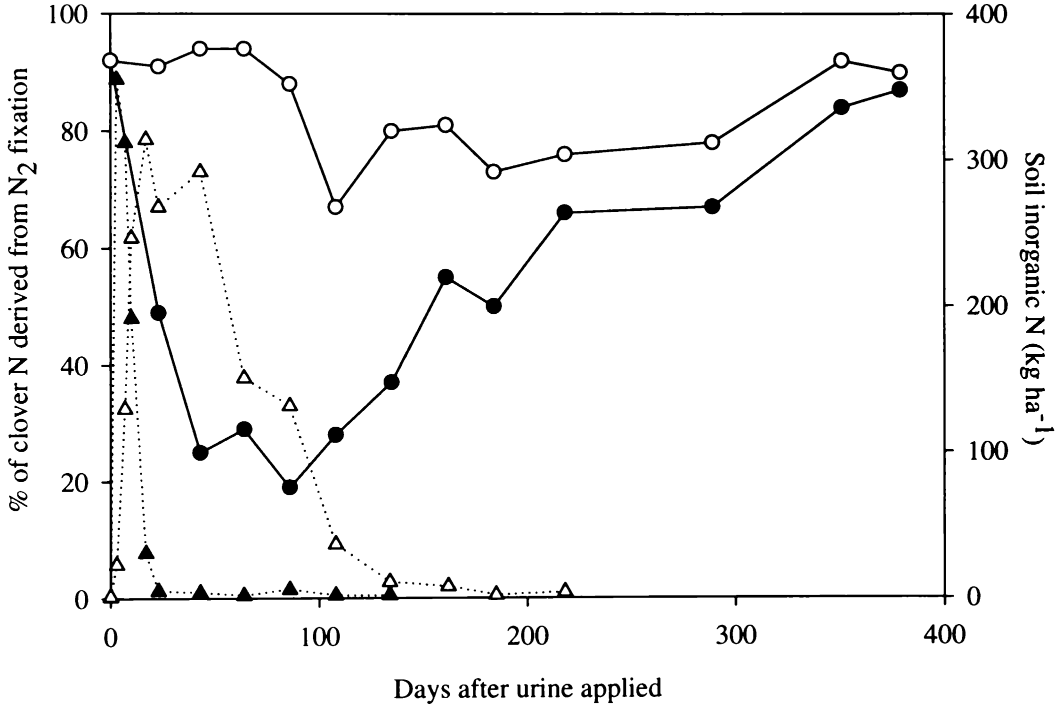

Figure 5. Effect of a single application of urine (740 kg N ha^{-1}) on N_2 fixation using the ^{15}N- labelling technique (•), compared to a non-urine control (○), and on soil inorganic NH_4^+-N (▲) and NO_3^--N (△), during a 12 month field study. (redrawn from Menneer et al., 2003).

V. Strategies to minimise the impacts of grazing animals

Optimising white clover performance and N_2 fixation by minimising grazing animal effects is only possible through judicious pasture and soil management and the implementation of effective farm management practices. This may require the adoption of special grazing management strategies (e.g. mixed grazing) and the integration of novel farm management practices (e.g. animal stand-off pads) to achieve the desired goal. However, a proactive approach such as this is highly dependent on the management skill and decision making ability of the grazier, and only by blending on-farm experience with off-farm support can informed decisions be made (Figure 6).

A. Pasture management to aid legume production

One of the key aspects of pasture productivity in mixed legume-grass swards is the need to adequately manage defoliation by grazing animals so legume performance and N_2 fixation are optimised. In farm systems the frequency and intensity at which livestock graze, their dietary preference/grazing attributes and the impact these processes have on legume performance can be regulated through strategic grazing or cutting management practices that overlie the principal grazing method employed (e.g. continuous versus rotational). Seasonal differences in the growth and development of legumes and grasses, and the subsequent effect this has on the legume-grass competitive interaction, require that grazing management strategies be flexible and suit the changing seasonal pattern of plant growth (Table 13). For example, recent work indicates that the sward condition of white clover (leaf area and plant size) during autumn is a key determinant of white clover content in the following spring (Fothergill et al., 1997, 2000; Luscher et al., 2001; Wachendorf et al., 2001a, b).

1. Late-summer and autumn pasture management strategies

At about mid-late summer, pasture management strategies can take advantage of declining grass tillering and maximum stolon development to

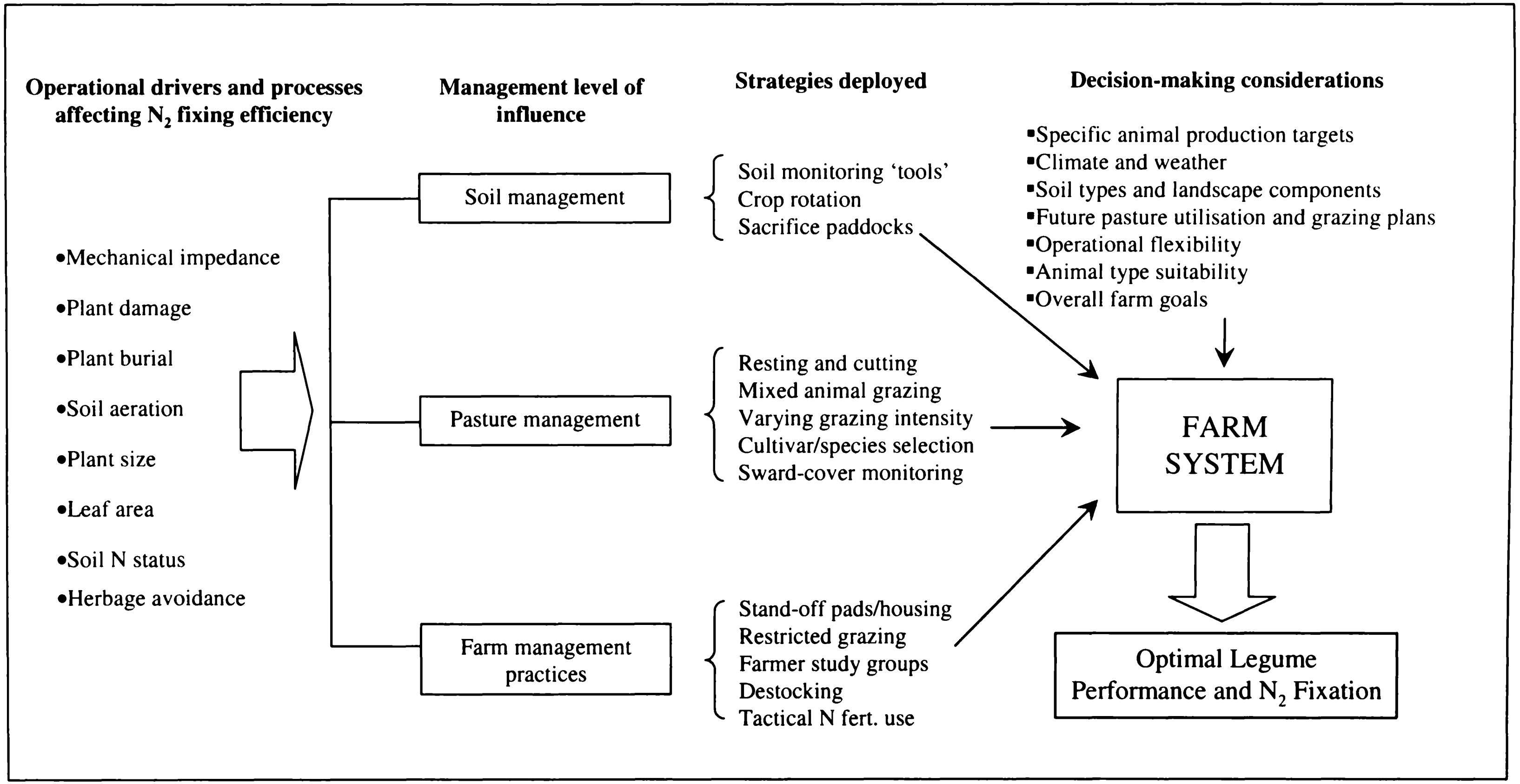

Figure 6. Farm management strategies and decision-based considerations for optimising legume-performance and N_2 fixation in mixed grass-legume based pastures to reduce detrimental impacts of grazing animals.

Table 13. Effect of seasonal timing of resting (conservation) on subsequent white clover abundance in mixed grass/legume pasture

References	Unrested control	Seasonal timing of conservation			
		Early spring	Mid-late spring	Late spring to early summer	Mid to late summer
White clover density[a]					
Gooding et al. (1996)	48	-	33	48	67
White clover content[b]					
Curl and Wilkins (1985)	-	-	31	37	40
Barthram and Grant (1995)	15	-	8	ns	40
Laidlaw et al. (1992)	15	22	-	-	-

[a] Number of 80 mm x 80 mm squares in which clover was present out of 100).
[b] White clover content (% DM basis).

Table 14. Yield of white clover grown with ryegrass after different spring grazing managements in a sheep system which is usually rotationally grazed[a]

Spring management	White clover yield (kg DM ha^{-1})	
	Summer	Annual total
Continuous	1865	2750
Grazing every 2 weeks	1500	2450
Grazing every 3 weeks	1165	2020
Grazing every 4 weeks	875	1820

[a] Data from Hay and Baxter (1984).

promote white clover presence in the sward (Laidlaw and Vèrtes, 1993). Relevant management strategies include a rest from grazing and a conservation cut in mid- or late-summer (Curll and Wilkins, 1985; Barthram and Grant, 1995; Gooding et al., 1996; Fothergill et al., 2000; Table 14), lenient grazing (Curll, 1982; Curll and Wilkins, 1982), and introducing mixed or alternate grazing by cattle rather than sheep only (Garwood et al., 1982; Gibb et al., 1989; Evans et al., 1992). All of these strategies can improve autumn white clover content, as well as reducing the chance of stolon exposure and 'burn-off' in drier climates (Brock and Hay, 1996). Implementing a late-summer silage cut also reduces the summer build-up of soil N in the root zone, thereby assisting with the benefit of increased white clover content in autumn. In addition, using a mid-late summer cutting strategy has the added gain of decreasing the potential for subsequent leaching losses of soil N during winter.

Although cutting of mixed clover-grass pasture removes a large proportion of white clover leaf tissue; growing points and newly developing leaves are much less affected than grass (Fothergill et al, 2000). This gives white clover a competitive advantage compared to the grass component which is slower to recover (depending on the season), and probably compensates for shading effects that occur during the initial resting phase of the cutting strategy (Fothergill et al, 2000). Establishing a high-quality white clover sward condition in autumn may help offset the winter period of stolon fragmentation and the reduced over-wintering capability of smaller plants into spring (Bouchart et al., 1998; Fothergill et al., 2000; Goulas et al., 2001). This may be critical if pastures are to be intensely grazed on a regular basis during the winter (e.g. Australia, New Zealand) when treading induced fragmentation can heighten the risk of plant loss.

2. Mid-spring to mid-summer pasture management strategies

From mid/late spring to mid-summer, grass growth is rapid and white clover is less able to compete for the upper layers of the mixed sward canopy (Woledge et al., 1990; Frame and Laidlaw, 1998). This is probably the reason for the

Table 15. Summary of grazing management strategies that provides either marginal (✓), good (✓✓), or significant (✓✓✓) gains in white clover performance

Grazing management	Strategic management technique	Seasonal timing	Effect on clover content	Reference
Continuous - sheep				
	Resting & cutting	Early summer	✓✓✓	Barthram and Grant (1994,1995)
	Resting & cutting	Early-mid summer	✓	Gooding et al. (1996)
	Resting & cutting	Late summer	✓✓✓	Curll and Wilkins (1985); Fothergill et al. (2000)
	Mixed grazing	All year	✓✓✓	Nolan et al. (2001)
Continuous - cattle				
	Hard grazing	Late spring to early summer	✓✓	Gibb and Baker (1989)
Rotational-cattle				
	Mixed grazing (sheep following cows)	All season	✓✓	Murphy et al. (1995b)
Rotational-sheep				
	Switch to rotational grazing	Spring	✓✓✓	Hay and Baxter (1984)
	Resting & cutting	late summer	✓✓✓	Sheldrick et al. (1993)

reported deleterious effect that resting for silage has on white clover content in late-spring or early-summer (see Barthram and Grant, 1995; Gooding et al., 1996). Because of increased grass growth at this time of year, grazing management becomes a critical factor in determining subsequent white clover content. Several New Zealand studies (e.g. Hay and Baxter, 1984) have found that continuous grazing (i.e. increased frequency of grazing) with sheep during this period, followed by rotational grazing for the remainder of the year has long-term benefits for white clover performance (Table 15). Similar advantages to white clover growth have been observed in Europe when grazing intensity has been increased to maintain low sward heights during spring and early-summer in mixed clover-grass pasture continuously grazed with cattle (Gibb et al., 1989; Teuber and Laidlaw, 1995). Intense grazing at this time of year was found to increase annual N_2 fixation by 33% in pastures in Argentina (Refi et al., 1989) and 10% in pastures in New Zealand (Brock et al., 1983). Increasing grazing intensity also increases competition between animals, lessens avoidance of grazing dung-affected areas and in doing so reduces shading of white clover by grasses. Furthermore, during late-spring and early-summer, soil has low susceptibility to pugging damage so high stocking rates are less likely to injure and/or bury stolon tissue.

B. Choice of white clover cultivar and companion grasses

1. White clover morphology

Even though white clover can adapt to defoliation through changes in plant size and density (as discussed in Sec.III.A.2), the degree of compensation is not always sufficient to maintain white clover productivity and persistence (Brock and Hay, 1996). This has led to a large research effort to produce cultivars that cover a range of morphology and has given farmers a variety of white clover cultivars from which to choose with improved suitability for different farm systems. Schematically presented in Figure 7 are guidelines for choice of suitable white clover cultivar in relation to the farm system. In general, the choice of cultivar is governed by their adaptability to particular grazing and cutting managements (e.g. Evans et al., 1992).

Under grazing, reduced white clover yield is usually due to removal of stolons which affect foliage growth, and so differences in branching ability of cultivars is important, particularly under frequent grazing. With the frequent defoliation of continuous grazing, smaller-leaved cultivars with their higher branching and growing point capabilities are more persistent and produce better than larger-leaved cultivars which have fewer stolons and a lower stolon density (Table 16). In contrast, the infrequent defoliation of rotational grazing favours production and persistence of larger leaved cultivars, which can compete with grasses for light as the sward height increases during the resting interval. However, the superiority of larger leaved cultivars under rotational grazing cannot be taken for granted, at least with sheep (Brock and Hay, 1996). In this case, selective grazing of large leafed cultivars which have fewer stolons reduces the capacity of plants to regenerate and persist.

Current work in selective breeding programs is starting to improve branching density while maintaining a particular leaf size and this is showing signs of increasing white clover productivity and persistency under rotational sheep grazing (e.g. *Trifolium repens* "Kopu II" and "Crusader"; Woodfield et al., 2001). A difficulty with small leaved cultivars is the lengthy time required for establishment in the sward and low initial white clover contents, but this can be overcome by blending small- and medium-leaved cultivars, thus increasing white clover yield in the establishment year (Evans et al., 1992).

2. Grass species and cultivar growth attributes

The choice of grass species and variety is also an important consideration given that differences in growth characteristics between grass types may influence the competitive interaction of the grass-white clover association. In particular, with ryegrass the lower tiller density of tetraploid versus diploid cultivars, and differences in the seasonal growth pattern between some types is worthy of consideration. Although studies are limited and restricted to sheep grazing, results have shown improved white clover growth when grown with early maturing ryegrass cultivars rather than late maturing cultivars, and with tetraploids rather than diploids (Swift et al., 1993; Gooding et al., 1996; Sanderson and Elwinger, 1999). These advantages stem from the timing of white clover's growth peak

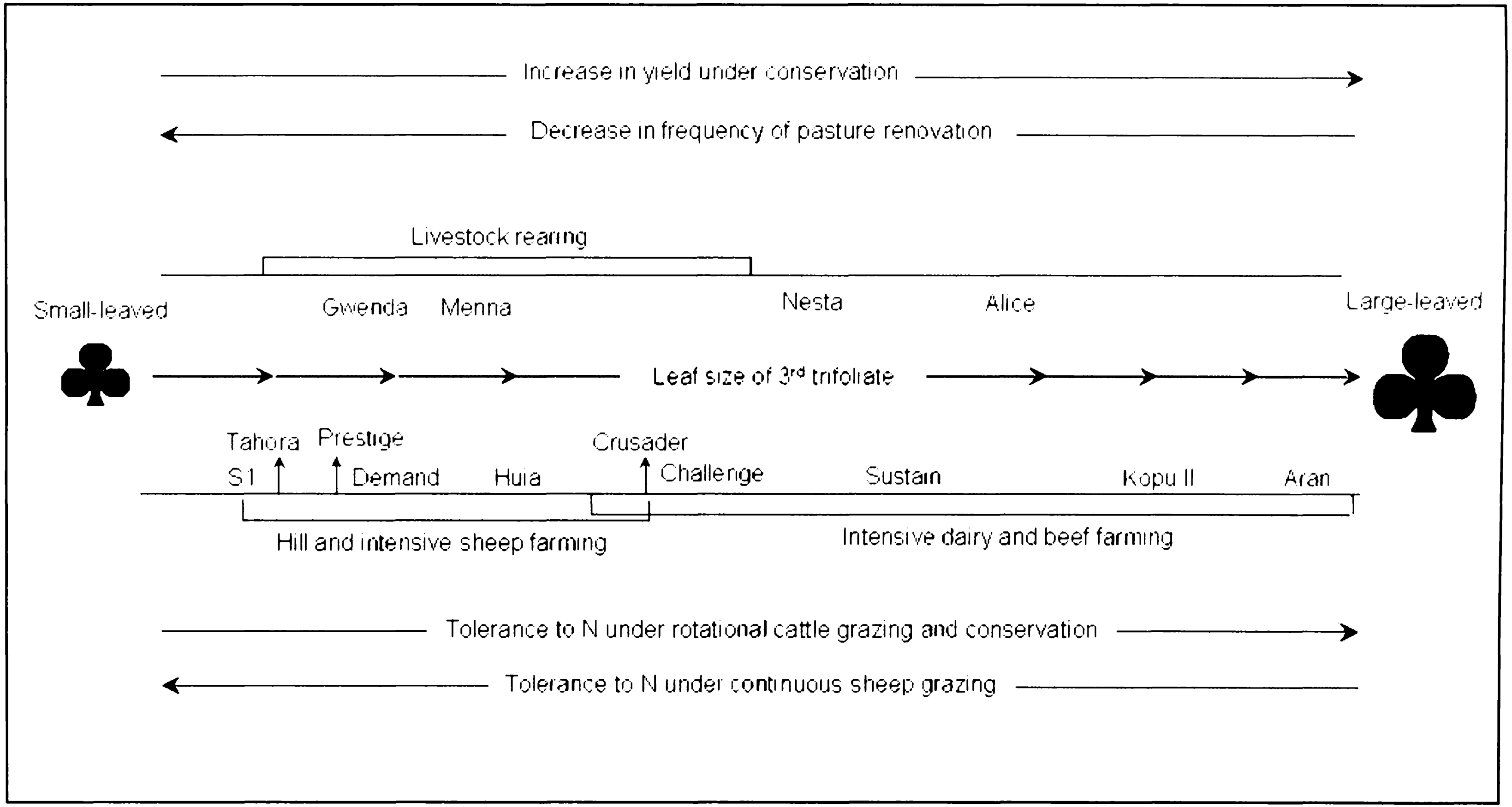

Figure 7. Guidelines for choice of white clover cultivar for farm systems (updated from Evans et al., 1992).

(in late season) with the less vigorous phase of ryegrass growth, and the greater openness of the sward for providing more space and light when using tetraploid ryegrass cultivars. Sowing ryegrass in new pasture at or below the recommended rate (c. 12 kg ha^{-1}) can also aid establishment and early growth of white clover.

Chestnutt and Lowe (1970) comprehensively reviewed the relative contribution made by clover when grown in association with a range of different grasses. The least compatible companion grass for clover was *Dactylis glomerata*, while *L. perenne* and *Festuca pratensis* were the most compatible. Subsequent work has shown that the various fescue species and cultivars (e.g. *F. pratensis*, *F. rubra* and *F. arundinacea*) as well as *Phleum pratense* and *Cynosurus cristatus* are often more compatible with clover than ryegrass (e.g. Pederson and Brink, 1988; Frame, 1990, Gooding and Frame, 1997). However, compared to ryegrass, fescue species typically lack persistence in pasture and *P. pratense* is less productive. Notwithstanding, these species are often grown in mixed legume-grass pastures where regional climate conditions limit the performance of ryegrass. For example, *F. arundinacea* is deeper rooting than ryegrass and is therefore more productive in dry summer conditions (Stevens and Hickey, 2000), whereas, *P. pratense* is a winter-active grass species more suited to moist soils in cool-temperate regions (Caradus, 1978, 1988; Maunsell and Scott, 1996). When either of these species has been grown in association with clover, the clover component is still able to make a significant contribution to both pasture production and animal performance (e.g. Ayres et al., 2000; Hyslop et al., 2000).

Compared to tillering grass types, stoloniferous and rhizomatous grasses have generally been shown to be counterproductive to clover performance when grown as its sward companion (e.g. Brougham, 1978; Frame, et al, 1990). Both *Agrostis stolonifera* and *Holcus lanatus* form a dense vegetative cover close to the ground and compete vigorously for light and space against clover (Brougham, 1978; Turkington et al., 1979; Frame, et al, 1990; Barthram, 1997). However, this effect is undeniably influenced by pasture management. Research (Stringer, 1997) with the stoloniferous grass species *Cynodon dactylon* showed that clover content was

Table 16. Effect of leaf size in different white clover cultivars on clover content of swards under continuous or rotational grazing, both at 22.5 ewes ha^{-1}[a]

Cultivar	Leaf size description	Individual leaf area (mm^2)		Clover content of pasture (% DM)	
		Rotational grazing	Continuous grazing	Rotational grazing	Continuous grazing
Grassland Tahora	Small	209	130	13.3	20.8
Grasslands Huia	Intermediate	275	115	11.0	13.1
Grasslands Pitau	Large-intermediate	408	130	15.1	7.0
Grasslands Kopu	Large	558	166	19.5	7.3
$LSD_{0.05}$[b]		35		2.8	

[a] Data from Brock (1988).

[b] Least significant difference of the mean at a $P<0.05$.

markedly increased as sward heights were reduced. This highlights the potential that grazing management can have on manipulating the legume-grass competitive interaction with grass species that are often considered incompatible with clover.

In general, the utility of combining different ryegrass or grass types and white clover cultivars needs further evaluation under new farm system managements and the implications for white clover performance more fully assessed. Furthermore, as new grass cultivars with differing vigour and persistence become available further testing of their compatibility with clover will be necessary (e.g. Pederson et al., 1999).

C. Tactical use of N fertiliser

An overwhelming amount of evidence exists showing the effects of N fertiliser on pasture production *per se*, as well as white clover performance and N_2 fixation (e.g. Curll et al., 1985b; Evans et al., 1992; Ledgard et al., 1996b; Elgersma et al., 2000). Typically, where N fertiliser is used, total pasture production is increased because of increased grass yield while that of the white clover component declines and N_2 fixation decreases markedly (Table 17). In some studies (e.g. Ledgard et al., 1996b; Table 17), the direct effect of N fertiliser on reducing N_2 fixing activity is largely responsible for causing the loss in annual fixed N (e.g. up to 60%) with reductions in white clover yield contributing to a far lesser extent. However, this is not always the case and in other studies (e.g. Frame and Boyd, 1987a; Nesheim et al., 1990; Elgersma et al., 2000), depending on the level of N application, white clover yield losses of between 50-80% have been observed within a year of frequent N fertiliser use, and can therefore be a major contributing factor in reducing the amount of N fixed in these systems. The reason for declining white clover growth under fertiliser N is due to a combination of factors brought about principally by competition for light and nutrient resources from its more vigorous and upright-growing grass associate. These factors include reduced photosynthesis, a reduction in growing point densities because of diminished assimilate allocation and stolon branching, and possibly increased competition for soil nutrients (Hoglind and Frankow-Lindberg, 1998; Laidlaw and Withers, 1989).

Table 17. Effect of N fertilization on annual white clover production, content, and N_2 fixation in a mixed legume/grass pasture rotationally grazed by sheep[a]

N fertilizer treatment (kg DM ha^{-1} yr^{-1})	White clover production (kg DM ha^{-1} yr^{-1})	White clover content (%)	Proportion of clover N fixed[b] (%)	Total N fixed[c] (kg N ha^{-1} yr^{-1})
0	3602	28	58.4	111
390	2974	19	33.4	47
SED	196	NR	2.6	9

[a] Data from Ledgard et al. (1996b).
[b] Estimated using the ^{15}N isotope method.
[c] Fixed N in herbage.
NR=not reported.

Table 18. Effect of spring and/or autumn N fertilization on white clover content (% DM basis) in a white clover/grass sward under mowing (mean of 3 years)[a]

Spring N application (kg ha^{-1})	Autumn N application (kg ha^{-1})			
	0	25	50	75
0	48	48	41	45
25	43	36	40	36
50	38	33	33	31
75	34	27	29	27

[a] Data from Frame and Boyd (1987b).

While the repetitive use of N fertiliser is often reported to be detrimental to white clover performance, some studies have shown that even under high regular N fertiliser applications white clover content can be maintained, but only if sward management prevents shading from the grass component (Frame and Boyd, 1987a). For example, in pasture grazed by dairy cows, Barr (1996) and Harris and Clark (1996) reported that under high N fertiliser use (up to 400 kg N ha^{-1} $year^{-1}$) white clover can persist and contribute usefully to production, provided the additional grass grown is fully utilised. In view of the significant direct effect of N fertiliser on N_2 fixation, maintaining a sward with high clover content but of greatly diminished N_2 fixing capability is of limited advantage from an N_2 fixation efficiency standpoint. To overcome this drawback the tactical use of N fertiliser is often advocated, particularly at times when rates of legume growth and N_2 fixation are low. If the tactical use of N is paired with specific grazing management strategies, it could provide the best compromise in maintaining optimal pasture production without substituting N fertiliser for N_2 fixation or jeopardising the long-term performance of white clover.

It is common for farmers to apply N fertiliser in early-spring to boost early-season grass production. Unfortunately, this tactic can have a negative effect on white clover performance (Frame and Boyd, 1987b), unless grazing management strategies are used to control subsequent grass growth and minimise shading effects on white clover. More recently, studies in the northeast USA (Stout and Weaver, 2001; Stout et al., 2001) predicted that a single application of fertiliser N (45 kg N ha^{-1}) in spring gave the largest gains in pasture production without compromising clover growth or N_2 fixation. This was provided that a target sward harvest height of 15 cm was not exceeded, since above this height clover content declined rapidly. Alternatively, applying N fertiliser in autumn seems to have less effect on white clover content than in spring (Table 18).

Thus, to realise the full potential of N_2 fixation in mixed ryegrass-white clover pastures, N fertiliser use should be minimised or strict grazing/cutting management strategies used to control total herbage height, particularly after spring-applied N.

VI. Farm-scale management practices

Within the farm system a number of opportunities exist for improving farm management practices and increasing legume performance and N_2 fixation in mixed legume-grass pastures. Generally, changes at the farm system level involve integrating new management practices with an increased awareness of the limitations and optimal utilisation of plant and soil resources. This is best carried out by using 'quantifiable assessment tools' to evaluate pasture and soil conditions, and by engaging the assistance of other farm colleagues and off-farm expertise. Because animals are an intrinsic part of any grazing system, realistic management options are required which do not compromise the business goals of the farming enterprise.

A. Soil management: Preventing treading and compaction

In farming systems, good soil management should aim to create optimal physical conditions for legume growth through preventative management strategies. To minimise treading damage to soils a sound knowledge is required of the different landscape units and soil types that comprise the farm and their susceptibility to structural damage in wet conditions. Information such as this should be incorporated into far-sighted grazing management plans to ensure restricted or zero grazing of vulnerable areas is easily implemented when conditions necessitate, without placing undue strain on other farm activities. Additionally, in pasture that suffers from waterlogging in winter, improving the drainage of the soil, for example by mole/tile draining, also reduces the likelihood of treading damage to soil (e.g. Davies and Armstrong, 1986).

Farm systems that include areas for forage cropping (e.g. maize silage) could provide an opportunity for renovating compacted pasture soils through breaking-down compacted layers, increasing soil aeration, and by providing an extended rest from animal grazing impacts. For this strategy to be effective the forage crop should be rotated and tillage only performed when soil moisture conditions are ideal, so to avoid any additional compaction by agricultural machinery and to optimise soil rejuvenation. A further benefit of legume growth and N_2 fixation would be incurred after pasture resowing as a result of soil N depletion during the cropping phase, and reduced insect pests (e.g. clover nematodes, Yeates, 1977; Yeates and Hughes, 1990).

Recently, indicators of soil physical condition have been developed (e.g. macroporosity, penetration resistance, microbial biomass, worm counts, e.g. Ditzler and Tugel, 2002; Drewry et al., 2002) and can be used on-farm alongside soil fertility measures to indicate limiting conditions for plant growth. For example in New Zealand some current research has resulted in a "macroporosity test" for on-farm use to determine limiting soil physical conditions for pasture yield (Drewry et al., 2001, 2002). This work is still in its infancy and requires further calibration across a range of soil types and farm management practices before it can be widely used.

B. Restricted grazing and supplementary feeding in winter/spring

An effective method of reducing treading damage to pasture in wet conditions is the practice of restricted grazing and supplementary feeding. This concept requires farmers to identify the risk of damage to pasture at key times during winter/spring and remove livestock from paddocks and onto an area(s) designated for animal stand-off and supplementary feeding. Alternately, animals may graze for 4-6 hours and then be put on a stand-off area for the remainder of the day. In dairy systems, stand-off areas may be highly sophisticated feed-pad systems such as 'herd houses' that are entirely covered, have concrete pads and collect and contain effluent in sub-surface storage tanks for later disposal. In practice, simpler uncovered feed-pads with either a concrete pad or a bark/saw dust pad on a road metal or lime base with drainage of effluent to storage ponds are more common in Australia (e.g. Moran and Wamungai, 1993) and New Zealand. Even less complicated is the practice of standing-off cows in races/lanes for part of a day during wet soil conditions. In sheep and beef farm systems a more rudimentary approach is sometimes taken in wet periods with animals stocked on a 'sacrifice paddock' and fed hay. This approach contains treading damage to one area of the farm rather than causing indiscriminate damage to larger areas. This strategy has largely been replaced in current times by the more deliberate method of destocking for winter and keeping livestock off paddocks that are susceptible to treading damage.

In New Zealand, the use of stand-off pads (cf. feed-pads) on dairy farms is becoming increasingly common, and gives the farmer the option of 'on/off' grazing during winter and early-spring when ground conditions may be overly wet. The extent of feed pad use varies with climate. In milder regions intermittent use in winter or early-spring is often all that is required to prevent treading damage to pasture. In wetter-cooler regions, feed-pads are sometimes occupied for extended periods in winter (e.g. 75 days in Southland, New Zealand) because of limited pasture growth and the greater potential for damage by treading. In Europe with the current move towards extended grazing in some milder regions similar tactics to those above could be applied. That is, using an extended but restricted or partial grazing regime through the winter in conjunction with cow housing and supplementary feeding. This strategy would minimise winter damage to pasture and have the added benefit of keeping sward heights in check to favour white clover growth and productivity for spring grazing.

Despite the increasing popularity of feed-pad use in Australasia, there is little research on the benefits of these systems on pasture productivity let alone white clover productivity. One Australian study (Moran and Wamungai, 1993) using partial restrictions to grazing during winter (6 hours per day) and supplementary feeding with maize silage on a feed-pad gave improvements in pasture quality (increased subterranean clover content) while still maintaining a high winter stocking rate and milk production. Other work by de Klein (2001) estimated that zero grazing for an extended period in winter (123 days) while supplying cut pasture to cows on a feed-pad could increase annual pasture production by 2-8%. However, this estimate only considered the effect of better utilisation of applied effluent collected from the feed-pad; the beneficial effects from lowering the impact of other grazing animal factors (e.g. treading and defoliation, Sec. II and III, respectively) were not considered and could arguably further boost this value. Furthermore, effects on white clover productivity were not considered in the de Klein (2001) study, but presumably the frequent cutting of pasture for feed-pad supply would be advantageous for white clover, giving the potential for higher white clover content in spring swards and increased N_2 fixation (Bax and Thomas, 1992; Roberts et al., 1989).

Schwinning and Parsons (1996b) estimated that white clover can take advantage of sward patchiness and areas of low soil N in grazed pasture for growth and survival. This behaviour of white clover allows it to escape light restrictions caused by shading of N stimulated grass. Substituting grazing with cutting and mechanically applying animal effluent to pasture both create a more even spatial distribution of soil N and therefore improved sward height uniformity, which eliminates opportunities for white clover to escape light limiting situations. To compensate for this, keeping pasture cover low during periods of nil grazing by using a frequent/hard cutting management regime would improve light penetration to the lower sward canopy and probably assist in white clover growth and persistence.

While the few studies reviewed here allude to the benefits of partial or zero grazing in winter, the increasing use of feed-pad systems necessitates further research with a broader investigative approach to consider the additional benefits for grass and legume that might occur through reduced treading and defoliation impacts, as well as by the improved utilisation of animal excreta. This future work should take into account the effect of variations in feed-pad use (intermittent, partial, or extended) brought about by differences in regional climatic conditions together with a fuller assessment of their economic viability.

Table 19. The results of a workshop at a New Zealand dairy farm study group documented the understanding, management and outcomes developed to prevent winter pugging[a]

Observations	Management strategies	Management options	Overcoming implications	Outcomes
1. Soil condition prior to grazing in the wetter months of: June, July, August, September, October. 2. Soil moisture content - How soft is the ground? - Is the area waterlogged? - Is water ponded on the ground surface? 3. Know the topography of the paddock. The above three give the basic paddock information and should be used in conjunction with 4 and 5. 4. Actual and forecasted weather, particularly previous rainfall. Also, wind speed and direction in combination with actual rainfall. 5. Grass colour i.e. dirt on grass within the area grazed beyond acceptable levels.	1. Recognise the potential for damage. 2. Grade paddocks according to their susceptibility to damage. Followed by one or more of the following options: a) Implement On/Off grazing using: Races, cow sheds, dry peat land or a sacrifice paddock. b) Grazing off farm, or carrying feed to cows on a stand-off pad. c) Decrease stocking pressure by using quicker rotation, short-term only. Increase the area cows are grazing e.g. no break feeding or use larger breaks.	1. ON/Off grazing: This will minimise damage and is convenient, beneficial, cheap, and allows easy feed rationing. Implications: Cows get sore feet, damage to races, possible loss of cow condition, damage to gateways and fences, labour intensive. 2. Grazing either off farm or use of a stand-off pad: no pugging. Implications: Going off farm is expensive, and depends on land availability and results in loss of control of cow condition and stock management. On farm pad has a high labour input of feeding, requires buying or making large quantities of feed, effluent has to be spread. 3. Speeding up rotation: Short-term for 1-2 days. Early in winter it is a quick short-term solution. Implications: Uses too much feed. Eating into reserve feed is the key point. Have reduced grass cover.	Put in longer hours and be prepared to change priority on your time. Short-term, take care and shift cows slowly, long-term do more repairs and maintenance. Keep yard clean and free of debris. Use a foot bath and put copper sulphate on the races or on mats. Separate and treat cows with severely sore feet. Restrict the movement on races to a small area. Research the cost of grazing off farm and supplements on farm. Forward planning to control sward heights at home.	Time well spent. Short term, there was less cost. Long term no negative effects. The problem was perceived to be greater than that which eventuated. No effect on cow condition. Success -no permanent damage, sore feet healed quickly without long term negative effects. Increase the area: Keep it short term, a few days at a time, and consider nitrogen as an option.

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[a] Data from Tarbotton et al. (1997).

C. Technical based decision making for improved management

In farm systems, management skill and decision making have a great bearing on white clover performance, N_2 fixation and overall pasture productivity. To assist the farmer in making accurate knowledgeable decisions regarding the legume productivity component of farm management a number of useful 'tools' and off-farm interactions (e.g. farmer study groups) offer options for optimising clover performance and N_2 fixation (Figure 6).

Participatory farmer study groups are a relatively recent concept (last 5 to 10 years), but have been successful in addressing complex farm system changes and environmental problems where expert-only driven approaches have failed (e.g. Engel, 1991; Okali, 1994). Typically, these study groups involve linking farmers, scientists, and policy agents to provide a broad-base of knowledge and experience to tackle on-farm issues and to facilitate technology transfer. In New Zealand, for example, a study group in a predominantly dairying region was formed in 1995 to identify relevant on-farm issues and determine ways of dealing with these (Tarbotton et al., 1997). The study group, comprising 8 dairy farmers, a farm systems analyst, a soil scientist, and 2 policy agents, soon identified pugging by dairy cows as causing considerable damage to pasture during winter and spring (up to 10% area affected on one farm). Using the pooled knowledge and expertise of all the group participants, a number of management strategies and options were identified to prevent winter and spring pugging (Table 19). These were successfully implemented and have achieved the desired outcome of greatly reducing pugging and damage to pasture (Tarbotton et al., 1997).

The study group is now in its 7th year and has continued to use the participatory process approach to solving on-farm problems, one of which is seeking to find ways of improving white clover performance in white clover based pastures (Bateup et al., 2001). Although multi-disciplinary on-farm study groups are not widespread the application of the group discussed here highlights what can be achieved by bringing together individuals having a breadth of experience and expertise. In Europe, some farmers are recognising the value of such an approach and have called for a greater participation of agricultural scientists and farm advisors in farm discussion groups, especially in the light of some recent changes to farm system operations (e.g. a move to grazing based dairy systems and extended grazing) (John and Bird, 2000). The challenge for these study groups will be in dealing with the multiple facets of farm optimisation under a new farm system, for example, livestock management in winter, pasture utilisation throughout the year, and white clover performance and N_2 fixation.

Some simple and inexpensive tools which aid decision making are available for farm-use. These include: soil tests (e.g. nutrient status, macroporosity, and penetration resistance), pasture yield meters (e.g. rising plate meters), recording on-farm meteorological information, and weather forecasting (short and long-term). In conjunction with other farm decision support mechanisms (e.g. practical computer models, advisors, study groups, and tacit knowledge) these tools, if used effectively, will further enhance the potential for optimising legume content as well as farm productivity. For example, adopting a standardised method of pasture herbage assessment (e.g. rising plate meter) provides for greater consistency of yield information and gives confidence in making reliable grazing management decisions for better utilisation of pasture (Lile et al., 2001). Inclusive to this decision making process should be all the key pasture production elements, including that of optimising legume performance and N_2 fixation. In achieving this, careful thought needs to be applied in deciding on which type of pasture management strategies are best utilised and their appropriate timing of implementation (e.g. seasonal timing of certain grazing regimes, duration of rest interval, and timing of silage cuts). Using an approach like this is especially useful where certain farm objectives (e.g. finishing lambs in autumn or increasing milk production in summer) are known to be benefited by swards containing high clover content (Askin et al., 1987; Harris and Clark, 1996; Papadopoulos et al., 2001; Wu *et al.*, 2001; Woodward et al., 2001). Thus, specifically targeting high legume contents on some parts of the farm to meet these objectives is likely to be most advantageous for farm productivity. Indeed, new research is examining the potential of growing grass and clover separately within the same paddock, to achieve strategic goals of high clover content for increased clover intake and animal production (e.g. Cosgrove et al., 2003; Marotti et al., 2002).

VII. Summary and conclusions

In this review the impacts of treading, defoliation, and excretion by grazing animals on the efficiency of N_2 fixation have been considered along with preventative management options to improve legume performance in mixed legume-grass pasture systems. Grazing animal activities have a significant impact on the efficiency of N_2 fixation, which is mediated by large-scale changes to legume morphology and physiology both at the individual plant level and/or by influencing the legume-grass competitive interaction. The magnitude of animal impacts, both individually and as a whole, vary greatly and are closely tied to farm management practices and the edaphic features of the entire farm system.

Future research focus should include increasing the competitiveness of white clover for light and space resources, and further evaluation of companion grasses with attributes such as lower tillering and earlier maturity so as to desynchronise their growth peaks and provide gap opportunities for clover exploitation.

Excretion by grazing animals has a major effect on the efficiency of N_2 fixation by altering soil N status. This in turn directly affects N_2 fixing activity for extended periods, or operates through reducing clover productivity from shading by grasses if grazing management allows. Exploring the potential for use of clover cultivars that can continue high N_2 fixation under elevated soil N status is tempting, but flow-on effects to N cycling would first need to be considered, in particular the likelihood of increased N losses to the environment.

With regard to treading and soil compaction, more detailed research is needed to determine the broader and longer-term implications of impacts on N_2 fixation and clover productivity in the field. Indications are that if soil aeration is limited by compaction over extended periods then white clover may be able to compensate by utilising cellular and structural adaptations to adjust to low O_2 levels. Damage and burial of plants by treading is also of considerable importance and appears to be a key factor in determining successful overwintering and subsequent spring white clover content in clover-grass associations. In farm systems where animal intensity is high but defoliation pressure is relatively low (due to supplementary feeding) treading damage may have a greater relative effect on white clover performance than defoliation and excretion processes. The use of models may provide the best means to unravel the complexity of treading processes, their interaction with defoliation and excretion, and the overall response of the legume system.

By far the greatest means of practical improvement of N_2 fixation efficiency in legume-based pasture systems is by evaluating the management decisions of the farm system and identifying alternate pasture management strategies or farm management practices to achieve optimal legume presence and N_2 fixation in the sward. These management strategies may include: varying seasonal grazing intensity, mixed animal type grazing, strategic silage cuts, and removal of animals from pasture in wet conditions. Future research will need to develop guidelines that facilitate the adoption/integration of these strategies and other novel approaches into different farm systems. In addition, research should more clearly define the potential economic and environmental advantages from greater reliance on N_2 fixation technology. Farmer involvement in collaborative study groups which blend together the expertise of farmer, scientist, farm advisor and other industry specialists in a participatory team process will provide the best opportunity to realise these goals and achieve improved legume performance and N_2 fixation.

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3

Effects of Urine and Defoliation Intensity on N_2 Fixation

3

The effect of a single application of cow urine on annual N_2 fixation under varying simulated grazing intensity, as measured by four ^{15}N isotope techniques[1]

Keywords: light defoliation, moderately-severe defoliation, N_2 fixation, urine patches, white clover, ^{15}N isotope dilution

I. Abstract

The effects of dairy cow urine and defoliation severity on biological nitrogen fixation and pasture production of a mixed ryegrass-white clover sward were investigated over 12 months using mowing for defoliation. A single application of urine (equivalent to 746 kg N ha^{-1}), was applied in late spring to plots immediately after light and moderately-severe defoliation (35 mm and 85 mm cutting heights, respectively) treatments were imposed. Estimates of percentage clover N derived from N_2 fixation (%Ndfa) were compared by labelling the soil with ^{15}N either by applying a low rate of ^{15}N-labelled ammonium sulphate, immobilising ^{15}N in soil organic matter, adding ^{15}N to applied urine, or by utilising the small differences in natural abundance of ^{15}N in soil.

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[1] Menneer J C, Ledgard S F, McLay C D A and Silvester W 2003 The effect of a single application of cow urine on annual N_2 fixation under varying simulated grazing intensity, as measured by four ^{15}N isotope techniques. Plant and Soil 254, 469-480.

Urine application increased annual grass production by 85%, but had little effect on annual clover production. However, urine caused a marked decline in %Ndfa (using an average of all ^{15}N methods) from 84% to a low of 22% by 108 days, with recovery to control levels taking almost a year. As a result, total N fixed (in above ground clover herbage) was reduced from 232 to 145 kg N ha^{-1} y^{-1}.

Moderately-severe defoliation had no immediate effect on N_2 fixation, but after 108 days the %Ndfa was consistently higher than light defoliation during summer and autumn, and increased by up to 18%, coinciding with an increase in growth of weeds and summer-grass species. Annual N_2 fixation was 218 kg N ha^{-1} yr^{-1} under moderately-severe defoliation compared to 160 kg N ha^{-1} yr^{-1} under light defoliation.

Estimates of %Ndfa were generally similar when ^{15}N-labelled or immobilised ^{15}N were used to label soil regardless of urine and defoliation severity. The natural abundance technique gave highly variable estimates of %Ndfa (-56 to 24%) during the first 23 days after urine application but, thereafter, estimates of %Ndfa were similar to those using ^{15}N-labelling methods. In contrast, in urine treated plots the use of ^{15}N-labelled urine gave estimates of %Ndfa that were 20-30% below values calculated using conventional ^{15}N-labelling during the first 161 days. These differences were probably due to differences in the rooting depth between ryegrass and white clover in conjunction with treatment differences in ^{15}N distribution with depth.

This study shows that urine has a prolonged effect on reducing N_2 fixation in pasture. In addition, defoliation severity is a potential pasture management tool for strategically enhancing N_2 fixation.

II. Introduction

In many temperate pasture systems, white clover (*Trifolium repens* L.) is a valued legume, not only for its high nutritional feed value, but also for the key role it has in providing nitrogen (N) to pasture through biological N_2 fixation. Estimates of the annual contribution of fixed N to pasture summarised by Ledgard and Steele (1992) are in the range of 55-296 kg N ha^{-1} $year^{-1}$. This broad range of values to some degree relates to differences in environmental and site-dependent factors, but is primarily due to the effect of pasture management and soil inorganic N status on clover growth and N_2 fixation.

In dairy farm systems intensive grazing may remove up to 80% of the total above ground foliage (Clark and Penno, 1996), and has the potential to decrease amounts of N_2 fixation in pasture. Defoliation has been found to decrease nitrogenase activity within several hours (Moustafa et al., 1969; Chu and Robertson, 1974; Ryle et al., 1985), with recovery anywhere from 5-21 days depending on the severity of defoliation.

In addition, in intensively grazed systems about 75-95% of ingested N is excreted, mostly in urine (~75% of the total excretal-N); N concentration in a urine patch may be the equivalent of 1000 kg N ha^{-1} (Haynes and Williams, 1993; Whitehead, 1995). The direct effect of urine N is to decrease N_2 fixation by up to 90% (Ball et al., 1979; Ledgard et al., 1982) within 2-10 days. Studies using both the acetylene reduction assay and ^{15}N isotope dilution techniques (Ball et al., 1979; Harris and Clark, 1996; Vinther, 1998; Ledgard et al., 1996) have reported full recovery of N_2 fixation after urine N or fertiliser N application by 30-120 days. Although the short to medium term effect of defoliation and urine N on N_2 fixation activity is well documented, insufficient information exists regarding the effect of these factors on the total fixed N contribution to pasture.

The most effective methods for measuring N_2 fixation in pasture systems involve the use of the ^{15}N isotope dilution and natural abundance methods

(Ledgard and Steele, 1992). The natural abundance technique depends on the small natural enrichment in ^{15}N concentration of soil N relative to atmospheric N_2, whereas with the ^{15}N dilution technique these differences are enhanced by the addition of a low rate of ^{15}N-enriched fertiliser.

An important assumption in the use of the ^{15}N isotope dilution technique, is that both the legume and the non-fixing reference plant must assimilate N from the ^{15}N-label and the indigenous soil N in the same ratio (Ledgard et al., 1985a; Witty, 1983). Erroneous estimates of N_2 fixation usually only occur if (a) the legume and reference plant differ in their temporal pattern of N uptake in association with a decreasing soil ^{15}N enrichment with time, or (b) the legume and reference plant roots uptake N at different depths and this corresponds with differences in ^{15}N enrichment of the plant-available soil N (Chalk, 1985). In intensively grazed systems, defoliation severity and urine N could potentially affect the key ^{15}N methodology assumptions.

In pasture systems, the natural abundance technique generally shows little variation in ^{15}N soil enrichment of the plant-available soil N over time and with soil depth compared to the^{15}N dilution technique where soil is typically enriched by surface application of ^{15}N-enriched fertiliser (Hogberg, 1997; Ledgard et al., 1985b; Peoples et al., 1995). However, the technique does require that the $\delta^{15}N$ of the soil is significantly different to the $\delta^{15}N$ of legume N derived from atmospheric N_2 (B value), of which reported B values range between -1.4 and -2.0‰ for white clover (Ledgard et al., 1985b; Eriksen and Hogh-Jensen, 1998).

Steele and Daniel (1978) measured the $\delta^{15}N$ of urine from dairy cows in the range of -1.2 to -1.7‰ compared to the original pasture diet of 0.6‰. Thus, fractionation within the animal leads to urinary N being excreted at a lower ^{15}N concentration than that in the diet feed. Hence, where urine patches occur in dairy pasture the $\delta^{15}N$ values of ryegrass and clover may become more negative and possibly not significantly different to the B value, resulting in inaccurate estimates of N_2 fixation.

The aims of the research presented in this paper were (1) to establish the effect of urine deposition and defoliation severity on pasture growth and clover N_2 fixation, and (2) to compare estimates of N_2 fixation using ^{15}N natural abundance and three different ^{15}N dilution techniques.

III. Materials and Methods

A. Experimental site and soil characteristics

The experimental soil was a Horotiu silt loam (Aquic Hapludand, Soil Survey Staff, 1994; Typic Orthic Allophanic Soil, Hewitt, 1992). The pasture contained a long-term (>30 years) permanent mixed stand of perennial ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.). The pasture had been grazed by dairy cows, except during the previous 6 months when it had been cut for silage.

A basal fertiliser (80 kg P ha^{-1}, 75 kg K ha^{-1}, 110 kg S ha^{-1}) was applied, by hand, to satisfy plant nutrient requirements.

B. Experimental design

Treatments were either urine or nil-urine, and under either light or moderately-severe defoliation. A randomised block experimental design was used with 4 replicates. Plots were 0.8 m wide x 2.5 m long with a 0.5 m wide buffer strip around each plot. Two pre-conditioning harvests were taken from the site to reduce variability and for potential covariate analysis.

The ^{15}N natural abundance method and 3 different ^{15}N enrichment methods were used to estimate the effect of defoliation and urine on N_2 fixation: conventionally ^{15}N-labelled soil, ^{15}N-labelled+glucose soil, and ^{15}N-labelled urine. The ^{15}N-labelled soil treatments were enriched with ^{15}N by applying a solution of ^{15}N-enriched (80 atom %^{15}N) ammonium sulphate [$(NH_4)_2SO_4$] to the plots at a rate of 1 kg N ha^{-1}. In the ^{15}N-labelled+glucose treatment (which was applied to the urine treatments only), glucose and ^{15}N-enriched (80 atom $\%^{15}N$) $(NH_4)_2SO_4$ were dissolved in water to obtain a C:N ratio of 10:1 to stimulate the immobilisation of N in soil, before application to plots at a rate of 1 kg N ha^{-1}.

Plate 1. Urine was applied to plots at a rate of 746 kg N ha^{-1} which is similar to that deposited by a dairy cow in a single urine patch.

After the second preconditioning harvest (21 days prior to commencing the study) the $(NH_4)_2SO_4$ solutions were applied evenly to each plot as a 3 L volume using a watering can with a rosette attachment. Any $(NH_4)_2SO_4$ residue on the herbage was washed off with 3 L of tap water applied to the plots with a knapsack sprayer.

After 21 days, the site was again mown, herbage samples were retained for covariate analysis, and the defoliation and urine treatments were applied. Defoliation severity was defined by the height of standing biomass after mowing at two different levels, which were 80 mm and 35 mm and were equivalent to a spring grazing of light and moderately-severe defoliation, respectively.

The urine that was applied to urine treatments was collected during a 3 day period from dairy cows housed in metabolism stalls and fed a pasture diet. The urine was stored in a single tank at 4 °C prior to application on the third day. The total N concentration of the urine was 5.5 g L^{-1}.

Application of the urine occurred immediately after the defoliation treatments had been imposed (Plate 1). Urine was either unlabelled or labelled with a small amount (0.17 g L^{-1}) of ^{15}N-enriched urea to give an enrichment of 1.13 atom % ^{15}N. The slight increase of N in the ^{15}N-labelled urine treatments due to the addition of urea was compensated for by applying a lesser volume of the labelled urine to those plots. Thus, urine was applied to the plots at 13.5 and 13.1 L m^{-2} for the unlabelled and ^{15}N-labelled urine plots, respectively. This represented an application rate of 746 kg N ha^{-1}, which is similar to that deposited by a dairy cow in a single urine patch (Haynes and Williams, 1993).

C. Harvest and analysis

To determine the immediate effects of urine addition and defoliation severity on N_2 fixation, grab samples of herbage (8 per plot) were collected, bulked for separation into ryegrass and clover, at 3, 7, 10, and 17 days after urine application. The first full mowing harvest of entire plots at either 80 mm or 35 mm cutting height (light and moderately-severe defoliation) and species separation occurred 23 days after treatment application, and thereafter at approximately 21-day intervals for 12 months (Plate 2). Soil samples (0-150 mm; 6 cores per plot) were taken and bulked for NO_3^--N and NH_4^+-N determination at all harvest dates from 0 to 218 days after urine application.

Herbage samples were dried and analysed for total N and ^{15}N concentration using an automated N analyser (Europa Scientific ANCA-SL) interfaced to a Europa Scientific 20-20 continuous-flow stable isotope analyser.

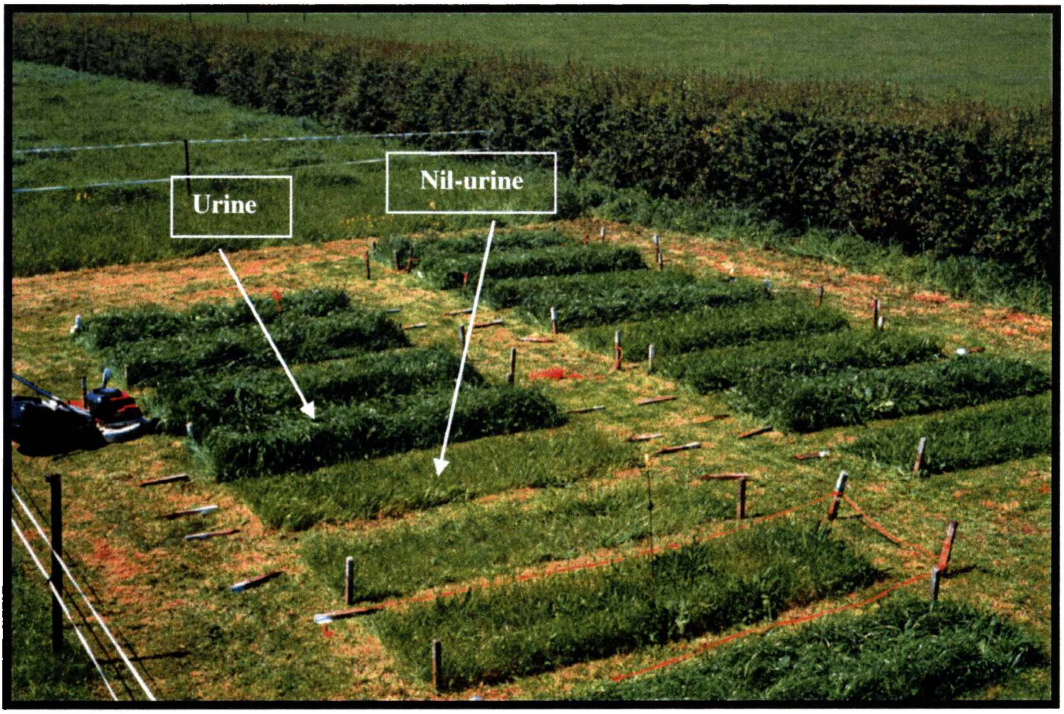

Plate 2. Field site showing the experimental plots and the response of pasture to urine application, prior to harvesting by mower.

D. Calculations of N_2 fixation

Calculation of the percentage of legume N fixed from atmospheric N_2 (%Ndfa) was as follows where the ^{15}N dilution technique was used (Ledgard and Steele, 1992).

$$\%\text{Ndfa} = 100 \times \left\{ \frac{\text{Atom } \%\,^{15}\text{N}_{\text{ryegrass N}} - \text{Atom } \%\,^{15}\text{N}_{\text{legume N}}}{\text{Atom } \%\,^{15}\text{N}_{\text{ryegrass N}} - \text{Atom } \%\,^{15}\text{N}_{\text{air N}_2}} \right\}$$

The same equation was used for the natural abundance technique, except that $\delta^{15}N$ values were used for calculation. An accurate value of the atom % of ^{15}N of N derived from air N_2 (B value) for the natural abundance technique was determined by comparison of estimates for %Ndfa obtained by enriched ^{15}N with those of the natural abundance method (Doughton et al., 1992). Adjacent control plots that had received no urine and were under the light defoliation treatment regime were used to compare %Ndfa at each harvest date. Values of %Ndfa estimated by natural abundance best matched those calculated by the ^{15}N enrichment method when the atom % of ^{15}N of N derived from air N_2 was 0.365932 atom % ^{15}N (B=-1‰). This B value is similar to the range of reported values (-1.4 and -2.0‰) for white clover (Ledgard et al., 1985b; Eriksen and Hogh-Jensen, 1998) and was used to calculate all estimates of N_2 fixation presented in this paper.

Recovery of urine N in plant tissue was calculated using the following equation:

$$\text{N recovery} = \left\{ \frac{\text{atom } \%\, ^{15}\text{N excess}_{\text{(plant)}}}{\text{atom } \%\, ^{15}\text{N excess}_{\text{(urine)}}} \times \frac{\text{total N}_{\text{(plant)}}}{\text{total N}_{\text{(urine)}}} \right\} \times 100$$

$$\text{Atom } \%\, ^{15}\text{N excess} = \text{Atom } \%\, ^{15}\text{N}_{\text{(plant or urine)}} - 0.365932$$

E. Statistical analysis

Analysis of variance was carried out using Genstat 4.2, Fifth Edition. $LSD_{0.05}$ was used to compare means, when main effects were found to be significant at the $P<0.05$ level.

IV. Results

A. Effects of urine on pasture production and N_2 fixation

Urine application increased annual grass dry matter production by 85% ($P<0.05$) in both light and moderately-severe defoliation treatments (Table 1). The increase in grass yield was mainly due to an initial rapid response to urine (up to 166% during the first 43 days) followed by a gradual decline to control levels after about 100 days (Figure 1a). However, under moderately-severe defoliation there was a second period of large response to urine through to day 135. In contrast, there was no significant effect of urine on annual clover dry matter production (Table 1). However, there was a significant ($P<0.05$) decline of up to 52% in clover growth after urine application, evident at days 64 – 108 under light defoliation (Figure 1b). A similar trend under moderately-severe defoliation was not significant.

Under urine the temporal pattern of clover growth closely corresponded to the changing pattern of the clover proportion in pasture (Figure 1c). In general, urine had a greater effect on lowering the clover proportion in pasture through time under light defoliation than under moderately-severe defoliation ($P<0.05$), and resulted in an overall reduction of annual average clover proportion in pasture in the urine plots from 44% to 33% (Table 1).

Table 1. Main effects of urine and defoliation on selected components of total dry matter yield, total N yield, pasture composition and N_2 fixation over the 12 month study

	Urine[a]			Defoliation[b]		
	No urine	Urine	$LSD_{0.05}$	Light	Mod-severe	$LSD_{0.05}$
Total DM yield (kg ha^{-1} yr^{-1})	12928	16461	2110	12655	16734	2110
Grass DM yield (kg ha^{-1} yr^{-1})	5042	9325	795	6614	7752	795
Weed DM yield (kg ha^{-1} yr^{-1})	2075	1993	NS	1078	2990	600
Grass N yield (kg ha^{-1} yr^{-1})	148	370	49	234	284	49
Clover proportion (%)	44	33	5.9	41	35	NS
Clover DM yield (kg ha^{-1} yr^{-1})	5789	5265	NS	5125	5929	NS
Clover N yield (kg ha^{-1} yr^{-1})	273	254	NS	236	290	NS
Clover N fixed (kg ha^{-1} yr^{-1})	232	145	32	160	218	32
N fixed (%)	85	57	6.5	67	75	6.5

[a] average of light and moderately-severe defoliation.

[b] average of nil and plus urine.

NS=not significant.

The percentage of clover N derived from atmospheric N_2 (%Ndfa; using conventional ^{15}N-labelling) decreased markedly during the first 43 days to a minimum of 25% after urine application and remained low during the following 65 days (Figure 1d). Thereafter, values increased until 351 days when control levels were reached. %Ndfa in the nil-urine plots generally varied between 75% and 90% throughout the study. On average (using estimates of N_2 fixation from all four ^{15}N methods), the annual total N fixed by clover was reduced by 38% when urine was applied (Table 1) and the decrease was similar for both light and moderately-severe defoliation.

Urine increased the total N yields of ryegrass by 150%, but had no significant effect on clover N yield (Table 1). Ryegrass N concentrations increased most during the first 108 days (from 3.3 to 5.7%) and highlight the importance of ryegrass in uptake of urine N (Figure 2a; Table 1).

In all annual yield and N_2 fixation estimates, there was no significant interaction between urine and defoliation treatments.

B. Effect of defoliation severity on pasture production and N_2 fixation

Moderately-severe defoliation resulted in 36% higher annual pasture dry matter production than light defoliation ($P < 0.05$; Table 1). This increase was largely obtained by an increase in weed dry matter production of 177% and an increase in grass dry matter production of 17%. Increases in weed growth (particularly *Plantago lanceolata* L.) occurred within 64 days of applying the moderately-severe defoliation treatments (with or without urine) and in general continued throughout the entire period of the study (data not shown). The growth rate of grass under moderately-severe defoliation was greater than light defoliation for a short period between days 86 and 135 ($P < 0.05$) (Figure 1a), and corresponded to a four-fold increase in the growth of the summer grass component of the grass species. During this period, summer grasses (*Bromus catharticus;*

Dactylis glomerata and Agrostis capillaris) dominated the moderately-severe defoliation treatments, with 66% of the sward comprising these grass species.

There was a delayed effect of defoliation on clover growth ($P < 0.05$), with a decline of up to 43% during days 64 - 108 under light defoliation, but this was only significant under urine (Figure 1b).

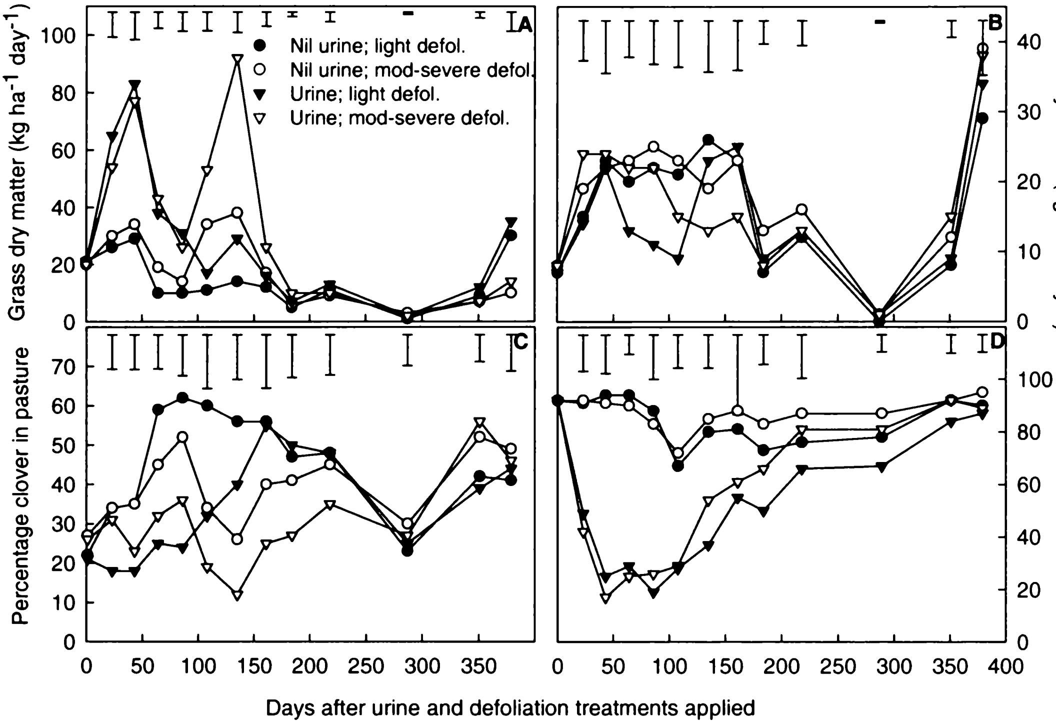

Figure 1. Effect of urine and defoliation on grass dry matter production (A), clover dry matter production (B), percentage of clover in pasture (C), and N_2 fixation (using the ^{15}N-ammonium sulphate labelling method) (D), during the 12 month study. Error bars represent LSD values (P=0.05; 4 replicates).

During the following 53 days (until day 161), this difference was reversed, and thereafter clover growth was the same in both the defoliation treatments. On an annual basis, increasing defoliation severity did not affect annual clover dry matter production.

In the moderately-severe defoliation treatments the proportion of clover in pasture decreased ($P < 0.05$) between days 108 – 181 (Figure 1c). This reflected a change in pasture composition as measured by the increase in the proportion of summer grasses and weeds (reported above).

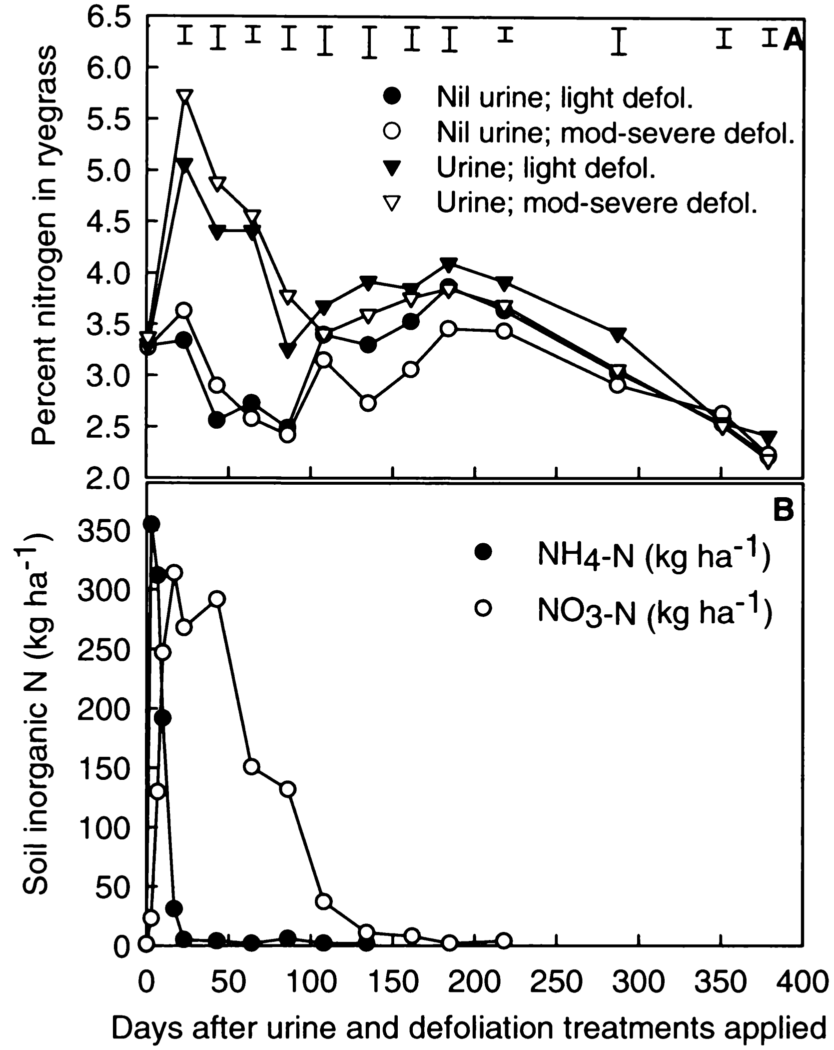

Figure 2. Effect of urine and defoliation on percentage of nitrogen in ryegrass herbage (A), and for urine treatments only; soil inorganic N in 0-150 mm soil depth (B). Error bars represent LSD values (*P*=0.05; 4 replicates).

Initially, estimates of %Ndfa were not affected by defoliation severity. However, after 108 days, %Ndfa increased significantly (*P* <0.05) under moderately-severe defoliation (up to 18% higher) in both urine and nil-urine plots

(Figure 1d). This increase in %Ndfa was paralleled by a decrease in ryegrass N concentration after 108 days under moderately-severe defoliation (Figure 2a).

Increasing defoliation severity significantly ($P < 0.05$) increased the annual amount of N fixed from 160 to 218 kg N ha^{-1} yr^{-1} (Table 1).

C. Plant ^{15}N enrichment as affected by urine and defoliation

The application of urine to plots previously labelled with ^{15}N caused a rapid decrease (within 3 days) in ^{15}N enrichment of the soil inorganic N (as reflected in the atom% ^{15}N excess of the ryegrass), to values below that of nil-urine plots (Figure 3a). These lower values of ^{15}N enrichment persisted for the duration of the study ($P < 0.05$), but still provided an adequate level of soil ^{15}N enrichment.

In the slow-release treatment, where glucose was added to stimulate the immobilisation of N, the ^{15}N enrichment showed a similar decline over time (data not shown) to the ^{15}N-labelled plots without carbon. Both methods gave similar estimates of %Ndfa (Figure 4).

Under moderately-severe defoliation the ^{15}N enrichment in clover fell below that for light defoliation after 108 days (in both urine and nil-urine plots), and remained lower until the end of the study ($P < 0.05$; Figure 3b). This period of decreased ^{15}N enrichment in clover under moderately-severe defoliation coincided with increased estimates of %Ndfa in both urine and nil-urine plots during the same time-frame (Figure 1d). Defoliation severity did not have a significant effect on the ^{15}N enrichment of ryegrass (Figure 3a). The natural abundance of plant-available ^{15}N (as reflected in the $\delta^{15}N$ of ryegrass tissue) was most variable during the first 23 days after urine application, with a wider range of $\delta^{15}N$ values (1.6 – 7.1‰; Figure 5) recorded than before urine was applied (2.8 - 3.3‰). The coefficient of variation for the $\delta^{15}N$ of ryegrass was 11% prior to urine application, but up to 57% during the 17 days after urine was applied and down to 10% thereafter, indicating large short-term spatial variability of ryegrass $\delta^{15}N$ due to urine. After 23 days, the range of $\delta^{15}N$ values in ryegrass was less variable

(Figure 5). Natural abundance data from harvests between days 3-23 in nil-urine plots were omitted in figure 5 because of inadvertent batch-sample contamination.

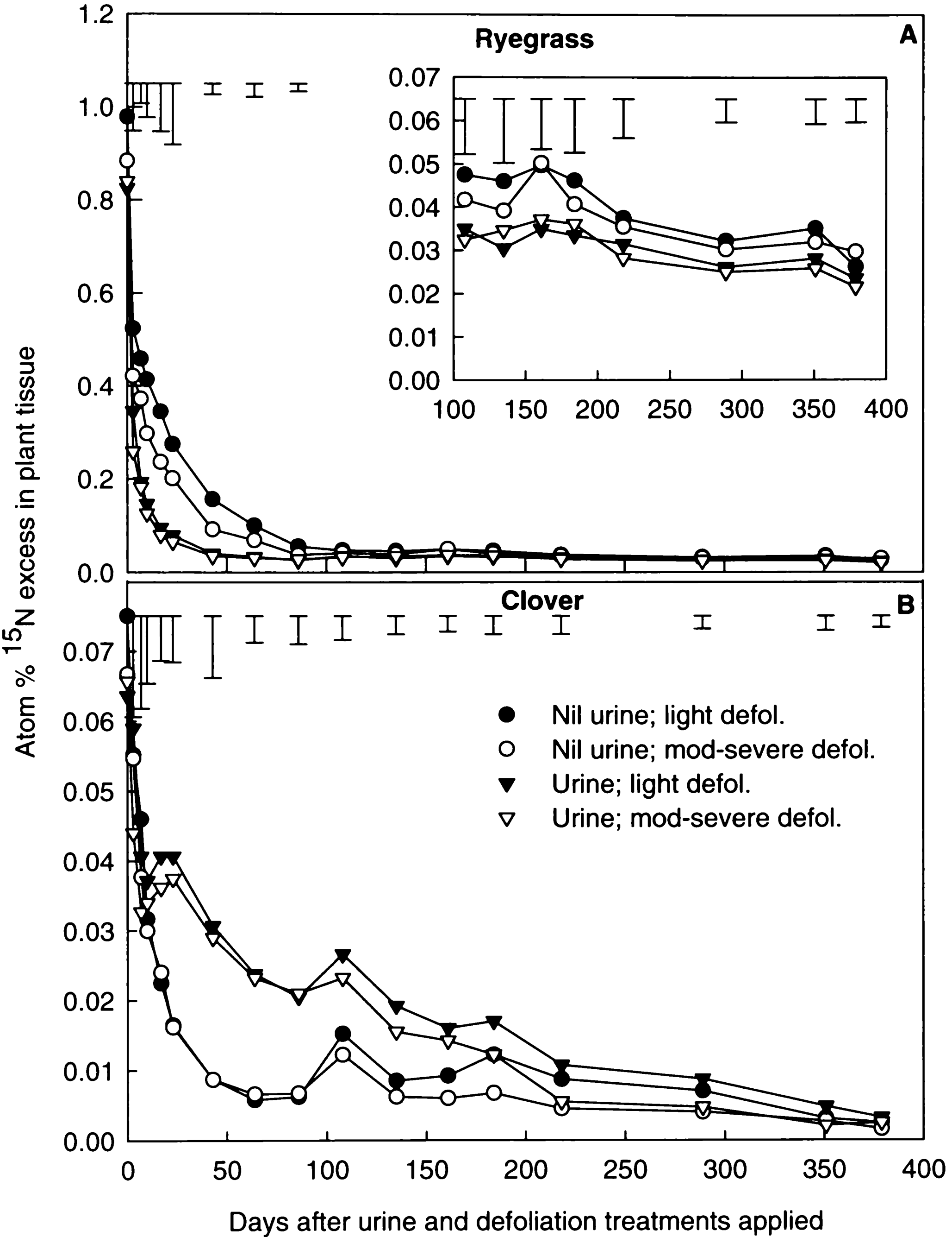

Figure 3. Effect of urine and defoliation on the ^{15}N enrichment (atom % excess) of ryegrass (A), and clover (B) over the 12 month study period, in plots initially amended with 1 kg N ha^{-1} of ^{15}N-labelled ammonium sulphate (80 atom% ^{15}N). Error bars represent LSD values (*P*=0.05; 4 replicates).

D. Estimates of N_2 fixation under urine using different ^{15}N-isotope techniques

Estimates of %Ndfa under urine were the same when soil was labelled using ^{15}N ammonium sulphate alone or the slow-release formulation with glucose (Figure 4). In contrast, the use of ^{15}N-labelled urine led to significantly lower estimates of %Ndfa (as low as 6%) compared to the conventional soil ^{15}N-labelling method (minimum of 25%) and the ^{15}N-label+glucose slow-release method (minimum of 21%) (Figure 4). These differences were greatest during the first 23 days ($P < 0.05$), and had diminished by day 43. However, subsequent to day 43 the %Ndfa estimates using the ^{15}N-labelled urine technique continued to be lower until day 161 and thereafter they were similar to the other ^{15}N-isotope techniques ($P < 0.05$). In the short-term (0-23 days), estimates of %Nfda using the natural abundance technique did not always produce meaningful values (e.g. large negative values of %Nfda; data not presented), but thereafter values were more reliable and closer to that of the conventional ^{15}N-labelling method (Figure 4).

As a result of the differences in estimates of %Nfda, the measured impact of urine on annual N fixed by clover varied depending on the ^{15}N-isotope technique used. When the soil ^{15}N-labelling technique was used, annual N fixed by clover was calculated to decrease by 31% under urine, but when the ^{15}N-labelled urine technique was used the reduction was much greater at 51% (Table 2).

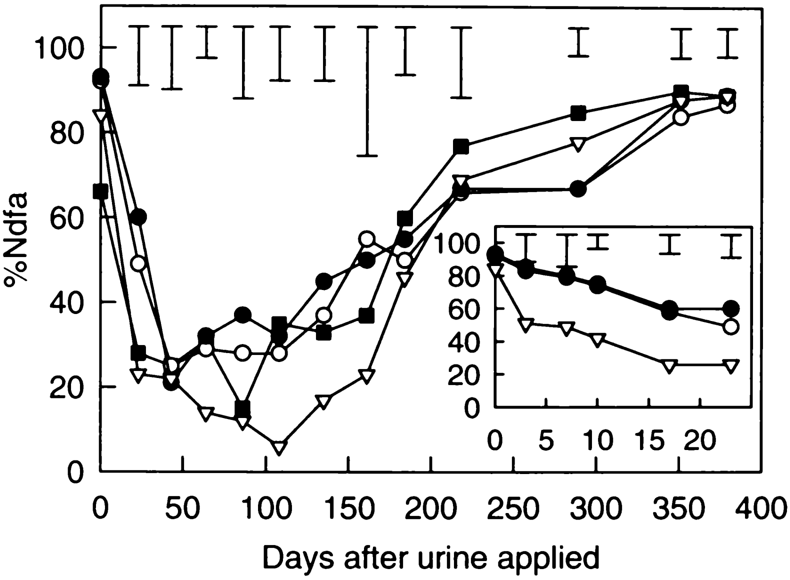

Figure 4. Estimates of N_2 fixation in urine treated plots under light defoliation over the 12 month study using four ^{15}N isotope methods: ^{15}N-labelled soil (+1 kg ammonium-N ha^{-1}) (○), ^{15}N-label+glucose (+1 kg ammonium-N ha^{-1}+glucose) (●), ^{15}N-labelled urine (▽), and natural abundance (■). Error bars represent LSD values (*P*=0.05; 4 replicates).

E. Soil inorganic N and urine recovery

In urine treated plots, urea was rapidly hydrolysed to NH_4^+-N and reached maximum values of 355 kg N ha^{-1} after 3 days (Figure 2b). Subsequently, the NH_4^+-N was nitrified to NO_3^--N and reached maximum values of 314 kg N ha^{-1} 17 days after urine was applied. Plant uptake and possibly leaching to below the 150 mm sampling depth returned soil NO_3-N to background levels 161 days after urine application (Figure 2b).

Ryegrass and clover recovered a total of 28% and 10% of the urine N, respectively. Plant recovery of ^{15}N-labelled urine occurred mostly (92%) during the first 161 days, and was associated with a decrease in soil NO_3-N to near control levels during the same period. The applied urine-N not accounted for was probably volatilised, immobilised in the soil microbial biomass and/or leached below the root zone.

V. Discussion

A. Effects of urine on N_2 fixation

In this study, N_2 fixation was dramatically reduced after urine application and recovery to control levels was prolonged, taking up to one year. The reduction in N_2 fixation due to urine was measured within 3 days and was greatest between 43-108 days. This large negative effect on %Nfda is attributed to the elevated levels of soil inorganic N measured in the 0 – 150 mm layer subsequent to urine application and shows the clover preference for uptake of N from soil over the more energy dependent process of N_2 fixation (Phillips et al., 1982). The initial rapid decline of %Nfda we observed under urine can be attributed to the negative effect of increased soil inorganic N on nodule activity (Streeter, 1988). Although the underlying mechanisms involved still require further elucidation they include (i) carbohydrate-deprivation in nodules (via reduced photosynthate supply), (ii) nitrite toxicity in nodules (via nitrate reduction), and (iii) reduced O_2 supply to N_2 fixing bacteriods (due to an increase in the resistance of the O_2 diffusion barrier). These short-term mechanisms interfere with the respiratory capacity of the bacteriods causing an inhibition of nitrogenase activity. Recent research (e.g. Parsons et al., 1993; Minchin, 1997) has shown that the operation of the O_2 diffusion barrier could be the key regulator of nitrogenase activity in the presence of high inorganic N. Potentially, other urine induced changes to soil chemical properties (e.g. pH and electrolyte concentration) may also have short-term adverse effects on nodule activity and N_2 fixation. However, the principle factor governing N_2 fixation (via nitrogenase activity and nodule growth) under urine patches is most likely related to the presence of elevated soil inorganic N. Over the longer-term (several weeks) soil inorganic N effects on clover root *Rhizobia*-infection, nodule growth and development are generally greater than soil inorganic N effects on N_2 fixation directly (Streeter, 1988).

While other workers have also measured short to medium term decreases in %Nfda in response to urine and fertiliser N (e.g. Vinther, 1998; Ledgard et al., 1996), few have measured the effect of urine through to complete clover recovery. Soil inorganic N (0-150 mm) returned to background levels after about 161 days whereas the subsequent recovery of %Ndfa to control levels took nearly one year. This delayed effect may have been due to the slow re-establishment of active nodules. Alternatively, the remobilisation of stored N from roots to shoots and/or the uptake of inorganic N from below 150 mm soil depth may have also delayed the recovery of N_2 fixation (Munns, 1977; Marriott et al., 1987).

It is known from many studies (e.g. Ledgard et al., 1990; Ledgard and Steele, 1992), that the application of nitrogen fertilisers and the associated increase in soil inorganic N reduces the amount of N_2 fixed by reducing the abundance of clover in pasture, and also, by reducing the proportion of clover N derived from atmospheric N_2. Similarly, we found that urine N reduced the proportion of clover in pasture (and clover growth rate as a result) through enhanced growth of ryegrass, until soil inorganic N had been depleted and returned to control levels (161 days after urine was applied).

The overall effect of urine on N_2 fixation (using an average of N_2 fixation from all four ^{15}N-methods) measured during the 12 months of the study was an annual reduction of 38% under both light and moderately-severe defoliation (from an average total fixed N in the harvested clover herbage from 232 to 145 kg N ha^{-1}; Table 1). This measured reduction in total fixed N was due largely to the direct effect of urinary N on nodule activity, as annual clover production was little affected by urine application.

Several workers (Jorgensen and Ledgard, 1997; McNeill et al., 1997) have shown that potentially 70% more N is fixed via the below cutting-height tissue (i.e. roots, stolons and some stem material). Although the below cutting-height fixed N contribution was not measured in this study, based on this value of 70%, estimates of total N fixed reported in this study could be as high as 394 and 247 kg N ha^{-1} for the control and urine treatments, respectively.

On New Zealand dairy farms, the average stocking rate is 2.8 cows ha^{-1} $year^{-1}$ (Livestock Improvement, 2000). Haynes and Williams (1993) reported a typical urination frequency of 10 urinations per day, and the average area covered of 0.30 m^2. Using these reported values and a Poisson distribution to allow for the overlapping of urine patches (Petersen et al., 1956), we calculated that urine would be deposited on 25% of the grazed area each year. However, the area affected by urine usually extends well beyond the wetted urine patch (Whitehead, 1995). Affected areas of 0.5 – 0.7 m^2 have been reported for dairy cows (Lantinga et al., 1987; Richards and Wolton, 1979), and could potentially result in up to 46% of grazed pasture being affected by urine deposition each year.

Based on the estimate of annual N_2 fixation in the present study with no input of urine (232 kg N ha^{-1} yr^{-1}), we calculate N_2 fixation to decrease by 10% (22 kg N ha^{-1} yr^{-1}) using the area covered by urine of 25% and the percent decrease of annual N fixed reported in this paper (38%). This reduction due to urine could be as high as 40 kg N ha^{-1} yr^{-1} by accounting for the total area affected by urine.

B. Effect of defoliation severity on N_2 fixation and pasture production

Although moderately-severe defoliation did not affect annual clover dry matter production or total clover N yield, it did have a significant effect on estimates of %Ndfa during the course of the study. Initially, values of %Ndfa were the same for both defoliation treatments (regardless of urine inputs), but between 108 and 289 days the %Ndfa increased significantly in the moderately-severe defoliation treatment both with and without urine present resulting in a 36% increase in the total N fixed compared to light defoliation (160 to 218 kg N ha^{-1} yr^{-1}).

Our data indicates that changes in N availability due to differences in N uptake between light and moderately-severe defoliation is the most likely cause of the greater estimates of %Ndfa under moderately-severe defoliation after 108 days. In the moderately-severe defoliation treatments, increases in the dry matter and N yield of drought-tolerant summer grasses and weeds during the period of days 86-135 and their greater ability to grow and take up soil inorganic N than ryegrass probably led to reduced N availability. This explanation is supported by lower levels of ^{15}N enrichment in clover tissue and lower ryegrass N concentration under moderately-severe defoliation after 108 days. Although the increase in summer grasses and weeds under moderately-severe defoliation had a positive effect on N_2 fixation, this may be of limited benefit for critical winter/spring productivity if summer species are not replaced in autumn by winter-active grass species such as ryegrass and annual poa.

The marked increase in summer grasses (up to 76%) that occurred under moderately-severe defoliation during the summer season (days 86-161) is probably also the main explanation for the measured increase in annual grass dry matter production (17%). More dramatic was the large increase in annual weed dry matter yield (177%) that also occurred under moderately-severe defoliation. This effect of moderately-severe defoliation on increasing weed production began within 64 days of applying the defoliation treatments and persisted for the duration of the study (data not shown). Brougham (1959) showed that an increase in weed abundance (up to 35% of dry matter yield in our study) under moderately-severe defoliation is the result of less competition for light with other upright species such as ryegrass, or more bare ground for weed ingress.

C. Effect of different ^{15}N labelling techniques on estimates of N_2 fixation

Of the four ^{15}N-labelling techniques used, the most marked difference in %Ndfa estimates occurred between the ^{15}N-labelled urine and the conventional technique of soil ^{15}N-labelling (using ^{15}N-ammonium sulphate). Differences between estimates of %Ndfa for the two techniques were up to 30% during the first 23 days. Subsequent to this, estimates of %Ndfa in the ^{15}N-labelled urine treatment were still between 20 – 30% lower than that of the ^{15}N-labelled soil for up to 161 days. The effect of these lower %Ndfa values using the ^{15}N-labelled urine culminated in the total annual amount of N fixed in urine plots being 29% lower than that estimated using the conventional soil ^{15}N-labelling technique (101 vs. 143 kg N ha^{-1} yr^{-1}).

The main potential errors in ^{15}N-derived estimates of %Ndfa occur because the legume and reference plant differ in the ratio of N assimilated from added ^{15}N to N assimilated from plant-available soil N (e.g. Chalk, 1985). Differences usually only occur if (a) the legume and reference plant differ in their temporal pattern of N uptake in association with a decreasing soil ^{15}N enrichment with time, or (b) the legume and reference plant roots uptake N at different depths and this corresponds with differences in ^{15}N enrichment of the plant-available soil N.

The calculated decline rate of soil ^{15}N enrichment (D values) in our treatments was gradual with D values of 0.03 (from Figure 3) for the first two full harvests, and thereafter D values were always below 0.01. These values are less than those which can cause incorrect estimates of %Ndfa (e.g. 0.05; Witty, 1983). Thus, any errors in estimates of %Ndfa may have been due to ryegrass and clover

Table 2. Estimates of annual percent clover N derived from N_2 fixation in urine treated plots under lax defoliation using four ^{15}N isotope methods: ^{15}N-labelled soil (+1 kg ammonium-N ha^{-1}), ^{15}N-label+glucose (+1 kg ammonium-N ha^{-1}+glucose), ^{15}N-labelled urine, and natural abundance

	Non-urine		Urine				
	^{15}N-labelled soil	Natural abundance	^{15}N-labelled soil	Natural abundance	^{15}N-labelled soil (+glucose)	^{15}N-labelled urine	$LSD_{0.05}$
%N fixed	84	76	54	59	62	42	11
Decrease in annual fixed N due to urine (%)[a]	-	-	31	41	38	51	-

[a] % Change in clover N fixed under urine was calculated using the mean clover N fixed from the ^{15}N-labelled non-urine plots under light

N uptake occurring at different depths, which corresponded with differences in the ^{15}N enrichment of soil.

In our study, the ^{15}N-labelled soil treatments received $^{15}(NH_4)_2SO_4$ two weeks prior to commencing the study (i.e. application of the urine and defoliation treatments) to avoid possible effects from an initial rapid decline in ^{15}N concentration of soil inorganic N. In this initial two week period, most of the ^{15}N labelled NH_4^+-N would have been nitrified to $^{15}NO_3^-$-N (with some plant uptake and soil immobilisation also occurring). During this two week interval, rainfall at the site was minimal. However, at the time of urine application (labelled and non-labelled) and during the subsequent 3 days substantial rainfall fell at the site. Estimates of water movement during the first 3 days of the study (using soil porosity and rainfall data) suggest that the large volume of urine applied and concurrent rainfall could have displaced labelled $^{15}NO_3^-$-N in the ^{15}N-labelled soil treatments to below 100 mm soil depth, while the ^{15}N-labelled urine probably remained in the upper 50-100 mm soil layer. Such differences in ^{15}N distribution with soil depth may have significantly affected estimates of %Ndfa (Ledgard et al., 1985b; Steele and Littler, 1987).

Steele and Littler (1987) injected ^{15}N at increasing soil depths and measured increased %Nfda due to greater relative recovery by roots of ryegrass than white clover at depth. If this also occurred in our study, it is likely that the application of non-labelled urine to the ^{15}N-labelled soil treatments and the greater depth of movement of $^{15}NO_3^-$-N would have favoured greater uptake and ^{15}N enrichment of ryegrass than clover. Such a scenario could explain the differences in %Ndfa values and suggests that where non-labelled urine was applied to ^{15}N-labelled soil the %Ndfa was overestimated. In addition, studies using the acetylene reduction assay to calculate relative differences in N_2 fixation have shown a rapid decline in N_2-fixing activity (up to 90% in the first 2 to 10 days) immediately following application of urine (Ball et al., 1979; Ledgard et al., 1982) which is nearer that

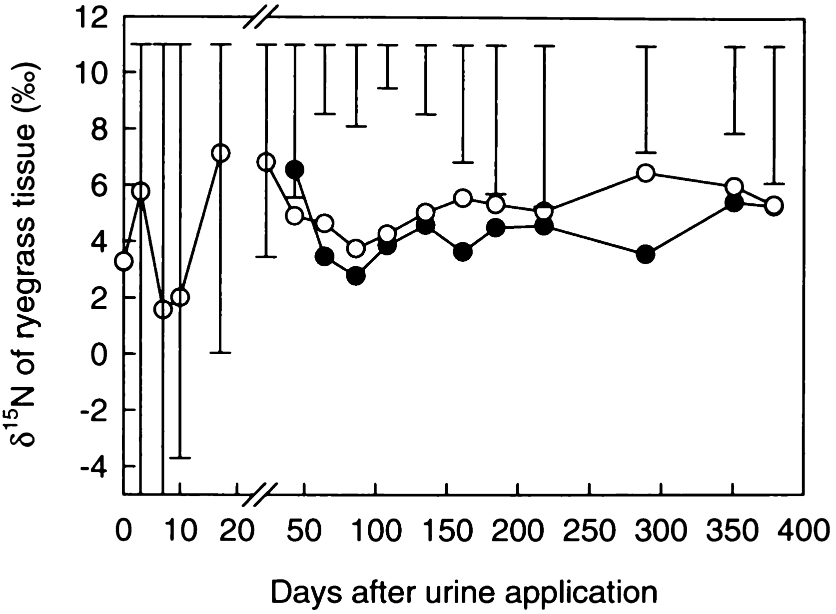

Figure 5. ^{15}N natural abundance ($\delta^{15}N$) of ryegrass in nil-urine (•) and plus urine (○) treatments, during the 12 month study. Error bars represent LSD values (*P*=0.05; 4 replicates).

measured in the ^{15}N-labelled urine treatment than in the soil ^{15}N-labelling treatments.

Potentially, the natural ^{15}N abundance technique could give more accurate estimates of %Nfda because the $\delta^{15}N$ of plant-available N is generally more uniform with soil depth compared to soil artificially enriched using surface applied ^{15}N-fertiliser (Ledgard et al., 1984). However, the application of urine with a different $\delta^{15}N$ value to plant-available soil N and any subsequent differentiation of $\delta^{15}N$ values with depth would make accurate assessment of %Ndfa in urine affected areas difficult.

When using the natural abundance technique to estimate N_2 fixation, it is generally recommended that the $\delta^{15}N$ of plant-available soil N be above 6‰, because below this value the accuracy in estimating N_2 fixation decreases markedly (Ledgard and Peoples 1988). Even though values measured in this study were generally below 6‰, estimates of %Ndfa after 23 days using the natural abundance technique produced similar values to those calculated using ^{15}N-labelled soil in the nil-urine and urine plots. However, in the short-term (0-23 days) after urine application, estimates of %Ndfa were highly variable (-56 to 34%), and reflected the large spatial variability and low values of δ^{15}N (1.6 – 7.1‰) that initially occurred after urine was applied.

Given the large spatial variation of δ^{15}N of plant-available soil N measured immediately after urine application in this study and elsewhere under grazing (e.g. -3.3 to 11.6‰; Eriksen and Hogh-Jensen, 1998), along with the low δ^{15}N values found in many pasture soils (e.g. this study; Ledgard et al., 1984; Riffkin et al., 1999), and the lower value of δ^{15}N of urine N relative to pasture from which it was derived (Steele and Daniel, 1978), it is suggested that the natural abundance method in intensively grazed dairy pasture where frequent inputs of urine affect large areas, should only be used with caution.

VI. Conclusions

Our results show that the application of urine to pasture can have prolonged effects on N_2 fixation, even after high levels of inorganic N in the root zone have been depleted. Defoliation management was also shown to influence N_2 fixation and interestingly, moderately-severe defoliation encouraged the invasion of vigorous growing summer grasses and weeds, which reduced plant available soil N and increased N_2 fixation. From an on-farm prospective, varying grazing severity could be a useful pasture management tool for manipulating sward composition and enhancing N_2 fixation and production during key regrowth periods.

In this study it appears that changes to the soil ^{15}N enrichment with depth, and differences in the rooting depths of clover and ryegrass was responsible for differences in N_2 fixation estimates in urine-affected areas between the ^{15}N-labelled urine and ^{15}N-labelled soil techniques, and warrants further investigation.

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4

Effects of Treading on White Clover Growth

4

The effects of treading by dairy cows on white clover productivity, growth and morphology in a mixed clover-grass pasture[1]

Keywords: animal treading, stolon fragmentation, plant burial, soil compaction, soil aeration.

I. Abstract

A single pugging event of moderate or severe pugging intensity was initiated in plots during spring by using dairy cows at varying stocking rates (4.5 cows 100 m^{-2} for 1.5 or 2.5 hours, respectively), and changes in white clover growth and morphology investigated over the following 12 months. Defoliation was carried out by mowing. Total pasture production was reduced under moderate and severe pugging by 16% and 34% compared to the non-pugged control. The corresponding decrease in clover production was 9% and 52%, respectively. Total ryegrass yield was reduced by 37% under severe pugging. This indicates that clover is more susceptible to severe treading than ryegrass. Effects of pugging on clover growth persisted for up to 156 days, and coincided with a large decrease in sward clover content over the same period (e.g. 40%

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[1] Menneer J C, Ledgard S F, McLay C D A and Silvester W 2004 The effects of treading by dairy cows on white clover productivity, growth and morphology in a mixed clover-grass pasture. Grass and Forage Science, (Submitted).

versus 12% on day 112, under nil-pugging and severe pugging). Analysis of individual clover plants extracted from turves (300 mm x 300 mm) showed that direct hoof damage, fragmentation and burial of stolons were the major factors which reduced clover performance, rather than measured changes in soil physical properties. Morphological characteristics associated with plant size (e.g. stolon length, growing points, and leaf numbers) all decreased under pugging. The situation had reversed by late summer, with larger plants (e.g. 3rd order plants) dominating pugged plots, and coincided with the recovery of clover content in pasture. Strategic pasture management practices such as restricted grazing and the use of stand-off pads when soils are overly wet can minimise losses of fixed N.

II. Introduction

Many farm systems, including those utilising mixed legume-grass pastures, operate at intensive levels of grazing in a desire to achieve higher productivity and profitability. However, in these systems increased levels of animal treading has the potential to impact on a range of soil and plant properties. For example, in wet soils, pugging or poaching may occur with remoulding of the soil around animal hooves causing deformation at the soil surface and compaction at depth (Horne, 1992; Scholefield and Hall, 1985; Scholefield et al., 1985). This can result in pasture soils with increased soil resistance and decreased soil aeration (Davies et al., 1989; Greenwood and McNamara, 1992; Mulholland and Fullen, 1991). These soil effects can have negative consequences for plant growth, with decreases in both shoot and root growth observed in pot experiments where grass and pasture legumes have been grown separately in artificially compacted soil (Cook et al., 1996; Houlebrook et al., 1997; Naiden et al., 1996, 1997). In addition to the indirect effects of soil physical changes, animal treading in pasture can have direct effects on plant growth by plant injury, fragmentation, and burial (Brown and Evans, 1973; Edmond, 1962; Vertès, et al., 1988).

In mixed clover-grass pastures, a limited amount of early research (Brown, 1968a, b; Edmond, 1958a, b; 1962) showed that treading and its associated effects on soil and plant properties can lead to losses of grass production of 2% to 69% and white clover production from 5% to 95%. However, these reported studies carried out using sheep were of short-term duration (less than 1 year), and so did not measure the full effect of treading on plant growth response across the entire season. Furthermore, most studies under treading have not provided detailed observations of soil physical changes and plant damage, and attempted to differentiate these effects. In particular, detailed work on white clover growth and morphology as affected by treading has yet to be carried out.

Research in Europe (Fothergill et al., 1997; Gooding and Frame, 1997; Marriott and Smith, 1992) and New Zealand (Hay and Chapman, 1984; Hay et al., 1987; Harris, 1994) has demonstrated that white clover undergoes a seasonal cycle of stolon burial (mainly by worm castings) in winter (up to 80-90%), stolon fragmentation and re-emergence of growing points in spring, followed by surface stolon development over summer-autumn. Consequently, in spring the clover population of pasture consists mainly of small fragmented plants. Several researchers (Fothergill et al., 1997; Hay et al., 1989) have reported that clover populations comprising a high proportion of small, simple-structured plants have an increased vulnerability to environmental stresses. Losses of small plant units often occur in spring/early summer (e.g. spring or early summer drought; Brock et al., 1988), but if grazing management does not encourage plant size to rebuild during summer, plant death can also occur in late summer/early autumn (Fothergill et al., 1997).

The main factor involved in the process of spontaneous stolon breakdown is very low plant carbohydrate reserves going into spring (Collins and Rhodes, 1995; Hay et al., 1989). Although the process of stolon breakdown is plant initiated, spring grazing management can also have a significant effect on the degree of fragmentation that occurs. For example, in a short-term study during spring, Vertès et al. (1988) observed a large increase in the proportion of small, simple-structured plants under intense cattle treading in wet soil conditions compared to areas where no treading occurred. Other research (e.g. Laidlaw et al., 1992), has also shown that overly wet conditions during spring can lead to treading damage and detrimental effects on clover production.

The purpose of the research presented in this paper was, first, to determine the effects of treading and associated changes in soil physical properties on the productivity of both grass and white clover over a full year, and second, to describe the detailed changes that occurred in white clover growth and morphology subsequent to treading.

It should be noted that the results presented in Chapters 4, 5, and 6 are derived from a single field study that was carried out in September 1999.

III. Materials and Methods

A. Experimental site and soil characteristics

The experiment was established on a Te Kowhai silt loam (Typic Ochraqualf, Soil Survey Staff, 1994; Typic Orthic Gley Soil, Hewitt, 1993) with impeded subsoil drainage. The pasture was a long-term (>30 years) permanent mixed stand of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.).

A basal fertiliser (80 kg P ha^{-1}, 75 kg K ha^{-1}, 110 kg S ha^{-1}) was applied, by hand, to satisfy plant nutrient requirements.

B. Experimental design

Treatments consisted of a single pugging event of three severities (nil, moderate and severe; Plate 1). A randomised block experimental design was used with 8 replicates. Plots were 2.5 m x 7 m long with a 0.5 m buffer strip around each plot. Two pre-conditioning harvests were taken from the site during the month prior to commencing the study to reduce variability and for potential covariate analysis. In early spring (21 September) 1999, moderate and severe pugging treatments were established (after the site had been harvested) as follows: Plots for the treading treatments were grouped into for 4 separate areas (units A, B, C and D) and enclosed with a parameter fence (Figure 1). Subsequently, each unit was simultaneously pugged by walking dairy cows through the plots at a typical grazing intensity equivalent to 450 cows ha^{-1} (approximately 3-5 cows per unit depending on the unit area). After 1.5 hours the moderately pugged treatments were fenced-off and the relevant number of cows removed to maintain the required stocking rate, thereafter the pugging event continued only in the severe pugging plots until 2.5 hours when the desired level of treading damage was achieved.

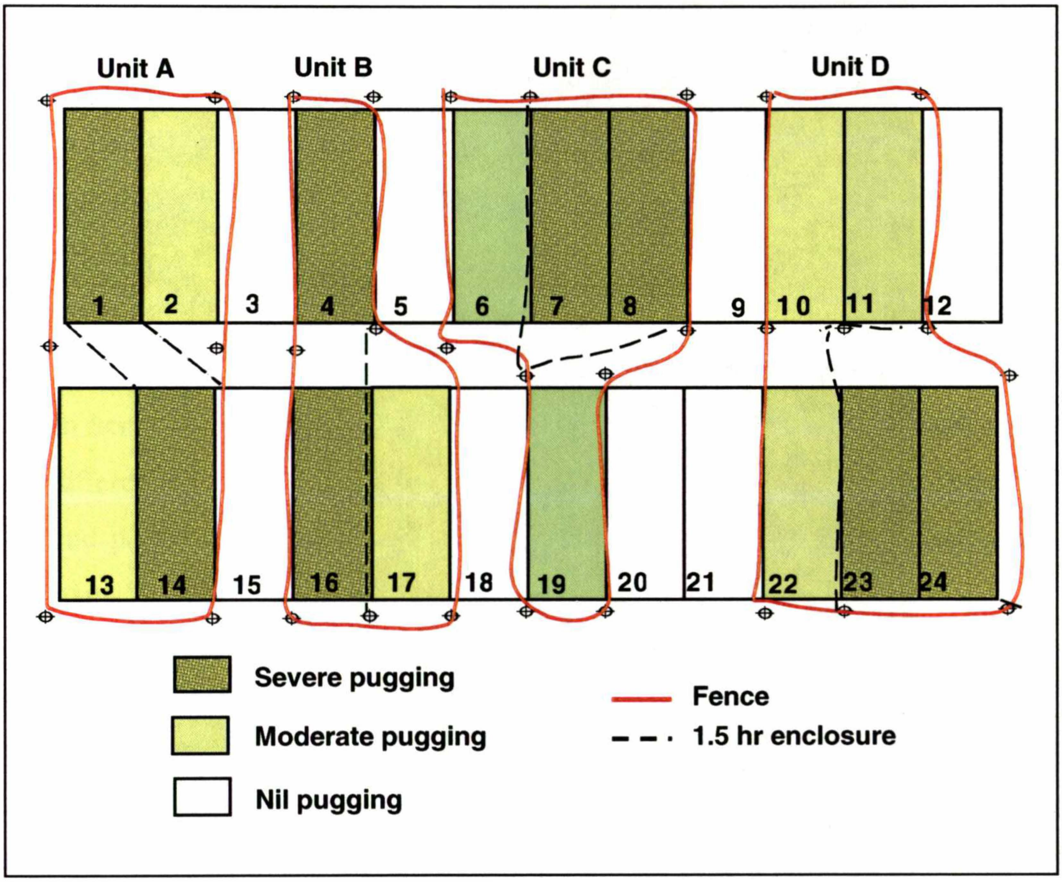

Figure 1. Experimental layout of the treading study showing the grouping of the plots (red lines) to allow the simultaneous application of the pugging treatments.

Rainfall and irrigation in the days before commencing the experiment meant that the soil was near saturation when the pugging treatments were applied. Prior to applying the pugging treatments the cows were kept in stockyards overnight so as to minimise inputs of dung and urine onto the plots during the treading event. In the rare event that excreta inputs did occur, these were intercepted and removed.

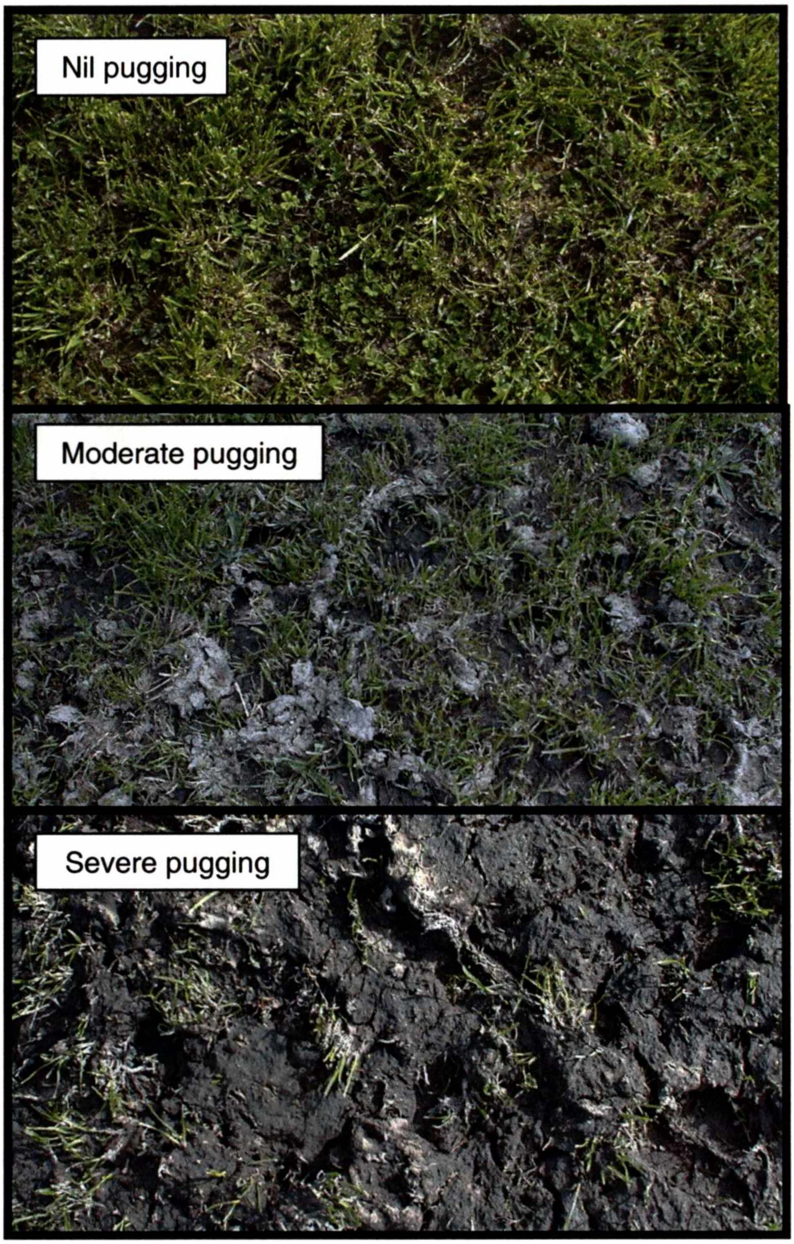

Plate 1. Pugging treatments 6 days after treading at 4.5 cows 100 m^{-2} for 1.5 and 2.5 hours, for moderately and severely pugged treatments, respectively.

C. Pasture productivity measurements

1. Harvest and analysis

To determine the full effect of pugging on pasture productivity the study was carried out over a 12-month period, and used mowing for defoliation. The first harvest of plots occurred 28 days after treatment application, and thereafter at approximately 21-day intervals. The wet weight of herbage from each harvest was recorded and a subsample used to determine the dry matter (DM) yield. Immediately prior to each harvest, herbage was sampled from within a 3 m x 1 m microplot in each plot for separation into species.

2. Percentage of ground without pasture cover

The percentage of ground without pasture cover (% bare ground) was measured on days 1, 31 and 84, after pugging treatments were applied. Digital photos covering an area of about 1.5 m^2 were taken in each plot and used to estimate the proportion of soil surface occupied by vegetation or bare ground. Images were analysed using Adobe Photoshop version 6. Image pixel counts for green herbage were deduced by using the colour selection tool, and bare ground by difference of this number from the total pixel count of the image. Thus, bare ground percent was calculated as the proportion of pixels covering bare ground compared to the total number of pixels in the entire image.

3. White clover growth characteristics and morphology

Three turves were removed from random locations within each plot immediately prior to harvests on days 48, 156, and 356 after pugging for individual plant sampling and analysis of white clover morphology. Soil was gently washed from the roots and whole clover plants separated from the vegetation mat. Any plants severed by the quadrat edge were rejected.

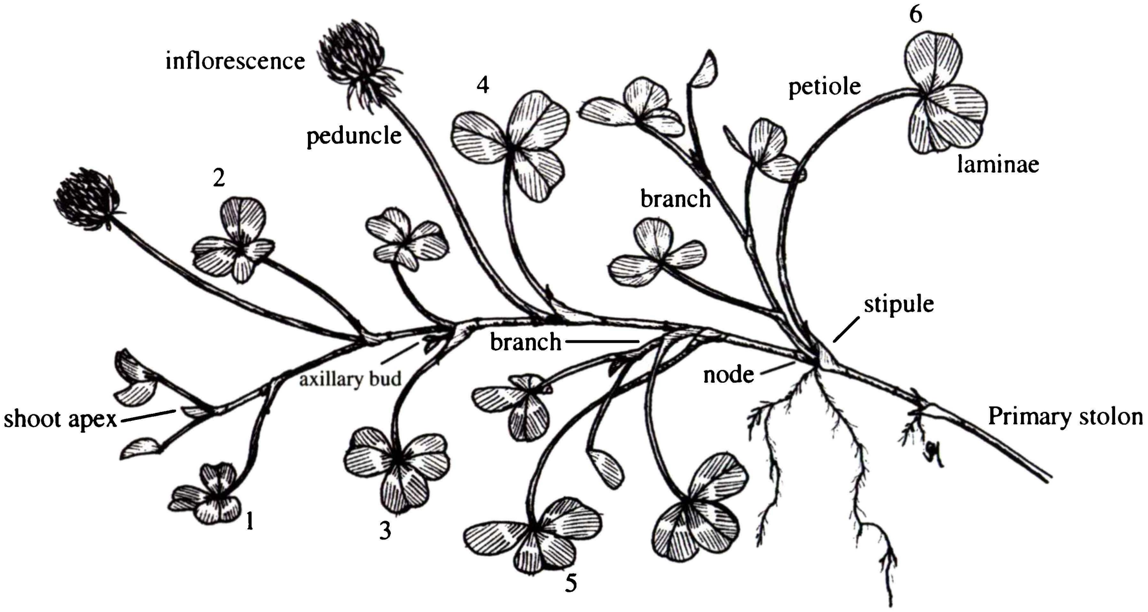

Figure 2. Drawing of a white clover stolon showing the morphological features of an adult plant. Emerged leaves on the primary stolon, and the nodes bearing them, are numbered 1 to 6. Features measured in the present study include, the number of growing points, branches, leaves (petiole+laminae), and stolon length. The above plant has one level of lateral branching off the primary stolon, and is therefore classified as a 2nd order plant.

A suitably large quadrat size (300 mm x 300 mm each) was used to minimise any bias against large plants caused by the removal of severed plants (Harris, 1994; M. Hay pers comm.).

The number of severed plants per turf was recorded and a maximum of 20 plants (randomly selected when more than 20 were present) were dissected, with each plant described as follows. Each plant was assigned an order according to the level of stolon branching; plants with a single parent stolon were 1st order; plants with one level of branching off the parent stolon were 2nd order, etc. Stolons within plants were classified on the basis of morphological age such that the primary stolon was the oldest or parent stolon. The numbers of leaves, branches, nodes with roots, growing points, and length, were determined for each stolon present (Figure 2). Finally, the leaf and stolon components for the total number of plants dissected in each turf were bulked for drying and subsequent weighing.

D. Soil physical measurements

Soil physical properties (bulk density and macroporosity) were measured using undisturbed soil cores carved out of the topsoil (0-5 cm) from the different treatments at 3 and 356 days after pugging. Cores were placed in plastic bags and stored at 4°C prior to measurement to prevent moisture loss and slow biological activity. Soil macroporosity was assessed by measuring the drainage from all pores greater than 30 µm (as defined by Greenwood and McNamara, 1992) at a suction of 100 cm (–10kPa) using tension tables constructed of sand and silica flour, following the methods of Ball and Hunter (1988). Cores were first saturated then left on the tension tables to equilibrate at –10kPa. Equilibrium was indicated when the cores ceased to loose weight. They were then oven dried at 105°C for 24 hrs to determine moisture content at the potential of –10 kPa, and the dry bulk density. The volume of air filled pores greater than 30 µm was than calculated as a measure of macroporosity from the following equation:

$$\%\text{air filled pores at} - 10\text{kPa} = \{\varepsilon - \theta_{-10} \times \rho_b\}$$

Where ε = total porosity (%), $\theta_{.10}$=volumetric water content at -10 kPa, and ρ_b = dry bulk density.

Total porosity was calculated from the bulk density and the average particle density of the soil using the following equation:

$$\varepsilon = 1 - \frac{\rho_b}{\rho_p}$$

Where ρ_p is particle density.

E. Statistical analysis

Analysis of variance was carried out using Genstat 4.2, Fifth Edition. No covariate adjustment for preconditioning harvests was made because of lack of significant differences. SED values are presented for comparing means when main effects were found to be significant.

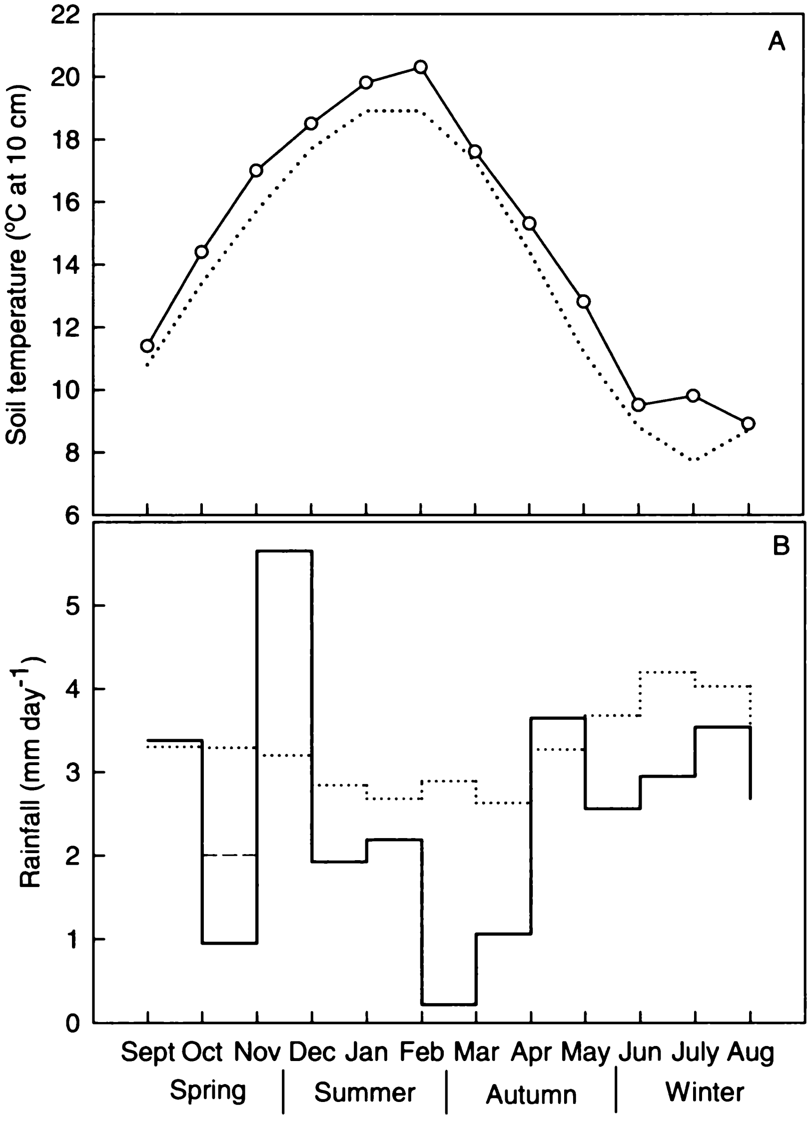

Figure 3. Mean monthly soil temperature (A) and rainfall (B) relative to the longer term average (‥‥‥). Dashed line in October represents irrigation.

IV. Results

A. Climatic data

Total annual rainfall during the study was 964 mm, and was 20% below the long-term average (1201 mm year^{-1}) (Figure 3). With the exception of one month in late-spring (November) and another in mid-autumn (April), rainfall in all other months was below average (Figure 3). Most noticeable were two periods of very low rainfall; one in mid-spring (October) and the other in late-summer (February), when rainfall was only 8% and 28%, respectively, of the long-term monthly average.

B. Effects of pugging on pasture production

Annual pasture production decreased ($P<0.001$) under moderate and severe pugging by 16% and 34%, respectively (Table 1). This was due to combined losses of both grass and white clover production, which were greatest in the severely pugged plots (37% and 52%, respectively; Table 1). In the moderately pugged plots, annual production of grass and white clover decreased by 19% and 9%, respectively.

The decrease in grass yield from pugging occurred as a result of two separate periods of reduced grass growth (Figure 4a). During the first 48 days, grass growth decreased by up to 90% in severely pugged plots before recovery to control levels, and this was later followed by a more prolonged second phase (days 192-356) of reduced growth in the pugged treatments (Figure 4a).

The temporal pattern of white clover growth (Figure 4b) showed marked reductions under both moderate pugging (by up to 53%) and severe pugging (by up to 82%) which lasted for 156 days after pugging. Thereafter, clover growth under pugging was not statistically different from non-pugged plots. However, in severely pugged treatments a trend of reduced growth continued until day 259. There was no significant effect of pugging on annual weed dry matter production.

Pugging affected the botanical composition of pasture mostly through the white clover component, especially when pugging was severe (Figure 4c). In the severely pugged plots the white clover content was markedly less than in the control plots (e.g. 12% versus 40% on day 112; $P<0.05$); this effect was prolonged lasting up to 220 days. However, the average annual white clover content in pasture was not significantly affected by pugging (Table 1). During the summer months the contribution of summer annual grasses and weed species in pasture (data not shown) increased under severe pugging compared to control plots (e.g. 31% versus 9% for weeds, on day 112).

Table 1. Main effects of pugging on selected components of total dry matter production and pasture composition over the 12 month study.

	Pugging severity			
	Nil	Moderate	Severe	SED[a]
Total DM yield (kg ha^{-1} yr^{-1})	10149	8562	6683	417***
Grass DM yield (kg ha^{-1} yr^{-1})	6817	5497	4264	558***
Weed DM yield (kg ha^{-1} yr^{-1})	1248	1210	1430	297NS
Clover DM yield (kg ha^{-1} yr^{-1})	2046	1854	989	479†
Clover proportion (%)[a]	20	22	15	3.6NS

[a]based on total clover DM yield.

***, † significant at $P<0.001$ and $P<0.1$, respectively, unless designated NS, not significant (8 replicates).

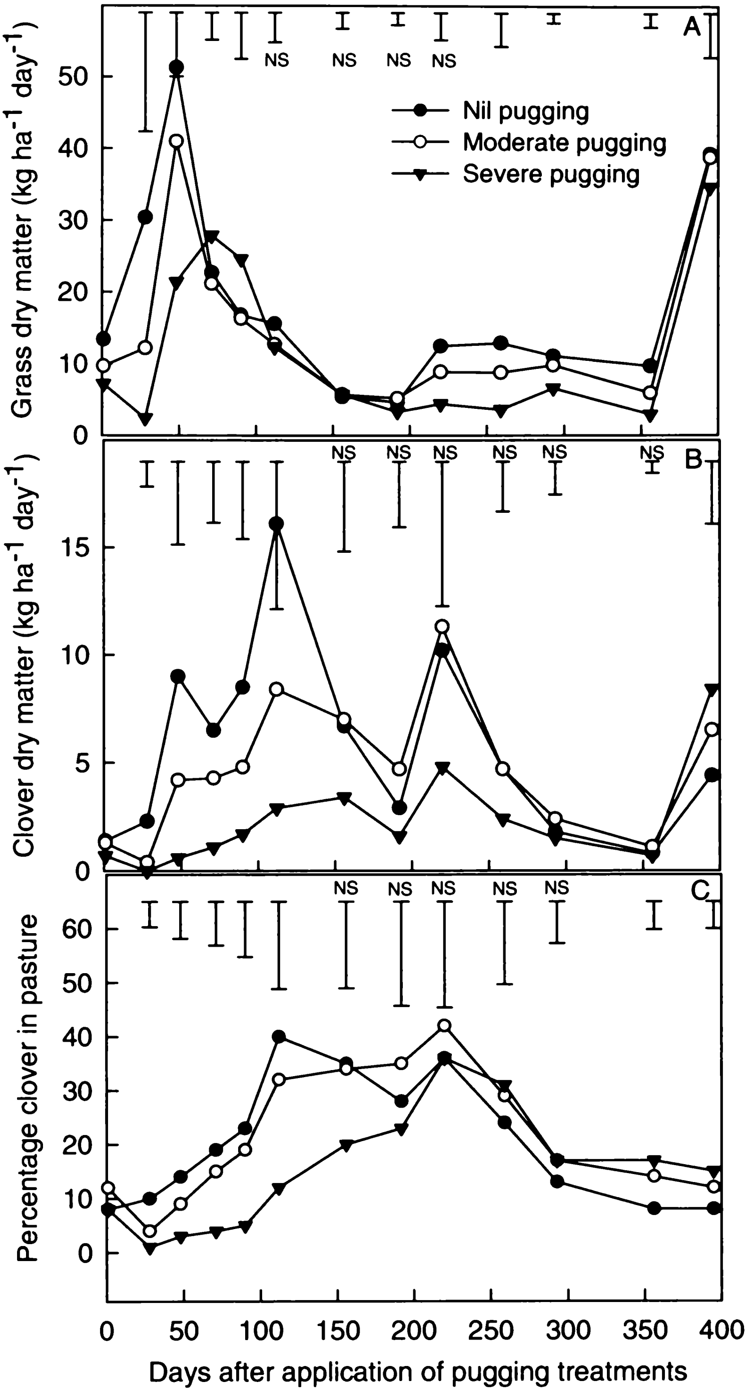

Figure 4. Effect of pugging on grass dry matter production (A), clover dry matter production (B), and percentage of clover in pasture (C), during the 12 month study. Error bars represent SED; *P*<0.05 unless designated NS, not significant (8 replicates).

Table 2. Effect of pugging severity (nil, moderate, or severe) on morphological characteristics of white clover at selected times during the year following a single pugging event by dairy cows in late spring.

	Day 48 (late spring)				Day 156 (late-summer)				Day 356 (early spring)			
Plant parameter	Nil	Moderate	Severe	SED	Nil	Moderate	Severe	SED	Nil	Moderate	Severe	SED
Plant density (m^{-2})[a]	492	311	72	128*	1000	636	222	142**	689	939	595	104*
Seedling density (m^{-2})	0	8.2	23.5	8.92*	0	0	19.2	5.42**	0	0	0	-
Leaf number ($plant^{-1}$)	ND	ND	ND	-	8	13.7	22.4	3.9*	3.9	5.1	9.9	1.41*
Growing points ($plant^{-1}$)	2.8	2.5	1.4	0.57†	1.99	4.0	6.2	1.42†	1.3	1.7	3.1	0.39*
Branch number ($plant^{-1}$)	1.5	1.0	0.9	0.34NS	0.8	2.0	3.6	0.76*	0.4	1.8	2.5	0.66*
Stolon length ($mm\ plant^{-1}$)	128.0	97.0	36.0	26.9*	215	382	234	72.2NS	103	130	209	30.3*
Stolon density ($m\ m^{-2}$)	50.1	36.1	3.9	17.7†	200	223	51	32.9**	70.6	164.4	121.6	21.6†
Total stolon length buried (%)	15.4	19.7	43.3	8.70*	3.7	3.6	2.9	1.24NS	6.9	7.3	6.4	2.8NS
Leaf dry weight ($mg\ plant^{-1}$)	78.0	47.5	18.5	22.4†	67	137	234	49.6*	22.2	41.0	59.2	10.3*
Stolon dry weight ($mg\ plant^{-1}$)	69.0	49.6	25.8	17.7†	129	222	223	50.6NS	53.2	123.5	146.5	24.2NS
Root dry weight ($mg\ plant^{-1}$)	23.4	16.4	7.2	4.79*	39.4	65.7	100.4	26.3NS	59.4	106.8	119.3	6.29†
Rooted nodes ($plant^{-1}$)	12.1	10.4	3.7	1.95***	18.0	25.2	14.2	3.03†	11.7	13.8	17.5	2.92NS

[a] seedling plants included.

***, **, *, † significant at $P<0.001$, $P<0.01$, $P<0.05$, and $P<0.1$, respectively, unless designated NS, not significant (4 replicates).

ND=not determined.

Table 3. Effect of pugging severity (nil, moderate, or severe) on branching order (% of total population) of white clover at selected times during the year following a single pugging event by dairy cows in spring.

Branching order	Day 48 (late spring)				Day 156 (late-summer)				Day 356 (early spring)			
	Nil	Moderate	Severe	SED	Nil	Moderate	Severe	SED	Nil	Moderate	Severe	SED
1st – order[a]	39	56	76	10.7**	63	44	24	7.4**	70	61	33	7.1**
2nd – order	51	39	24	11.9†	35	46	59	4.9**	30	32	49	6.9*
3rd – order	9	3	0	3.4*	3	12	15	7.7NS	0	7	15	2.3***
4th – order	1	1	0	0.96NS	0	0	3	1.6NS	0	0	3	1.0*
5th – order	0	1	0	0.82NS	0	0	0	-	0	0	0	-

[a] includes seedling plants.

***, **, *, † significant at $P<0.001$, $P<0.01$, $P<0.05$, and $P<0.1$, respectively, unless designated NS, not significant (4 replicates).

C. Percentage of ground without pasture cover

Estimates of the percentage of ground containing no vegetation (bare ground %) are presented in Figure 5, and show that under severe pugging up to 90% of the soil surface contained no visible vegetation, compared to 20% in the non-pugged control. By day 84 the bare ground percent in pugged plots was similar to that of non-pugged control plots and averaged 3.5%.

D. White clover growth characteristics and morphology

White clover morphological characteristics measured in this study 48 days after pugging were all negatively affected by pugging (Table 2). For example, stolon length and growing points were reduced by 72% and 50%, respectively, under severe pugging. Plant densities were reduced by 37% and 85% in the moderately and severely pugged treatments, respectively. In addition, under severe pugging, a large proportion of the clover population was comprised of juvenile seedling plants (33%; Table 2), compared to no seedlings in non-pugged plots. This increased seedling abundance fell to 9% by the second plant sampling (day 156), after which seedling numbers were nominal in all treatments.

By 156 days, there were indications that plant size had increased in the pugged treatments with measured increases in stolon length, branch numbers, growing points, and leaf numbers of individual plants. This was also reflected in the order of plant branching; at the first plant sampling (day 48) there was a greater proportion (76%) of smaller 1st order plants present in the pugged treatments (Table 3). However, at subsequent plant samplings (days 156 and 356) this trend had reversed and in the pugged treatments there was a greater proportion of 2nd, 3rd, and 4th order plants ($P<0.05$) compared to the non-pugged control. Higher values of per-plant leaf dry weight and stolon dry weight in pugged treatments at days 156 and 356 also highlighted the greater abundance of larger sized plants in these treatments that occurred later in the study (Table 2). However, plant densities in both the moderately and severely pugged treatments still remained low on day 156 at 64% and 22% of the non-pugged control, respectively.

E. Burial of stolon material

By 48 days after pugging the percentage of stolon length buried from the turf-sampled plants was over two times higher under severe pugging, compared to moderately pugged and non-pugged control treatments ($P<0.05$; Table 2).

F. Effect of pugging on soil physical properties

At day 3, soil physical properties (bulk density, macroporosity and soil moisture) were adversely affected by pugging, but this was only significant ($P<0.05$) for estimates of macroporosity and soil moisture (Table 4). For example, in the pugged treatments, macroporosity decreased to an average of 15.5% compared to the non-pugged control value of 21%. There was no long-term effect of treading on soil physical properties, with soil physical indicators similar in all treatments 1 year after pugging (Table 4).

V. Discussion

A. Effects of pugging on pasture production and soil physical properties

Treading by dairy cows resulted in annual pasture production decreases of 16% and 34% under moderate and severe pugging, respectively, and is similar to reported values in other studies of cattle treading (see review of Menneer et al., 2004a; Chapter 2, Sec. II.A). Both clover and grass were affected, but under severe pugging the reduction in clover was large and more prolonged than for grass growth. These effects were potentially due to soil physical deterioration and/or plant damage and loss. Indicators of soil physical deterioration measured 3 days after pugging showed a treatment effect which was significant for estimates of macroporosity and soil moisture. Nonetheless, values of macroporosity were still well above the 10-12% threshold that is often used to indicate limiting conditions for plant growth (Carter, 1988; 1990; Grable, 1971; Greenland, 1981). Indeed, in recent work by Drewry et al. (2001) an optimum macroporosity for

Table 4. Effect of pugging severity (nil, moderate, or severe) on soil physical properties at selected times during the year following a single pugging event by dairy cows in spring.

	Day 3				Day 356			
Soil physical parameter	Nil	Moderate	Severe	SED	Nil	Moderate	Severe	SED
Bulk density (g cm^{-3})	0.77	0.85	0.83	0.037NS	0.70	-	0.72	0.30NS
Macroporosity (%)	21	15	16	0.011**	24	-	22	0.18NS
Soil moisture (%)[a]	39	45	44	1.68*	46	-	46	0.69NS

[a] θ_v, at field capacity (-10 kPa)=46%.

**, * significant at $P<0.01$, and $P<0.05$ unless designated NS, not significant (4 replicates).

ryegrass growth was proposed to be in the range of 16-17%, whereas critical levels were suggested to be approximately 9-11%. Thus, soil physical degradation was unlikely to be a major contributing factor in the reduced pasture productivity measured in the present study.

A large decline in clover plant numbers, and increased burial of stolons indicated that the direct impact of pugging on plants was the main cause of reduced production. Immediately after pugging (day 1), estimates of the percentage of ground containing no vegetation (bare ground %) were up to 90% in pugged treatments compared to 20% in the non-pugged control. This indicates a major component of the above ground herbage biomass had been buried by hoof action. These effects of hoof action were initially met with a dramatic medium-term reduction (during the first 48 days) of grass growth (up to 92%) before recovery to control levels.

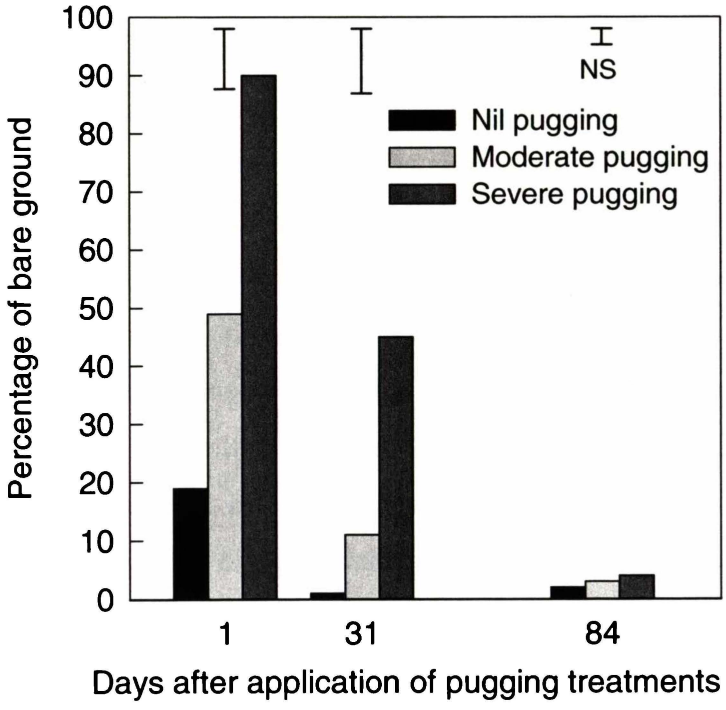

Figure 5. The effect of pugging severity on the percentage of exposed bare ground containing no pasture. Error bars represent SED, $P<0.001$ unless designated NS, not significant (3 replicates).

During autumn and winter a reduction in grass growth, which accounted for 47% of the annual loss in grass production, seemed to be mediated by prior changes in the pasture species composition that occurred during summer. Grass species composition measured 112 days after pugging showed that summer grass species abundance (e.g. *Digitaria sanguinalis*, *Paspalum distichum*, *Panicum dichotomiflorum*) was 3 times greater than in non-pugged plots (data not shown). In addition, very low rainfall occurred at the site later in the study (late-summer; Figure 3) and meant that conditions were more favourable for the continued growth of summer grass species relative to the more drought-sensitive C3 grasses (Menneer et al., 2003). This scenario is probably responsible for the reduction in winter grass growth measured, due to a lower abundance of winter-active grass species such as ryegrass and annual poa in the pugged treatments. In the pugged treatments, areas of bare ground probably provided space that enabled the ingress of summer grasses during the summer period of low soil moisture (Brougham, 1959).

Early research (e.g. Edmond, 1964; Brown, 1968), indicated that white clover could be more susceptible to treading damage than associated grass species. Similarly, in the current study the effects of pugging on clover growth were more prolonged than on grass, and persisted for up to 161 days and 220 days under moderate and severe pugging, respectively (Figure 4a,b). Compared to the upright tufting growth habit of grass species, white clover with its sprawling stoloniferous growth form appears to be prone to burial and plant fragmentation (Cluzeau et al., 1992; Edmond, 1964). This seems to be particularly the case when treading is severe and soil conditions are wet as was the case in the current study. The greater susceptibility of clover to treading damage could partly explain the often reported difficulty with which clover is retained in clover-grass swards (Fothergill et al., 1996). In clover-grass systems competitive interactions between grass and clover are important in governing clover performance and content in mixed pastures, e.g. self-regulation by clover-grass swards of soil inorganic N concentration (Chapman et al. 1996; Schwinning and Parsons, 1996). Consideration, therefore, should be given to the possibility that animal treading may be inferring a greater growth advantage to associated grasses than clover, by way of limiting clover growth and development in grazed pastures.

B. Effects of pugging on clover morphology and stolon burial

The temporal pattern of stolon growth in this study was typical (Gooding and Frame, 1997; Hay and Chapman, 1984; Hay et al., 1987; Harris, 1994; Marriott and Smith, 1992) with a general pattern of abundant small plants in spring (e.g. day 48; Tables 1 and 2) which grew and developed into larger plants by late-summer (day 156). By the following spring (day 356) stolon fragmentation resulted in plant units of smaller size than those in late-summer. However, overlying this general pattern of stolon cycling was a marked effect of pugging on increasing stolon fragmentation, which caused large differences to occur in the characteristics of white clover growth and morphology between treatments over the duration of the study (Table 1 and 2). Initially, plants were much smaller and less complex in pugged plots compared to non-pugged plots (e.g. reduced stolon length and growing points in pugged plots on day 48) indicating treading had induced fragmentation of the stolon network. However, despite an increase in fragmentation due to pugging, plant densities were still lower in pugged plots than in non-pugged plots. This indicates that a large proportion of the fragmented plant units resulting from treading must have perished during the initial 48 days after pugging treatments were applied. In addition, an increase in stolon burial (by up to 3-fold; Table 2) measured on day 48, and the large areas of bare ground immediately after pugging, imply large-scale burial of the stolon network.

In other studies (e.g. Brock et al., 1988; Fothergill et al., 1997; Chapman and Anderson, 1987) small clover plants have been shown to be more vulnerable to environmental and management stress and can lead to decreased white clover content (Brock et al., 1988; Hay et al., 1989). During the 48 days between commencing the experiment and the first clover plant sampling, average rainfall at the site was 70% below average. Under these climatic conditions, along with other possible factors (e.g. stolon damage and burial), many of these smaller plants (including observed seedlings) which lacked a maternal support system would have perished. In addition, the starch content of stolons during spring is at its annual lowest, with plant energy reserves are only 10-15% of those in autumn (Frankow-Lindberg et al., 1997; Hay et al., 1989), and this would have further heightened the risk of plant failure to recover from pugging induced fragmentation.

Despite the large negative impact of treading on clover, subsequent growth was rapid and by late-summer (day 156) plant densities had increased in all treatments. This may have been due to either continued stolon fragmentation and/or by the contribution of seedling plants during late spring (after day 48). Plant densities were still lowest in pugged treatments on day 156, but by this stage individual plants were generally larger and more complex (2nd, 3rd and 4th order plants) under pugging. This trend of increasing plant size and density probably explains the recovery of the white clover content that occurred between 156 days and 220 days after pugging (Figure 4c). By the following spring (day 356), plants had reduced in size compared to late-summer, and plant densities no longer showed any effect of pugging. However, individual plants were still larger and more complex under pugging, and this probably contributed to the observed greater white clover content in these treatments at the time.

The observed differences in plant characteristics across treatments in this study, indicates that treading initiated a change in the life-cycle structure of the original clover population (Brock et al., 2000). This was characterised by clover populations in treading treatments that initially (day 48) comprised both clonal plants (fragmented) and seedling plants (see below), whereas in non-pugged plots a fully clonal population was present. As a result, plant growth and morphological development was desynchronised across treatments. Thus, with time and progressive plant growth and development, clover populations of diversely different morphology occurred which were individual to each treatment.

C. Seedling recruitment

In established clover-grass pastures, white clover seedling recruitment is usually rare and plants spread by vegetative growth (Gustine and Sanderson, 2001). Data is scant regarding the quantity of seedling recruitment in clover-grass pastures, though some studies (Barrett and Silander, 1992; Chapman and Anderson, 1987) indicate average seedling recruitment of 2-6 plants m^{-2} $year^{-1}$. In late spring, seedling recruitment is apparently lower than average (Barrett and Silander, 1992; Chapman and Anderson, 1987) with reported values of 2.5 plants m^{-2} in mixed clover-grass pastures (Barrett and Silander, 1992). Seedling recruitment in the present study was high with seedling densities of 24 plants m^{-2} in severely pugged treatments after 48 days, compared to no observed seedlings in the non-pugged control. This increase in seedling density probably occurred as a result of soil disturbance by hoof action (Barrett and Silander, 1992; Turkington et al., 1979), which assisted in breaking the dormancy of buried seeds and provided a disturbed soil layer optimal for seed germination.

The data reported here suggests that seedling recruitment could be important where treading damage causes loss of clover from pasture. However, this would be dependent on the long-term survival of seedlings, which was not measured in our study. Other research by Barrett and Silander (1992) has shown that increased seedling recruitment does not necessarily guarantee the re-establishment of adult plants in grazed pasture, as defoliation and treading can cause high seedling mortality.

D. Implications for pasture management

In practice, it has been suggested that moderate and severe pugging damage may affect about 50% and 10% of an entire farm area, respectively (P. Singleton pers comm.). Based on the losses of pasture production measured in this study under moderate and severe pugging (16% and 34%), we estimate that total farm pasture production could decrease by about 7%. For the white clover component, the decrease in whole farm clover production was estimated to be an average of about 5%. For farm systems dependent on white clover for inputs of fixed nitrogen, these losses of legume production will have important implications for farm productivity (see Menneer et al., 2004b; Chapter 6).

Under conditions of regular grazing (cf. mowing), factors such as defoliation by animals, animal excreta, grazing management, and/or repeated treading activity could influence the pattern of clover growth recovery after pugging. For example, rotationally grazed clover-grass swards, with their inherent rest periods and associated lower frequency of defoliation, tend to encourage greater clover plant size than in continuously grazed swards (Brock et al., 1988). Thus, certain pasture management strategies could assist with clover recovery after damage to pasture by treading.

Where possible, strategies should be implemented that minimise the time animals spend grazing when soil conditions are overly wet. In New Zealand, winter grazing is a standard practice, and animals are often moved onto stand-off pads or yards/lanes during wet periods in winter or spring to reduce treading damage. Some early work (Brougham, 1975), has indicated that stand-off pads may increase clover content, but as yet, the full benefit of these practices on white clover production remains unknown and necessitates further research. In Europe, with the increasingly common practice of extending outdoor grazing in some milder regions (e.g. Fox, 2000), using a restricted or partial grazing regime through winter in conjunction with cow housing and supplementary feeding will also help reduce treading damage. This strategy would have the added benefit of keeping sward heights in check during winter and improve light penetration to the lower sward canopy, and favour white clover growth and survival for productive spring swards (Laidlaw et al., 1992).

In cases where winter or spring treading damage has occurred, paddock-localised remedial measures may have to be identified that provide the best opportunity for assisting white clover recovery (e.g. resting and cutting for conservation). The development of simple field-based diagnostic tools to identify white clover sward condition (e.g. visual estimates of stolon length; Jones et al., 2000) may also provide useful information from which more accurate management decisions can be made.

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5

Effects of Treading on N_2 Fixation by White Clover

5

Animal treading reduces N_2 fixation in mixed clover-grass pasture[1]

Keywords: animal pugging, soil nitrogen, white clover, ^{15}N isotope dilution, nitrogenase activity, soil aeration.

I. Abstract

A field experiment was carried out to determine the effect of animal treading on N_2 fixation in a mixed clover-ryegrass pasture over 12 months. A single pugging event of moderate or severe pugging intensity was initiated in plots during spring by using dairy cows at varying stocking rates (4.5 cows 100 m^{-2} for 1.5 or 2.5 hours, respectively). Thereafter, defoliation was by mowing as required. Annual N_2 fixation in clover herbage decreased significantly from 76 kg N ha^{-1} yr^{-1} in the non-pugged control, to 66 kg N ha^{-1} yr^{-1} and 36 kg N ha^{-1} yr^{-1} under moderate and severe pugging, respectively. Associated reductions in clover dry matter production were 9% and 52%, respectively. The loss of fixed N occurred mainly during the first 156 days after pugging, but under severe pugging the depression persisted for 259 days. The proportion of clover N derived from atmospheric N_2 (%Ndfa) was initially reduced (to a lower limit of 40%) by severe pugging (days 28-71) before recovery to control levels by day 91. Soil aeration,

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[1] Menneer J C, Ledgard S F, McLay C D A and Silvester W 2004 Animal treading reduces N_2 fixation in mixed clover-grass pasture. Plant and Soil (Submitted).

expressed as air-filled porosity, decreased from 21% in the non-pugged control to non-critical levels of 15-16% in pugged treatments by day 3. Soil inorganic N concentration increased in pugged treatments, and was 4 fold greater on day 28 in severely pugged plots compared to non-pugged plots. Increased N concentration in ryegrass over the medium-term (days 28-71) reflected this greater availability of soil inorganic N. The greater soil N concentration probably led to the measured medium-term reduction of N_2 fixation. On a whole farm basis we calculate the annual fixed N contribution to decrease by 9% when taking into account typical areas of farmland subjected to moderate (50%) and severe (10%) pugging by dairy cows during any one year. Over the long term, reduced inputs of fixed N associated with animal treading could gradually drive soil organic N to lower equilibrium levels and further impact on farm productivity.

II. Introduction

In many temperate pasture systems, white clover (*Trifolium repens* L.) plays a key role in providing nitrogen (N) to pasture through biological N_2 fixation. Estimates of the annual contribution of fixed N in clover-grass pastures are in the range of 55-296 kg N ha^{-1} $year^{-1}$ (Ledgard and Steele, 1992). This wide range of values reflects to some extent differences in environmental and site-dependent factors, but is primarily due to grazing management practices which control clover persistence, production and N_2 fixation.

In intensively managed legume-based pastures, factors relating to grazing animals (e.g. treading, defoliation, and excreta) and their management have the potential to significantly affect clover growth and N_2 fixation. Previous studies (Brock et al., 1983; Ledgard et al., 1996; Menneer et al., 2003; Vinther, 1998) have largely focussed on defoliation and excreta effects on clover growth and N_2 fixation. For example, Menneer et al. (2003) reported a prolonged (nearly 12-months) decrease in N_2 fixation in urine treated clover-grass swards. In contrast, animal treading, although an important feature of intensively grazed systems, has received little research attention.

In general the response of soil to animal treading depends on the soil moisture status. In wet soils, pugging or poaching may occur with remoulding of the soil around animal hooves causing deformation at the soil surface and compaction at depth (Horne, 1992; Scholefield and Hall, 1985; Scholefield et al., 1985). Under very wet conditions puddling followed by surface crusting and compaction can also occur (Greenwood and McKenzie, 2001). If soils are drier, soil compaction can still result, but with little deformation of the soil surface.

Various studies, in intensively grazed pasture suggest that animal treading has the potential to impact on a range of soil and plant properties (Brown and Evans, 1973; Davies et al., 1989; Edmond, 1962; Greenwood and McNamara, 1992; Mulholland and Fullen, 1991; Vertès, et al., 1988). For example, soil compaction resulting from animal treading can lead to increased soil resistance and decreased soil aeration (Davies et al., 1989; Greenwood and McNamara, 1992; Mulholland and Fullen, 1991). Studies of pasture and crop legumes grown in pots containing artificially compacted soil and/or under conditions of low aeration often show a reduced shoot and root yield, and an observed depression in N_2 fixation (Cook et al., 1996; Naiden et al., 1996, 1997; Pugh et al., 1995; Tu and Battery, 1988). However, these effects are yet to be observed in clover-grass swards affected by compaction resulting from treading.

In addition to the negative effects of soil compaction, animal treading in pasture can also reduce plant growth by plant injury, fragmentation, and burial (Brown and Evans, 1973; Edmond, 1962; Vertès, et al., 1988). In the field, plant damage effects have been difficult to differentiate from the indirect effects of soil compaction. Several studies have measured the combined effects of treading on clover growth and production (Brown and Evans, 1973; Edmond, 1962; Vertès, et al., 1988). For example, early research (Brown, 1968; Edmond, 1958, 1962) in mixed clover-grass pastures, showed that treading and its associated effects on soil and plant properties can lead to individual losses of white clover production ranging from 5% to 95%. These reported studies were of short-term duration (less than 1 year), and so did not measure the full seasonal effect of treading on legume growth response, nor did they measure effects on N_2 fixation.

To help fill this gap in the literature we recently (see Menneer et al., 2004a; Chapter 4) provided detailed observations of the temporal changes in clover growth and morphology over 1 year, in clover-grass pasture subjected to treading by dairy cows. The aims of the research presented in this study were to (1), quantify the effects of treading and associated changes in soil physical properties on white clover production and amounts of annual N_2 fixation, and (2), to investigate the underlying processes involved in regulating N_2 fixation subsequent to treading.

III. Materials and Methods

A. Experimental site and soil characteristics

The experiment was on a Te Kowhai silt loam (Typic Ochraqualf, Soil Survey Staff, 1994; Typic Orthic Gley Soil, Hewitt, 1993) with impeded subsoil drainage. The pasture contained a long-term (>30 years) permanent mixed stand of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.).

A nil-N basal fertiliser (80 kg P ha^{-1}, 75 kg K ha^{-1}, 110 kg S ha^{-1}) was applied, to ensure non-limiting nutrient levels.

B. Experimental design

Treatments consisted of a single pugging event of three severities (nil, moderate and severe). A randomised block experimental design was used with 8 replicates. Plots were 2.5 m x 7 m long with a 0.5 m buffer strip between plots and blocks. In early spring (21 September) 1999, moderate and severe pugging treatments were established (after the site had been harvested) by walking dairy cows through the plots at a typical grazing intensity of 4.5 cows $100m^{-2}$ for 1.5 and 2.5 hours, respectively, to achieve the desired level of treading damage. Rainfall and irrigation in the days before commencing the experiment meant that the soil was near saturation when the pugging treatments were applied. Prior to applying the pugging treatments the cows were kept in stockyards overnight so as to minimise inputs of dung and urine onto the plots during the treading event. In the rare event that excreta inputs did occur, these were intercepted and removed.

C. Harvest and analysis

To determine the full effect of pugging on pasture productivity the study was carried out over a 12-month period, and used mowing for defoliation. The first harvest of plots occurred 28 days after treatment application, and thereafter at approximately 21-day intervals. The wet weight of herbage from each harvest was recorded and a subsample used to determine the dry matter (DM) yield. Immediately prior to each harvest, herbage was sampled from within a 3 m x 1 m microplot in each plot. Samples were separated into species, and the clover and ryegrass components were dried and analysed for total N and ^{15}N concentration using an automated N analyser (Europa Scientific ANCA-SL) interfaced to a Europa Scientific 20-20 continuous-flow stable isotope analyser.

D. Plant analysis

Three turves (300 mm x 300 mm each) were removed from random locations within each plot for individual plant sampling and analysis immediately prior to harvesting on day 48. Soil was gently washed from the roots and whole clover plants separated from the vegetation mat. Any plants severed by the quadrat edge were rejected. A suitably large quadrant size (300 mm x 300 mm each) was used to minimise any bias against large plants caused by the removal of severed plants (Harris et al., 1994; M. Hay pers comm.). The number of severed plants per turf was recorded, and a maximum of 20 non-severed plants (randomly selected when more than 20 were present) were dissected, and the leaf, stolon, and root components bulked for drying and subsequent weighing. The number of nodules on roots was also recorded.

E. Calculations of N_2 fixation

1. ^{15}N isotope dilution technique

Long-term time averaged estimates of total amounts of N_2 fixation were measured using the ^{15}N-isotope dilution technique on microplots (3m x 1 m) within each plot.

^{15}N-enriched (40 atom %^{15}N) ammonium sulphate solutions were applied evenly to each microplot as a 2.5 L plot^{-1} volume using a watering can with a rosette attachment at a rate of 1 kg N ha^{-1} two weeks prior to application of the pugging treatments. Any $(^{15}NH_4)_2SO_4$ residue on the herbage was washed off with 2.5 L plot^{-1} of tap water using a knapsack sprayer.

This method of applying ^{15}N in advance of study commencement allows the initial rapid decline in ^{15}N concentration of soil inorganic N to be avoided. It has been shown that such a decline can sometimes lead to inaccurate estimates of N_2 fixation, (e.g. Witty, 1983). Later in the study (day 112), a second application of $(^{15}NH_4)_2SO_4$ was applied to ensure that an adequate level of soil ^{15}N enrichment continued to be available to accurately estimate N_2 fixation.

Calculation of the percentage of legume N fixed from atmospheric N_2 (%Ndfa) was as follows where the ^{15}N dilution technique was used (Ledgard and Steele, 1992).

$$\%\text{Ndfa} = 100 \times \left\{ \frac{\text{Atom } \%\, ^{15}\text{N}_{\text{ryegrass N}} - \text{Atom } \%\, ^{15}\text{N}_{\text{clover N}}}{\text{Atom } \%\, ^{15}\text{N}_{\text{ryegrass N}} - \text{B}} \right\}$$

where:

B is the atom % of ^{15}N of N derived from atmospheric N_2 (0.365762 atom % ^{15}N; Ledgard et al., 1985).

2. *Nitrogenase activity*

Nitrogenase activity was determined during the first 28 days (on days 3, 8, 14, 21 and 28) after pugging using a field soil incubation system, involving acetylene reduction (Knowles, 1981). Assays were performed between 0900 – 1200 hrs. Minimally disturbed soil cores (65 mm diameter x 70 mm depth) were taken from plots and wrapped in tinfoil (to minimise diffusion of atmospheric O_2 into the core), but with the soil surface still exposed, and placed in 1 litre preserving jars. Three jars containing two cores each were taken from each plot. The jars were closed with a steel lid, fitted with a silicon septum, and sealed with a rubber gasket and a screw band. Acid-scrubbed acetylene (80 ml, 10% v/v) was injected into each jar through the septum, after ejecting an equal volume of headspace to maintain atmospheric pressure. The jars were then incubated in situ in a shallow covered trench, on site, for 24 hours.

After incubation, the headspace of the jars was sampled in duplicate using double-ended sampling needles and pre-evacuated Vacutainer (Becton, Dickinson and Co., Rutherford, New Jersey, U.S.A.) blood-sampling tubes (10 ml volume). Direct analysis of the gas sample for ethylene was carried out immediately after collection using gas chromatography. Results for nitrogenase activity are expressed on a per area basis, and specific activity (for day 21) is expressed on the basis of the clover leaf dry weight of the previously assayed cores.

F. Soil analysis

After sampling the headspace of each jar, the soil cores were thoroughly mixed and soil NH_4^+-N and NO_3^--N concentrations were determined by extracting 20 g of fresh soil with 50 ml 2M KCl. After 1 hour on an end-over-end mixer the soil suspension was filtered and colourmetrically analysed using a flow injection analyser (Blakemore et al., 1987). Soil water content was determined gravimetrically after drying fresh subsamples at 105°C for at least 24 hours. Soil cores were taken from the topsoil (0-50 mm) 3 days after pugging and used to estimate bulk density, particle density and total porosity using the methods of the

New Zealand Soil Bureau (1972). Gravimetrically determined water contents were multiplied by bulk density to calculate volumetric water contents. Air-filled porosity (AFP) at a potential of -10kPa was calculated by subtracting the volumetric water content from the total pore space.

G. Statistical analysis

Analysis of variance was carried out using Genstat 4.2, Fifth Edition. SED or LSR values were used to compare means when main effects were found to be significant.

Table 1. Main effects of pugging on selected components of total dry matter yield (DM), total N yield, pasture composition and N_2 fixation over the 12 month study.

	Pugging severity			
	Nil	Moderate	Severe	SED
Total DM yield (kg ha^{-1} yr^{-1})	10149	8562	6683	417***
Grass DM yield (kg ha^{-1} yr^{-1})	6817	5497	4264	558***
Grass N yield (kg ha^{-1} yr^{-1})	197	154	116	16***
Clover DM yield (kg ha^{-1} yr^{-1})	2046	1854	989	479†
Clover proportion (%)[a]	20	22	15	3.6NS
Clover N yield (kg ha^{-1} yr^{-1})	89	78	40	21†
Clover fixed N yield (kg ha^{-1} yr^{-1})	76	66	36	18†
N fixed (%)	82	85	85	1.4NS

[a] based on total clover DM yield.

***, †significant at $P<0.001$, and $P<0.1$, respectively, unless designated NS, not significant; 8 replicates.

IV. Results

A. Climatic data

Total annual rainfall of 964 mm was 20% below the long-term average of 1201 mm year^{-1} (Figure 1). With the exception of one month in late-spring (November) and another in mid-autumn (April), rainfall in all other months was below average (Figure 1). Most noticeable were two periods of very low rainfall; one in mid-spring (October) and the other in late-summer (February), when rainfall was only 8% and 28%, respectively, of the long-term monthly average. Irrigation (30 mm) was applied during October to avoid atypically dry conditions.

B. Effects of pugging on pasture production and amount of N fixed

Moderate or severe pugging decreased ($P<0.001$) annual pasture production by 16% or 34%, respectively (Table 1). This was due to decreases in both grass and white clover production, which were greatest in the severely pugged plots at -37% and -52%, respectively (Table 1). In the moderately pugged plots, the production of grass and white clover decreased by 19% and 9%, respectively.

N yield in ryegrass decreased by 22% and 41% under moderate and severe pugging, respectively. A period of reduced ryegrass N concentrations between days 112 – 259 ($P<0.1$; Figure 2b) after pugging, contributed to the lower N yield of ryegrass in pugged treatments.

During the first 48 days, non-legume (grass+weeds) growth showed a pronounced reduction of up to 90% in pugged plots ($P<0.01$; Figure 2c) compared to non-pugged control plots. Subsequently, non-legume growth returned to control levels, except in severely pugged plots which experienced increased growth over the subsequent 64 days ($P<0.01$; Figure 2c). Later in the study, a more prolonged second phase (days 220-356) of reduced grass growth occurred in the pugged treatments (Figure 2c).

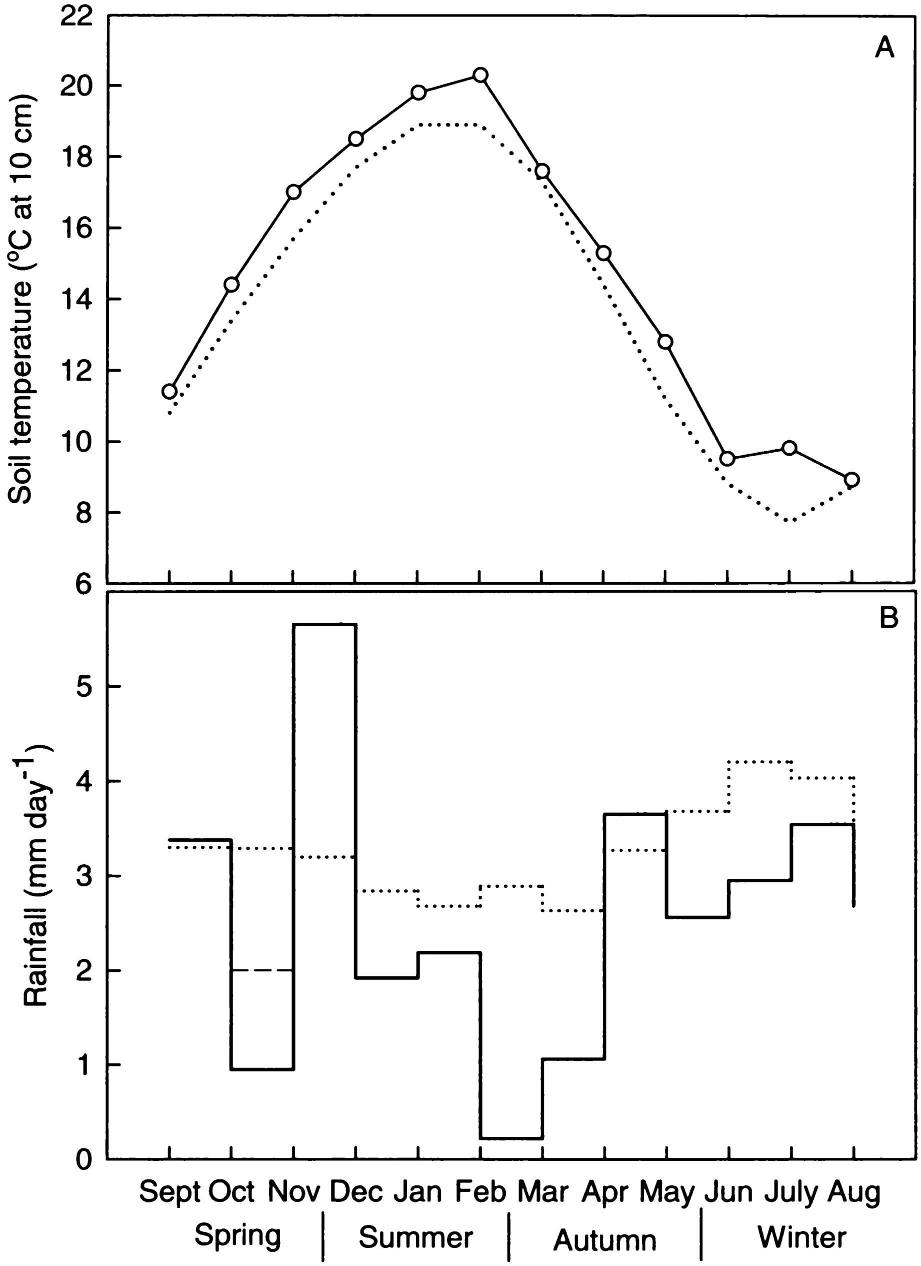

Figure 1. Mean monthly soil temperature (A) and rainfall (B) relative to the longer term average (·······). Dashed line in October represents irrigation.

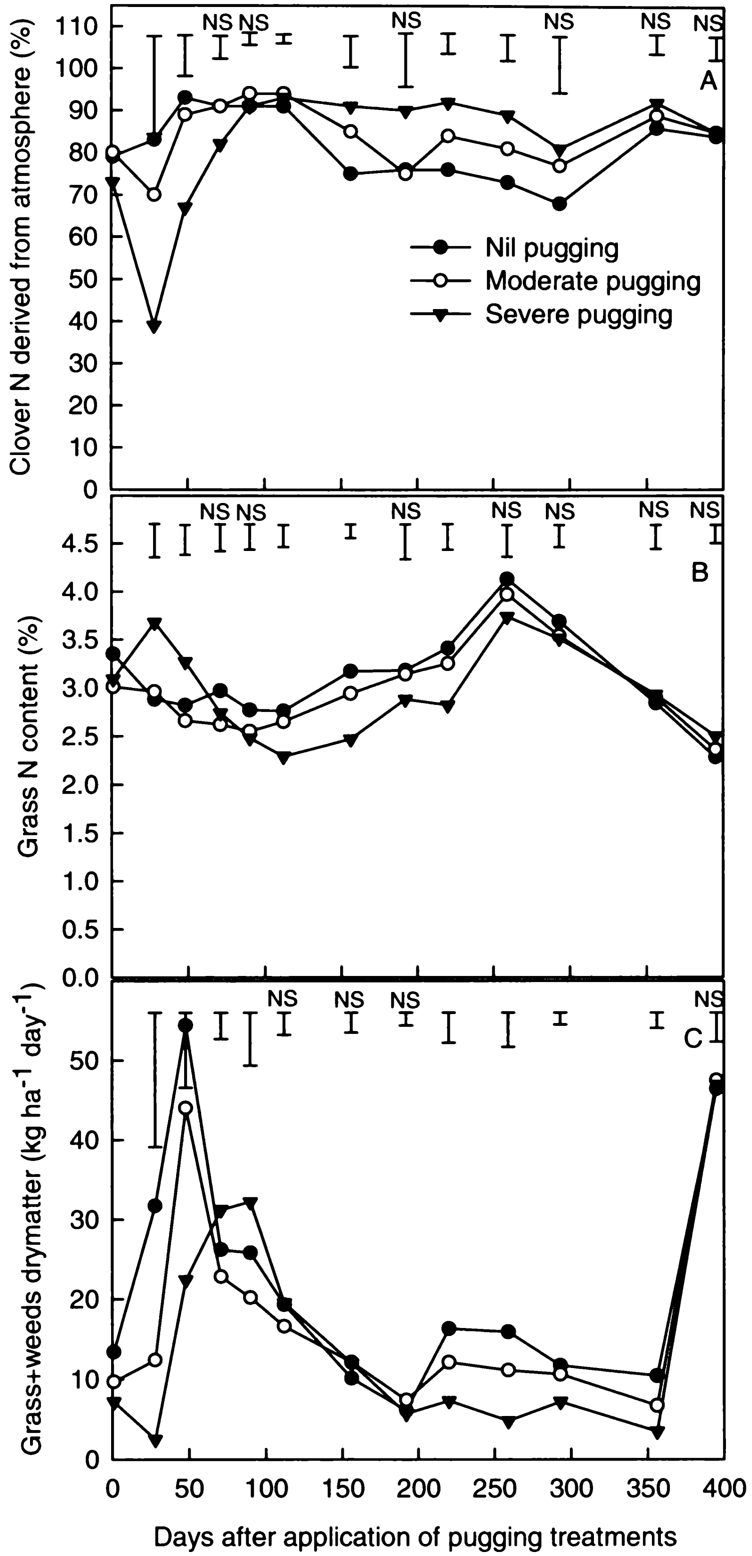

Figure 2. Effect of pugging on N_2 fixation (A), grass N concentration (B), and grass+weed growth (C), during the 12 month study. Error bars represent SED; $P<0.05$ unless designated NS, not significant (8 replicates).

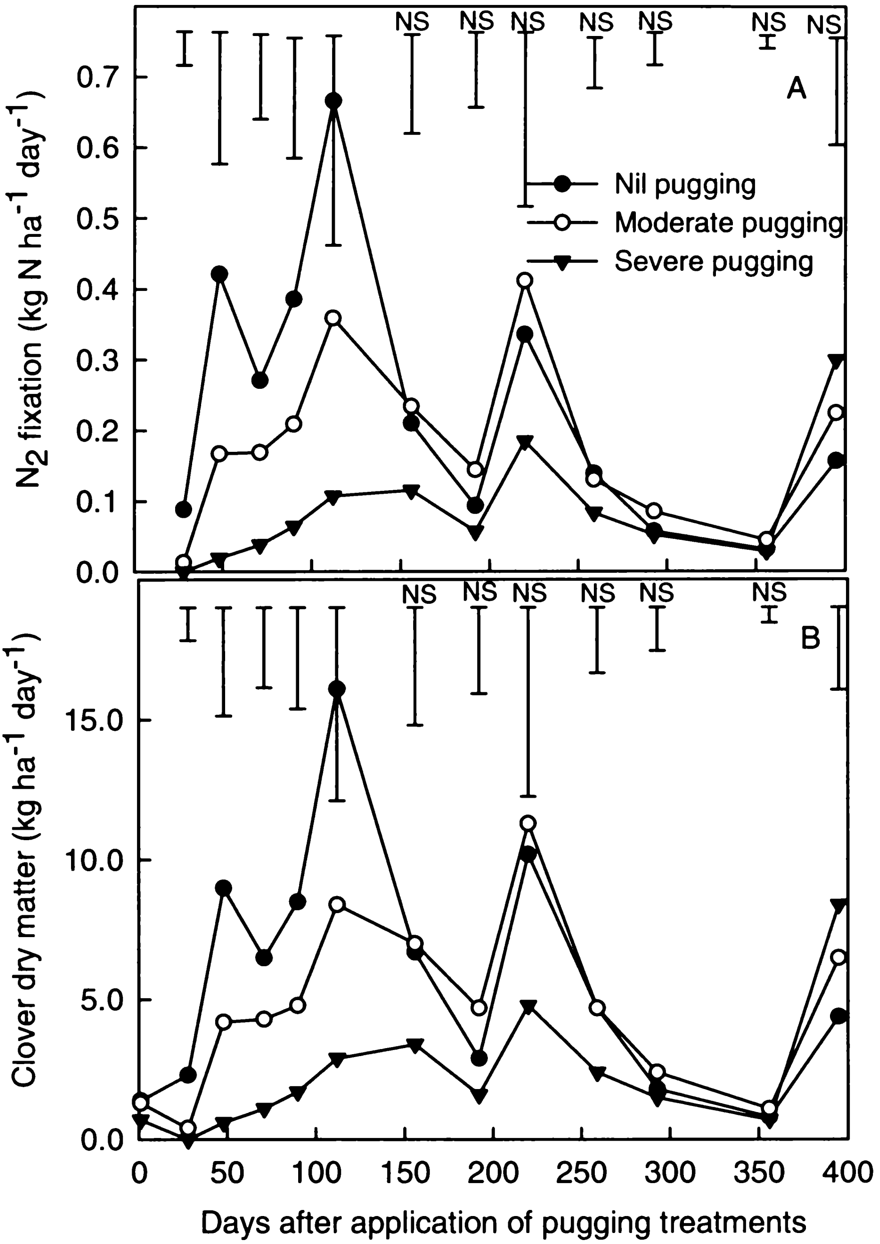

Figure 3. Effect of pugging on N_2 fixation (A), and clover growth (B), during the 12 month study. Error bars represent SED; $P<0.05$ unless designated NS, not significant (8 replicates).

The temporal pattern of white clover growth (Figure 3b) showed marked and prolonged reductions (156 days) due to pugging. This decrease was most marked on day 112 when clover growth was 53% and 82% less under moderate and severe pugging, respectively. Associated with this loss in clover yield was a large decrease in annual fixed N in clover herbage of 13% and 53% in moderately and severely pugged plots, respectively (*P*<0.1; Table 1). The effect of pugging on N_2 fixation rate was greatest during the first 156 days (up to 90%; *P*<0.1; Figure 3a), coinciding with the period of greatest clover growth reduction.

C. Effects of pugging on individual white clover plants

Moderate and severe pugging significantly decreased the dry weight of individual clover plants by 48 days after pugging (Table 2). Under severe pugging, this effect was represented by a 70% reduction in per plant dry weight, from 170 mg plant^{-1} in the control, to 52 mg plant^{-1} in severely pugged plots. Equivalent losses of leaf and stolon+root dry weight accounted for the reduction in total plant yield.

Pugging also had a significant effect on plant densities which decreased by 37% and 85% in the moderately and severely pugged treatments, respectively. In addition, root nodule numbers also decreased under severe pugging (Table 2).

D. Direct effects of pugging on N_2 fixation

Nitrogen fixation (per area basis) estimated by acetylene reduction declined rapidly by up to 90% within 3 days in both moderate and severe pugging treatments (*P*<0.05; Figure 4a), and probably reflected the loss in clover yield that was apparent after pugging. During the remainder of the measurement period (25 days) acetylene reduction in pugged treatments increased slightly, but still remained low at 13% to 40% of the non-pugged control on day 28. When acetylene reduction was calculated on a shoot dry weight basis (specific activity on day 21), values were still significantly (*P*<0.05) lower in pugged treatments

Table 2. Effect of pugging on selected clover plant properties at 48 days after pugging by dairy cows in spring.

	Pugging severity			
Plant parameter	Nil	Moderate	Severe	SED
Plant density (m^{-2})	492	311	72	128*
Total dry weight (mg $plant^{-1}$)	170	114	52	40*
Leaf dry weight (mg $plant^{-1}$)	78	48	19	22.4†
Stolon dry weight (mg $plant^{-1}$)	69	50	26	18†
Root dry weight (mg $plant^{-1}$)	23	16	7	4.8*
Nodule number ($plant^{-1}$)	21	19	7.3	4.9*

*, † significant at $P<0.01$, $P<0.05$, and $P<0.1$, respectively, unless designated NS, not significant (4 replicates).

compared to non-pugged treatments (Figure 4b). Thus, reduced fixed N yield was due to both reduced clover growth and reduced specific activity.

Long-term, time integrated estimates of N_2 fixation using the ^{15}N isotope dilution technique showed that the percentage of clover N derived from atmospheric N_2 (%Ndfa) decreased markedly ($P<0.05$-0.001) under severe pugging to a low of 40% on day 28 (Figure 2a). Subsequently, values of %Ndfa steadily increased until control levels were reached by 90 days. However, %Ndfa showed a prolonged (200 days) second period (days 112-356) of treatment effect which was the reverse of the earlier trend and characterised by estimates of %Ndfa that were greater in pugged plots compared to the non-pugged plots (Figure 2a).

E. Soil analysis

Within 3 days of pugging, soil NH_4^+-N concentration had increased in severely pugged plots to a value 2.5 fold greater than that of the control ($P<0.10$; Table 3), while soil NO_3^--N concentration was unaffected ($P<0.05$-0.10; Table 3). However, by day 28, soil NH_4^+-N in the severely pugged treatment had returned to control levels, and soil NO_3^--N concentration had increased 4-fold.

Pugging adversely affected both air-filled porosity and soil moisture content by 3 days after pugging ($P<0.05$; Table 3). The effect was similar in both pugging treatments, with air-filled porosity decreasing to an average of 15.5% under moderate and severe pugging, compared to 21% in the non-pugged control. There was no long-term effect of treading on soil physical properties, with soil physical indicators similar in all treatments 1 year after pugging (data not shown).

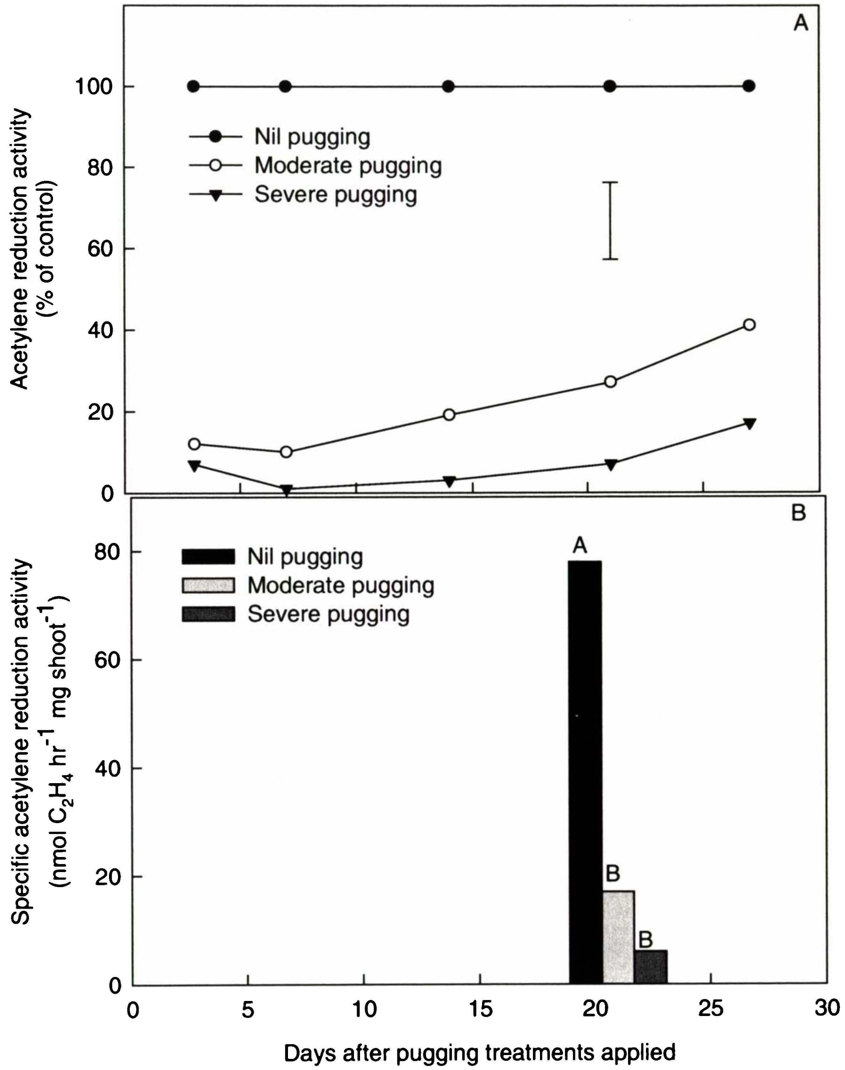

Figure 4. The effect of pugging on acetylene reducing activity, based on nmol C_2H_4 m^{-2} (A), and specific acetylene reducing activity per unit clover shoot dry matter (B). For (A), on average error bar represents SED; $P<0.05$, 8 replicates. For (B), geometric means are presented and the LSR = 6.29, bars with different letters are significantly different from each other; $P<0.05$).

Table 3. Effect of pugging severity (nil, moderate, or severe) on selected soil properties at 3 and 28 days after a single pugging event by dairy cows in spring.

	Day 3				Day 28			
Soil parameter	Nil	Moderate	Severe	SED/LSR	Nil	Moderate	Severe	SED/LSR
Air-filled porosity (%)	21	15	16	0.011**	NR	NR	NR	-
Soil moisture (%)[a]	39	45	44	1.68*	27	30	25	0.021NS
NH_4^+-N (kg ha^{-1})[b]	3.4	3.9	8.4	2.43†	1.8	2.1	2.4	1.87NS
NO_3^--N (kg ha^{-1})[b]	2.5	2.8	2.7	1.35NS	2.2	5.2	8.8	2.79*

[a] θ_v, at field capacity (-10 kPa)=46%.

[b] data are geometric means, and relevant error terms are LSR.

**, *, † significant at $P<0.01$, $P<0.05$, and $P<0.1$, respectively, unless designated NS, not significant (4 replicates).

NR=not recorded.

V. Discussion

A. Effects of pugging on amount of N fixed and pasture production

This study is the first to show the large adverse impact animal treading can have on N_2 fixation in the absence of added N from dung and urine, with recorded decreases in annual N_2 fixation of 13% and 53% under moderate and severe pugging, respectively. The decrease in N_2 fixation was due mainly to substantial losses of clover DM production that occurred under moderate and severe pugging (9% and 52%, respectively) as a result of major treading damage and burial of clover plant tissue by hoof action (see Menneer et al., 2004a; Chapter 5). The adverse effect of both moderate and severe pugging on clover growth was most noticeable (up to 90% decrease) during the first 156 days after pugging, and accounted for the majority of the loss in total fixed N during the 12-month study.

Research (Jorgensen and Ledgard, 1997; McNeill et al., 1997) has shown that fixed N in stolon+roots (below cutting-height tissue) can potentially be 70% of the fixed N in leaves. Although the contribution of below cutting-height fixed N was not directly measured in this study, stolon+root yields in control plots represented 54% of the total plant dry weight, which is similar to that measured by Jorgensen and Ledgard (1997). In pugged plots, individual plant dry weight showed significant decreases (e.g. 70% reduction under severe pugging), but the proportion of leaf and stolon+root dry weight in whole plants remained similar. Thus, based on a contribution from fixed N in below-cutting height tissue at 70% of that in leaves, estimates of total annual N_2 fixation in our study would be 129, 112, and 61 kg N ha^{-1} under nil, moderate, and severe pugging, respectively.

In addition to treading, other factors relating to grazing animals (e.g. grazing severity and excreta) can also affect N_2 fixation in clover-grass pastures (Menneer et al., 2003; Vinther, 1998). For example, Menneer et al. (2003) found that moderately-severe cutting, compared to lenient cutting, caused an increase in annual N_2 fixation of 36%, whereas a single application of cow urine caused annual N_2 fixation to reduce by 38%. These effects were largely mediated by changes in soil N status over time (see Menneer et al., 2003). In comparison, pugging, at least when severe, appears to have a greater effect on fixed N inputs than urine, but mainly through its impact on clover growth rather than from effects on soil N (but see below).

B. Direct effects of pugging on N_2 fixation

An initial decrease (up to 71 days) in the percentage of total clover N derived from N_2 fixation (%Ndfa) under severe pugging implies that changes in the efficiency of nodule functioning is a factor in the short-medium term after a severe pugging event. This effect was potentially due to soil physical deterioration (e.g. reduced aeration) leading to reduced N_2 fixation, and/or increased soil N availability. For example, various studies (Pugh et al., 1995; Tu and Battery, 1988; Voorhees et al., 1976) using both pasture and crop legumes have shown that soil compaction and/or reduced aeration can cause a reduction in specific nitrogenase activity.

Indicators of soil physical deterioration measured 3 days after pugging showed a deleterious effect which was significant for estimates of air-filled porosity and soil moisture. Nonetheless, values of air-filled porosity were still well above the 10-12% threshold that is often used to indicate limiting conditions for plant growth and function (Carter, 1988; 1990; Grable, 1971; Greenland, 1981). Furthermore, rainfall during the month after pugging was well below average, and soil water content fell from an average of 45% in pugged treatments to 28% by day 28 (Table 3). At this lower limit of soil moisture content it is doubtful that poor soil aeration would provide any limitation to plant growth and function. This indicates that the seasonal timing of pugging events, and the extent of subsequent rainfall, probably has an importance influence on the potential for decreased N_2 fixation due to poor aeration.

In our study, a one-off pugging event was carried out in spring, which is generally typified by less rainfall than in winter, and soils which are gradually drying out. In comparison, if pugging occurs in late autumn or winter when rainfall and soil moisture contents are generally greater, soil aeration could potentially limit N_2 fixation. Future research, under field conditions, during wetter seasons will assist in determining the importance of seasonal climatic differences on pugging-induced changes in soil aeration and N_2 fixation.

Recent studies, (e.g. Menneer et al., 2003) have shown that pasture growth mediated changes in soil N availability under clover-grass pastures can influence %Ndfa. In the present study, soil inorganic N concentrations increased in severely pugged plots (e.g. 4 fold increase of soil NO_3^--N on day 28) compared to non-pugged plots during at least the first 28 days of the study. This increased concentration of plant-available soil N was probably due to a reduction in plant N uptake associated with the marked decreases in grass growth (up to 90%) measured under moderate and severe treading, respectively, during the first 28 days of the study. Greater ryegrass N concentrations in severely pugged treatments during these initial stages of the study also imply that plant-available soil N concentrations had increased in these low yielding treatments. Typically, under an increasing soil inorganic N pool, N_2 fixation is depressed because of the inhibitory effect of soil N on nitrogenase activity (e.g. Moustafa et al., 1969; Mundy et al., 1988; Hunt and Layzell, 1993). In legume-grass systems that depend solely on N_2 fixation for N inputs, even subtle increases in the indigenous plant-available N pool have been found to adversely affect nitrogenase activity (Halliday and Pate, 1976; Hoglund and Brock, 1978). Thus, in the present study, the medium-term reduction (days 28-71) in %Ndfa under severe pugging was probably due to increased soil N uptake by clover over the more energy dependent process of N_2 fixation (Phillips et al., 1982). Similarly, the depressed levels of nitrogenase activity per unit of clover growth on day 21 in pugged treatments probably reflected the greater concentration of plant-available soil N in these treatments.

An apparent increase in estimates of %Ndfa in severely pugged plots during the latter part of the study was probably also generated by a change in plant-available N levels. Increased growth of non-legumes between days 48 and 112 in severely pugged treatments, may have driven soil inorganic N to a new lower level. Soil inorganic N concentrations were not measured during this period, but foliar N concentrations in ryegrass showed a prolonged period (days 112-220) of reduced values in severely pugged plots, indicating a possible reduction in soil inorganic availability. This would have had flow-on effects for N_2 fixation. On average, the %Ndfa between days 112 and 356 in severely pugged plots was 15% above that of the non-pugged control. However, this increase did little to compensate for the large loss of fixed N that occurred as a result of reduced clover yield.

C. Implications for management

In intensively grazed New Zealand dairy systems, the average stocking rate is 2.7 cows ha^{-1} $year^{-1}$ (Livestock Improvement, 2003), and in these systems it has been suggested that moderate and severe pugging damage may affect about 50% and 10% of an entire farm area, respectively (P. Singleton pers comm.). Based on the estimate of N_2 fixation in the present study in non-pugged plots (76 kg N ha^{-1} yr^{-1}), we calculate that N_2 fixation would have decreased by 12% on a whole farm basis using the land area covered by moderate and severe pugging above, and the measured decreases in annual N fixed (13% and 53% under moderate and severe pugging, respectively). This decrease in whole-farm fixed N due to pugging is similar to recently reported losses of fixed N (about 10% on whole farm basis) that occur in clover-grass swards affected by cow urine (Menneer et al., 2003).

In intensively managed legume-grass systems relying on N_2 fixation for their main N input, annual losses of fixed N due to treading could impact on the N economy of pasture. For example, treading damage to pasture during wet periods is a seasonal feature of many intensive grazing systems, and over the long-term the associated reduced annual input of fixed N could gradually drive soil organic N to lower equilibrium levels and impact on farm productivity. Future research using modelling approaches are required to assess the effect of reduced fixed N inputs, associated with regular seasonal treading events, on N cycling in legume-grass pastures.

Clearly, farm management practices should be identified that minimise animal grazing time on pasture when soil conditions are wet and susceptible to treading damage. Examples of management strategies include winter stand-off pads and restricted grazing (Menneer et al., 2004b; Chapter 2. Sec. VI.B). These practices would need to be linked with protocols to identify when soil conditions are susceptible to treading damage, and a greater understanding of the possible feed-back for animal productivity.

VI. Acknowledgements

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6

Animal Treading Stimulates Denitrification

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Animal treading stimulates denitrification in soil under pasture[1]

Keywords: denitrification; animal treading; soil nitrate; water-filled porosity; soil aeration; plant N uptake.

I. Abstract

The current study was carried out to measure the effects of animal treading on denitrification in a mixed clover-ryegrass pasture. A single treading event of moderate and severe treading intensity was initiated in plots during spring by using dairy cows at varying stocking rates (4.5 cows 100 m^{-2} for 1.5 and 2.5 hours, respectively). Treading caused a significant short-term (21 days) increase in denitrification. Denitrification rates reached a maximum of 52 g N_2O-N ha^{-1} day^{-1} 8 days after severe treading compared to 2.3 g N_2O-N ha^{-1} day^{-1} under nil treading. Thereafter, denitrification rates declined, and were similar to non-trodden control plots after 28 days. Soil aeration, as expressed by water-filled porosity, was significantly increased by treading during the study. In addition, soil NH_4^+-N and NO_3^--N concentrations were also increased by treading. We propose that the underlying processes involved in increasing denitrification under treading were two-fold. Firstly, treading caused a temporary (<21 days) reduction in soil

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aeration through soil physical damage, and secondly, reduced soil N utilisation prompted by reduced plant growth led to increased soil NH_4^+-N and NO_3^--N availability. This study shows that treading, without the influence of other grazing animal factors (e.g. excretion), can stimulate denitrification in grass-clover pastures.

II. Introduction

Losses of nitrogen (N) from soil by denitrification are known to be affected by many soil properties, such as soil water content, forms and amounts of N, soil temperature, pH and available carbon (Groffman et al., 1987). In intensively grazed pastures, the principal regulators of denitrification are soil aeration and soil NO_3^--N availability, which commonly limit denitrification (Barton et al., 1999; Groffman et al., 1987, 1993; Jarvis et al., 1991). In legume-based systems not receiving fertiliser N, inputs of excreta N by grazing animals potentially provide a large source of soil NO_3^--N for denitrifying microbes. Indeed, studies using synthetic urine applied to soil cores, lysimeters, and directly onto pasture soils (e.g. Clough et al., 1994; Fraser et al., 1994; de Klein and van Logtestijn, 1994; Ryden, 1986) have shown greatly enhanced rates of denitrification.

Under field conditions, various workers (Carran et al., 1995; Luo et al., 1999; Ruz-Jerez et al., 1994) have measured high denitrification rates soon after grazing. In these studies the stimulation of denitrification has been mainly attributed to the direct influence of the N returned in excreta. However, other factors associated with grazing are often speculated to contribute to higher denitrification after grazing, and include, increased soil NO_3^--N due to limited plant N uptake after defoliation, increased soil carbon availability by deposition of animal excreta, and reduced soil aeration resulting from animal treading and soil compaction (e.g. Carran et al., 1995; Luo et al., 1999; Ruz-Jerez et al., 1994). Very little is known about the effect of these grazing related factors on denitrification or the underlying regulating processes involved.

The aims of the current study were, firstly, to determine the effect of treading on denitrification without the influence of animal excreta inputs, and secondly, to describe the underlying processes that regulate denitrification in soils affected by treading.

III. Materials and Methods

A. Experimental site and soil characteristics

The experiment was established on a Te Kowhai silt loam (Typic Ochraqualf, Soil Survey Staff, 1994; Typic Orthic Gley Soil, Hewitt, 1993) with impeded subsoil drainage. The pasture was a long-term (>30 years) permanent mixed stand of perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.). Fertiliser N had not been applied to the site for at least 5 months.

B. Experimental design

Treatments consisted of a single treading event of three severities (nil, moderate and severe). A randomised block experimental design was used with 8 replicates. Plots were 2.5 m x 7 m long with a 0.5 m buffer strip around each plot. The site was taken out of grazing 5 months prior to commencing the study, and all dung pats removed. Two pre-conditioning harvests were taken from the site during the month prior to commencing the study to reduce variability and for potential covariate analysis. In early spring (21st September) 1999, moderate and severe treading treatments were imposed (after the site had been harvested) by walking dairy cows through the plots at a typical grazing intensity of 4.5 cows $100m^{-2}$ for 1.5 and 2.5 hours, respectively, to achieve the desired level of treading damage. Rainfall and irrigation in the days before commencing the experiment meant that the soil was near saturation when the treading treatments were applied. Prior to applying the treading treatments the cows were kept in stockyards overnight to avoid inputs of dung and urine onto the plots during the treading event. Subsequently, grazing animals were excluded from the site for the duration of the study. In the rare event that excreta inputs did occur these were either intercepted (for urine) or removed from the plots (for dung).

C. Denitrification measurements

Denitrification rate was determined over 28 days (on days 3, 8, 14, 21 and 28) after treading using a field soil incubation system, involving acetylene inhibition (Aulakh et al., 1992; Ryden et al., 1987). Minimally disturbed soil cores (65 mm diameter x 70 mm depth) were taken from plots and wrapped in tinfoil (to minimise diffusion of atmospheric O_2 into the core), but with the soil surface still exposed, and placed in 1 litre preserving jars. Three jars containing two cores were taken from each plot. The jars were closed with a steel lid, fitted with a silicon septum, and sealed with a rubber gasket and a screw band. Acetylene (50 ml, 10% v/v) was injected into each jar through the septum after ejecting an equal volume of headspace to maintain atmospheric pressure. The jars were then incubated in situ on site for 24 hours.

After incubation, the headspace of the jars was sampled using double-ended sampling needles and pre-evacuated vacutainer (Becton, Dickinson and Co., Rutherford, New Jersey, U.S.A.) blood-sampling tubes (10 ml volume). Samples were collected in duplicate in case of needle blockages and as a check on variability. Direct analysis of the gas sample for N_2O concentration was carried out immediately after collection by gas chromatography.

D. Soil analysis

After sampling the headspace of each jar, the incubated soil was thoroughly mixed, and 20 g of fresh soil was extracted with 50 ml 2M KCl. Soil NH_4^+-N and NO_3^--N concentrations were determined using a flow injection analyser (Blakemore et al., 1987). Soil water content was determined gravimetrically after drying fresh subsamples at 105°C for at least 24 hours. Soil cores were taken from the topsoil (0-50 mm) 3 days after treading and used to estimate bulk density, particle density and total porosity. Gravimetrically determined water contents were multiplied by bulk density to calculate volumetric water contents.

Water-filled porosity (WFP) in soil was calculated by dividing the volumetric water content by the total pore space.

E. Plant analysis

At the end of the denitrification measurement period (day 28), plots were harvested by mower, and pasture dry matter yield determined. Immediately before harvesting, herbage was sampled from within each plot for separation into grass and clover. Herbage, sampled from within a 3 m x 1 m microplot in each plot, was dried and analysed for total N.

F. Statistical analysis

Analysis of variance was carried out using Genstat 4.2, Fifth Edition. SED or LSR values were used to compare means, when main effects were found to be significant.

Table 1. Main effects of pugging on grass dry matter and N yield, grass N concentration, and total soil inorganic N 28 days after pugging.

	Pugging severity			
	Nil	Moderate	Severe	SED/LSR
Grass DM yield (kg ha^{-1})	852	342	67	232*
Grass N yield (kg ha^{-1})	23.9	10.1	1.4	7.0*
Grass N concentration (%N)	2.9	3.0	3.6	0.17***
Total soil inorganic N^{bc} (kg ha^{-1})	4.0	7.7	11.4	2.39†

***, *, † significant at *P*<0.001, *P*<0.05, *P*<0.1, respectively, unless designated NS not significant (8 replicates).

[b] data is geometric mean, and relevant error term is LSR.

[c] total inorganic N = NH_4^+-N plus NO_3^--N.

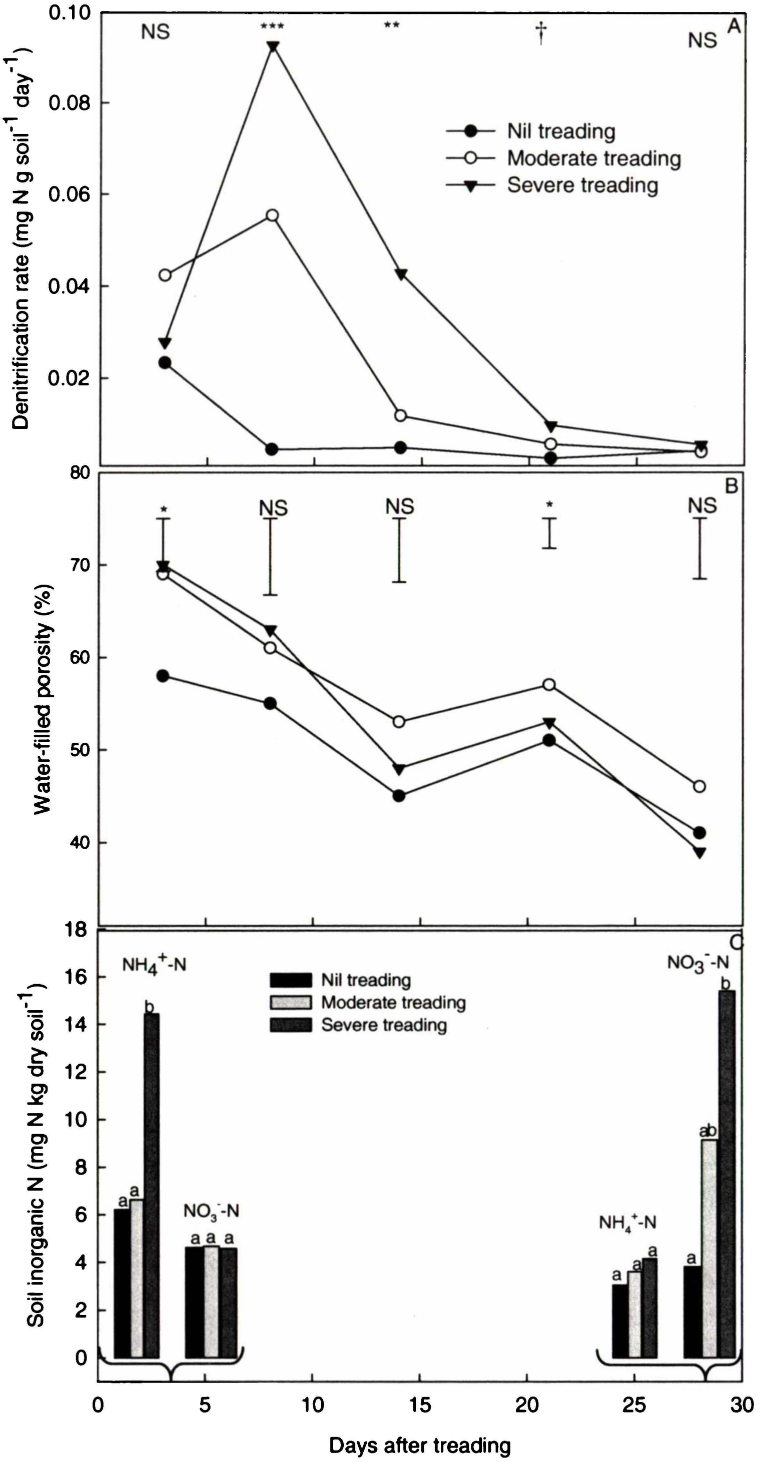

Figure 1. The effect of treading on denitrification rate (A), and water-filled porosity (B), and soil nitrate concentration (C). For (A) and (C), geometric means are presented, and LSRs are 1.93-3.06 and 2.78, respectively; $P<0.05\text{-}0.1$. For (C), bar plots with different letters are significantly different from each other; $P<0.05$. For (B) error bars represent SED; $P<0.05$ unless designated NS, not significant (4 replicates).

IV. Results and discussion

A. Denitrification as affected by treading

Denitrification rates showed significant ($P<0.001$-0.1) increases up to 21 days after both moderate and severe treading (Figure 1a). The highest rates of denitrification were measured 8 days after treading, reaching a maximum rate equivalent to 52 g N_2O-N ha^{-1} day^{-1} under severe treading. Subsequently, denitrification rates in treading treatments declined sharply, and were similar to control soils after 21 days. Similarly, Luo et al. (1999) reported a short-term increase (14 days) of denitrification in a grass-clover pasture after grazing.

Integration of the daily rates of denitrification over time resulted in total denitrification losses of about 105, 376, and 593 g N_2O-N ha^{-1} under nil, moderate and severe treading, respectively, for the 4 week study. Thus, losses of N through denitrification were 3 to 5 fold greater in treading plots than in nil-treading plots.

In pasture soils the principal regulators of denitrification are soil oxygen and soil NO_3^--N, both of which may limit denitrification depending on pasture management and site-dependent environmental factors (Groffman et al., 1987; Jarvis et al., 1991; Mosier et al., 1986; Smith and Tiedje, 1979). In intensively grazed systems, deposited excreta can cover a large proportion of pasture, and make a significant contribution to the soil N pool (Haynes, and Williams, 1993; Menneer et al., 2003). Increased soil NO_3^--N from excreta has been suggested as the main cause of increased denitrification rates following animal grazing (e.g. Carran et al., 1995; Luo et al., 1999; Ruz-Jerez et al., 1994). However, these authors also speculated that treading and compaction may have reduced soil aeration creating an environment more conducive to denitrification (Carran et al., 1995; Luo et al., 1999; Ruz-Jerez et al., 1994). In our study, pasture had not been grazed for 5 months prior to commencing the experiment, and had been under a cutting regime. Hence, any influences of excreta on soil inorganic N and carbon availability should have been avoided, thereby enabling the potential effects of treading on soil aeration and denitrification to be determined. This experiment, therefore, separates the two factors relating to grazing, that of treading and compaction, from the addition of N in excreta.

B. Regulators of denitrification under treading

Soil aeration was adversely affected by treading, with an immediate (within 3 days) increase in water-filled porosity (WFP) compared to the untrodden control treatment (Figure 1b). For example water-filled porosity increased ($P<0.001$) from 58% in the control to an average of 70% under moderate and severe treading (Figure 1b). Notably, elevated ($P<0.001$) values of WFP on day 3 were not associated with any increase in denitrification between treatments. However, an apparently higher denitrification rate in control plots on day 3 compared to subsequent days, suggests that a WFP value of between 55-60% in this soil is adequate to stimulate denitrification. After day 3, values of WFP gradually declined in all treatments, and although values were greatest under treading, this difference was only significant on day 21. By day 28 WFP had reached an average lower limit in treading treatments of 43%. Reported threshold values of WFP causing denitrification differ according to soil type, but for soils similar to those used in our study (silt loams), threshold WFP ranging from 50% to 74% have been reported (e.g. Barton et al., 1999; Nelson and Terry, 1996; Sexstone et al., 1988).

Grass dry matter production was dramatically reduced by 60 and 94% under moderate and severe treading, respectively (Table 1). In contrast, soil NH_4^+-N and NO_3^--N concentrations in the soil 0-70 mm sampling depth increased ($P<0.05$-0.10) during the first 28 days of the study (Figure 1c, and Table 1). Within 3 days of treading, soil NH_4^+-N had increased under severe treading to a value 2.5 fold greater than that of the control ($P<0.1$; Figure 1c). However, soil NO_3^--N concentrations on day 3 were similar in all treatments. Notwithstanding, by day 28 soil NO_3^--N concentration had increased ($P<0.05$) from 4.0 mg NO_3^--N kg^{-1} dry soil, in the control treatment, to 10 and 16 mg NO_3^--N kg^{-1} dry soil, for the moderate and severe treading treatments, respectively (Figure 1c). Greater ryegrass N concentrations in severe trodden plots on day 28 also imply that plant-available soil N concentrations had increased in these lower yielding treatments (Table 1). Apparently, nitrification was not markedly inhibited by the reduction in soil aeration, and soil NH_4^+-N was readily converted to NO_3^--N. Given the much reduced grass growth and therefore low soil N uptake under treading, it is not surprising that soil NH_4^+-N and NO_3^--N increased in these treatments. Although soil NO_3^--N concentrations on day 28 were above reported values that generally limit denitrification in clay loams (1-2 mg NO_3^--N kg dry soil^{-1}; Estavillo et al., 1994; Jordan, 1989) denitrification rates were similar between treatments, and probably reflected the low WFP (43%) at the time (spring). Further research in wetter seasons (e.g. autumn/winter) is necessary, to determine if treading induced reductions of soil aeration will have more prolonged effects on denitrification under these potentially more favourable conditions.

The data presented in this study shows that treading alone, without any influence of excreta, can lead to an increase in denitrification in grass-clover pastures. We propose that the underlying processes involved are two-fold. Firstly, treading causes a temporary (<21 days) reduction in soil aeration and, secondly, reduced soil N utilisation prompted by reduced plant growth increases soil NH_4^+-N and NO_3^--N availability. Future research is required to determine the relative effects of these grazing factors (e.g. excreta and treading) on denitrification and the amounts of total N loss involved.

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7

Synthesis, Modelling and Conclusions

7

Synthesis, modelling, and conclusions

I. Introduction

In legume-based pasture systems, pasture production and animal performance are directly linked to the efficiency of N_2 fixation by the legume. By and large, the contribution of fixed N by legumes to pasture depends on the ability of the legume to grow and persist, and more directly, on N_2 fixation itself. Numerous factors (e.g. nutrient deficiencies, pests and diseases, and climatic stresses) can affect both clover growth and N_2 fixation, and thus the overall performance of the legume-based system. Many of these environmental/edaphic related factors have been the subject of extensive research, the results of which have been covered in reviews (e.g. Ledgard and Steele, 1992; Woodfield and Caradus, 1996).

During the last decade, an overall need by farmers, globally, for greater productivity and profitability has led to increased intensification of farming, and this presents an additional factor that could affect legume performance and N_2 fixation. In particular, prior to the research presented in this thesis, there have been few detailed studies on the response of N_2 fixation to increased intensification and grazing animal impacts via i) treading, ii) defoliation, and iii) excreta. This study has shown that intensive grazing of animals can have a dramatic impact on N_2 fixation, either directly by affecting nodule function, or indirectly by influencing clover growth and persistence.

II. Overview and synthesis of results

A. A conceptual model of the processes regulating N_2 fixation in intensively managed legume-based pastures

A conceptual model is proposed in Figure 1 which illustrates the possible mechanisms and underlying processes that affect N_2 fixation in intensively managed legume-based pasture systems subjected to treading, defoliation, and inputs of urine (dung has a lesser effect and is discussed in chapter 2). Soil and plant processes are both involved, either as a direct consequence of the applied treatment, or as feedback mechanisms that affect N_2 fixation. The conceptual framework described here centres on the main issues investigated and identified in this thesis.

1. Effects of treading on N_2 fixation

When treading occurs in legume-based pastures, changes in both plant and soil processes/factors are affected and act to reduce N_2 fixation. Plant factors involve significant plant organ damage, tissue burial, and stolon fragmentation (Blue box A). The indiscriminate fragmentation of stolons by treading results in a population of small clover clonal fragments, many which are buried. The small and highly disrupted clonal plants are susceptible to environmental stresses and many perish following treading (Blue box B). Over the long-term (up to 150 days), this leads to low clover content and reduced clover production (Blue box C). This direct impact of treading on the legume plant was the chief cause of reduced annual N_2 fixation (-53%) in the treading experiment (Chapters 4 and 5).

Soil related factors include a major disturbance and mixing of the soil surface layers by hoof action and penetration in wet soil (Yellow box A) which affects soil physical properties, and leads to a short-term (21 days) decrease in soil aeration (Yellow box B). Low soil aeration affects soil biological processes such as denitrification (Yellow box C), and possibly N_2 fixation (via inhibition of nitrogenase activity) for a brief period after treading (21 days in the treading experiment, chapter 5; Yellow box D).

Figure 1

A third process, involves a plant-soil interaction that feeds back to inhibit N_2 fixation. This process occurs in response to reduced grass production and decreased N uptake, which allows soil inorganic N to increase (Green box A). Under high soil N, clover 'switches' from N_2 fixation to soil inorganic N uptake for a greater proportion of its N requirement for a medium-term period until soil N is reduced again by the recovery of grass growth. This occurred after 70 days in this study (Chapter 5; Green box B).

2. *Effects of urine on N_2 fixation*

When urine is applied to pasture, both plant and soil factors are involved in regulating N_2 fixation. In soil, applied urine N is rapidly converted to plant-available N (NH_4^+-N and NO_3^--N) (Brown box A). When this occurs, clover switches from N_2 fixation to the uptake of soil inorganic N over a prolonged period (e.g. 160 days in chapter 3) for a significant proportion (up to 80%) of its N requirements (Brown box B). In time, soil inorganic N is reduced in the root zone (Brown box C), but N_2 fixation shows a delayed recovery (e.g. a further 150 days in chapter 3), due to the slow reestablishment of an N_2 fixing nodule population, or possibly plant uptake of N from below the soil sampling depth (Brown box D). The direct impact of urine N on nodule function was the main cause of reduced annual fixed N in clover herbage (-38%) in this study (Chapter 3).

Increased soil N from applied urine also causes a dramatic increase in grass growth (Purple box A). This gives grass a competitive growth advantage (for light and space resources) over clover (Purple box B). Under light defoliation this competition causes a short-term (e.g. 50 days in chapter 3; Purple box C) reduction in clover production and decreases annual N_2 fixation.

3. *Effects of defoliation severity on N_2 fixation*

Increasing the severity of defoliation in legume-based pastures may cause N_2 fixation to increase via a plant-soil N interaction mediated by an increase in summer grasses and weed species in the sward. Under moderately-severe defoliation the grass and weed species changes over summer to favour more drought resistant summer grass species (Pink box A). Better soil N utilisation by the more aggressive growing summer species (Pink box B) reduces the availability of soil N in the root zone (Pink box C). This causes a greater dependence on N_2 fixation by clover over a prolonged period during summer and autumn (e.g. up to 180 days in chapter 3; Pink box D). Overall, moderately-severe defoliation can lead to an increase in fixed N in clover herbage, and this equated to a 36% increase in this study (Chapter 3).

III. Implications of intensive grazing management for N_2 in legume-based pasture systems

A. Animal treading in legume-based pastures

The dominating effect of treading on clover production in legume-based pastures is a key determinant affecting the magnitude of fixed N inputs in intensively managed systems (Chapters 4 and 5). The adverse effect of treading on clover production is mediated by large-scale stolon fragmentation and burial caused by hoof action (Chapter 5). Normally, stolon fragmentation is a natural plant initiated process occurring in spring when low carbohydrate reserves kick-start a process of stolon breakdown and the supply of remobilised nutrients to new growth. What is apparent is that when fragmentation is artificially induced (e.g. treading; Chapter 5) the survival of clonal fragments is poor which reduces clover content and production.

Important plant attributes that govern the survival and subsequent growth and development of stolon fragments include the capability to photosynthesise, the presence of a viable growing tip, and favourable soil conditions during the early stages of establishment. Treading works against these factors by burying and/or destroying leaves and growing tips (Chapter 4). As a result, plants lack the photosynthetic capability to drive subsurface growth and the redirection of buried stolon apices to the surface that might occur in less intensively managed systems with lower treading impacts. In addition, the indiscriminate nature of fragmentation by treading is likely to result in a large population of clonal plant fragments that have been severed at sites unsuitable for propagation. The severity of treading will have an overriding influence on the size and viability of individual stolon fragments.

Treading impacts on clover can be further compounded by climatic conditions in the weeks following treading (Chapter 4). For example, in this study (Chapter 4), lower than average rainfall following treading in spring resulted in low soil moisture and sub-optimal conditions for plant growth. No doubt, small and recently separated clonal plants that result from treading, are more susceptible to environmental stresses than larger plants with more leaf area and greater root complexity. Conversely, if prolonged wet conditions had occurred after pugging the potential negative impacts of reduced soil aeration could have impacted on plant re-establishment and reduced N_2 fixation activity in existing plants. Thus, climatic conditions have a role in affecting the recovery of pasture damaged by treading. Research assessing the impact of treading in different seasons to determine that effect on the recovery of plants during different growth phases and/or growing conditions would aid in understanding the different processes affecting clover recovery and N_2 fixation. This would be particularly important for clover which has, during winter for example, less leaf growth and petiole extension, and greater leaf turnover and so is more vulnerable to stress than grass. From a management perspective, minimising treading and its deleterious impacts of fragmentation and burial would help retain adequate clover leaf area, an intact root system, and greatly enhance the survival of plants.

Issues relating to the competitive interaction of grass and clover also arise when considering treading impacts. The results in this thesis show that clover growth is apparently less susceptible to moderate treading than grass, but when treading is severe, clover is more susceptible. In reality, a continuum of treading impacts probably exists across grazed pasture which is dependent on animal grazing patterns, and varies with animal grazing preferences, animal grazing behaviour, and the intensity of grazing. In theory, treading could be viewed as a series of discrete footfall events that differ in intensity and have differential effects on plant and soil properties in space and time. This would result in patches of pasture that are in variable states. If such a phenomenon does exist in intensively managed pasture, this could partially explain (in addition to dung/urine) the patchiness of clover content and N_2 fixation in mixed clover-grass swards. Differential treading effects on grass and clover growth will lead to complex competitive interactions that would display strong temporal and spatial heterogeneity.

In addition to direct treading effects on plant growth, competition between grass and clover could also be partially affected by plant-driven changes in the soil inorganic N pool (Chapter 5). When grass growth is reduced by treading, soil N utilisation decreases causing soil inorganic N to build up in the root-zone as mineralisation continues, which down-regulates N_2 fixation (via nitrogenase inhibition). Although this apparently has only a short-term effect, under normal grazing conditions where treading *per se* is always present at some level of impact (cf. this study; Chapters 4 and 5), this process could be another 'competition factor' that affects clover-grass interactions.

Surprisingly, in spite of what appeared to be a dramatic disturbance of the soil surface layers by treading, only a marginal change in soil physical properties was measured (Chapter 5). This was reflected by a short-term (21 days) decrease in values of air-filled porosity and soil moisture, indicating low soil aeration. Even so, values of air-filled porosity were well above the 10-12% threshold that is often used to indicate limiting conditions for plant growth and function. However, denitrification, which is stimulated by low soil aeration, did increase for a short period (21 days) following treading (Chapter 6). In theory, aerobic biological processes that are dependent on a constant supply of O_2 could have also been affected (e.g. nitrogenase activity). I cannot say with certainty whether N_2 fixation was or was not affected by the short period (first 21 days) of low soil aeration.

Given that the treading experiment began in spring and was followed by below average rainfall and dry soil conditions it is unlikely that any limitation to N_2 fixation resulting from low soil aeration would have been long-lasting, and certainly less than the effects due to fragmentation and burial. Under grazing though, successive grazing events during wet soil conditions may lead to low aeration for longer periods and limit N_2 fixation. This could be a potential problem during wetter seasons (e.g. autumn/winter) when rainfall and soil moisture are generally high.

B. Animal urine deposition in legume-based pastures

Urine inputs to pasture lead to a dramatic increase in soil inorganic N and causes clover to 'switch' from N_2 fixation to soil N uptake for a significant proportion of its N requirements (e.g. see Chapter 2). The longevity of this effect is much greater than previously thought, and in this study (Chapter 3) N_2 fixation remained lower than urine treated areas for a further 200 days after soil inorganic N had been depleted from the main root zone (0-150 mm). This delay in the recovery of N_2 fixation may have been caused by senesced nodules not being replaced during high soil N conditions, and then slow nodule re-establishment following soil N depletion in the root zone.

This lag-phase of sub-optimal N_2 fixation, coincident with low soil N, could represent a significant inefficiency in the cycling of N whereby growth limitations are imposed on both grass and clover via reduced N supply. Since the productivity of legume-based pasture systems depends on the efficiency of both N_2 fixation and N supply to associated grasses, there may be a need to address this problem. This could be achieved by plant breeding or molecular manipulation, to produce plants that rapidly reinstate nodulation after soil N depletion, and show a more rapid 'switching' from uptake of soil N to N_2 fixation. By overcoming this 'N void', additional benefits for clover production would occur by the improved meeting of growth and N demands, and increased competitiveness with grass.

It is well known that one of the key drivers of legume production in pasture is the outcome of competition with grass for light and space resources, and that this is mediated through a feedback mechanism between soil N and N_2 fixation. Thus any opportunities that exist to optimise N_2 fixation when soil inorganic N is low will improve the competitiveness of clover, and therefore, should help clovers' persistence in the sward – the failure of which, is an attribute of clover often maligned.

C. Animal defoliation in legume-based pastures

Where urine patches are deposited, high soil N almost always induces an aggressive growth response from grass which can reduce light penetration and gap opportunities, thereby limiting clover performance. Results from this study (Chapter 3) indicate that this competitive effect can be manipulated by appropriate defoliation management; that is, when defoliation management controls urine N boosted grass growth, clover productivity is maintained.

In this study, increasing the severity of defoliation had other 'pastoral' benefits too, such as enabling the ingress of drought resistant summer grass species during summer which enhanced pasture production, and surprisingly N_2 fixation (Chapter 3). The increase in N_2 fixation observed in this study was associated with low soil N conditions, which occurred as a direct result of greater soil inorganic N uptake by summer grasses compared to the winter active species (e.g. ryegrass) that dominated under more light defoliation. Given that this effect is strongly related to climatic conditions, it is unlikely to be as important in cooler temperate regions (e.g. South Island of New Zealand, and Europe) where summer rainfall is greater. In addition, while severe defoliation did have some positive effects in this study, typically, overly frequent grazing can limit legume regrowth, photosynthesis, herbage production, and N_2 fixation (e.g. see Chapter 2).

If the influence of grazing/cutting management is ignored, it would be expected that under high soil N, grasses will dominate and have a competitive advantage over legumes, whereas under low soil N, legumes will dominate and derive most N from N_2 fixation. Results from this study show that this N self-regulation feedback is likely to vary seasonally in intensively grazed systems, due to fluctuations in grass species composition and associated differences in soil N utilisation. This could be a contributing factor in the strong temporal variability of clover content often observed in legume-based pastures.

Knowledge of the different grass species (e.g. summer vs. winter dominant species) and their competitive interaction with clover could provide valuable information for making informed pasture management decisions for optimising N_2 fixation and clover content. For example, boosting N_2 fixation in mid to late-summer (Chapter 3) by manipulating grass species composition (via defoliation management), could give clover a competitive edge and increased productivity going into autumn. This may provide flow-on benefits for overwintering survival and clover growth during the following spring.

D. Estimating N_2 fixation in intensively managed legume-based pastures using ^{15}N isotope methodology

The results in this thesis (Chapter 3) show that, in general, ^{15}N isotope dilution methods are effective for measuring N_2 fixation in intensively managed legume-based pastures. Some differences in estimates of N_2 fixation did exist between the different ^{15}N dilution methods used (e.g. ^{15}N-labelled soil vs. ^{15}N-labelled urine; Chapter 3), which may have been due to changes in soil ^{15}N enrichment with depth, and the uptake of N from different depths by clover and ryegrass. Using the natural ^{15}N abundance method in pastures that have frequent inputs of urine affecting large areas may lead to erroneous estimates of N_2 fixation because of the large spatial variation in $\delta^{15}N$ of plant available soil N (e.g. Chapter 3). Thus, in intensively managed legume-based systems receiving regular inputs of urine, the natural ^{15}N abundance method should be used with caution.

Notwithstanding, all methods have some potential limitations and ideally two (or more) ^{15}N isotope methods should be used to give estimates of N_2 fixation that can be compared and related to associated measurements of soil N status.

E. Integrating grazing animal factors and considerations for modelling

Discussion in this chapter so far has highlighted that grazing animals influence N_2 fixation through interactions of soil-plant-management factors. When you add to this the complexity of temporal climatic influences and spatial heterogeneity of plant/soil responses to animals, then the difficulty of attaining a 'complete' understanding of legume-grass systems becomes apparent. Further experimentation is necessary to increase our understanding of these processes and to enable some of these relationships to be quantified. However, mathematical models may provide the best opportunity and framework for unifying the complexity of legume-grass systems.

To be effective, a legume-based pasture model would require particular characteristics that differentiate it from other non-legume ecosystem models. Most critical, would be the inclusion of a clover-grass dynamic interaction that takes into account the N self-regulation feedback mechanism between soil N and N_2 fixation. Several grassland models have been jointly developed by AgResearch and others (e.g. EcoMod and DAMN) that include this feature. The dynamic dairying model of N cycling (DAMN; Ledgard et al., unpublished) was used to assess the impact of grazing animals on pasture growth and N_2 fixation.

The DAMN model is mechanistic, dynamic, and deterministic and has been developed to give an insight into the potential long-term effects of different management practices (e.g. N fertilisation and cutting for silage) on legume growth and N cycling, by taking into account the dynamic interaction of grass and clover with changes to soil inorganic and organic N. The model includes a modified version of the dynamic clover-grass model of Schwinning and Parsons (1996) which accounts for seasonality and effects of herbage mass (via N accumulation rate in grass tissue) on legume growth and N_2 fixation.

Model simulations were undertaken to predict the response of clover yield and N_2 fixation over 1 year to: (1) urine application under different defoliation managements, (2) urine application and different defoliation managements following a single moderate or severe treading event. In addition, a simulation was run to determine the long-term effect (10 years) of a single annual severe treading event on farm productivity under grazing.

1. Response of legume production and N_2 fixation to urine and defoliation management

In the simulation presented in this section, DAMN was used to investigate the effect of four different defoliation managements (24, 12, 6, and 3 harvests per year by cutting) on clover production and N_2 fixation over 1 year following a single uniform application of urine (equivalent to 746 kg N ha^{-1}) at the start of the modelling simulation.

The model simulation predicted that when grass growth was increased by high soil N from urine, lengthening the interval between harvests had a detrimental effect on clover production and annual N_2 fixation (Table 1). However, whereas the study reported in this thesis (Chapter 3) showed that applied urine had a large impact on annual N_2 fixation (-38%), the modelled response of N_2 fixation to urine predicted a relatively small decrease in N_2 fixation (up to 26%; Table 1), suggesting that this component of the model may require further refinement.

Table 1. Response of selected pasture properties to a single application of urine at 3 different defoliation managements using the DAMN model (Ledgard et al. unpublished). Data are relative to a 'default' simulation which was a clover-grass sward under cutting with 24 harvests per year

	Number of harvests over 12 months			
	24	12	6	3
Modelled Parameter				
Clover yield	100	89	63	58
Grass yield	100	108	122	126
N_2 fixation	100	98	87	74

The predicted decline in legume production from infrequent defoliation is not atypical (Chapter 2) and in the field is due principally to the much greater standing biomass present during the longer harvest intervals, which decreases gap opportunities and light interception and photosynthesis by clover. This was observed in the study in Chapter 3, when clover production was reduced for a medium-term period after urine application to plots under lenient cutting. Figure 2 illustrates the predicted effect of mean preharvest standing biomass on clover production.

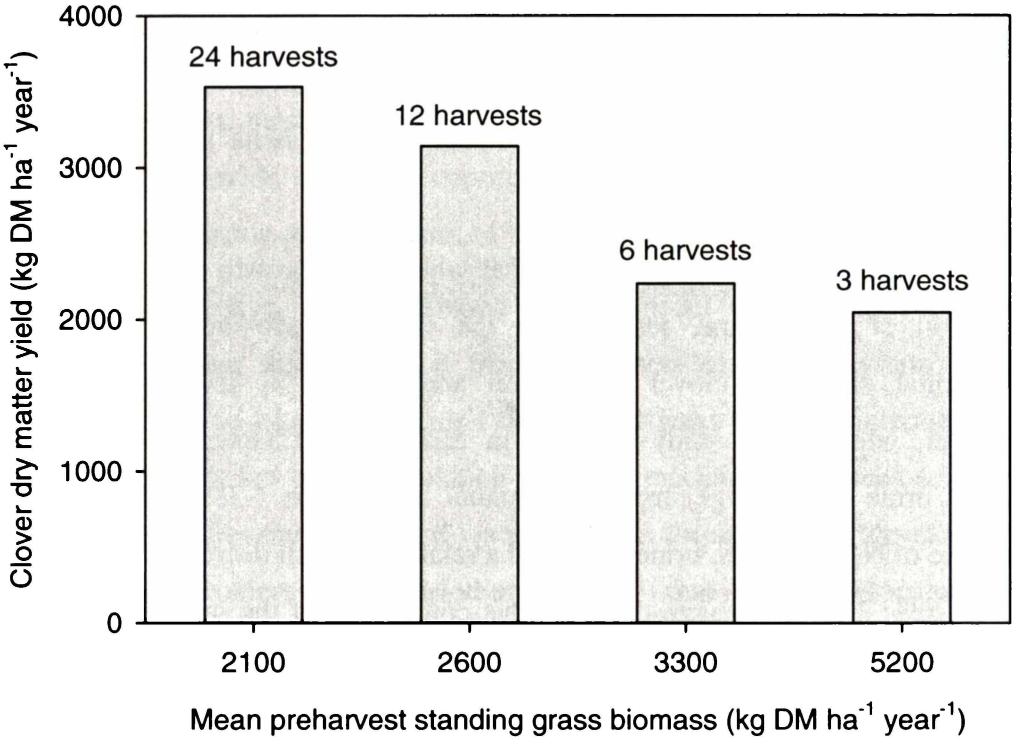

Figure 2. Predicted effect of mean preharvest standing grass biomass on clover production under 4 different harvest frequencies in a mixed clover-grass pasture that had received a single application of urine (equivalent to 746 kg N ha^{-1}), using the DAMN model.

The predictions from this model simulation emphasise the need to have appropriate pasture management strategies, as previously discussed (see Chapter 2), that do not cause clover to be overly disadvantaged by companion grass species. However, the DAMN model only includes ryegrass and white clover and does not account for the changes in species composition in multiple-species pastures and associated effects on soil inorganic N that can occur when defoliation severity is increased. In the study reported in Chapter 3, N_2 fixation was consistently lower under moderately-severe defoliation compared to light defoliation for most of the year, which coincided with an decrease in soil inorganic N and a greater abundance of weed and summer-grass species.

2. Response of legume production and N_2 fixation to urine and defoliation management after moderate or severe treading

In the simulation presented in this section, the response of clover and N_2 fixation to urine and defoliation management was estimated for a clover-grass pasture that had been subjected to either a moderate or severe 'treading event'. Clover and grass growth potentials within the model were adjusted to approximate the temporal magnitude of productivity loss reported in this thesis due to severe or moderate treading (Chapters 4 and 5). A single application of urine and differing defoliation managements (12, 6, and 3 harvests by cutting) were then applied.

Table 2. Predicted response of selected plant properties to a single moderate 'treading event', with or without urine application under 3 different defoliation managements using the DAMN model (Ledgard et al. unpublished). Data are relative to a 'default' simulation which was a moderately trodden clover-grass sward under 12 cuts per year

Modelled Parameter	Moderate treading (12 cuts)	Moderate treading (6 cuts)	Moderate treading +urine (12 cuts)	Moderate treading +urine (6 cuts)	Moderate treading +urine (3 cuts)
Clover yield	100	117	94	106	98
Grass yield	100	100	131	130	140
N_2 fixation	100	105	20	21	18

Table 3. Predicted response of selected plant properties to a single severe 'treading event', with or without urine application under 3 different defoliation managements using the DAMN model (Ledgard et al. unpublished). Data are relative to a 'default' simulation which was a severely trodden clover-grass sward under 12 cuts per year

Modelled Parameter	Severe treading (12 cuts)	Severe treading (6 cuts)	Severe treading +urine (12 cuts)	Severe treading +urine (6 cuts)	Severe treading +urine (3 cuts)
Clover yield	100	114	96	108	102
Grass yield	100	99	122	122	128
N_2 fixation	100	106	23	25	23

As expected, grass production was predicted to increase after urine application, with greater gains in grass yield under moderate versus severe treading (Tables 2 and 3). Due to the low grass production under treading, the longer interval between harvests was predicted to have little effect on clover growth when urine was applied. There was a subtle indication that clover yield reductions due to urine could be accentuated when treading was less intense (e.g. moderate vs. severe treading), but confirmation of these interactions would require further experimentation and more detailed modelling. The low grass growth in the treading+urine treatments was associated with a predicted decrease in soil N utilisation by grass and low N_2 fixation due to greater clover uptake of urine N (c.f. Table 1).

3. Predicting the long-term effects of treading on farm productivity

In addition to increasing our understanding of the main drivers affecting N_2 fixation in legume-based pastures (e.g. see above examples), modelling also has the potential to give insight into the impacts of grazing animals on long-term pasture performance and farm productivity. For example, in the simulation presented here, DAMN was used to predict the effect of decreased N_2 fixation caused by treading on N availability and long-term pasture productivity and milk production under grazing (Table 4).

Using a 10-year scenario with single annual events of moderate and severe 'treading damage', whereby annual N_2 fixation was reduced by 15% and 56% (via the clover yield component; Chapter 5), grass yield was predicted to decrease by 14% and 24% under moderate and severe treading, respectively, after 10 years. Soil organic N was also predicted to decline to lower equilibrium levels by 3% and 7% under moderate and severe treading because of decreased inputs of N from N_2 fixation. The corresponding predicted loss in milk production was 11% and 35%.

Table 4. Predicted effects after 10 years of a single annual 'treading event' (moderate or severe) on selected plant and soil parameters using the DAMN model (Ledgard et al. unpublished). Data are relative to the 'default' simulation which was a rotationally grazed Waikato dairy farm producing 11,800 litres milk ha^{-1} $year^{-1}$ with nil N fertiliser. Clover growth potential inputs for the moderate and severe 'treading' treatments were varied to simulate the proportional decease in N_2 fixation measured in the field study

		'Treading severity'	
Modelled Parameter	Default	Moderate	Severe
Clover yield	100	87	40
Grass yield	100	86	76
N_2 fixation	100	85	44
Soil organic-N	100	97	93

On a whole farm basis with moderate and severe treading on 50% and 10% of the farm (P. Singleton pers comm.), respectively, this could represent a decrease in milk production of 9% (e.g. from a New Zealand average of 11,800 to 10,740 litres ha^{-1} $year^{-1}$). This modelling simulation has indicated that, over the long-term, reduced fixed N inputs can lead to a gradual decline in soil organic N to a lower equilibrium level. This can result in a long-term reduction in total pasture and milk production.

4. Some key requirements for future models of legume-based pastures

For future modelling of legume-based pasture systems the simulations presented above have highlighted some key requirements that should be incorporated into new or existing models (Table 5). For example, as regard to treading, the DAMN model lacks an elaborate soil physical component, has no representation of treading impacts, and does not account for stolon fragmentation or burial by hoof action. Other weaknesses of the DAMN model relevant to excreta and defoliation include, no representation of urine patch spatial heterogeneity, and no response of sward botanical composition to changes in defoliation severity.

Table 5. Key requirements for modelling animal impacts on N_2 fixation in intensively managed legume-based pasture systems

Main ecosystem components	Factors/subcomponents	Essential 'process' components
Climate	Rainfall, soil temperature	• Temporal changes in grass species • Different growth optima for grass and clover
Animal	Treading, defoliation, excreta	• Dynamic clover-grass competitive interactions • Sensitivity of N_2 fixation to changes in soil N
Plant	Grass and clover growth and function	• Differing preference for grass and clover by animals
	N_2 fixation	• Temporal pattern of stolon cycling and turnover
Soil	Soil physical, chemical, and biological properties	• Soil structural responses and soil aeration

These constraints certainly do not just apply to the DAMN model as the author of this thesis is not aware of any other grassland ecosystem model that includes the above components while specifically dealing with legume-based pastures. The future challenge for modellers will be to build a model that includes many of these key factors, and thus, has the ability to more fully simulate intensively grazed legume-based pasture systems. This would greatly assist, in addition to further experimentation, in increasing our understanding of the main operational drivers affecting N_2 fixation, and enable predictions to be made about the future efficiency of the legume-based system, and appropriate intervention strategies for optimising N_2 fixation.

IV. Conclusions

The main conclusions from this thesis were:

1. Treading by dairy cows in a clover-grass pasture led to a substantial reduction in the contribution of fixed N by white clover. The loss in N_2 fixation was almost solely due to reduced clover production during the first 6 months after treading. This was mediated by large-scale stolon fragmentation and burial resulting from hoof action, which led to poor plant survival.

2. Treading also reduced grass growth which led to an increase in soil inorganic N because of low N uptake. A short period of low N_2 fixation in clover corresponded with the higher soil inorganic N, until grass growth recovered and soil inorganic N declined.

3. Treading had only a marginal effect on soil physical properties. This was reflected in a brief period (21 days) of low soil aeration, which stimulated soil denitrification, and possibly contributed to reduced N_2 fixation.

4. A whole farm model predicted regular annual treading events decreasing annual N_2 fixation could lead to a decline in soil organic N and long-term farm productivity.

5. The application of urine to pasture had prolonged effects on N_2 fixation, which lasted for nearly 12-months even though high levels of urine N in the root zone had been depleted within about 4 months. The delayed recovery of N_2 fixation after soil inorganic N had returned to low levels may have been due to the slow reestablishment of root nodules.

6. Increasing the severity of defoliation enhanced N_2 fixation during summer and autumn, and resulted in a 36% increase in annual fixed N in clover. This was due to a greater proportion of summer grasses and weeds under moderately-severe defoliation, which had a greater ability to grow and take up soil N than ryegrass, thereby reducing the inhibitory effect of soil inorganic N on N_2 fixation.

7. Accurate estimates of N_2 fixation in intensively managed legume-grass pastures should be based on using two, or more, ^{15}N isotope dilution techniques. Due to the large spatial variation of $\delta^{15}N$ in soil under urine, the natural ^{15}N abundance method should be used with caution in intensively managed systems.

V. References

Woodfield D R and Caradus J R 1996 Factors affecting white clover persistence in New Zealand pastures. Proceedings of the New Zealand Grassland Association 58, 229-236.

Ledgard S F and Steele K W 1992 Biological nitrogen fixation in mixed legume/grass pastures. Plant and Soil 141, 137-153.