Aspects of grazing management to improve productivity and persistence of white clover in Irish grassland

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Doctor of Philosophy

By

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List of Abbreviations

ADF = acid detergent fibre

AFP = air filled porosity

Al = aluminium

AMF = arbuscular mycorrhizal fungi

ANCOVA = analysis of covariance

ANOVA = analysis of variance

AR = acetylene reduction method of measuring BNF

BNF = biological nitrogen fixation

BW = bodyweight

C = carbon

CAN = calcium ammonium nitrate

CP = crude protien

CT = condensed tannin

C_2H_2 = acetelyne

C_2H_4 = ethylene

cm = centimetre

CSO = central statistics office (Ireland)

DM = dry matter

DH = defoliation height

DHA = daily herbage allowance

DHI = daily herbage intake

EU = European Union

FADN = farm accountancy data network (Europe)

FIN = final defoliation date in winter

HMnt = herbage mass in non-treaded area

HMtr = herbage mass in treaded area

HT = highly treaded

GHG = Green - House Gases

GLM = general linear model

Grass-clover = unless otherwise stated, a sward dominated by perennial ryegrass and white clover

GWC = gravimetric water content

H = hydrogen

ha = hectare

hr = hour

ID = isotopic determination method of measuring BNF

INT = defoliation interval

IVOMD = *in vitro* organic matter digestibility

K = potassium

kDa = kilodaltons

kg = kilogram

LAI = leaf area index

LU = livestock unit

LP = light penetration

LW = liveweight

m = meter

Mn = managanese

N = nitrogen

N_2 = dinitrogen

NC = non-clover

ND = nitrogen difference method method of measuring BNF

NDF = neutral detrgent fibre

NE = net energy

NFS = national farm survey (Ireland)

NHA = net herbage accumulation

NH_3 = ammonia

N_2O = nitrous oxide

nm = nanometer

NS = not significant

NT = non treaded

NZ = New Zealand

OM = organic matter

OMD = organic matter digestibility

P = phosphorus

PAR = photosynthetically active radiation

PGH = post-grazing height

pNdfa = proportion of nitrogen derived from the atmosphere

PRHM = proportional reduction in herbage mass in treaded areas

REPS = rural environmental protection scheme

R:FR = red light to far-red light ratio

s = second

SBD = soil bulk density

SD = standard deviation

SDC = size-density compensation

SEM. = standard error of the mean

t = tonne

UAA = utilisable agricultural area

UK = United Kingdom

USA = United States of America

VSP = vegetative storage protein

VWC = volumetric water content

WSC = water soluble carbohydrate

yr = year

Declaration

This thesis has not previously been submitted for any degree at this, or any other institution and unless otherwise acknowledged, the work embodied in it is my own.

Signature: ________________________________

Date: ______________________________

Abstract

White clover houses symbiotic *Rhizobia* bacteria that make atmospheric nitrogen (N) available for plant growth. This can reduce agriculture dependency on synthetic fertilizer N and thereby reduce financial and environmental costs of agricultural production. This study investigated certain aspects of grazing management of grass-clover swards on sward clover content, herbage production and animal performance on a wet clay-loam soil at Solohead Research Farm, Tipperary, Ireland.

Defoliation height (DH), defoliation interval (INT) and final winter defoliation date (FIN) were investigated under simulated grazing in summer and autumn. Treading damage was investigated within three dairy grazing systems that differed in fertilizer-N input, stocking rate and grazing season. The effects of post-grazing height (PGH) on sward clover content, herbage production and milk yield per cow was investigated in grazing systems over three entire grazing seasons.

Lowering DH under simulated grazing increased sward clover content and herbage production. Under actual grazing conditions, lowering PGH did not increase sward clover content but did increase herbage production. Unlike most previous experiments, lowering PGH was not associated with any reduction in milk yield per cow. The main reasons for this were that (i) the current experiment was on grass-clover, rather than grass-only swards and (ii) treatments in the current experiment were imposed from turnout in spring to housing in winter, rather than for briefer periods in late spring/summer imposed after the grazing season had begun. Therefore, low (4 to 5 cm) DH and PGH are recommended to increase clover content and/or herbage production in

grass-clover swards. However, to avoid losses of milk production, target PGH should be practiced from first turnout in spring and should not be abruptly lowered during the grazing season with unsupplemented animals.

Simulated grazing intervals of 21 days in summer and 42 days in autumn gave the best results in terms of balancing herbage production, herbage quality (CP and OMD) and sward clover content into the following year. These are similar to current recommendations for grass-only swards in Ireland and white clover may actually assist these aspects of grazing management by maintaining herbage nutritive value for longer periods of regrowth than grass-only swards.

Extending FIN from 23 September to 16 December in the mown plots had no effect on sward clover content or clover herbage production. However, in the grazing systems, a later grazing season (April to January instead of February to November) was associated with higher sward clover contents and herbage production in the following year. The difference between the results of the two experiments may have been due to the higher grass competition generally found in grazed swards.

Treading damage was associated with reductions in herbage production of both grass and clover. The effect of treading damage was greatest in spring, with reductions of herbage production by up to 58%. Particular care should be taken at this time of year, when silage ground has been removed and stocking rates are higher. More research is needed to find practical solutions that enable farmers to reduce treading damage of pasture (both grass and grass-clover) on Irish farms.

Acknowledgements

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I wish to thank my mother for putting up with an adult son returning home and lodging for three years! I also wish to thank my uncle, Paddy, the entire Sweeney family and my close friends for their support and understanding throughout. Finally I wish to thank my fiancé Emma for her endless encouragement and support and for never once punching me when I said I’m going to have to work on the PhD this evening/weekend (again)!

This thesis is dedicated to the memory of my father, Jim.

1. Introduction

1.1. General Introduction

Grazed-grassland is the most economic feed source for milk production (Dillon, 2006; Finneran *et al.*, 2010; O'Donovan *et al.*, 2011). Grazed grassland in Ireland is generally dependant on fertilizer N inputs to achieve high levels of herbage productivity and quality. However, the cost of fertilizer N has been rising relative to farm product price for the last two decades at an increasing rate (CSO, 2012a). The average use of fertilizer N on Irish grassland has declined 40% in this period, falling from a mean application rate of 145 kg ha^{-1} in 1999 to 86 kg ha^{-1} in 2008 and is now below Teagasc recommended rates for every soil class, stocking rate and farming system (Lalor *et al.*, 2010). Similar reductions (from 110 to 55 kg N ha^{-1}) have been recorded over the same time period in the UK (Thomas, 2009).

Biological N fixation (BNF) via the increased use of white clover in Irish grassland is an economically and environmentally beneficial alternative to fertilizer N (Humphreys, 2008; Dewhurst *et al.*, 2009; Humphreys *et al.*, 2009a; Ledgard *et al.*, 2009). White clover is the best suited N-fixing plant for grazing under temperate conditions due to its high productivity, high palatability, nutritive value and sprawling, stoloniferous growth habit (Frame and Newbould, 1986; Rattray, 2005). Organic grazing systems rely heavily on white clover (Baars, 2002) and the Irish government has targeted a 500% increase in land area under organic production by 2020, primarily in response to domestic and export market demand (Department of Agriculture Fisheries and Food (DAFF) Ireland, 2010; 2011).

In general, the wetter land areas in Ireland cannot carry the same dairy stocking rates as their free-draining counterparts and are limited to approximately 2.0 cows ha^{-1} (O'Loughlin *et al.*, 2001). Stocking rates are currently below this level on approximately 70% of Irish dairy farms and 92% of Irish beef farms (Lalor *et al.*, 2010). At a similar stocking rate (2.1 cows ha^{-1}), Humphreys *et al.* (2009a) has found that increasing white clover content (in a predominantly perennial ryegrass sward) from 60 g kg^{-1} to 219 g kg^{-1} can achieve the same level of milk production while reducing fertilizer N requirement from 220 kg ha^{-1} to 90 kg ha^{-1}.

The amounts of white clover in Irish grassland are generally low and most of the N requirements on Irish livestock farms could potentially be achieved with the increased use of white clover, allowing conversion to organic production or simply a reduction of fertilizer N costs (O' Mara, 2008). However, white clover is generally perceived as difficult to manage correctly, with problems in year-to year persistence (Rochon *et al.*, 2004). Therefore, further research is required to enhance understanding of grazing management that promotes clover productivity and persistence in grazed grassland.

1.2. Aims and Objectives

The objective of this thesis was to review the biology and management of white clover, identify knowledge gaps and investigate the effects of various aspects of grazing management on sward clover content and herbage production from grass-clover swards. Consequently, the effects of autumn grazing interval, autumn closing date, autumn cutting height, grazing system and treading were investigated with a mix of simulated grazing and actual grazing systems.

1.3. Thesis Layout

The thesis contains eight chapters. Following this introduction, Chapter 2 is a literature review of white clover biology, use in agriculture, its interactions with grasses and its grazing management. Chapters 3 to 7 describe experiments conducted during the study. Chapter 3 investigates the effects of simulated summer grazing management on white clover flowering and herbage production in a grass-clover sward. Chapter 4 investigates the effects of simulated summer-to-winter grazing management herbage production and persistence in a grass-clover sward. Chapter 5 investigates the ability of a literature meta-analysis to predict N-fixation in a grass-clover sward. Chapter 6 investigates the effects of treading by dairy cows on soil properties and herbage production in three white clover based grazing systems. Chapter 7 assesses the effect of target post-grazing height on sward clover content, herbage production and dairy production from grass-white clover pasture. Finally, Chapter 8 gives a general discussion of the findings in the thesis, along with overall conclusions and recommendations for future work.

2. Literature Review

2.1. Biology of White Clover Plants

2.1.1. Development from seed

After germination, white clover embryos develop into seedlings with epigeal cotyledons and a single primary taproot (Frame and Newbould, 1986). The root system in young seedlings develops faster than the plumule and three rows of lateral roots are usually present by the time the first leaf appears. Root nodules for the plant-*Rhizobia* relationship also start to form very early, appearing around the same time as the first lateral roots (Thomas, 1987b). Leaf emergence and unfolding occur after the development of the root system. The first leaf is unifoliate and heart shaped. All leaves that follow are larger and trifoliate (Burdon, 1983). Figure 2.1 (a to f) shows the initial development of seedling morphology.

Initially, young white clover plants have a rosette growth habit (Figure 2.2) with a single central stem that doesn't elongate much. The development of the young seedling can be divided into two distinct periods (Thomas, 1987b):

1. The plant does not change much visually as leaf emergence rates are low. During this period the young plant initiates, but for the large part does not develop, leaf primordia and axillary bud primordia.

2. After approximately six leaves have formed the visual appearance of the plant begins to change rapidly as the leaves expand and axillary buds develop into horizontally

growing and branching stems called stolons. After approximately seven weeks under ideal growing conditions, the primary stem commonly stops growing and all subsequent growth, including root formation, occurs from nodes on the stolons. At this stage the plant is considered to have matured beyond the seedling stage.

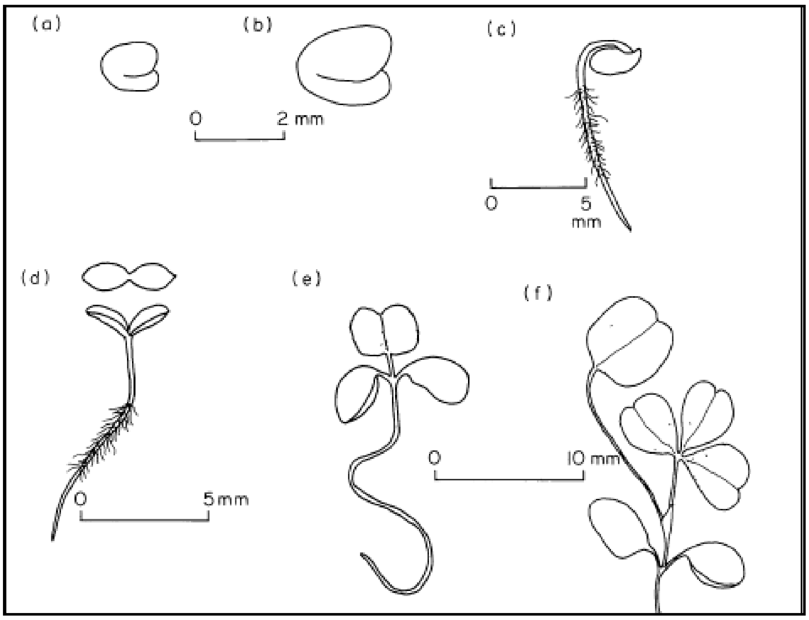

Figure 2.1. Seedling morphology of white clover: (a) dry seed, (b) seed soaked for 24 hours, (c) three-day old seedling, (d) five-day old seedling, (e) two-week old seedling and (f) three-week old seedlings (lateral roots and nodules not shown) (Burdon, 1983).


Figure 2.2. A young white clover plant in the initial rosette stage.

2.1.2. Structure of the mature plant

The structure of a mature white clover plant is shown in Figure 2.3. The stolons are considered to be the basic structural unit of the mature plant. These are the stems that grow horizontally along the ground and give the plant the *repens* ('creeping') part of its Latin name. Stolons are usually found on, or just below the soil surface and consist of a series of nodes and internodes produced as the apical bud grows (Frame and Newbould, 1986).

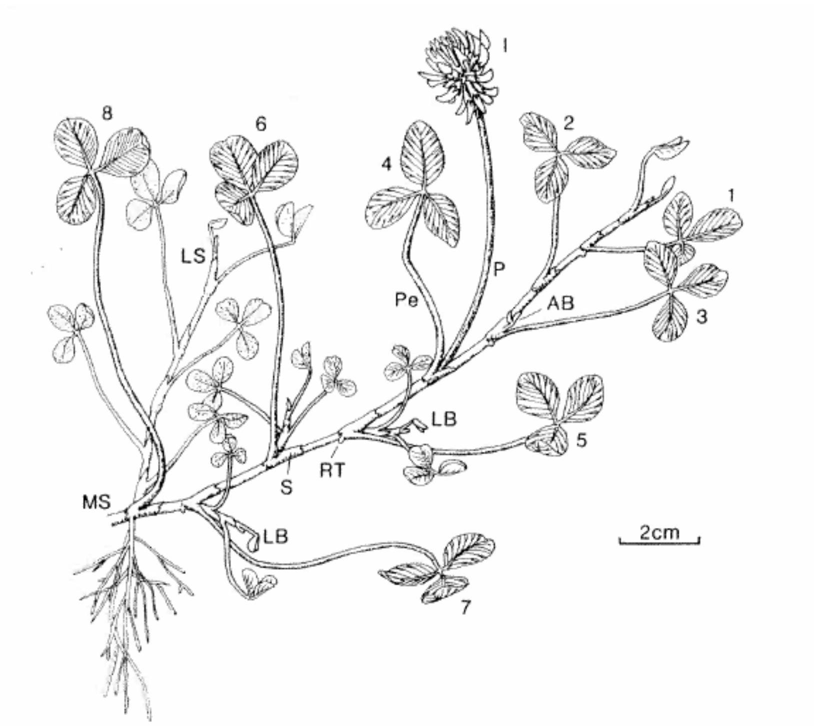

Figure 2.3. Structure of a mature white clover plant. MS = main stolon; AB = axillary buds; LB = lateral branches; LS = stolon; S = stipule; Pe = petiole; RT = nodal root primordium; I = inflorescence; P = peduncle. Emerged leaves on the main stolon are numbered 1 to 8, 1 being the youngest (Thomas, 1987b).

The nodes and apical buds house most of the active meristematic tissue in the mature plant (Thomas, 1987). Each node produces one upright petiole bearing a leaf and either a vegetative or reproductive bud; the vegetative buds can form lateral stolons which branch off from the sides of the original stolon and the reproductive buds produce flowers (Burdon, 1983).

Leaves are held on petioles that grow upwards from alternating sides of the stolons at the nodes. They are typically trifoliate, can be serrated or smooth and often have a whitish V-shaped mark on each leaflet. White clover leaves can rotate slightly throughout the day to keep their uppermost surface facing the sun (heliotropism) and can close slightly at night (Thomas, 1987b).

Each node on a stolon also bears two adventitious root primordia; one at each side. In this manner one primordium is always closer to the ground and can form a nodal root under suitably moist soil conditions. In some varieties the nodal roots remain fibrous (e.g. Kentish Wild White) while in others (e.g. Ladino) they form obvious taproots (Thomas, 1987b). The most distinctive feature of white clover (and other legumes) roots is the formation of nodules that house symbiotic N-fixing bacteria. The bacteria are usually present in the soil and start to proliferate at points on the rhizosphere of white clover roots. The plant responds by curling a root hair around the bacterial colony and that root hair then grows to encase the bacterial colony in a rough oval (sometimes fan-shaped) nodule approximately 1.5 to 3 mm in diameter (Crush, 1987).

2.1.3. Biological nitrogen fixation (BNF)

As a legume, white clover develops root nodules that house symbiotic N-fixing bacteria, *Rhizobium leguminosarum biovar trifolii* L. Biological nitrogen fixation is essentially the reduction of atmospheric dinitrogen gas (N_2) to ammonia (NH_3) through the action of the nitrogenase enzyme system (Crush, 1987). The relationship between the *Rhizobia* and the host plant is mutualistic and in return for fixed N, the plant provides the bacteria with carbon assimilated from photosynthesis which places a carbon burden on the host plant (Schulze, 2004). The cost to the plant in terms of carbon is estimated across most legumes at approximately six g C per g of fixed N (Vance and Heichel, 1991).

Per gram of N acquired by the plant, BNF is approximately 5% greater in terms of required photons and 6% greater in terms of water than N-assimiliation (Andrews *et al.*, 2009). This higher metabolic cost has been used to explain the observation that white clover plants growing in pots with no soil N (therefore relying entirely on BNF) produce 20 to 40% less growth than clover plants with fertilizer N surplus to requirement (Chapman *et al.*, 1996). However, clover never relies solely on N uptake from the soil and tends to retain at least 15% of its N uptake from BNF even at optimal soil N levels (Davidson and Robson, 1986). White clover tends to be less efficient at N-uptake than the companion grasses it usually grows with and therefore tends to be more reliant on BNF when grown in grass-clover mixtures (Carlsson and Huss-Danell, 2003).

Using ^{15}N isotope determination, Vinther (2000) estimated that white clover derived 87 to 99% of it's N from the atmosphere, fixing 150 to 277 kg N ha^{-1} yr^{-1} in Denmark, which corresponds to about 34 to 45 mg N fixed per gram of white clover dry matter.

Approximately 10 kg N ha^{-1} yr^{-1} were transferred from clover to grass. More recently, Black *et al.* (2009) stated that the amount of N fixed by a sward with an annual clover content of 20% in Ireland is about 150 kg N ha^{-1} yr^{-1} (range of 50 to 170 kg N ha^{-1} yr^{-1} from studies in UK and Ireland) which is equivalent to about 11 bags (50 kg each) of calcium ammonium nitrate (CAN) (27% N) per hectare.

There are various methods for measuring BNF and these have been reviewed extensively elsewhere (McNeill and Wood, 1990; Hardarson and Danso, 1993; Haystead, 1993; Carranca *et al.*, 1999; Unkovich and Pate, 2000; Loges and Taube, 2002; Carlsson and Huss-Danell, 2003; Fustec *et al.*, 2011; Liu *et al.*, 2011).

In a brief summary, there are three main methods:

1. The nitrogen difference (ND) method is the most basic and involves the comparison of nitrogen yields from control swards and N-fixing swards. The control swards involved are typically either non-nodulating/sterile legumes or, in the case of grass-clover combinations, a grass-only sward. A simple equation is then used:

Amount of N fixed = Total N yield in N-fixing sward – Total N yield in control sward

2. The isotopic determination (ID) method is based on the presence of lower ^{15}N:^{14}N ratios in N derived from the atmosphere and is measured either using the natural abundance of ^{15}N typically found in the soil or the application of ^{15}N enriched fertilizer.

3. The acetylene reduction (AR) method is based on the fact that nitrogenase also reduces acetylene gas (C_2H_2) to ethylene (C_2H_4). Therefore, by incubating a sample

(usually an intact sod, either *in situ* or removed) in pure acetylene gas and measuring the rate of reduction with a gas chromatographer, nitrogenase activity can be calculated.

The AR method measures short-term rates of N-fixation which are difficult to scale up to longer time scales (Crush, 1987). The ID methods are the most common in the last two decades because they can give measurements of the proportion of N derived from the atmosphere (pNdfa) in clover tissue. However, the methodology involved requires expensive equipment (mass spectrometers) and materials (isotopically labelled N). The ND method is a relatively simple and cost-effective method but may be subject to error if the two swards differ in their uptake of non-fixed N.

Most of the variations in BNF estimates are due to variations in clover biomass as BNF has been positively correlated with stolon mass, leaf DM and number of rooted nodes in spring (Marriott, 1988). However, BNF is also influenced by temperature, available soil moisture and the N content of the soil. Soil N, in particular nitrate, inhibits nitrogenase activity in an example of product feedback regulation (Schulze, 2004). Many researchers have shown that increasing soil N with excreta or nitrogenous fertilizers can reduce BNF. For example, Ledgard *et al.* (2001) found that applying 400 kg N ha^{-1} yr^{-1} reduced pNdfa from 0.77 to 0.46. Viruses and fungi can also infect nodules and reduce their functioning (Burdon, 1983). However, in the majority of experiments in grass-clover swards, the high competition for N from the grass portion of the sward results in relatively high stability of pNdfa when compared to clover monocultures (Høgh-Jensen and Schjørring, 1997; Carlsson and Huss-Danell, 2003). For this reason, BNF in grass-clover swards tends to be closely related to the total N yielded in clover tissue and (because N concentrations in clover herbage are relatively constant) clover herbage production (Carlsson and Huss-Danell, 2003). The relationship between annual clover herbage production and BNF is examined through meta-analysis in Chapter 5.

One of the challenges to accurately quantifying BNF is that calculations are usually based on removed herbage. The amount of fixed N that is withheld in white clover stubble, stolons and roots, transferred to soil and grass, and lost from the system in various pathways is much more difficult to measure. There is little direct excretion of nitrogen from live clover to the grass component of the sward (Murphy and Sherwood, 1989). Instead, it occurs through decomposition of plant material and the excretion of grazing animals. Vinther (2006) measured root:shoot ratios of fixed N in Denmark and found that, on average 28% of the fixed N in harvested material was found in clover stubble, stolons and roots. However, this did not account for tissue turnover and it was concluded that using such fixed root:shoot ratios were insufficient for calculating the total amount of BNF. Jorgensen and Ledgard (1997) included measurements of the growth of stolons and roots and concluded that 70% of the fixed N harvested in clover herbage was accumulated in stubble, stolons and roots during the growing season.

Sturite *et al*. (2007) found that the longevity of leaves and petioles ranged from 21 to 86 days (mean 59), of main stolon sections from 111 to 677 days (mean 411) and of roots from 27 to 621 days (mean 290). About 60% of the leaf tissue produced had turned over by the end of the growing season and another 30% had died or disappeared by the subsequent spring. Harvesting reduced the longevity of stolons and increased plant fragmentation, but did not decrease leaf or root lifespan or increase soil N availability. From the plant organ turnover data, it was estimated that the gross N input to the soil–plant system from white clover during two growing seasons was 2.5 times the total N in harvestable shoots (Sturite *et al.*, 2007). However, this was a clover monoculture that was harvested relatively infrequently (three times per year) in Southern Norway. A recent review by Fustec *et al.* (2011) concluded that the proportion of fixed N that was transferred to the soil generally varied from 4 to 70% for temperate regions.

In a continuously grazed grass clover sward in Northern Ireland, Laidlaw *et al.* (1996) found that small-leaved clover cultivars transferred more of their N to grass (34%) than large leaved clover cultivars (15%), although there was large year to year variation. The processes that have a negative affect on clover such as shading, defoliation and low temperatures, promote the transfer of N from clover to the soil (Murphy and Sherwood, 1989).

Høgh-Jensen *et al.* (2004) has developed literature-derived equations for estimating the total BNF under different sward ages (< 2 years or > 2 years), managements (cutting or grazing) and soil type (clayey or sandy) based on clover herbage production. While simplistic, it is one of the few models that include the various non-harvested herbage sinks for fixed N, such as stubble, stolons, roots, soil and grass. Using this equation for a four year old grass-clover sward on a clayey soil gives an estimate of 53 kg N per t of clover DM annual herbage production if under grazing and 59 kg N per t of white clover DM if under cutting. There is a need to expand this modelling work to include the greater variation in climate, sward age, soil parameters and management variables to derive accurate quantification of BNF.

2.1.4. Growth and vegetative reproduction

In the majority of cases the stolons are considered to be the primary means by which the white clover population persists from year to year (Brock *et al.*, 2000; Davies, 2001; Guckert and Hay, 2001). They grow from the active meristematic tissue on axillary and apical buds of an older stolon. While many plants rely heavily on vertical growth to compete for light, white clover's prostrate habit means that it also relies on lateral growth through either stolon elongation or stolon branching. Therefore while many plants evolutionary strategy has been to grow upwards to reach light, white clover has adapted to move horizontally to seek out gaps in the canopy (Burdon, 1983). This characteristic suits clover to grazing conditions as it keeps growing points below the grazing horizon and thereby enables recovery after each grazing (Davies, 2001).

Stolon growth occurs as either branching or elongation (Frame and Newbould, 1986). Stolon branching is when new stolons develop from axillary buds of a pre-existing stolon. Stolon elongation is when the pre-existing stolon becomes longer through growth of its apical bud. Growth is usually a mixture of the two and whether a stolon branches or elongates is influenced by environmental factors such as light, temperature, defoliation and season. These are discussed further in Chapter 2.2.

As the new stolons develop they form leaves and adventitious roots which enable nutritional independence from the older plant material (Thomas, 1987b). In this manner newer plant material can survive as ramets when older plant material dies or becomes fragmented. Under grazing conditions, fragmentation frequently occurs due to damage by grazing animals, pests and diseases, weather and seasonal senescence (Brock *et al.*, 2000). Therefore white clover growth in most grass-clover swards is considered clonal and the size of individual ramets is dictated by the equilibrium between the rate of stolon formation at the apices and the rate of death of older basal stolon material (Hay *et al.*, 1987; Cain *et al.*, 1995).

It has been suggested that the fragmentation of the plant from that of an integrated network of branched stolons anchored by a central taproot to that of many, unconnected stolons relying on adventitious roots is associated with crashes in clover populations in grassland (Brock *et al.*, 2000; Bonesmo and Bakken, 2005). However, this is an unavoidable occurrence that usually occurs during the first or second winter in undisturbed plants (Bonesmo and Bakken, 2005), and much sooner under most grazing conditions (Brock *et al.*, 2000).

Stolon growth patterns result in complex populations that are difficult to differentiate into individuals and ages. As stated above, many plants within the population are ramets and due to the axillary growth habit, can be some distance from each other (Cain *et al.*, 1995). There is also a problem in measuring plant population density as there can be large variation in plant size and extracting complete plants can be difficult. Therefore many studies on population parameters measure growing point density (number of active apical and axillary buds per unit area) as a measure of population density (Hoglind and Frankow-Lindberg, 1998). Collins *et al.* (1991) define a growing point as a “stolon apex bearing an expanding leaf”. Other commonly used measurements are stolon length or stolon mass per unit area (e.g. Hay *et al.* (1989)).

Mature plants also present a problem in assigning chronological age (Thomas, 1987b).

In a single, unbranched stolon, this is simple, the youngest node being closest to the apical bud. However, in branched plants, it is more difficult as individual branches on the plant may have developed and elongated at different rates depending on their individual environmental influences. Despite these difficulties, Burdon (1983) measured stolon extension rates of 0 to 2.5 cm per week from a single pasture in Wales and concluded that an average yearly extension rate of 18 cm was likely in pastures.

White clover populations follow a typical seasonal trend in which many plant parameters (leaf size, petiole size, stolon length, stolon thickness and individual plant size) increase between spring and autumn and decrease over winter (Frame and Newbould, 1986). This is associated with lower temperature and radiation budgets. There are also seasonal fluctuations in percentage of buried stolon tissue; in summer and autumn most stolon tissue lies on the soil surface while in winter and spring most of it is buried (Hay *et al.*, 1987). In older studies this has been attributed to earthworm activity (Frame and Newbould, 1986) but more recent studies have found that root contraction (Cresswell *et al.*, 1999) and treading by stock animals (Menneer *et al.*, 2005a) are also important factors. The role of stolon burial is unclear and it has been suggested that it protects stolons from frost damage during winter (Frame and Newbould, 1986). However, it has contrastingly been associated with lower stolon mass following burial by treading during spring (Menneer *et al.*, 2005a). The effect of treading by grazing animals on white clover stolons requires more research.

2.1.5. Seed production

Round clusters of 20 to 40 white flowers (Figure 2.4) are borne on stalks that originate from the axillary buds on the stolons (Burdon, 1983). Two or more vegetative buds are usually found between each reproductive bud and the apical buds usually remain vegetative (Brock *et al.*, 2000). The ratio of reproductive to vegetative buds is obviously of great interest to seed producers and is influenced by growing conditions, cultivar and management (Thomas, 1987b).

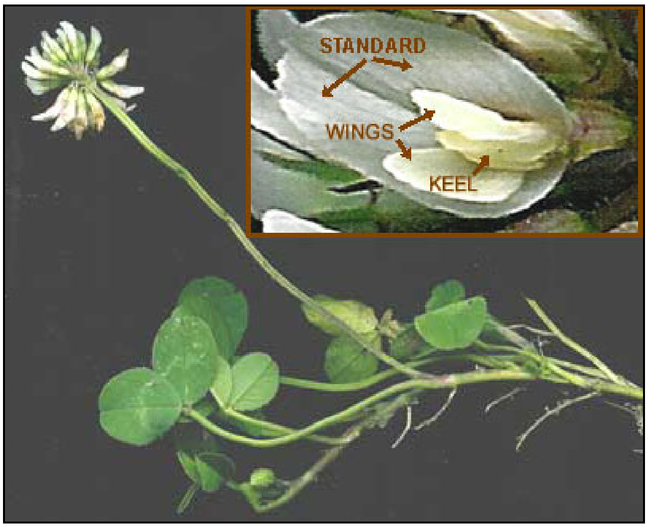

Figure 2.4. A white clover flower head with individual flower inset.

When an axillary bud first develops inflorescence, rather than 'normal' leaf primordia, flowering is said to be initiated in that bud (Thomas, 1987b). Flowers are initiated in March and April and usually appear between May and July in the UK and Ireland (Burdon, 1983). Flowering is enhanced by long days (14 to 16 hrs) and has a broad optimum temperature range (20 to 30 ºC) (Takeda and Agata, 1965; Frame and Newbould, 1986). However, between and even within, cultivars, there is considerable variation in the plants response to day length, temperature and light intensity (Zaleski, 1964). Williams and Abberton (2004) found that white clover tends to flower earlier when soil temperatures are higher. Flowering follows a distinct pattern after initiation with three flowering nodes developing on each stolon, interspaced with two or three vegetative nodes (Thomas, 1962).

Thomas (1981) found that flowering initiation declined with time after the start of the growing season but defoliation of the sward could cause an increase in initiation later in the year (May and June). The increased light levels after defoliation may be responsible for reinitiating flowering as Zaleski (1964) found that increasing plant density in white clover monocultures resulted in less flowers per plant. Zaleski concluded that the low light intensity at the base of dense plant populations retarded both vegetative and reproductive growth.

White clover is generally self incompatible and needs cross fertilization between individuals. Clover is pollinated by a large variety of insects, but particularly honey bees (*Apis mellifera* L.) and bumble bees (*Bombus* spp.) (Burdon, 1983). Following pollination, seeds can ripen after 3 to 4 weeks in good growing conditions (Thomas, 1987a). The seeds are usually around 1 mm diameter, approximately 0.0004 to 0.0008 g in weight (Connolly, 1990), yellow-brown in colour and are heart shaped (Figure 2.1). The seed has no specific adaptation for dispersal and most is not spread very far, although it can pass through cattle, deer and horse guts unharmed (Burdon, 1983).

A proportion of all white clover seed develops an impermeable coat and becomes known as ‘hard seed’. These seeds cannot readily germinate due to the impermeable coats preventing water infiltration. The impermeable coats can be removed with scarification, acid treatment, soaking, freezing or heating and naturally break down over time in grazed swards (Burdon, 1983). The proportion of hard seed produced is negatively related to the relative humidity (Hyde and Suckling, 1953) but also tends to increase as harvesting is delayed. Most commercially produced seed is harvested before the seed has fully completed development. However, under natural conditions the proportion of hard seed continually increases until seed is shed (Harris, 1987). Chapman and Anderson (1987) found that 75% of seed produced in NZ hill pastures was hard and of this, 22% germinated within the first seven months. Pederson and Brink (2000) measured an average of 97% hard seed across cultivars in the south eastern USA. The production of hard seed may contribute to the accumulation of a seed bank under grass-clover pastures that, as Burdon (1983) suggested, could be important following some sort of disturbance that exposes the soil.

There are few studies into natural reseeding of white clover in Ireland or the UK. Burdon’s (1983) review concluded that viable seed densities in the upper 10 cm of British soils ranged from 200 to 2,000 m^{-2} while it could reach 15,000 m^{-2} in NZ. Connolly (1990) measured seed production of ten white clover cultivar monocultures under Irish conditions and found that harvested seed yield per ha was between 36 and 228 kg ha^{-1}. However, losses at seed recovery at harvest was very low (20 to 30%) and the actual seed production was much higher, at 120 to 910 kg ha^{-1} with 54 to 158 seeds per head and 4.2 to 11.4 million heads per ha. These results are for Connolly’s monocultures (1990) and have not been investigated in grass-clover swards. However, taking the basic assumption that a monoculture is 100% sward clover content and assuming flowering rates and density are relative, a grass-clover sward containing 200g kg^{-1} white clover content might produce 24 to 182 kg of clover seed per ha, or between approximately 42,105 and 319,298 seeds per ha given the required conditions and time periods to develop and set seed.

Chapman (1987) measured natural reseeding of white clover on a hill pasture grazed by sheep in New Zealand and recorded approximately 55,000 seedlings per ha but found that only 4.4% (2,420) of them survived to become mature, stolon-baring plants. This was equivalent to only one new plant per 5.5 m^2 per year. However, in that experiment, annual sward clover contents were also quite low (90 g kg^{-1}) and inflorescences were removed very frequently under the continuous grazing (within 12 days of appearance). In contrast, in rotationally grazed New Zealand dairy pastures with clover contents of approximately 200 to 300 g ha^{-1}, Harris *et al.* (1999) recorded 0.33, 0.53, 1.47 and 2.27 million seedlings per ha where summer grazing had been deferred for 25, 50, 75 and 100 days, respectively. Even assuming Chapman's (1987) very low seedling survival rate of 4.4%, this would still result in the addition of 1 to 10 new clover plants per m^2.

The ability of white clover to naturally re-establish via seed in Irish grazed swards is generally considered to be low because of competition from the established plants in the sward and the likelihood that flowerheads/seedheads will be consumed by the livestock before seed develops (T. Gilliland, personal communication). However, this does require further research.

2.1.6. Vegetative production vs. seed production

Since each node on a stolon can produce either a flower or a lateral stolon but not both, profuse flowering can have a negative effect on stolon production (Frame and Newbould, 1986; Hart, 1987; Williams, 1987a). Kawanabe *et al.* (1963) showed strong evidence of competition between inflorescence and vegetative growth of distal stolons. In that experiment, the weight of lateral stolons and roots was lower in plants where the inflorescences were allowed to develop to seedheads than in plants where the inflorescences were snipped off, the reduced stolon/root weight being approximately equal to that of the seedhead and peduncle.

Piano and Annicchiarico (1995) found that persistence of white clover ecotypes over a five year period was positively correlated with stolon density and internode length and negatively correlated with flowering and seed production. Burggraaf *et al.* (2006) also found that increased flowering in a clover cultivar ‘HT’ resulted in poor agronomic performance. Although the fact that nodes can either develop into stolons or flowering buds (but not both) is generally identified as one of the main reasons for the inverse relationship between flowering and vegetative persistence, Thomas (1987b) identifies three other mechanisms that explain this negative correlation:

1. Most other growth parameters are hormonally inhibited at flower initiation.

2. The allocation of resources to the growing flowers.

3. The allocation of resources to the developing seeds.

Two different survival strategies can therefore be identified in white clover, primarily according to climate and/or cultivar: Under hot dry conditions, it tends to flower more and produce more hard seed but under cool moist conditions it relies more on vegetative growth (Harris, 1987; Williams, 1987a; Pederson and Brink, 2000). The second option is generally considered to be more likely under Irish conditions, as discussed at the end of Chapter 2.1.5.

Flowering characteristics in clover cultivars therefore appear to place a conflict of interest between seed producers and the farmers that use their products: high flowering and seed production is obviously a desirable trait for a seed producer but not necessarily for the livestock farmer to which that seed is going. A solution would be a form of grazing management intervention that reduced flowering. Although flowering in white clover is primarily determined by genetic and environmental factors, management factors can also influence flowering. For example, Thomas (1981) found that flowering in a seed-producing white clover monoculture could be encouraged by defoliation once in June as opposed to once in May. Under grazing conditions, grass-clover swards are defoliated much more frequently. However, there is still flexibility for management in terms of defoliation frequency (grazing rotation length) and defoliation intensity (post-grazing height). The effects of these parameters on flowering in white clover, and subsequent implications for clover herbage production and persistence are investigated in Chapter 3.

2.2. Effects of Temperature, Moisture, Light and Soil conditions on White Clover

2.2.1. Temperature

The minimum soil temperature for white clover growth is around 5 ºC and the optimum is 24 ºC (Frame and Newbould, 1986; Hart, 1987; Brock *et al.*, 1989; Junttila *et al.*, 1990). However, the minimum temperature for BNF is approximately 9 ºC with a broad optimum range of 13 to 26 °C (Frame and Newbould, 1986; Hart, 1987). Nitrogenase activity is inhibited above 30 °C (Hart, 1987). There may be some adaptation for BNF in low temperatures as Marriott (1988) recorded low levels of BNF (measured by Acetylene reduction) in an upland grass-clover in Scotland after soil temperature (at 10 cm) increased above 3 °C.

The temperature required for initiation of the development of a plant part is usually lower than that required for growth, for example the minimum temperature for growing point production is lower than the minimum temperature for stolon growth (Collins *et al.*, 1991) and leaf initiation has a threshold of around 2.6 °C (Hart, 1987). Temperature can also influence flower formation in white clover. Takada and Agata (1965) found that flower production was enhanced at higher temperatures (25 to 30 ºC) whereas stolon branch formation and leaf area declined.

White clover survival at temperatures less than -10 °C can be very low (Frame and Newbould, 1986). However this depends on the rate at which temperatures drop as clover displays cold hardening. Cold hardening is when the plant restricts water uptake and mobilises vegetative storage proteins (VSP) and water soluble carbohydrates (WSC) at the onset of cold weather in order to avoid damage from freezing (Hart, 1987;

Frankow-Lindberg, 2001; Goulas *et al.*, 2001). Concentrations of these compounds typically accumulate in clover stolons during the autumn and increase cold tolerance and regrowth ability in white clover (Svenning *et al.*, 1997; Goulas *et al.*, 2001).

Frost injury to clover is higher in plants with fewer rooted nodes and lower levels of carbohydrate reserves (Burdon, 1983). Root nodules usually overwinter and resume growth in the spring (Hart, 1987). Low temperatures also activate fragmentation of taprooted white clover seedlings into mature stolon based plants and this usually happens after the first winter in undisturbed seedlings (Brock *et al.*, 2000; Bonesmo and Bakken, 2005). However, after initial fragmentation of the taprooted plant, the process can increase with temperature (Bonesmo and Bakken, 2005).

2.2.2. Soil moisture

While white clover can grow well at relatively high (24 °C) temperatures, this is assuming sufficient soil moisture is present. Plant death in hot summers is usually associated with low soil moisture levels and white clover is relatively susceptible to drought (Burdon, 1983; Brock *et al.*, 1989). Clover survival drops at temperatures greater than 20 °C on dry soils but can survive temperatures of 35 °C if sufficient moisture is present (Rattray, 2005).

Hart (1987) states that white clover has poor transpirational control of water loss from the leaf surface and is relatively inefficient at conserving water. The majority of literature found in this review discusses white clovers susceptibility to low soil moisture status with relatively few studies on the effects of the other extreme (water-logging and soil saturation), which are more common in Ireland. In general, root metabolism and BNF are inhibited due to lack of air in the soil when soil has very high moisture content (Crush, 1987). However, in one experiment, root nodules in white clover seedlings showed an ability to adapt morphologically to repeated soil flooding over a nine week period, developing larger cell vacuoles and increased aerenchyma production (Pugh *et al.*, 1995). These plants also had higher BNF rates compared to control (normally watered) plants when drained. However, control plants exposed to a sudden flooding event showed a marked decrease in BNF.

Certain cultivars may be more/less susceptible to flooding. For example, Hoveland and Mikkelson (1967) compared a medium- (S.1) and large-leaved (Ladino) cultivar when they were both periodically flooded and drained over a four month period and found that herbage production of the medium-leaved type was reduced more than the large-leaved type. Ireland receives high levels of rainfall and has low levels of field drainage (Collins and Cummins, 1996). Therefore, the ability of clover to perform under wet soil conditions should be considered important for many Irish farms.

2.2.3. Soil nutrients and soil pH

The effects of soil nutrients on clover were reviewed by Dunlop and Hart (1987). Clover is a legume and relies on BNF, therefore it is not as susceptible to low soil N as most plants. However, BNF itself has certain nutrient requirements. For example, effectiveness of *Rhizobia* in BNF is positively correlated with percentage base saturation, pH and exchangeable calcium and magnesium (Burdon, 1983). Clover also requires good soil P and K status and is considered a poorer competitor than the co-habiting grasses (see Chapter 2.4) therefore most agricultural advisors advise that non-N soil nutrient levels should be at least as high as those required for optimum grass production (Frame and Newbould, 1986; Bailey and Laidlaw, 1999).

White clover distribution is generally associated with relatively high pH and calcium levels (Burdon, 1983; Williams, 1987a). Simpson *et al*. (1987) found that clover distribution in hill swards in Wales was not related to soil P or K levels but was positively correlated with soil pH. White clover is considered to be more susceptible to low pH than perennial ryegrass (Frame and Newbould, 1986). Bailey and Laidlaw (1999) found that increasing pH from 5.4 to 6.1 resulted in a doubling of clover herbage production from glasshouse pots. Low pH can inhibit BNF and Andrew (1976) found that nodulation of clover growing in sand culture was nil at pH 4.0 and increased when pH was above 5.0. Although some older texts recommend optimum pH values of 5.3 to 5.5, many of these are based on water and sand cultures and do not take into account Al^{3+} and Mn^{+2} toxicity which can occur in soils at pH below 5.5 (Frame and Newbould, 1986; Dunlop and Hart, 1987) therefore soil pH should be maintained above this level. H^+ ions are produced as a by-product of the BNF process in *Rhizobia* bacteria (Crush, 1987), therefore pH should be monitored over time where high clover production is to be maintained.

2.2.4. Soil

Soil type and texture (sand:silt:clay) can influence moisture, temperature, macroporosity and nutrient retaining ability (Marshall and Holmes, 1988). In general, soils with higher clay contents are associated with higher soil moisture, higher nutrient retaining ability, lower temperatures (if soil water is present) and lower macroporosity whereas soils with higher sand contents are associated with lower soil moisture, lower nutrient retaining ability, higher temperatures and higher macroporosity. The effects of soil moisture, temperature and soil nutrients on white clover plants have been discussed in Chapter 2.2. White clover requires relatively high levels of macroporosity and low levels of bulk density as sufficient soil-air movement is required for BNF in the root nodules (Vidrih and Hopkins, 1996).

2.2.5. Light

Light is obviously necessary for photosynthesis and the photosynthetically active radiation (PAR) wavelengths of light are those in the range of 350 to 725 nm (Gay, 1991). However, light levels both within and outside the photosynthetically active range have important roles as photomorphogenic signals for plants. Three major wavelengths have been defined as having important photomorphogenic effects in plants (Woodward, 1983).

1. Blue; 400 to 500 nm.

2. Red; 500 to 700 nm.

3. Far-red; 700 to 800 nm.

These light signals are detected by the phytochrome in plants and used to avoid shading. The phytochrome in white clover is specifically located at the apical and lateral stolon buds, and the tip of the petiole (in the middle of the three leaves) (Thompson, 1995). Most discussions on shade avoidance in plants discuss the red to far-red (R:FR) light ratio as this decreases when light passes through or is reflected from leaves, therefore it is a useful signal to the phytochrome that neighbouring leaves are encroaching (Héraut-Bron *et al.*, 2001). In daylight, the R:FR ratio is about 1.2, which is more than sufficient to stimulate stolon branching. However, under grass-clover swards it can be as low as 0.1 to 0.3, which inhibits branching (Robin *et al.*, 1994). Thompson and Harper (1988) found that decreasing the R:FR light ratio that reaches the petioles also results in increased petiole length as the plant attempts to place it's leaves above the shade. Thompson (1993) found that reducing the R:FR that reaches stolon apices resulted in reduced stolon internode length, nodes per stolon, branching and rooting. Robin *et al.* (1994) had similar findings, with the exception that shaded R:FR reduced stolon branching but not stolon elongation and Lötscher and Nösberger (1997) found that branch initiation and stolon elongation could still occur as long as the developing leaves were not shaded. Furthermore, Héraut-Bron *et al.* (2001) found that although R:FR shading reduced stolon growth, C^{14} assimilate increased in the stolon buds, in preparation for increased growth if the shade was removed. Shading of white clover leaves can also reduce root growth as the plant diverts resources to producing shoot material in order to access light (Hart, 1987).

Reduction in blue light also occurs under plant canopies and can have a similar effect on petiole elongation and stolon growth as reduction in R:FR, i.e. reducing blue light levels inhibit stolon growth and increase petiole elongation (Gautier *et al.*, 1997; Gautier *et al.*, 1998; Christophe *et al.*, 2006). Reductions in PAR have similar effects on clover growth as the reductions in blue light and R:FR light, however the results for PAR can be complicated by lower growth rates due to lower photosynthesis when PAR is reduced (Solangaarachchi and Harper, 1987; Christophe *et al.*, 2006).

Photoperiod also has important effects on plant development such as flowering and branching, as discussed in Chapter 2.1.6 and Chapter 2.2.5. Woledge *et al.* (1989) found that gross photosynthesis rates of a grass-clover sward were only slightly reduced in winter (temperatures 5 to 7 ºC) as opposed to summer in southern England. However, net photosynthesis was as low as a tenth of summer values, due to short photoperiods and low irradiance levels. Temperature can also influence stolon responses to light, for example shading has no effect on stolon elongation at 12 °C but inhibits it at 22 °C (Hart, 1987). The research cited above separates the effects of the three major wavebands, however in natural conditions shading by a given canopy usually reduces all three (Gautier *et al.*, 1998). For example Figure 2.5 shows the relationship between PAR and the R:FR light.

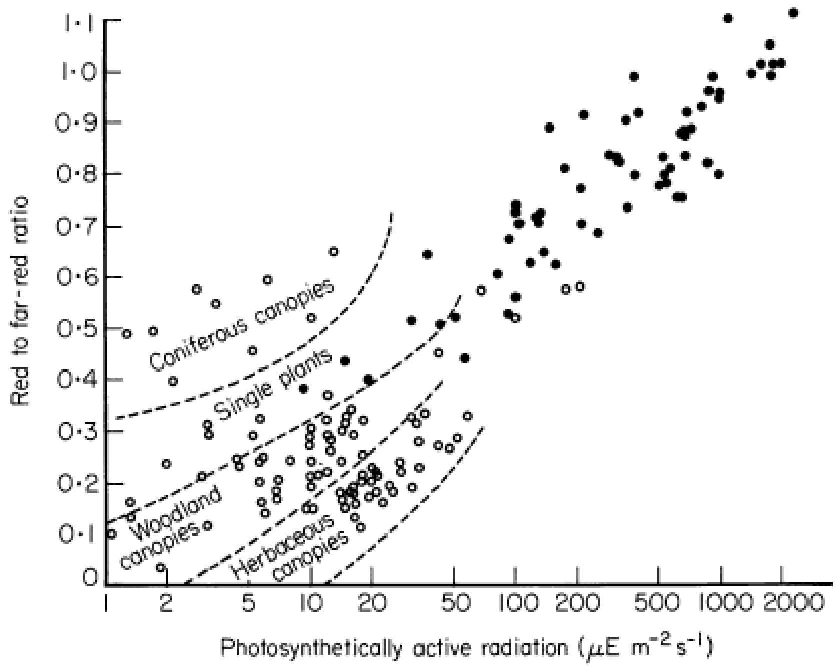

Figure 2.5. The relationship between photosynthetically active radiation (PAR) and the red to far-red (R:FR) light ratio, under different plant canopies (Woodward, 1983).

Therefore numerous studies have shown that, in general, increased light levels are associated with increased rates of leaf appearance and leaf expansion, while shading increases petiole elongation and can inhibit branching and stolon elongation. This is the simplest of plant strategies 'grow towards the light' and has mostly been studied in laboratory or small, mown plots. However, clover plants in grazed swards are in a patchy environment and usually span a range of light conditions and one section of a given stolon can be more shaded than another section on that same stolon (Teuber and Laidlaw, 1995). The plant still has the principle to grow towards the light so now has two options: either extend petioles within the shaded patch to try and reach the light or divert resources to branching and leaf production in the less shaded area. The plant response appears to be dose-dependant on the light intensity reaching the phytochrome on individual leaves and stolon apices (Robin *et al.*, 1994; Thompson, 1995; Teuber and Laidlaw, 1996; Hay, 2001).

2.3. White Clover in Agriculture

2.3.1. Introduction to the agricultural use of white clover

White clover (*Trifolium repens* L.) is generally considered to be the commonest and most agriculturally important grazing legume in all temperate regions of the world (Frame and Newbould, 1986; Zegwaard *et al.*, 2000; Abberton and Marshall, 2005; Black *et al.*, 2009). There are four main reasons for this:

1. The symbiotic relationship that clover forms with *Rhizobia* bacteria that biologically fix nitrogen from the atmosphere. This can reduce agricultural requirement of fertilizer N, along with its associated financial and environmental costs.

2. White clover is one of the few legumes well suited for grazing systems due to its prostrate growth habit which enables to plant to spread out through a sward and retain most of its growing points beneath the grazing/cutting horizon. This enables the plant to survive repeated grazing/cutting events to a much greater extent than more vertically growing legumes such as red clover (*Trifolium pratense* L.).

3. White clover has an extremely high feed value for grazing animals, with low fibre, high protein, high digestibility and high intake rates (Dewhurst *et al.*, 2009).

4. White clover has a long agronomic history, which has resulted in well established breeding programs and a large amount of acquired information available to farmers (Frame and Newbould, 1986).

White clover is most frequently grown in permanent pastures in a mixture with perennial ryegrass (*Lolium perenne* L.). These grass-clover swards are usually grazed *in situ* by either cattle or sheep (Davies, 2001; Black *et al.*, 2009).

2.3.2. Worldwide use of white clover

Legumes were the main way to increase soil N fertility in permanent grazed pastures for centuries before fertilizer N use became so predominant. For example, records show reference to the agricultural use of white clover in Britain in the early 1600's (Burdon, 1983). However, since the 1980's, fertilizer N has replaced the importance of legumes in most countries.

In the last 60 years, New Zealand is a country in which the widespread use of white clover has been most prominently documented. However, clover use in NZ has declined over the last 20 years, due to pests such as the clover root weevil (*Sitona lepidus* L.) and the intensification and increased use of fertilizer N (+ 700% in the last 20 years) (Woodfield and Clark, 2009).

Abberton and Marshall (2005) estimated that grass-clover swards covered approximately 15 million ha in Australasia and 5 million ha in the USA (but gave no such value for Europe) with global sowing totalling 3 to 4 million ha annually. Within Europe, however, it was noted that the greatest use was in the northern and western countries and seed sales within the UK were an estimated 288 tonnes between July 2002 and June 2003 (Abberton and Marshall, 2005). The area covered by forage legumes in Europe has decreased in the last 30 years in most regions, although notable exceptions are Switzerland and the west of France (Peyraud *et al.*, 2009). In Switzerland, leys are usually a mixture of perennial ryegrass and white clover. Swiss seed companies allocate a large amount of resources towards grass-clover mixtures and over 90% of grassland seed is sold as ‘Standard Mixtures’ with a quality label (‘AGFF’, Gütezeichen) awarded by the Swiss Grassland Association (Lehmann and Kessler, 1988). In the West of France, approximately 50% of sown pastures are currently grass-clover, an increase from 10% in 1985 (Peyraud *et al.*, 2009).

Seed sales in Northern Ireland have shown that legumes consistently accounted for approximately 6% of grass and clover seed weight that was bought each year between 1980 and 2004 (Gilliland *et al.*, 2007). Of that legume seed, almost all (99%) was white clover. However, the total area that white clover seed was used over would have actually been similar to the area that perennial ryegrass was used over, because the vast majority of the clover seed was sold in seed mixtures at rates of approximately 1 kg of clover seed in every 14 kg of seed. White clover use in the Republic of Ireland is probably similar, being mostly sown at low rates in seed mixtures. In the majority of cases, these seed mixtures are unlikely to result in high sward clover contents, given the low proportion of clover seed in them and the fact that the swards are generally managed for their grass species, with post emergence herbicides and N fertilizer being used commonly.

White clover is a vital component in organic grazing systems (Kuusela, 2004) due to the factors mentioned above. Within the EU27, Ireland has the third lowest proportion of UAA devoted to organic production at 1% (European Commission, 2010a). It could be argued that Ireland has large potential for organic meat and dairy production, given the predominance of low input grazed grassland and low stocking rates relative to other European countries (Thorne and Fingleton, 2006). In recent years in Ireland, there have been high price premiums (150% of conventional) available for organic farmers that can supply 55% of their milk in the winter period. However, organic feed concentrates also tend to be very expensive. Therefore the ability to extend grazing on grass-clover swards into winter is an important management objective for Irish organic dairy farmers. The implications of such grazing management for subsequent clover persistence and herbage production are discussed further in Chapter 2.5.4 and Chapter 6.

2.3.3. Agricultural varieties

Wild or 'naturalised' white clover was described by Burdon (1983) as being 'a highly variable species' with large differences in morphological characters both between and within populations. Over the years this variation has been exploited to breed varieties/cultivars that perform better in agricultural systems. New Zealand led the way in clover breeding in earlier years, improving agronomic performance (mainly DM production ha^{-1}) by about 30% between 1930 and 1970 (Chapman *et al.*, 1996). One particular cultivar 'Huia' was developed in NZ and became a benchmark for testing newer cultivars (Williams, 1987b). White clover cultivars are normally classified by leaf size relative to Huia, which would be considered medium leaf size today. However the trait of leaf size is positively correlated with the size of most of the other plant organs such as petiole length and stolon thickness. Table 2.1 shows 2009-2010 recommended list varieties from Northern Ireland with information on leaf size, herbage production and persistence.

Small-leaved cultivars tend to have a higher stolon growing point density and less annual herbage production than larger leaved cultivars. This has been suggested as an explanation for the inverse relationship between productivity and persistence that has been reported between cultivars (Wilman and Asiegbu, 1982b; Wilman and Asiegbu, 1982a; Brock and Hay, 1996; Deptartment of Agriculture Fisheries and Food (DAFF) Ireland, 2010) (Figure 2.6).

Table 2.1. Department of Agriculture and Rural Development 2009-2010 recommended list varieties for white clover, Northern Ireland 2009 (Gilliland, 2009).

		Grazing yield Potential			Grazing persistence**	
Cultivar	Leaf size	Total	Clover	Grass	Low N	High N
	%*	%*	%*	%*	(0 – 9)	(0 – 9)
AberAce	41	90	55	108	6.6	4.6
Gold’s Demand	77	97	81	105	6.4	5.2
Crusader	85	101	101	101	5.8	4.9
Avoca	95	101	99	102	5.9	5.1
Aberdai	99	101	107	98	5.5	4.7
Chieftain	105	104	124	94	5.4	4.5
Alice	124	102	113	97	5.1	4.2
Barblanca	125	104	121	95	5.7	4.6

* Relative to Huia.
** Index estimated from area of ground covered by clover plant tissue.

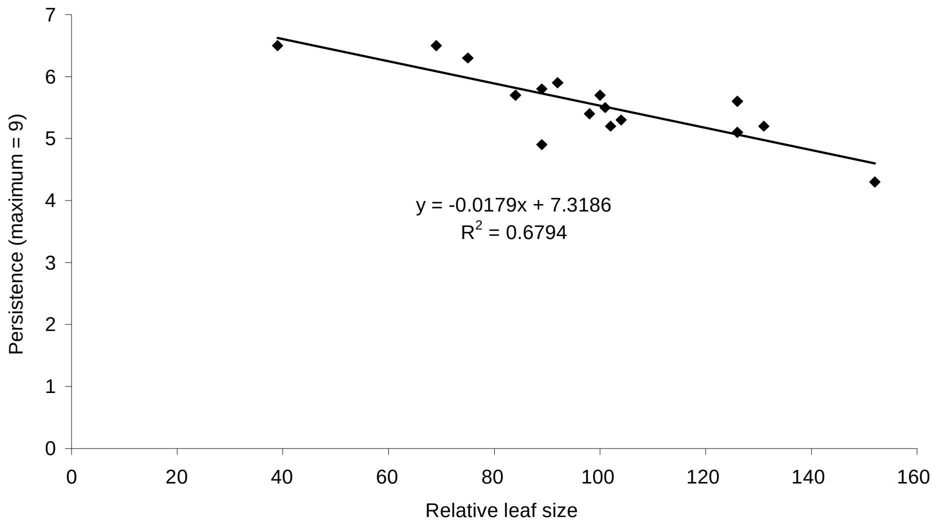

Figure 2.6. Relationship between leaf size (% relative to Huia) and grazing persistence of white clover cultivars grown in grass/clover swards under low fertilizer N (Department of Agriculture and Rural Development, 2008) (Gilliland *et al.*, 2009).

The inverse relationship between leaf size and clover growing point density has been described as an example of ‘size density compensation’ (SDC) in plant breeding. Plant breeders have used this relationship to design cultivars for specific situations (Dewhurst *et al.*, 2009). For example, small-leaved dense cultivars are commonly considered better suited for the frequently defoliated swards under continuous grazing by sheep. Conversely, large-leaved, productive cultivars are believed to be better suited to the more infrequently defoliated swards in rotational cattle grazing and silage systems as they produce longer petioles and can compete better for light in the taller swards (Gilliland *et al.*, 2009).

It should also be noted that each white clover cultivar has a large capacity for phenotypic plasticity (Seker *et al.*, 2003), i.e. a small-leaved cultivar will develop the characteristics of a larger-leaved cultivar when subjected to rotational grazing and *vice* *versa*; a large-leaved cultivar subjected to continuous grazing will develop the characteristics of a small-leaved cultivar. However, the potential for plasticity also differs between cultivars and better performance and persistence are usually achieved when using a clover cultivar that suits the system it is to be used in. For example Brock *et al.* (1988) found that a small-leaved cultivar (Tahora) increased its content in a sward continuously sheep-grazed while a larger leaved cultivar (Kopu) declined under the same management.

Aside from the leaf-size continuum which dominates white clover cultivar classification, there is room for identification of novel traits that improve clovers agronomic performance. Probably the most important breeding objective for white clover in Ireland is to improve spring growth (Gilliland *et al.*, 2009). This could be achieved by improving clovers ability to overwinter as less loss over winter = more clover material present in spring (Wachendorf *et al.*, 2001); however this may also be restricted by the leaf-size continuum as there appears to be relationships between morphology and ability to tolerate low winter temperatures. Cold-tolerant plants from northern Europe are usually smaller leaved and more prostrate than the larger, frost-sensitive Ladino types that usually originate from Mediterranean populations (Frame and Newbould, 1986; Collins *et al.*, 1991). Harris *et al.* (1983) also noted that the small-leaved cultivar S.184 overwintered better in a monoculture than medium- and large-leaved cultivars. However, some medium leaf sized Swiss genotypes have been shown to have good spring growth due to their ability to maintain higher stolon masses over winter (Collins *et al.*, 1991). Gilliland *et al.* (2009) identified large- and medium- leaved cultivars such as Triffid, Barblanca and Crusader that have increased spring production.

Therefore, while cultivar selection for a particular system should follow the leaf size recommendations discussed, there are other traits at least as important (such as spring growth) that need to be considered.

It may be prudent to use mixtures of cultivars in order to buffer against unforeseen circumstances and changes in management practices. The researchers and staff at Solohead Research Farm have found that a 1:1 ratio of Chieftain and Crusader gives good production and persistency and is well suited to the conditions there. However, persistency is not guaranteed with any mix or cultivar and over-sowing clover seed onto 20% (at a rate of 5 kg per ha) of the farm each year in rotation (such that each area is over-sown every five years) has also been recommended to ensure productive clover levels (Humphreys and Lawless, 2008).

2.3.4. Nutritive value

The nutritive value of white clover has recently been reviewed by Dewhurst *et al*. (2009). White clover is known as having an extremely high nutritive value with high protein, low structural fibre values and relatively high *in vitro* organic matter digestibility (Wilman and Riley, 1993), even when compared with perennial ryegrass (Table 2.2). It has also been found to maintain relatively uniform levels of total N, neutral-detergent fibre, acid-detergent fibre and *in vitro* digestibility during autumn through to mid-winter (Ayres *et al.*, 1998). However, the main benefit of white clover is a higher intake rate and a higher digestion rate (Dewhurst *et al.*, 2009). Sheep and cattle both show a partial preference of approximately 70% for clover when grazing grass-clover swards (Rutter *et al.*, 2004; Rutter, 2006).

The lower fibre content of clover cell walls is the main reason for its higher intake and digestion (Søegaard, 1993). This is the main reason that *ad libitum* feeding studies using white clover have obtained improved animal performance. For example, Castle *et al.* (1983) found that the daily intake of silage offered *ad libitum* to dairy cows increased from 13.6 to 15.1 kg per cow as the percentage of clover in the silage increased from 0 to 70% with parallel increases in milk yield (19.0 to 21.0 kg per cow per day).

Table 2.2. Chemical composition (% of DM) of plant fractions in perennial ryegrass mixed with white clover fertilized with 200 kg N ha^{-1}, mean values of the growing season (Søegaard, 1993).

	Perennial ryegrass		White clover leaves and petioles	LSD 0.05
	Stems and sheaths	Leaves		
N	1.9	3.1	4.1	0.3
NDF	43.3	37.8	26.2	3.6
ADF	26.6	23.7	22.5	6.6
Lignin	2.1	1.7	3.9	0.6
IVOMD	73.8	78.0	77.3	2.6

European studies generally report that white clover does not lose palatability with maturity to the same extent that grass does (Dewhurst *et al.*, 2009). However, in the more arid climate of Australia, Ayres *et al.* (1998) found that neutral-detergent fibre (NDF) increased from 184 to 301 g kg^{-1} dry matter (DM), nitrogen (N) declined from 36 to 20 g kg^{-1} DM, *in vitro* digestibility declined from 0·74 to 0·65 and metabolizable protein content declined from 144 to 67 g kg^{-1} DM for white clover herbage during the transition from early flowering in spring to ripe seed stage in summer.

The two main problems associated with feeding white clover are (i) risk of bloat (Hart,

1987) and (ii) significant loss of crude protein (35%) between ingestion and absorption at the intestines (Ayres *et al.*, 1998). These are both due to the high content of rapidly degradable protein in clover tissue (Dewhurst *et al.*, 2009). Bloat is caused when foam in the rumen tract blocks the escape of gases through eructation (Hart, 1987). Pressure then accumulates in the rumen and can cause animal death (Rattray, 2005). This foam is caused by the formation of stable protein complexes in the rumen as a result of the highly soluble nature of the dietary constituents, especially the protein (Brock *et al.*, 1989).

In general, grass-clover pastures with high clover contents are associated with a higher risk of bloat (Clarke and Reid, 1974). However, Carruthers and Henderson (1994) found that bloat in a sample of NZ farms over three years was not influenced by clover content but was positively associated with the proportion of perennial ryegrass and negatively associated with the proportion of other (weed) grasses. However, the average clover contents of these farms over the study period were quite low (11%) and the effects of individual paddocks were not assessed. Carruthers and Henderson (1994) also found that incidence of bloat was negatively associated with pre-grazing sward height and in a related experiment found that increasing the pre-grazing height by 500 kg DM ha^{-1} could halve the incidence of bloat (Carruthers, 1993). On-off grazing may increase the risk of bloat as observed with cattle grazing alfalfa (Majak *et al.*, 1995). Research has been conducted at Solohead farm, Co. Tipperary for the last ten years into dairy production from grass-clover swards and has yet to encounter a case of bloat that required intervention (Humphreys *et al.*, 2009a). This has been attributed to not allowing the cows to get too hungry before grazing and the fact that the sward clover contents rarely exceed 400 g kg^{-1} under grazing conditions in Ireland, particularly in spring.

Condensed tannins (CT) are plant compounds that bind to plant protein, protecting it from microbial degradation in the rumen, and the resulting increase in amino acid flow to the intestines can benefit animal health and productivity (Woodfield and Clark, 2009). Condensed tannins can reduce the risk of bloat, increase N-utilization in the digestive tract and protect against parasites (Rochon *et al.*, 2004). Unfortunately white clover produces very low levels of condensed tannins; in fact, the flower heads are the only parts of a white clover plant that contain significant levels of CT (Hart, 1987).

Increasing the content of condensed tannins in white clover is a major breeding objective (Woodfield and Clark, 2009) that has not been commercially successful to date. For example Burggraaf *et al*. (2006) evaluated a white clover cultivar ('HT') that had been selected for increased CT levels via increased flower production. Although the higher flower densities in HT did increase CT levels in herbage, this only occurred in the short time period that the clover was flowering profusely (1 to 2 months in summer) and the agronomic performance was negatively affected, producing 1,000 kg DM ha^{-1} yr^{-1} less than the control cultivar, Huia (Burggraaf *et al.*, 2006). Breeding cultivars with CT production in the leaves is the more recent goal, possibly through the use of genetic modification (Woodfield and Clark, 2009). However, even if this is achieved, it is probable that CT production comes at a metabolic cost to the plant and another aspect of agronomic performance, such as DM production could be compromised. However, CT have insecticidal properties (Waiss *et al.*, 1981) so there could also be reduced loss to insect pest species if it was present in the leaves.

The benefits of white clover on herbage intake and milk yield have generally been most prominent when herbage with high proportions of white clover (> 500 g kg^{-1} DM) was offered *ad libitum* (Harris *et al.*, 1998) or where grass-clover swards were compared with grass-only swards receiving no fertilizer N (Wilkins *et al.*, 1994). In grazed grass-clover pasture, the proportion of clover in the sward obviously influences any potential benefit of clover on nutritive value and intake. Ribeiro Filho *et al*. (2003) found that grass-clover swards with average clover contents of approximately 420 g kg^{-1} increased grazing dairy cows herbage intake by 16% and milk yield by 12%, compared with fertilized grass-only swards. However, Ribeiro Filho *et al*. (2005) reported no such increase when the grass-clover swards had clover contents of 270 g kg^{-1} of herbage DM. A four-year study by Humphreys *et al*. (2009a) found no difference between grass-clover swards (annual clover contents of 218 g kg^{-1}) receiving 90 kg fertilizer N ha^{-1} and grass only swards receiving 226 kg fertilizer N ha^{-1} for in vitro organic matter (OM) digestibility and the crude protein content was slightly higher in the grass-only sward (219 compared to 209 g kg^{-1}). Therefore, although white clover has good potential to increase sward nutritive value and intake rates, achieving the necessary sward clover contents to achieve this is challenging and if they are achieved, the potential for increased risk of bloat needs to be addressed.

2.3.5. Production potential of grass-clover swards

Previous experiments have found that mixtures of grassland species, including grass-clover swards, generally have higher annual DM yields over a broad range of fertilizer

N inputs than monocultures (Frankow-Lindberg *et al.*, 2009; Nyfeler *et al.*, 2009). However, high rates of fertilizer N have a negative impact on BNF and can reduce sward clover content over time (see Chapter 2.5.1.). Because of this, lower amounts of fertilizer N are usually used on grass-clover swards and usually at times when BNF is less active, such as early spring.

In the Netherlands, Elgersma *et al.* (2000) found that mown grass-clover swards with high levels of clover (>500 g kg^{-1} DM) could produce as much annual herbage as grass monocultures receiving 280 to 360 kg fertilizer N ha^{-1}. Roberts *et al*. (1989) found that, in a three-cut silage system in Scotland, a grass-clover sward receiving 0 fertilizer N produced 71% as much herbage DM as a grass-only sward receiving 320 to 340 kg fertilizer N ha^{-1}. However, both swards were grazed after the final silage cut in September and grazed herbage was not included in results. In New Zealand (Ledgard *et al.*, 2001), grass-clover swards receiving no fertilizer N had herbage production that was 89% of swards receiving input of 200 kg ha^{-1} and 80% of swards receiving input of 400 kg ha^{-1}.

The production potential of grass-clover swards is obviously influenced by the clover content in the sward. A large study of over 400 fields on commercial farms in France found that annual herbage production from swards with summer clover contents of less than 200 g kg^{-1} was, on average, 75% that of grass-only swards receiving an average of 200 kg fertilizer N ha^{-1} (Pflimlin *et al.*, 2003). However, grass-clover swards with 400 to 600 g kg^{-1} had 96% that of the fertilized grass-only swards.

One of the problems with white clover is that it has low BNF rates and herbage production in early spring, in comparison with fertilized perennial ryegrass. For this reason, strategic inputs of fertilizer N are often applied to grass-clover swards in early spring to increase grass production at that time only. In the Netherlands, Schils *et al.* (2000a; 2000b) found that grass-clover receiving fertilizer N input of 17 kg ha^{-1} in spring produced 95% of the herbage of a grass-only sward receiving annual fertilizer N input of 208 kg ha^{-1}. Likewise in Ireland, Humphreys *et al.* (2009a) have shown that grass-clover pastures receiving between 80 and 90 kg ha^{-1} of fertilizer N in spring had herbage production that was 92% of grass-only pastures receiving 226 kg N ha^{-1} and 80% of grass-only pastures receiving 353 kg N ha^{-1}.

2.3.6. Economic benefits of white clover

The primary economic benefit of white clover is its ability to increase BNF in grazed grassland and thereby reduce the fertilizer N costs needed for agricultural production. Caradus *et al*. (1996) estimated that white clover contributed NZ$ 3 billion per year to NZ's economy in the early 1990's through BNF (NZ$ 1.49 billion), pasture production (NZ$ 1.55 billion), seed sales (NZ$ 25 million) and honey production (NZ$ 30 million). However, it can be argued that BNF and pasture production should not have been counted separately and that other factors, such as offset energy and environmental costs, were not included. In a similar manner, Woodfield and Clark (2009) estimated that each 1% increase in white clover production brought about by improved cultivar breeding is worth NZ$ 20 million to the NZ economy.

In the Netherlands, Schils (1996) found that a grass-clover farmlet receiving 70 kg fertilizer N ha^{-1} yr^{-1} had lower gross margins per ha (NZ$ 7,000 as opposed to NZ$

7,800) than a grass-only farmlet receiving 275 kg fertilizer N ha^{-1} yr^{-1}, due to the lower stocking rate on the grass-clover (1.9 instead of 2.2 cows ha^{-1}). However, stocking rate might not have matched herbage production. This was indicated by the difference in silage surpluses: the grass-clover sward had an annual surplus of 707 kg DM ha^{-1} yr^{-1} whereas the grass-only sward had an annual silage deficit of -353 kg DM ha^{-1} yr^{-1}. The economic benefit of BNF is generally proportional to the price of fertilizer N, which has been increasing relative to farm produce price over the last two decades (Figure 2.7).

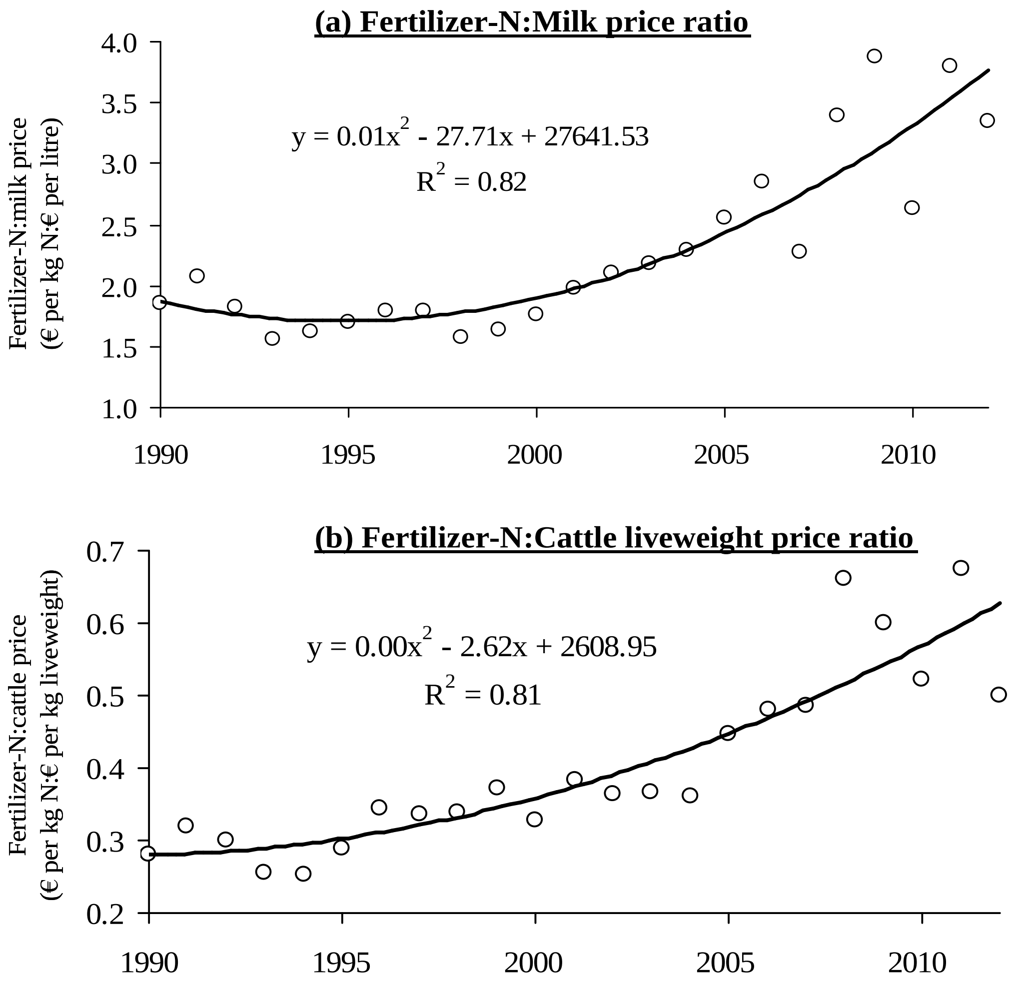

Figure 2.7. The price ratio of synthetic fertilizer N to (a) milk and (b) beef cattle liveweight between 1990 and 2012 (Central Statistics Office (CSO), 2012a).

In the U.S.A., Falconer *et al*. (2011) compared the cost of suckler beef production from a grass-clover sward with grass-only swards receiving either 140 or 0 kg fertilizer N per ha (stocked at 2.5, 3.8 and 1.3 LU ha^{-1}, respectively). Per kg of meat produced, the grass clover sward was 43% and 25% more cost effective than the grass-only swards receiving fertilizer N inputs per ha of 140 and 0 kg, respectively. The cost-effectiveness of the grass-clover sward was primarily due to lower fertilizer N inputs and a longer grazing season, which was not explained further. Stochastic modelling also found that the grass-clover sward had a lower range in variation of costs compared to the grass swards receiving 140 or 0 kg fertilizer N (range of $ 0.51 per kg of beef as opposed to $1.14 and $0.76,respectively) (Falconer *et al.*, 2011).

More recently, Humphreys *et al*. (2012) compared economic performance of dairy production from either white clover based systems (WC: grass-clover swards receiving 80 to 100 kg fertilizer N ha^{-1} yr^{-1}) or fertilizer N based systems (FN: grass-only swards receiving 180 to 353 kg fertilizer N ha^{-1} yr^{-1}) over a ten year period at Solohead Research Farm in Ireland. In that study, the stocking density, milk yield per ha and total sales from grass-clover were approximately 90% of FN reflecting the generally lower productivity of WC. Overall variable costs on WC were 82% of FN and 58% of this difference was due to differences in the fertilizer N input; the remainder was mostly due to differences in scale. Consequently, there tended to be little difference in the gross margins between the two systems at intermediate milk and fertilizer N prices. The fixed costs tended to be marginally higher on FN, which was attributable to activities associated with higher stocking densities and higher milk output such as electricity use, labour and repayments on capital investments.

Humphreys *et al.* (2012) also examined the sensitivity of net margin to high, intermediate and low fertilizer N and milk prices that were within the range experienced by Irish farmers between 2008 and 2010, which was a period with relatively volatile fertilizer N and milk prices. In scenarios with high fertilizer N price combined with intermediate or low milk prices WC was more profitable than FN. As shown in Figure 2.7, the price of fertilizer N has been increasing at a higher rate than milk price. As can be seen in Figure 2.8, FN was more profitable than WC between 1990 and 2005. This could explain the generally low levels of clover use discussed in Chapter 2.3.2. However, with the steady increase in fertilizer N prices relative to milk price, the difference between FN and WC was less clear cut between 2006 and 2010. Projecting into the future, assuming that the trends in fertilizer N and milk prices continue, this analysis indicated that WC is likely to become an increasingly more profitable alternative to FN for pasture based dairy production (Humphreys *et al.*, 2012).

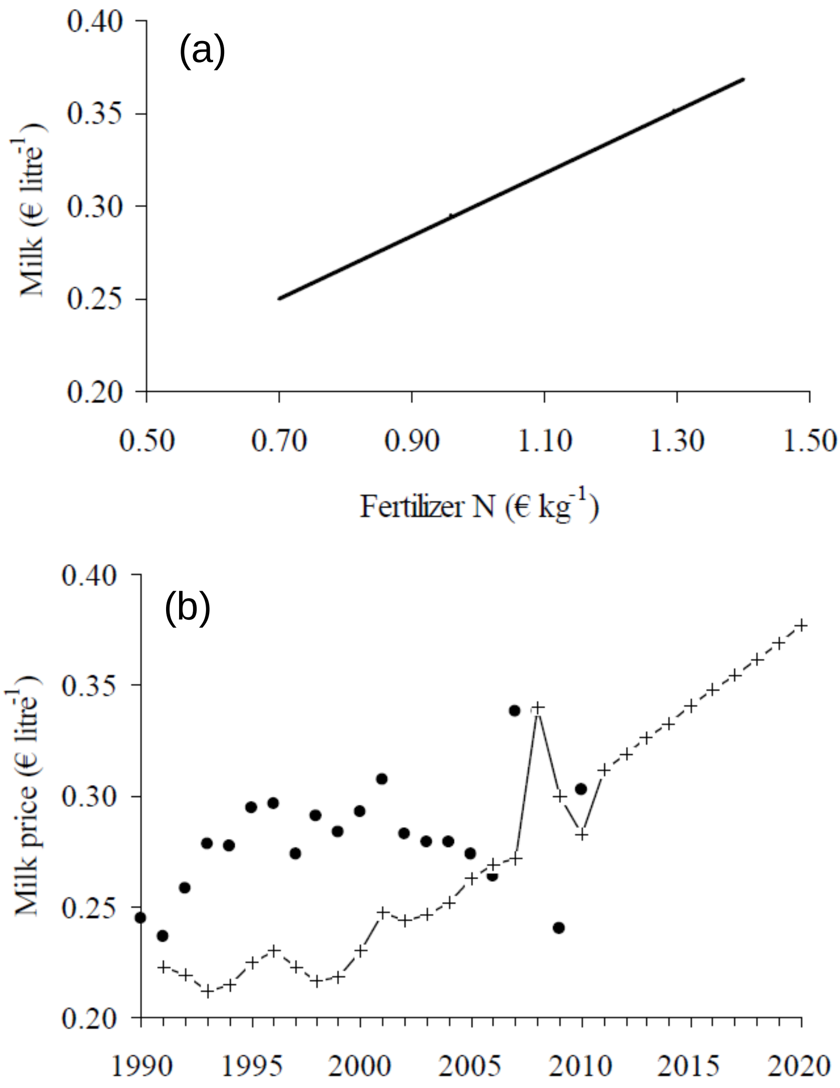

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Figure 2.8. (a) The combination of fertilizer N and milk prices at which the profitability of dairy production based on grass-white clover (WC) equals that based on N fertilized grass (FN): Above the line FN was more profitable and vice versa, and (b) actual milk price (●) and the milk price (+) at which the profitability of WC would have equalled FN between 1990 and 2010 and projected to 2020 based on the increase in fertilizer N price between 1997 and 2010 (R^2 = 0.77; P < 0.001; n = 14) (Humphreys *et al.*, 2012).

2.3.7. Environmental benefits of white clover

The overuse of fertilizer N in agriculture over the last three decades has been associated with increased losses of N to the environment in the form of pollutants such as nitrates leached to ground and surface waters, and greenhouse gases such as ammonia and nitrous oxide. Fertilizer N production also currently uses large amounts of non-renewable energy sources. The European Union has implemented the EU Nitrates and Water Framework Directives (European Council, 1991; European Parliament and Council, 2000), and the EU 2020 strategy targets (European Commission, 2010b) in order to reduce the negative environmental affects of intensive use of fertilizer N.

Ledgard *et al.* (2009) has reviewed the environmental benefits of white clover. Nitrogenase activity is reduced when available soil N increases (Schulze, 2004). Therefore (unlike fertilizer N applications) BNF responds directly to the levels of available soil N, increasing N inputs when soil N is low and reducing them when soil N is high. This means that BNF can function as a self-regulating negative feedback loop which should increase N efficiency and reduce environmental losses, relative to fertilizer N. However, the concentrations of N, and the N:C ratio in white clover tissue can often be higher than perennial ryegrass (Table 2.2), resulting in potentially higher concentrations of N in urine and subsequent environmental losses (Ledgard *et al.*, 2009).

Experiments have shown that, for a given system, soil and climate, nitrate leaching from grazed grassland increases exponentially with total N input, regardless of whether the source of that N is BNF or fertilizer (Figure 2.9). However, in grazed grass-clover swards, BNF is usually less than 200 kg ha^{-1} which limits their ability to leach nitrates and makes them a suitable option for nitrate-vulnerable regions (Andrews *et al.*, 2007; Ledgard *et al.*, 2009). White clover can also benefit the environment by increasing the economic feasibility of lower intensity farming by reducing fertilizer N costs and enabling access to organic markets (with their higher product prices).

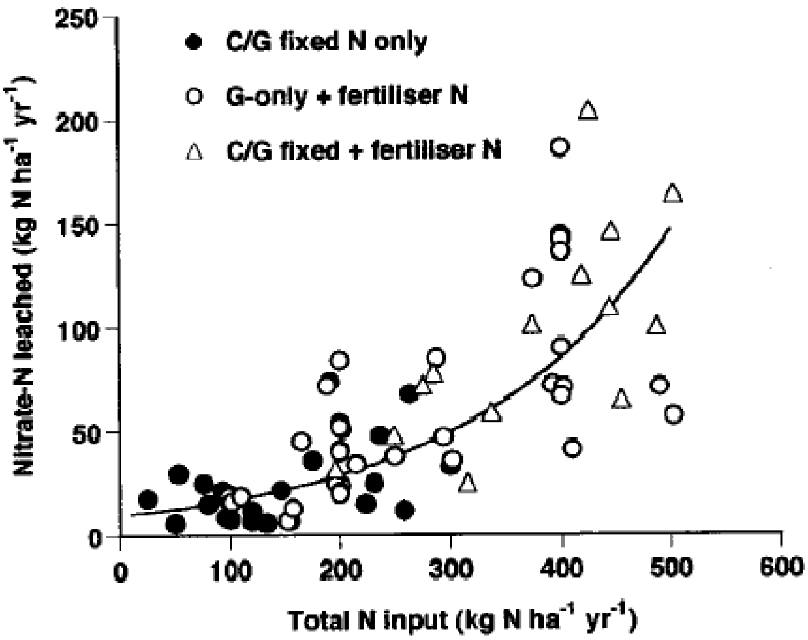

Figure 2.9. Nitrate leaching from grazed clover/grass (C/G) or grass-only (G-only) pastures, as affected by total N input from N_2 fixation and/or N fertilizer application (Ledgard, 2001).

There are also lower green house gas emissions associated with BNF than with fertilizer N. Production of fertilizer N is an energy intensive process using approximately 60 MJ of energy to produce one kg of fertilizer N (Jenssen and Kongshaug, 2003). This energy is currently provided by large amounts of fossil fuel consumption, in particular, natural gas (Figure 2.10).

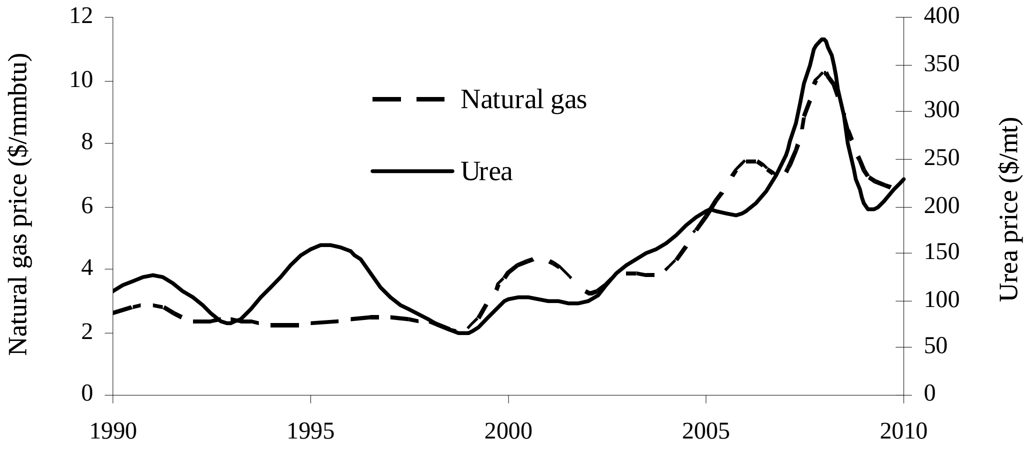

Figure 2.10. Trend in the international prices (adjusted for inflation) of natural gas and urea over time (World Bank, 1990-2012).

Green house gas emissions, primarily CO_2 are associated with the production and transport of fertilizer N, at rates of approximately 3.5 to 4.0 kg of CO_2 released for every kg of fertilizer N (Williams *et al.*, 2006). Fertilizer N applications also increase N_2O emissions from grassland. The IPCC (International Panel for Climate Change) currently do not consider BNF to be source of direct emissions of N_2O, unlike fertilizer N (Metz *et al.*, 2007). Li *et al.* (2011) has shown this assumption to be correct for grass-clover swards in Ireland, with emissions being similar from grass-clover swards and unfertilized grass-only swards, and increasing following fertilizer N application. Ammonia emissions are also likely to be lower where fertilizer N is replaced/reduced by BNF with clover (Ledgard *et al.*, 2009) but this requires further research.

There is a lack of research on the effects of white clover on biodiversity. Curry *et al.* (2008) found that earthworm abundance and biomass under cattle grazing increased with fertilizer N input at three sites in Ireland (although diversity was not measured). Changing from grass-only to grass-clover swards may therefore reduce earthworm abundance if overall N-inputs are lower, although this needs further research. It is generally assumed that biodiversity and biomass of below-ground biota is more heavily influenced by nutrient turnover and C:N ratios than the pasture species *per se* (Andrews *et al.*, 2007).

The effects of sward clover content on other aspects of biodiversity are also generally not investigated, however one obvious benefit that is surprisingly absent from reviews and the literature in general is potential increases in pollinator abundance and diversity. White clover is insect pollinated and therefore, grass-clover swards have greater potential to provide habitat for pollinators than fertilized grass-only swards (grass is wind-pollinated). Pollinating insects are vital for the production of many fruit, vegetable, seed, biofuel, floral and other horticultural products. As an ecosystem service, pollination has an annual economic value estimated at €153 billion worldwide (Gallai *et al.*, 2009), €15 billion for the European Union (European Parliament and Council, 2012), €210 million for the UK (Carreck and Williams, 1998) and €54 million for Ireland (Bullock *et al.*, 2008).

The worldwide decline in abundance and biodiversity of pollinating insects in recent years is a well recognised phenomenon for concern (Kluser and Peduzzi, 2007; Gallai *et al.*, 2009; Potts *et al.*, 2010). Disease and pesticide use have contributed to this decline, particularly in the case of honeybees (*Apis* spp.). However, habitat loss is believed to be the major cause for the decline of many wild pollinators, particularly in the case of bumblebees (*Bombus* spp.) (Goulson *et al.*, 2006). Bumblebees are particularly important commercial pollinators because they are larger, carry more pollen, increase flower vibration and are active in cooler temperatures and for longer time periods (both seasonally and daily) than honeybees. Bumblebees also pollinate certain flowers (with longer corollas) that honeybees do not (Goulson *et al.*, 2006).

Power and Stout (2011) have shown that organic farms tend to have greater numbers and diversity of pollinating insects than conventional farms, which was mostly attributed to the higher abundance of red and white clovers on organic farms. Therefore, increasing the use of white clover in conventional grazed grassland clearly has the potential to dramatically increase the habitat area for important pollinator species in temperate regions such as the UK and Ireland. This could assist the EU 2020 strategy target to "halt biodiversity loss and degradation of ecosystem services by 2020" (European Parliament and Council, 2012).

Many solutions to environmental issues in agriculture have financial costs, generally associated with either lower production (lower stocking rates, lower fertilizer use, taking land out of production for set-aside) or specific amendments (hedgerow planting, manure storage facilities). In contrast, the use of white clover can reduce financial costs by replacing fertilizer N with BNF.

2.4. Interactions with Grasses

2.4.1. Introduction to the clover-grass interaction

White clover is usually grown with a grass species (most commonly perennial ryegrass). The main reason they are grown in association is their contrasting relationships with nitrogen, along with differences in seasonal growth patterns and nutritive value. As stated in previous Chapters, the *Rhizobia* in clover roots fix atmospheric N and clover is relatively inefficient at taking up mineral N from the soil in comparison to most grasses (Tow and Lazenby, 2001). Therefore, the plan for a grass-clover sward is that clover acts as a source of N, while grass acts as a sink and because N fixation is negatively correlated to the available soil N, efficient uptake of that soil N by the grass stimulates BNF in the clover (Crush, 1987). The other main agronomic advantage to perennial ryegrass is higher cool-season growth than clover, thereby providing herbage for grazing in the cooler months of the year. These differences result in the competition between grass and clover being a bit more complex than it would be between two more similar co-habiting species.

2.4.2. Nitrogen and clover persistence.

The relative competitiveness of grass and clover towards each other is heavily influenced by the amount of soil N available for plant growth. Increases in soil N generally reduce competitiveness of clover because grass is more efficient at N-uptake (see sections 2.5.1 and 2.5.5). Clover generally increases soil N over time through release of fixed-N from dead clover plant tissue and excretion of ingested soil N by grazing animals (Haystead, 1993). Therefore, clover's competitiveness in a grass-clover sward usually diminishes over time, particularly in permanent grazed grassland, which is the commonest form of land use in Ireland (CSO, 2012b).

Chapman (1996) compared the grass-clover interaction to a predator-prey relationship where the grass is the predator, feeding on the clover's ability to fix N. This is a useful analogy because the components in a grass-clover sward behave similarly to simple predator-prey populations. In population ecology, an increase in the prey population causes a subsequent increase in the predator population, which feeds back to reduce the prey population. This causes oscillations over time. Populations of clover and grass can oscillate in a similar manner over time, giving long term dominance to each species of 3 to 5 years (Chapman *et al.*, 1996). For example, Turkington and Harper (1979) found that relative clover abundance in a permanent pasture in Wales was determined by soil N levels and the aggressiveness of the competing grass species and these changed over time. A white clover and perennial ryegrass sward may initially have low soil N and relatively high clover content. However, as BNF increases N in the soil, the ryegrass becomes more dominant and clover content decreases. Over time, the soil N may decrease with uptake by grass and clover can return (Schwinning and Parsons, 1996a).

In grazed systems, fluctuations in the relative dominance of each species is impacted by the actions of the grazing animal, competition for light and other resources as well as the effect of soil N (Tow and Lazenby, 2001). Therefore the population oscillations described above are somewhat of a simplification as a grazed pasture of grass and white clover is not a uniform mixture of the two species, instead they can be very heterogeneous due to animals grazing habits and excretion patterns (Turkington and Jolliffe, 1996). This results in mosaic patterns of patches temporarily dominated by either clover or grass (Burdon, 1983). In this way the population oscillations described in the previous paragraph occur over much smaller spatial scales and can be more stable over a larger spatial scale (Schwinning and Parsons, 1996b). In agricultural systems, it is generally desirable to minimise such oscillations as falling clover content and decreasing soil N have obvious negative impacts on sward performance. Therefore, it is necessary to examine other aspects of the grass-clover competition that may be manipulated to reduce the outcompeting of clover by grass when soil N increases.

2.4.3. Competition for light

Plants in most communities compete with each other for light (Tow and Lazenby, 2001). As stated in Chapter 2.2.4, light also provides photomorphogenic signals that regulate plant organ development, usually with the purpose of improving a plants ability to reach light if shaded by a neighbour. Clover is generally considered to have poor tolerance of shade compared to grass, and the fact that light is required to stimulate stolon branching and elongation means that lateral growth of clover can be inhibited when shaded (Davies, 2001).

Leaf area is an important determinant of how much light is being intercepted by a sward. Clover and grass compete for light by attempting to have a higher proportion of leaf area intercepting light. The leaf area index can be defined as the area of leaf per unit of ground area (LAI). Older methods measure this directly from the plants with callipers, and equations have been developed (Gay, 1991): For grass the equation is $A = 0.905 (L \times B)$ where A is leaf area (cm^2), L is leaf length (cm), B is leaf breadth (cm) and k is a constant. Equations for clover are more complex; $A = b (M \times W) + c$ where A is leaf area (cm^2), M is midrib length and W is maximum leaf width. The values of b and c however, can vary depending on cultivar/leaf size. For example b and c are respectively 0.74 and 0.12 for Ladino (large-leaved) and 0.68 and 0.04 for Blanca (small leaved) (Gay, 1991).

The area of leaf per unit ground area can be misleading as the amount of light captured will depend on what angle the leaves are at (Hart, 1987). Therefore a more accurate description is to project the leaf area, at the angles that they are at *in situ* onto a flat horizontal plane. Clover has horizontal leaves whereas grass leaves are more vertical and because of this, it has been argued that clover has a lower critical LAI (LAI that captures 95% incident light) than grass and should have higher photosynthesis at the same LAI (Hart, 1987). However, projecting LAI onto a horizontal plane is also misleading unless the light source is directly overhead. Therefore, it is the LAI projected onto the zenith angle of the sun that should be used but this is not the case in the majority of the literature. Considering this, the angle of the sun would have implications in competitiveness between clover and grass, the grass becoming more competitive as the angle of the sun decreases.

2.4.4. Competition for soil resources

In general clover is considered to be a poorer competitor for soil water and soil nutrients than most grasses (Davies, 2001). This is due to a much lower root hair area (490 mm^2 per mg for clover as opposed to 1,230 for perennial ryegrass) (Evans, 1977). Critical plant tissue nutrient levels are the minimum concentrations needed in a plant to maintain 90% of maximum growth. In white clover critical plant tissue nutrient levels tend to be lower in monocultures than when grown with ryegrass due to less competition in monocultures, reflecting the increased competition from grass (Table 2.3).

Table 2.3. Critical plant tissue concentrations for white clover (Dunlop and Hart, 1987).

Element	*Critical level in association with ryegrass*[*]	*Critical level in monoculture*[*]
Nitrogen	4.5 - 5.5%	
Phosphorus	0.3 - 0.4%	0.1 - 0.25%
Sulphur	0.25 - 0.30%	0.1 - 0.29%
Potassium	1.8 - 2.3%	0.8 - 1.1%
Magnesium	0.12 - 0.14%	< 0.14%
Calcium	0.5 - 0.8%	0.4 - 1.0%
Iron	50 - 70 ppm	
Manganese	50 - 70 ppm	20 ppm
Boron	25 - 35 ppm	
Zinc	12 - 18 ppm	12 - 17 ppm
Copper	4 - 6 ppm	
Molybdenum	0.05 - 0.15 ppm	

[*] Critical levels are the minimum concentration in a plant necessary to produce > 90% of maximum growth. ppm = parts per million.

The problem of poor competitiveness for soil nutrients due to clover's relatively low surface area of root hairs may be overcome somewhat if arbuscular mycorrhizal fungi (AMF) are present in the soil. AMF form a symbiotic association with plant roots, where the fungal hyphea spread out through the soil and facilitate uptake of plant nutrients from the soil in exchange for carbon assimilates from the plant (Albrecht *et al.*, 1999). Hayman and Mosse (1979) reported a 100% production increase in British hill swards when using clover seed inoculated with AMF as opposed to non-inoculated seed. Crush (1974) and Powell (1979) both found that when grown in sterile P-limited soil, the addition of AMF improved growth of both perennial ryegrass and white clover but tended to preferentially influence the clover growth. Zhu *et al.* (2000) also found that clover is more mycotropic (i.e. more prone to develop root-mycorrhizal interactions) than grass. Although AMF can improve clovers ability to absorb nutrients from the surrounding soil, there is a lack of literature on what influences the presence/absence of the relevant fungi in actual grazing systems.

2.4.5. Grass species and cultivar

White clover is considered less competitive with some grass species than with others. For example, Simpson *et al.* (1987) found that clover distribution in hill swards in Wales was positively associated with perennial ryegrass (*Lolium perenne* L.) and negatively associated with bent grass (*Agrostis tenuis* L.). However this is probably more to do with management influences than competitive interactions. Thompson and Harper (1988) found that the reduced R:FR light ratio under Yorkshire fog (*Holcus lanatus* L.) canopies had a significantly more detrimental effect to white clover than perennial ryegrass or bent grass. This supports the finding by Turkington and Harper (1979) that white clover grew better with perennial ryegrass or bent grass than Yorkshire fog.

Perennial ryegrass is the most commonly grown associate of white clover and even perennial ryegrass cultivar type can affect clover's competitiveness. For example Gooding *et al.* (1996) found that tetraploid grass cultivars and earlier heading date grass cultivars were associated with higher clover contents than diploid cultivars and cultivars with later heading dates. Heading date had a greater effect than ploidy and the lower grass production in June was believed to complement white clover's period of increasing growth (Gooding *et al.*, 1996; Laidlaw, 2005).

Some of the difficulty in maintaining the grass-clover association has arisen because breeding objectives for both species have progressed independently. An example of this is when novel advances in one species outpace the other. For example, the success of endophyte-infected ryegrasses in Australasian dairy farms has increased competition pressures on white clover (Thom, 2008; Woodfield and Clark, 2009). Another problem lies in the early spring growth and high spring production in perennial ryegrass, which white clover breeding has not managed to keep pace with (Abberton and Marshall, 2005). It has been shown that white clover persistence influenced by assigning the correct clover cultivars to the farming system which they are suited to (e.g. small-leaved cultivars to continuous sheep grazing, large- and medium- leaved cultivars to rotational grazing with cows (Gilliland *et al.*, 2009) but suitable associate grass species/cultivars for each clover leaf size category should also be evaluated.

2.4.6. Seasonal changes in the interaction

It has been suggested that clover's ability to cohabit with ryegrass despite competition for resources is due to the differences in their yearly respective growth rates (Burdon, 1983; Hodgson, 1990). Perennial ryegrass shows a peak of growth between March and June and again between August and September while clover usually has one large peak between June and September (Figure 2.11). However, the poorer growth rates of clover at temperatures below 10 ºC place it at a severe competitive disadvantage in the winter and early spring (Hart, 1987; Brock *et al.*, 1989; Rattray, 2005). In general, white clover has been observed to start growth approximately 2 to 3 weeks later in spring than most ryegrasses (Frame and Newbould, 1986).

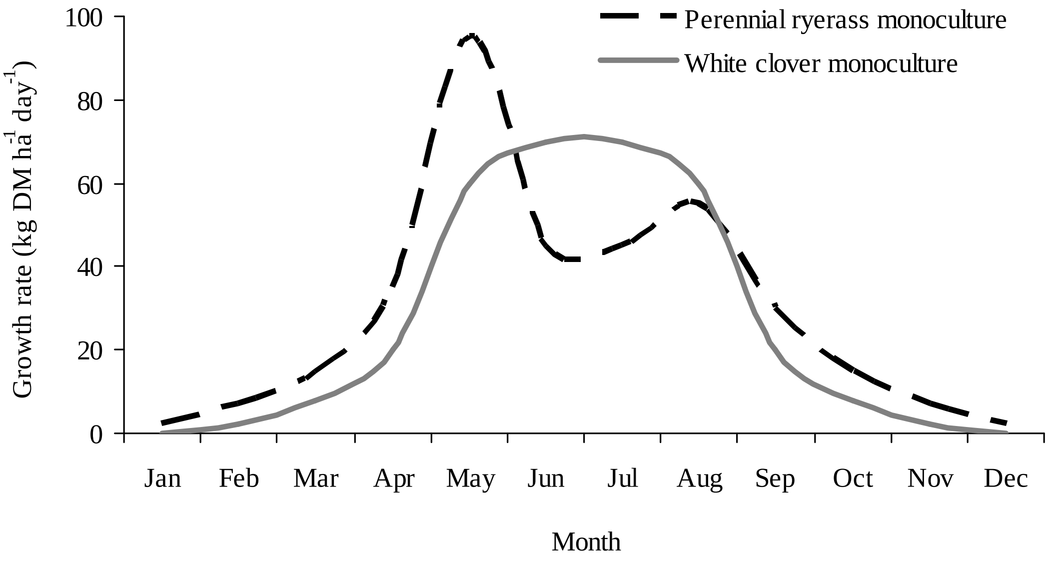

Figure 2.11. Seasonal growth rates of perennial ryegrass and white clover monocultures (Hodgson, 1990).

The reasons for clover's reduced competitiveness during the winter and early spring is linked to clover's reliance on BNF, which usually ceases below 9 ºC (Frame and Newbould, 1986; Hart, 1987). White clover growth rates in winter can be more limited by temperature, whereas grass growth rates are usually more limited by N supply (Castle *et al.*, 2002). However, the seasonal competitiveness of clover and grass is a bit more complex due to seasonal peaks in grass growth (Figure 2.11) and loss of clover leaf mass over winter. For example, clover has been observed to lose 60 to 95% of lamina weight over winter in the UK and tends to lose lamina in mild winters even where grass has been observed to be increasing lamina weight (Woledge *et al.*, 1990).

Clover leaves in winter also tend to be positioned lower in the sward than grass leaves, especially towards the end of winter/start of spring (Woledge *et al.*, 1989). The relative competitiveness of each species for light in the sward at any one time will be heavily influenced by their relative rates of leaf production. In summer the rate of petiole elongation in clover is similar to the rate of leaf and sheath production in vegetative perennial ryegrass and so, the clover is very competitive. However, during winter, clover petiole extension is considerably slower than the leaf and sheath extension of ryegrass; therefore the clover leaves are usually lower down in the sward and less competitive for light (Woledge *et al.*, 1989). The leaf angle of the two species could also influence their competitiveness as the vertical position of grass laminae would be more suited to the lower angle of the sun in winter (see Chapter 2.2.5.).

Clover stolon mass declines over winter and the proportion of buried stolon increases (Hay *et al.*, 1987). Clover growing point density decreased from 5,542 m^{-2} in September to 2,602 m^{-2} in May in an upland hill sward (Marriott, 1988). Stolon carbohydrate reserves are also at their lowest in spring (Hay *et al.*, 1989). There is also a higher proportion of small plants and these can be more susceptible to individual plant mortality (Brock *et al.*, 2000). Therefore clover is at its most severe competitive disadvantage to grass during winter and early spring and can be relatively competitive towards grass during summer and autumn (Figure 2.12).

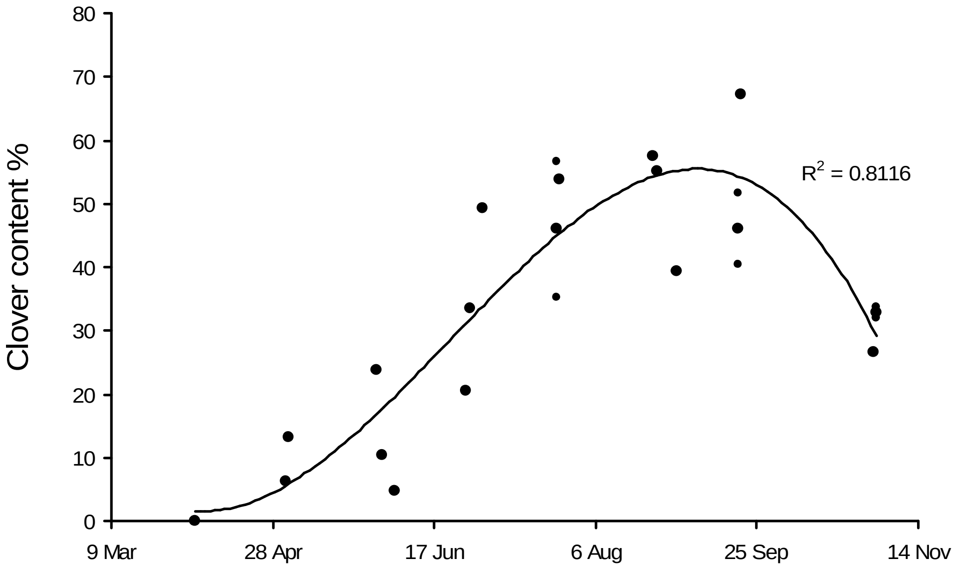

Figure 2.12. Seasonal distribution of clover content in grass clover mixtures at Athenry, Co. Galway, and Raphoe, Co. Donegal (Gilliland *et al.*, 2009).

Spring is a crucial time for clover and subsequent annual clover production is heavily influenced by the content of clover in the sward in spring (Collins *et al.*, 1991). However, the competitiveness of clover in spring is influenced by it's competitiveness over winter and this depends on grass growth rates, for example clovers disadvantage to grass during winter is more pronounced in mild, rather than severe, winters in the UK (Davies, 2001). Minimising clover losses over winter could therefore maximise clover competitiveness and production in spring. However, this would depend on reducing competition from grass and the challenge for research is to find ways to do this without a loss of grass productivity.

2.5. Management of Grass-Clover Swards

2.5.1. The use of fertilizer N

Fertilizer N usually decreases nitrogenase activity and BNF rates, as reflected in reduced pNdfa in clover tissue (Whitehead, 1995; Ledgard *et al.*, 2001; Schulze, 2004). However, the effect of fertilizer N on pNdfa is generally short lived (< six weeks), with a more long-term reduction in BNF typically being caused by lower sward clover contents (Høgh-Jensen and Schjørring, 1997; Jorgensen and Ledgard, 1997).

Applying fertilizer N to grass-clover swards generally puts clover at a disadvantage because clover is less competitive at N uptake than grass. In the case of a perennial ryegrass and white clover sward, applying N will increase the rate of leaf expansion and tiller formation in the grass, which increases it's ability to compete for light (Davies, 2001). More recently, simulation modelling of five years of data in Switzerland concluded that annual fertilizer N inputs greater than 100 kg ha^{-1} initiated grass dominance by first enabling rapid dominance of the root system and later through increased shoot growth (Lazzarotto *et al.*, 2009). This is in agreement with clovers generally lower root hair area in comparison to grass (see Chapter 2.4.3.).

Whatever the mechanism, long-term studies show consistent reductions in sward clover contents in response to fertilizer N use. For example in a five-year study described by Ledgard *et al*. (2001), increasing input of fertilizer N from 0 to either 200 or 400 kg N ha^{-1} on grass-clover pastures grazed by dairy cows resulted in the sward clover content being reduced from 152 g kg^{-1} to 107 g kg^{-1} and 49 g kg^{-1}, respectively, and BNF from 154 to 99 and 39 kg ha^{-1}, respectively. Another five-year study in Kiel, Germany (Trott *et al.*, 2004) showed that sward clover content decreased rapidly with increasing fertilizer N over a range of grazing/cutting management systems (Figure 2.13).

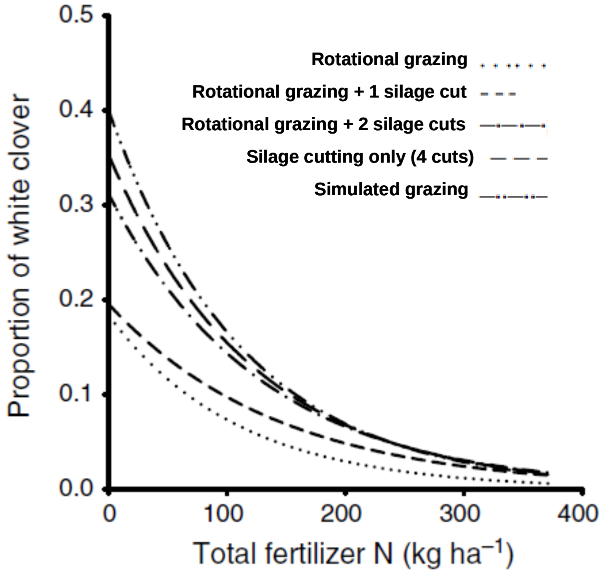

Figure 2.13. The effect of fertilizer N on the annual proportion of white clover in grassland under various grazing/cutting managements (Trott *et al.*, 2004).

A meta-analysis of the effects of fertilizer N in the available literature shows a similar response across a broad range of studies featuring different cutting, grazing and application regimes at various locations (Figure 2.14a). The effect of fertilizer N on BNF was determined with the same method (Figure 2.14b) and, below 250 kg fertilizer N, the response of BNF was generally a 0.34 kg reduction for every 1 kg input of fertilizer N. However, these are all short-term studies (< 5 years, most were 1 to 2 years) that do not include long-term reductions in clover content and BNF. These effects of fertilizer N can increase over time, as Ledgard *et al.* (1995) found that annual fertilizer N rates of 390 kg ha^{-1} reduced clover production by 8, 17 and 30% in years 1, 2 and 3, respectively.

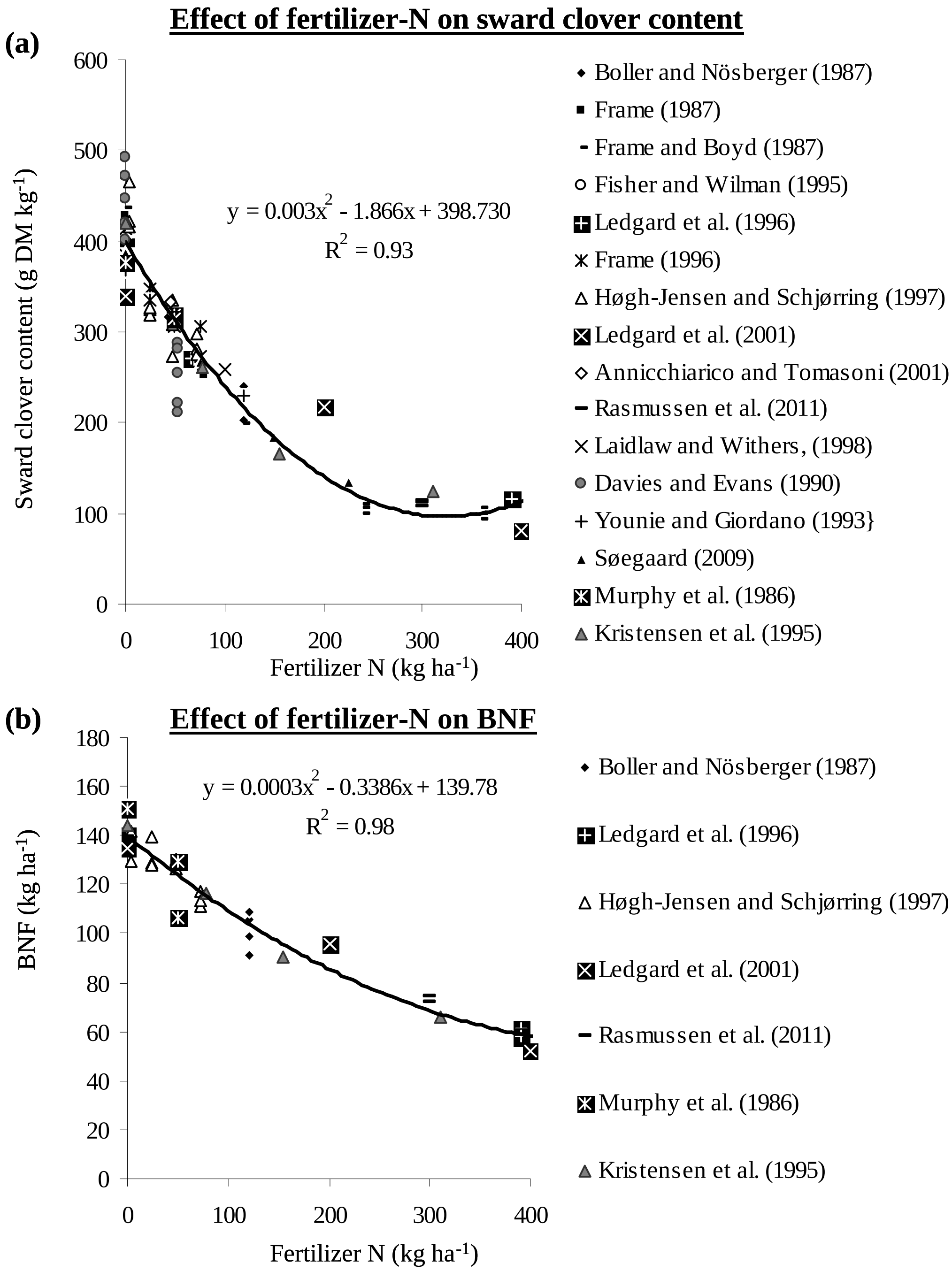

Figure 2.14. Meta-analysis of the effects of annual fertilizer N input on (a) annual sward clover content and (b) annual biological nitrogen fixation (BNF) in grass-clover swards. Relationships were significant ($P < 0.001$) in both cases. Regression performed in SAS using the methodology recommended by St. Pierre (2001).

Despite the negative effects of fertilizer N on sward clover content and BNF, it is still useful for increasing grass herbage growth rates at times of the year when clover contributes little to herbage production, particularly early spring (Clark and Harris, 1996). Furthermore, as Figure 2.14b shows, the response of BNF reduction to fertilizer N is generally not one-for-one. Therefore the input of fertilizer N does not appear to completely cancel out the BNF benefits of white clover in a sward (at least not within the time range of those experiments). Spring is the obvious time to strategically use fertilizer N on grass-clover swards in Ireland, given the low contribution of clover to herbage production, the high response rates of grass and the profitability of early spring grazing (Humphreys and Lawless, 2008).

Hogh-Jensen and Schjorring (1997) found that annual sward clover contents declined with spring application of urea at 72 kg N ha^{-1}, but that smaller amounts had no significant effect. Frame (1987) found that spring applications of 80 kg N ha^{-1} increased annual herbage production from 9.9 to 11.5 t ha^{-1} but reduced annual clover contents from 551 to 366 g kg^{-1}. Laissus (1983) found that N application of 90 kg ha^{-1} in March reduced clover content in September by 22 to 34% but concluded that N application is still worthwhile at this time due to the value of the increase in herbage production in spring. Laissus (1983) suggested that fertilizer N should be applied in early spring, (March to April in Normandy) as applying later in spring increases grass competition when clover is in the first stage of regrowth. Furthermore, Soegaard (2009) found that herbage DM production response to fertilizer N could be reduced by 4 kg DM kg^{-1} N for every 10% increase in sward clover content. Therefore, applying fertilizer N later in the year when clover contents are higher would be less efficient than applying it earlier in the year when clover contents are low.

Chapman *et al.* (1996) proposes a spatial rather than temporal solution to strategical fertilizer N use on grass-clover swards: A farm could use a mixture of grass-clover swards and grass-only swards. The grass-only swards would receive fertilizer N while the grass-clover swards would receive none and rely solely on BNF. This may have advantages in that fertilizer N could increase growth in the grass-only swards when needed, (e.g. early spring) without compromising the contribution from clover later in the year. However, the practicality of such systems has not been evaluated and there could be an increased risk of bloat when animals move from a grass-only field to a grass-clover field.

The form of N applied may influence clovers response. For example, Murphy *et al.* (1986) found that 50 kg N ha^{-1} applied as either urea or CAN in early spring reduced annual sward clover content and clover herbage production by similar amounts, but that annual BNF was lower with CAN than with urea (142 compared to 165 kg N ha^{-1}). Both slurry N and urinary N may inhibit clover less than mineral N (Frame and Newbould, 1986), although the reasons for this may be due to the differences in the rates of release of N and the proportion of applied N that becomes available for plant uptake.

Medium- and large-leaved varieties of white clover are generally able to compete with N-fertilized perennial ryegrass better than small-leaved varieties (Wilman and Asiegbu, 1982b; Wilman and Asiegbu, 1982a). Therefore, despite the lower persistence of these cultivars, they are considered to be better suited for grazing systems where fertilizer N is used. Davies and Evans (1990) also found that defoliation of a grass-clover sward could mitigate the negative effects of N-application on clover growing point density. Defoliation regimes that can mitigate the reduction response of sward clover content and BNF to spring applications of fertilizer N need to be further investigated in Irish grazing systems.

2.5.2. Grazing intensity/post grazing height

Lowering defoliation height in mowing/cutting experiments has generally been found to increase sward clover content and annual clover herbage production. For example, Clark *et al.* (1974) found lax cutting (10 cm above ground level) of an irrigated sward of mixed species (*L. perenne*, *Paspalum dilatatum*, *Bromus uniloides, D. glomerata* and *T. repens*) in south Australia produced 13 t DM ha^{-1} yr^{-1}, of which 27% was clover, while tight cutting (3 cm) produced 16 t DM ha^{-1} yr^{-1}, of which 42% was clover. Frame and Boyd (1987) compared cutting at 4 cm to cutting at 8 cm and found that the lower cutting height increased total annual herbage and clover production by 16% and 31% respectively.

Sereshine *et al*. (1994) found that BNF rates were not limited by cutting height and increased in line with the increased herbage production when defoliation (mowing) height was lowered from 10 cm to 4 cm in Switzerland. Schils and Sikkema (2001) found that lowering defoliation height from 7.5 to 4.5 cm increased sward clover content and clover herbage production under a range of cutting intervals (simulated grazing and simulated silage cutting).

A four year experiment by Acuña and Wilman (1993) examined the effects of cutting a grass-clover sward every four weeks (between April and October) at heights of either 2, 4, 6, 8, or 10 cm, along with P application (0 or 100 kg ha^{-1} yr^{-1}) and irrigation (0 or maximum soil water deficit 35 mm) treatments. Cutting height had a greater influence on clover content than fertilizer P application or irrigation. The closer cutting heights were associated with higher clover contents and production. The closer cut treatments initially caused an increase in grass tiller density but after the first summer this trend was reversed and the closer cut treatments were associated with lower ryegrass tiller density, which correlated with higher clover contents and higher productivity (Acuña and Wilman, 1993). In another paper on the same experiment, Wilman and Acuña (1993) found that there was a reduction in clover leaf size, petiole length and stolon diameter with the closer cut treatments but that stolon branching, length of stolon per m^2 and growing point density increased. The latter was proposed as the reason for the association between lower cutting heights and clover content.

Across a number of these previous experiments (Clark *et al.*, 1974; Briseno De La Hoz and Wilman, 1981; Frame and Boyd, 1987; Acuña and Wilman, 1993; Seresinhe *et al.*, 1994; Schils and Sikkema, 2001), each cm reduction in cutting height increased annual clover production by approximately 0.17 to 0.53 t DM ha^{-1} and annual total herbage production by approximately 0.26 to 0.51 t DM ha^{-1}, with generally linear responses to defoliation height. When analysed using the meta-analysis described by St. Pierre (2001), which treats the effect of study as a randomised block, the overall trend across studies can be tested (Figure 2.15). It can be seen that reducing defoliation height significantly increased annual sward clover content, clover herbage production and total herbage production but had no significant effect on annual grass herbage production.

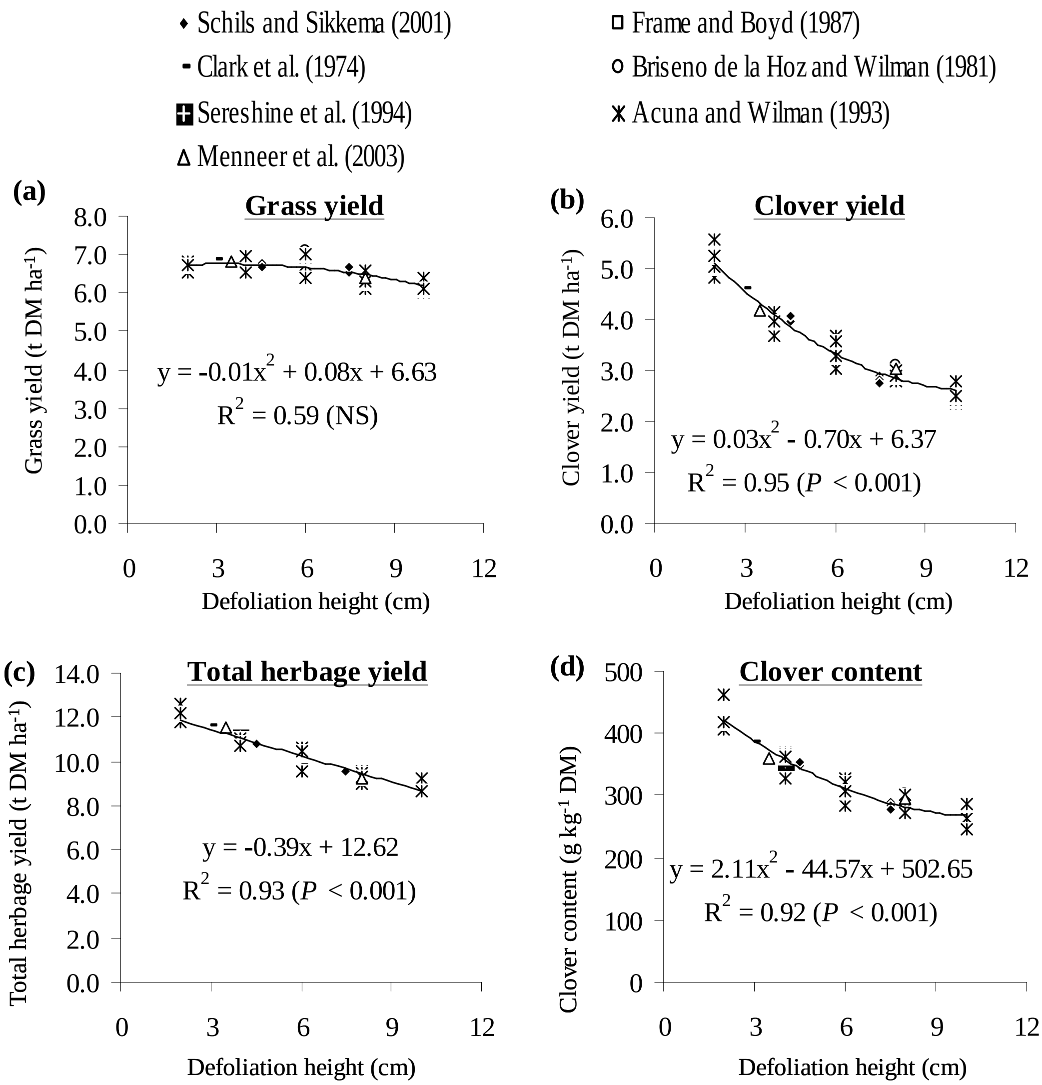

Figure 2.15. Meta-analysis of the effects of defoliation height of mowing/cutting on (a) annual grass herbage production, (b) annual clover herbage production, (c) annual total herbage production and (d) average annual sward clover content. Analysis was conducted with the mixed procedure in SAS, with results adjusted for between-study variation as described by St. Pierre (2001). Key is shown at top.

The results of Wilman and Acuña (1993) (increased stolon branching and growing point production) suggest that an increase in the R:FR light ratio was responsible for many of the morphological responses of clover to lower defoliation heights. Barthram *et al.* (1992) found that, in a continuously grazed grass-clover sward, receiving various levels of fertilizer N, the R:FR light ratio at ground level was negatively correlated with sward height (Figure 2.16) and positively correlated with stolon branching (but not elongation). Stolon branching is usually inhibited at R:FR ratios of 0.3 (Robin *et al.*, 1994), therefore it can be seen from Figure 2.16 that maintaining an average sward height greater than 7 cm could inhibit clover's ability to spread laterally through the sward. Of course, this is only applicable to continuously grazed sheep swards, which typically have very high grass tiller densities and it may not the case in rotationally grazed swards with their lower tiller densities (Hodgson, 1990).

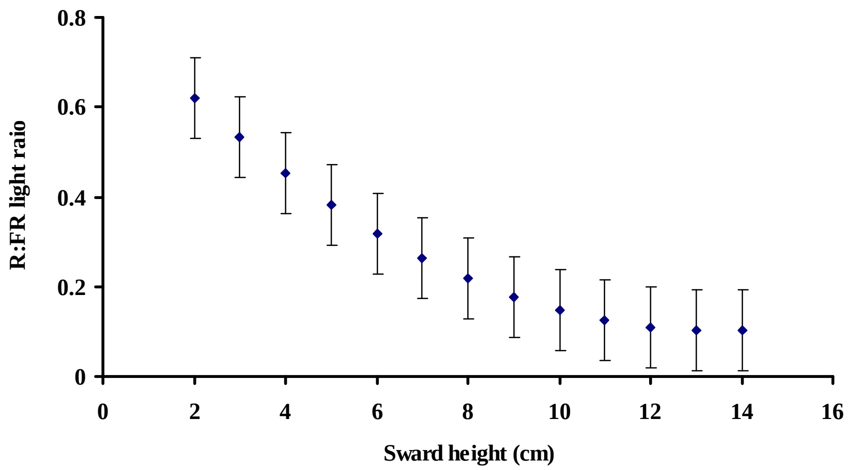

Figure 2.16. Relationship between sward height and the red:far-red (R:FR) light ratio in a grass-clover sward continuously grazed with sheep. Adapted from Barthram *et al.* (1992).

Another factor that can explain the differential response of clover and grass to cutting height is their difference in leaf morphology; clover leaves are on the end of long petioles, and both close and lax grazing will remove the majority of leaves, it is only the length of the defoliated petiole left behind that will be higher in the laxly grazed sward (Figure 2.17). Long petioles left after a lax grazing would not serve the clover plant much purpose as they cannot produce new leaves, the clover plant still has to produce new leaves from the stolon nodes at the base of the sward (Hart, 1987). However, the long, upright leaves of the grass can still photosynthesize after a lax grazing as the leaf area is only partially removed (Hodgson, 1990).

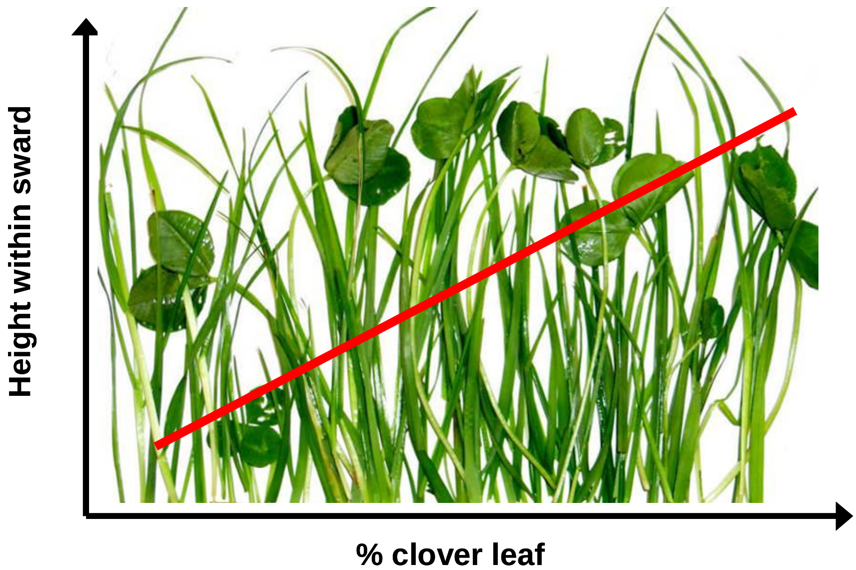

Figure 2.17. A schematic diagram of the relationship between height and % clover leaf in a grass-clover sward (Hodgson, 1990).

Recent work by Yu *et al.* (2008) has also shown that grass and clover have different size density compensation (SDC) responses; increased grazing intensity reduces the size and increases density of ryegrass tillers but has the opposite effect on clover, increasing individual stolon size and decreasing stolon density (measured as length per unit area).

Furthermore, the clover biomass can remain relatively constant across grazing intensities, compared to grass biomass which can decline with lower cutting heights (Yu *et al.*, 2008).

There is evidence of different responses to defoliation height in grass and clover. Lee *et al*. (2008a) found a quadratic response of herbage production to defoliation height in a mown grass-only sward in Tasmania. In that experiment, mowing at heights of 2, 4, 6, 8 and 10 cm resulted in annual herbage production of 12.2, 13.4, 13.7, 13.3, and 11.3 t DM ha^{-1}, respectively. It was concluded that the optimal defoliation height for a grass-only sward was 5.6 cm but that there was no biologically significant difference between 4 and 8 cm. The experiments in Figure 2.15 show that the same is not true for grass-clover swards and that lower defoliation heights (lowest was 2 cm (Acuña and Wilman, 1993)) generally increase herbage production.

The majority of the literature therefore concludes that low post-grazing heights should be recommended for grass-clover swards. However, the above experiments were primarily mowing or cutting experiments on small plots. Lowering actual post-grazing height (PGH) has not been investigated as thoroughly. Under continuous grazing, where animals had unrestricted access to swards, results have been much more variable. For example, continuous grazing experiments with sheep and cattle have found that lowering grazing height can increase (Laidlaw *et al.*, 1995), reduce (Gibb and Baker, 1989) or have no effect (Del Pozo *et al.*, 1996) on sward clover content. However, under continuous grazing, grazing height is linked to grazing frequency and the unrestricted access could increase animal’s ability to preferentially select clover leaves. The majority of Irish dairy farmers practice rotational or strip grazing, with cows being moved to new pastures or grazing strips every one or two days. The effects of post-grazing height under strip/rotational grazing by dairy cows on clover content and herbage production in a grass-clover sward is investigated in Chapter 7.

Ultimately, the effect of lowering PGH on animal performance needs to be considered. Daily herbage allowance (DHA) experiments involve measuring and rationing the amount of daily herbage available to each animal. This is generally done by adjusting the amount of pasture allocated to each experimental herd to achieve the required allowance for each animal. These DHA experiments on grass-only swards have generally found that reducing DHA results in lower PGH, herbage intake and milk yield per cow (Le Du *et al.*, 1979; Mayne *et al.*, 1987; Maher *et al.*, 2003; McEvoy *et al.*, 2009; Curran *et al.*, 2010; McEvoy *et al.*, 2010). A curvilinear positive response of both herbage intake rates and milk yield per cow to DHA have been reported across studies where the response declines as DHA increases (Greenhalgh *et al.*, 1966; Greenhalgh *et al.*, 1967; Greenhalgh and Reid, 1969; Combellas and Hodgson, 1979; Peyraud *et al.*, 1996).

One DHA experiment that was conducted on both grass-only and grass-clover swards (270 g kg^{-1} sward clover content in summer) was that of Ribeiro-Filho *et al.* (2005). That experiment found that PGH, herbage intake, milk yield and milk protein content all decreased when DHA was reduced from high to low (35 to 20 kg DM cow^{-1} to ground level, respectively) on both swards. However, for both DHAs, herbage intake, milk yield and PGH were also lower on the grass-clover sward than the grass-only sward, which was inconsistent with most other research. The authors attributed these differences to the much lower pre-grazing herbage mass/height on the grass-clover sward (the grass-only sward received fertilizer N and had higher growth rates). The difference in compressed pre-grazing sward height (15 cm on the grass-only and 10 cm on the grass-clover) could have accounted for the 2.1 kg difference in daily herbage DM intake between the two swards (Peyraud *et al.*, 1996). Furthermore, the DHA treatments were only imposed for four very short periods (10 days) in summer with cows grazing at common allowances before, and within the experimental period. The swards were also cut to a common (unspecified) height before, and in the middle of the experiment. As discussed below, such methodology makes it difficult to determine the effect of PGH, as opposed to DHA.

Many of the above experiments generally concluded that lower PGHs are associated with lower milk yield and/or milk fat and protein concentrations. However, this association needs to be treated with caution, as the link between DHA and PGH is not completely clear and can be influenced by the sward height above which DHA is determined, the pre-experimental sward management and animal behaviour. For example, Pérez-Prieto *et al*. (2012) have recently shown that the effect of herbage mass on herbage intake was positive, null and negative depending on if DHA was measured to 0, 2.5 or 5 cm (due to the differing proportions of available/unavailable herbage being included/excluded from the allowance measurement). Pre-experimental sward management is also important because the height of previous grazings influence the proportions of grass stem, pseudostem and leaf in the allocated herbage, which in turn influence sward nutritive value, palatability and intake (Holmes *et al.*, 1992). Repeated grazing/defoliation events establish a “grazing horizon” in a grass sward. Above this horizon, there are higher contents of more palatable grass leaf and below it, there are higher proportions of unpalatable grass stem (Holmes *et al.*, 1992). Assigning DHAs that allocate herbage above or below an already established horizon affects herbage intake (Pérez-Prieto *et al.*, 2012), especially if DHA treatments are only imposed for short time periods that do not re-establish a new grazing horizon. The dangers of assuming a relationship between PGH and herbage intake/milk yield from DHA experiments was shown by Lee *et al.* (2008b) who found that reducing PGH from 6 to 4 cm could actually increase milk yield per cow when DHA was (somehow) kept constant.

Therefore, the effect of PGH on sward clover content and herbage production has primarily been assumed from the results of mown plots whereas the effect of PGH on herbage intake and milk yield has been primarily assumed from DHA experiments on grass-only swards and/or for relatively short time periods. The effects of target PGH on milk production from dairy cows grazing grass-clover swards is investigated in Chapter 7.

2.5.3. Grazing frequency/pre-grazing height

There is some disparity in the literature on the effects of defoliation frequency on white clover content and persistence in grassland. In a review, Harris (1987) states that “in general more frequent defoliation of a grass-clover sward increases the content of white clover” and discusses this view with reference to experiments by Brougham (1959) who found that frequent grazing (every 2 to 5 weeks) increased white clover herbage production relative to infrequent grazing (every 3 to 8 weeks) in NZ. However, as Wilman and Asiegbu (1982b) point out, Brougham’s experiment involved a hybrid ryegrass which was better suited to longer defoliations and thereby may have been more competitive than perennial ryegrass under the longer intervals.

More recent studies show that successful clover management can actually require relatively long intervals between sward defoliations and that stolon length usually increases with interval between defoliation. In an experiment on a grass-clover sward Wilman and Asiegbu (1982b) found that increasing the interval between grazing throughout the growing season in the UK from “3 to 4” to “8 to 12” weeks increased total herbage and clover herbage production without negatively impacting the proportion of clover in the herbage. It was also found that medium and large-leaved clover varieties responded slightly better to the longer defoliation intervals. In another paper on the same experiment, Wilman and Asiegbu (1982a) reported that the longer (8 to 12 week) intervals increased stolon length per unit area, increased stolon diameter, petiole length and weight per leaf resulting in clover leaves being placed higher in the canopy than with the shorter cutting intervals.

Patterson *et al.* (1995) found that increasing interval between cutting from two to eight weeks in autumn increased stolon length per unit area and Harris *et al.* (1999) found that a rest interval of 50, 75 or 100 days in a dry summer in NZ increased clover herbage production, sward clover content, clover stolon survival and growing point density greater than the standard grazing interval of 25 days. Harris *et al.* (1999) attributed this to the higher soil moisture values over summer in the swards with rest intervals and higher numbers of clover seedlings. Elgersma and Schlepers (1997) found that cutting grass clover swards when herbage mass reached 2,000 kg DM ha^{-1} (7 to 9 cuts per year) rather than 1,200 kg DM ha^{-1} (5 to 7 cuts per year) increased herbage production, grass production and clover production with no effect on clover content. Fisher and Wilman (1995) also found that increasing the defoliation interval from 7 to 42 days between April and November increased total herbage production and did not affect clover content in the sward. Under rotational sheep grazing in the west of Ireland, Nolan and Grennan (1998) found that grazing every 7, 14, 21 or 28 days resulted in annual herbage production of 9.2, 10.2, 10.8 and 11.4 t DM ha^{-1} with average sward clover contents of 111, 160, 157 and 162 g kg^{-1} DM, respectively.

The effects of grazing/cutting frequency can be complex as increased defoliation frequency tends to increase some aspects beneficial to clover management such as growing point density but reduce others, such as stolon size. For example Simon *et al.* (2004) tested the effect of cutting interval (1, 2, 3, 6 and 8 weeks) in spring on pure stands of white clover. Increased cutting frequency increased residual leaf area and total growing point number per m^2 while reducing petiole length, stolon dry matter per m^2, stolon thickness, C and N reserves, and starch concentrations. Whether this could have a positive effect on clover content in a mixed sward has not been concluded but would depend on time of year, which is discussed in the next section (Chapter 2.5.4).

Plants in a continuously grazed sward are, on average, defoliated more frequently than plants in a rotationally grazed sward (Hodgson, 1990). Therefore it is worth considering some of the studies that compare the effects of rotational vs. continuous grazing on clover. For example, Hay *et al.* (1989) compared grass-clover swards grazed with ewes either rotationally or continuously in NZ and found that the rotationally grazed swards had higher mean annual clover content (25.6 compared to 6.2%), stolon DM mass (45.9 compared to 13.8 g m^{-2}) and growing point density (3,260 compared to 1,880 m^{-2}). Hay *et al*. (1989) argued that this was due to the lower grass tiller number and more 'clumped' grass distribution in the rotationally grazed swards but also due to greater clover plant size, rather than plant density. Swift *et al*. (1992) found that herbage production of a grass-clover sward was 50% lower when grazed continuously by sheep as opposed to five cuts per year. Davies (2001) reported that switching from continuous to rotational grazing caused an increase in clover content and clover stolon size (Figure 2.18) and Harris (1987) reported that allowing a continuously grazed sward a months rest in late summer/autumn could increase sward clover content 5 to 10 fold.

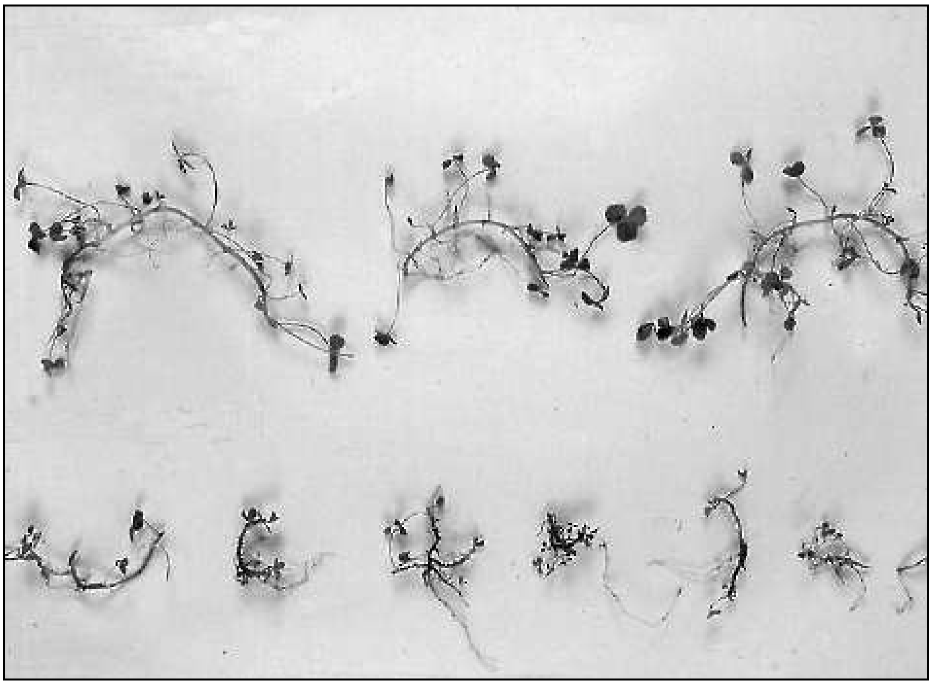

Figure 2.18. Effect of one year's rotational grazing on clover stolons previously subjected to three years of continuous grazing (top). Control (4 years of continuous grazing) is shown at the bottom (Davies, 2001).

Harris and Thomas (1973) analysed the effects of cutting frequency over two years using the de Wit (1960) model of competition. Infrequent cutting reduced clovers competitiveness in the first (establishment) year but increased it in the second year. This variability was attributed to the relative dominance of each species in each year: grass was dominant in the establishment year due to a high level of soil N and slower establishment of white clover. Therefore the more frequent cutting removed a higher proportion of grass than clover and increased clovers ability to compete. However, in the second year, clover became dominant (due to lower soil N status and full establishment) and frequent cutting removed a higher proportion of clover from the sward, increasing the grasses ability to compete. This research highlighted an important aspect of the effect of cutting/grazing frequency on the competitive interaction between clover and grass; it can depend on the competitive ability of each species. When clover is a relatively poor competitor (e.g. when temperature is low or fertilizer N is applied) frequent cutting can work in its favour as the preferential removal of the grass component will reduce shading of the clover, however, when clover is competing well (e.g. under low soil N status and in summer/autumn) frequent cutting may reduce clovers ability to compete (Davies and Evans, 1990).

The evidence from grazing/cutting intensity and frequency experiments described in this and the previous section suggest that grass-clover swards generally require intense but infrequent defoliation relative to grass-only swards. This was shown to some extent by Wolf and Schulte (2004) who found that smaller allocated paddock sizes for sheep, with their shorter associated grazing times, increased white clover content while having the opposite effect on a weed species (bent grass). The effects of simulated grazing intensity (defoliation height) and grazing frequency (defoliation interval) are investigated in factorial experiments in Chapters 4 and 5.

2.5.4. Timing of grazing

Considering the difference in seasonal growth rates of each species in a grass-clover sward, timing of appropriate grazing management is important. As clover is at a competitive disadvantage during winter and spring, it has been suggested that grass-clover swards should be grazed at this time to reduce competition from grass (Frame and Newbould, 1986; Davies, 2001). Collins *et al.* (1991) found that annual clover herbage production was positively correlated with stolon mass in spring, highlighting the importance of overwintering clover. A single grazing by sheep in November has been found to improve clover content relative to not grazing over winter (Laidlaw and Stewart, 1987). Following on from that experiment, Laidlaw *et al.* (1992) applied treatments of sheep grazing in either (a) November (b) November, January and March, (c) March and (d) no grazing. It was found that clover growing point density was negatively correlated with sward herbage mass in March, which was lowest in the swards grazed in November, January and March (treatment (b)). However, growth rates can be reduced by grazing too close to minimum temperatures, thereby not allowing cold-hardening to develop in the regrowing shoots (Jaindl *et al.*, 1991). Lüscher *et al.* (2001) found that more frequent defoliation (7 times between November and March) can have a stronger negative impact on stolon mass over winter than that caused by competition from grass in an ungrazed sward. It should be noted that the winter in the Laidlaw *et al.* (1992) study had milder winter temperatures than that of Lüscher *et al.* (2001), therefore grass competition would have been more of an issue for the clover in the former experiment. Harris *et al.* (1983) tested the effects of cutting frequency on clover monocultures in autumn. Infrequent (every 54 days) as opposed to frequent (every 32 days) caused the greatest loss of stolon mass over winter. However, the infrequently cut treatment had a greater amount of biomass before the winter, resulting in no difference the following spring.

The stress of defoliation on a clover plant needs to be considered; immediately after defoliation, BNF stops, and the plant mobilizes N reserves stored as soluble proteins in stolons and roots. This causes a mean reduction in reserves of about 30% within 6 days of regrowth (Goulas *et al.*, 2001). Among the soluble proteins mobilized, two prominent polypeptides of 15 and 17.3 kDa, which are vegetative storage proteins (VSP), exhibit a pattern of preferential utilization: a decline of up to 80% of their initial level during early regrowth of defoliated plants (Goulas *et al.*, 2001). Nitrogen fixation and re-accumulation of these reserves resume gradually with the appearance and photosynthesis of new leaves. It has also been found that N reserves in the form of storage proteins in white clover (like most plants) is seasonally regulated, accumulating in autumn and declining when spring growth commences. These VSPs are involved in cold-hardening, successful overwintering and spring growth in white clover as they can be readily mobilized when shoot N demand cannot be met with N uptake from soil or BNF (Bouchart *et al.*, 1998; Goulas *et al.*, 2001). Longer defoliation intervals can result in a greater allocation of resources in the form of N, P and K to stolons and growing point tips (Fisher and Wilman, 1995). Therefore the correct management of grass-clover swards in autumn and winter needs to balance the need for grazing to reduce grass competition and the need to allow the clover sufficient interval between grazing to accumulate stolon mass and reserves.

It has been stated that frequent grazing in spring can reduce competition for light and increase clover growing point densities (Woodfield and Clark, 2009) and lax grazing in spring should be avoided anyway due to the detrimental effects it can have on grass quality later in the year (McFeely *et al.*, 1975; Beever *et al.*, 2000). Older studies have found that in general, frequent and tight grazing in March, April and May increased clover content while light grazing reduced it (Burdon, 1983). However, later in the year higher DM production and clover contents can be obtained with longer cutting intervals with no negative effect on clover (Patterson *et al.*, 1995). Therefore, it can be argued from the work of Simon *et al.* (2004) discussed in the previous section that frequent grazing in spring should benefit white clover by increasing growing point density in spring which is linked to subsequent annual productivity (Davies, 2001). It can also be argued that from the work of Wilman and Asiegbu (1982a; 1982b), Patterson *et al.* (1995), Svenning *et al.* (1997) and Goulas *et al.* (2001) that longer defoliation intervals later in the year would lead to increased clover and total herbage production, while also allowing clover to accumulate WSC and VSP for cold-hardening, winter survival and spring regrowth. The beneficial effects on clover contents of a 'rest period' later in the year in continuously grazed swards support this view (Harris, 1987; Harris *et al.*, 1999; Davies, 2001).

However, changing the grazing intensity during the grazing season under continuous grazing can have negative effects. For example, Gibb and Baker (1989) changed the mean sward height throughout the grazing season in swards continuously grazed by beef steers and found that changing the sward height during the grazing season from 3 cm to 7 cm or *vice versa* had a detrimental impact on clover content, growing point density and stolon mass when compared to swards maintained at either 3 or 7 cm throughout the season.

Autumn management of rotationally grazed grassland in temperate regions such as Ireland usually involves increasing the intervals between grazing in order to build up the herbage mass available and thereby extend the grazing season into early winter (Hodgson, 1990). However, high herbage mass in winter can result in shading of clover leaves by the grass canopy (Laidlaw *et al.*, 1992). Low defoliation heights are associated with increased white clover contents in mixed swards (Acuña and Wilman, 1993) and this may enhance clovers ability to overwinter.

2.5.5. Effect of excretion

Excretion from grazing animals can increase soil N and this can have a negative effect on BNF and clover content as described for fertilizer N in Chapter 2.5.1. For example; at a stocking rate of 3.8 cows ha^{-1}, 40 to 50% of the area will be affected by urine at some time during the year (Richards and Wolton, 1976). Recently, Menneer *et al.* (2003) found that a single application of cow urine in late spring decreased annual BNF in that area by 37% and extrapolating that to the area affected in Richard and Wolton's (1976) study would give reductions of 15 to 18%. Vinther (1998) found that urine and dung applications increased grass growth and reduced the proportion of clover, while also temporarily reducing pNdfa in clover tissue from 0.85 to 0.30. The overall effect on BNF was a reduction by 45% and 20% for areas affected by urine and dung, respectively. It was estimated that, at a stocking density of 4 to 6 cows ha^{-1}, BNF would be reduced by 10 to 15% compared to the BNF in a grass-clover sward not exposed to animal excreta (Vinther, 1998).

Areas soiled by excretion are usually rejected by grazing animals (Hodgson, 1990) and this can result in longer regrowth periods and/or higher PGHs within those rejected areas. In a study of continuously grazed swards, Gibb (1991) found that the number of nodes per main stolon, number of branches on main axes and number of nodes on branches was higher in grazed than in rejected areas and both clover growing point and grass tiller density were up to three times higher in grazed than rejected areas. In continuously grazed swards, if grazing pressure is high rejected areas are relatively short lived, only being avoided when fouled by excretion. However, when grazing pressure is low, the rejected areas can last longer, being avoided not only because of fouling but also because of their maturity (Gibb, 1991). However, stolons in rejected areas can maintain viable nodes that become branches when those areas were eventually grazed (Teuber and Laidlaw, 1995).

In conclusion, excretion generally tends to reduce clover content and stolon growth due to the increased soil N, the rejection of herbage by grazing animals and the subsequent increase in competition from grass. This needs to be considered when making conclusions for grazing systems from the results of cut-plot experiments. Although excretion by grazing animals cannot be avoided, the proportion of rejected area may be mitigated to some extent by strip grazing and/or topping (Hodgson, 1990).

2.5.6. Animal treading

Treading by grazing animals can cause stress and reduced growth in plants through two main mechanisms:

1. Directly; by reducing the plants ability to function through fragmentation, crushing and/or burial of plant organs (Menneer *et al.*, 2005a).

2. Indirectly; through changes in soil physical parameters such as soil compaction and/or consolidation. This is usually measured by bulk density (mass of soil per unit volume) or macroporosity (volume of pores with a diameter greater than 30 µm per unit volume of soil) (Drewry *et al.*, 2008). Compaction/consolidation can cause increased resistance to root growth and impeded water/air infiltration (Drewry *et al.*, 2004; O'Connell, 2005).

Direct plant damage is usually more visual than indirect damage but this can be misleading. For example, Zegwaard *et al*. (2000) compared the effects of grazing a large number of cows (300 LU ha^{-1}) for 0, 3, 9 or 24 hours and found that the initial reduction in pasture growth was severe (51%) for the 24 hour treatment. The hydraulic conductivity recovered within 12 weeks and pasture growth fully recovered within 14 weeks, but it took 29 weeks for visually assessed pugging damage to recover to that of the control plots. This could be because an area with more bare ground (visually pugged) may have fewer plants, but those plants will have less competition and increase in size faster according to size density compensation (Yu *et al.*, 2008). The indirect effects such as soil compaction are often less visually prominent than direct plant damage but can have strong effects. For example, soil compaction by machinery can reduce annual herbage production of grass-clover swards by 18 to 74% by restricting the access of roots to water and nutrients (Phillips *et al.*, 2008). A review by Greenwood and McKenzie (2001) concluded that soil compaction under grazing conditions are generally not substantial enough to account for the observed reductions in herbage production, suggesting that direct plant damage is also partially responsible.

The way a soil responds to treading depends heavily on the soil water content (Figure 2.19). In wet soils, the soil tends to behave plastically, deforming and displacing around the hoof of the animal, leaving obvious hoof-prints (Greenwood and McKenzie, 2001). While on drier soils, compaction and macroporosity losses may occur in deeper layers without obvious visual damage. However, Herbin *et al.* (2011) found that soil compaction following a single treading event on a sandy loam soil in Ireland was influenced by soil moisture deficit, with bulk density increasing by 6% following treading under wet conditions, but only increasing by 0.5% under dry conditions.

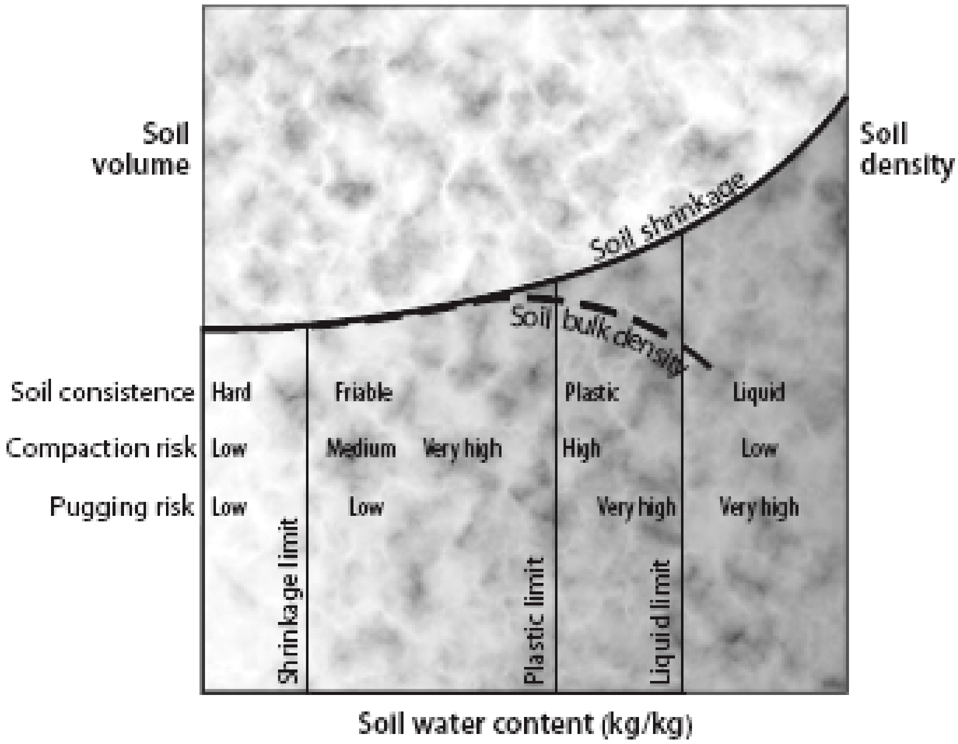

Figure 2.19. Schematic diagram of relationship of soil consistency and gravimetric water content (adapted from Marshall and Holmes (1988). The risk of compaction and pugging damage is also shown. Soil water range between plastic and liquid limits and damage risk varies with soil properties such as texture. The solid line shows soil shrinkage; the dashed line shows soil bulk density including maximum density (Drewry *et al.*, 2008).

In most practical applications, it is difficult if not impossible to separate the direct and indirect effects of treading on plant growth (Drewry *et al.*, 2008) and many studies add further complexity by discussing the effects of treading as pugging or poaching. Drewry *et al*. (2008) defined pugging as deep hoof-prints in soft, wet soil and poaching as the creation of slurry-like soil consistency in extremely wet soil. However, Davies and Armstrong (1986) defined poaching as 'a depression greater than 4 cm' and quantified it by the standard deviation of the soil surface along a transect. Greenwood and Mackenzie (2001) stated that shallow depressions and smears caused by remoulding of the soil can also be considered as poaching whereas Sheath (1998) further categorized wet soil damage into skid, ridge, loose clod and puddling.

From a grazing management perspective, the ultimate measure of treading damage to pasture is any reduction in subsequent herbage production/quality. The proportional reduction in herbage production attributed to treading varies widely across studies (0 to 88%) and is influenced by many factors such as size and number of animals, duration and frequency of occupation, plant species, soil strength properties and recovery period (Brown and Evans, 1973; Zegwaard *et al.*, 2000; Nie *et al.*, 2001; Drewry, 2003; Drewry *et al.*, 2008).

Many studies into the effects of treading damage to pastures are based on a single, severe treading event, usually on wet soils. In these cases, the immediate effect on pasture regrowth can be dramatic. For example, reducing subsequent regrowth rates in a grass-only sward by 40 to 42% and pasture utilisation by 34 to 40% (Nie *et al.*, 2001). Horne *et al.* (1990) found severe treading damage reduced regrowth in a grass pasture by 20 to 30% and utilisation by 20 to 40%. Most studies were also based on grass-only swards and it has been suggested that a grass-clover sward may recover better from treading damage due to the ability of clover to grow laterally and colonize the bare ground (Frame and Newbould, 1986). However, the majority of experiments have found clover to be more susceptible to treading damage than perennial ryegrass.

Brown (1968) found that a single severe treading event by sheep on a grass-clover sward reduced annual grass production by 8 to 25% but reduced annual clover production by 37 to 64%. Edmond (1964) compared ten common pasture species grown as monocultures for susceptibility to treading damage by sheep and found that, in terms of reductions of DM production caused by the sheep treading, perennial ryegrass was the most tolerant followed by white clover for most of the year but that both were equally tolerant in autumn. However, in that experiment, considerable amounts of unsown white clover grew in the grass monoculture plots and clover content was actually significantly higher in the moderately treaded grass plots than in the non-treaded grass plots. Vertes (1989) also found that white clover was less susceptible (lower reductions in stolon length) to treading with cows in autumn than it was in spring, suggesting that the more active stolon elongation in autumn enabled faster recovery.

The effects of severe treading by dairy cows in spring on a grass-clover sward in New Zealand was investigated by Meneer *et al.* (2005a). In that experiment, swards were actively treaded by 4.5 cows per 100 m^2 walked up and down grass-clover plots for 0, 1.5 and 2.5 hrs in spring and routinely mowed for the rest of the year. The severest (2.5 hrs) treatment initially reduced sward clover content by 70% for up to 100 days, reduced annual grass production by 37% and reduced annual clover production by 52%, the effects decreasing with time (Figure 2.20). Fragmentation and burial of stolons, rather than changes to soil physical properties, were concluded to be primarily responsible for the initial decline in clover content. However, clover content did recover and was actually significantly higher in the treaded areas one year later. Initially the treaded areas had smaller individual clover plants/ramets which would have had lower survival rates (Brock *et al.*, 1988; Brock *et al.*, 2000). However, those clover plants that did survive the spring and early summer benefited from less competition in the treaded areas so that by the following year individual clover plant size was greater in the treaded, rather than control areas (Menneer *et al.*, 2005a). In another paper on the same experiment, (Menneer *et al.*, 2005b) found that annual BNF was reduced by 53% in the severely treaded plots, in accordance with the reduction in annual clover herbage production.

Grant *et al.* (1991) investigated the effects of stolon burial and defoliation of clover plants growing in pots and found that simply burying stolons under 1 cm of soil had no effect on stolon extension, leaf appearance or the concentration of water-soluble carbohydrates (WSC) in the stolons. However, combining defoliation and stolon burial resulted in larger reductions in stolon extension, WSC concentration and leaf appearance rate than defoliation alone. This resulted in death of 42% of lateral stolons. In a grazing system, concurrent defoliation and burial of white clover is probable in wet conditions and could therefore have severe negative effects on white clover growth and/or survival.

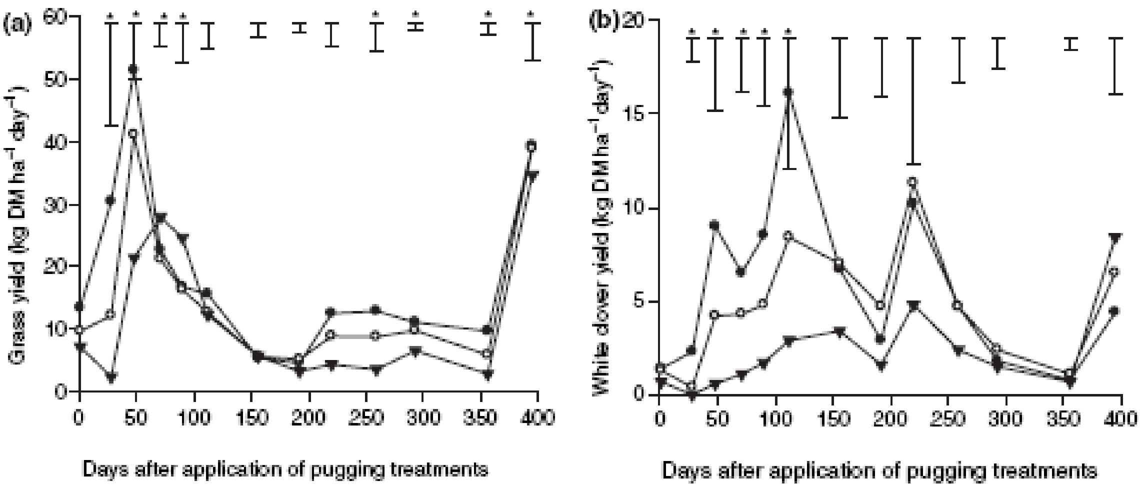

Figure 2.20. The effects of treading by 4.5 dairy cows per 100m2 in spring for (●) 0 hrs, (○) 1.5 hrs and (▼) 2.5 hrs on (a) grass production and (b) white clover production. Error bars represent s. e. of difference; * P < 0.05(Menneer *et al.*, 2005a).

One of the limitations of the above studies is that (with the exception of Edmond (1964)) they used a single, extremely severe treading event whereas in actual grazing systems, treading is a repeated event (and usually much less severe). Soil that has been poached once may be more susceptible to subsequent poaching because water can pool on the compacted end of the hoof-prints and is more easily absorbed by the remoulded areas around each hoof-print (Greenwood and McKenzie, 2001). Drainage can also be impeded by compaction in deeper layers of the soil (Horne, 1992; Greenwood and McKenzie, 2001).

Another limitation of previous plot-based experiments investigating the effects of treading on grass-clover swards is that they often manually remove herbage before imposing treading damage (e.g. Edmond (1964), Menneer *et al.* (2005a)). The importance of herbage mass in protecting swards from damage was shown by Brown (1968) where grass and clover production were reduced by 25 and 64% respectively, following treading by sheep after mowing (to a height 2.5 cm) but only reduced by 8 and 37% when the treading was done before mowing. Pande (2002) also found that the negative effects of light treading with dairy cows on a grass sward were 50% less when applied to tall (8 cm) rather than short (4 cm) grass swards. As cow hrs ha^{-1} in real rotational/strip grazing systems are generally dependant on the pre-grazing herbage mass, plot-based treading studies should adjust pre-grazing herbage mass in accordance with cow numbers/residence time.

Grazed permanent grassland in Europe tends to be concentrated in the regions where high levels of rainfall occur - the Atlantic zones and mountainous areas (Smit *et al.*, 2008). Ireland in particular has a high proportion of permanent grassland (90% of the utilisable agricultural area) and receives high levels of rainfall (750-1200 mm year^{-1}) (Central Statistics Office (C.S.O.), 2010). Coupled with geomorphic attributes this high level of rainfall has led to approximately 60% of the land area being classified as significantly wet with impeded drainage (Collins and Cummins, 1996). As a result approximately 50% of Irish farms are classified as being on soil with limited agricultural use due to land wetness (Gardiner and Radford, 1980; National Farm Survey (NFS), 2007, 2008, 2009). Previous research has shown that the grazing season length is limited by wet soil conditions on 60% of Irish dairy farms (Creighton *et al.*, 2011) and that profitability of dairying on a poorly drained heavy clay soil is considerably lower than on a free-draining, sandy-loam soil (Shalloo *et al.*, 2004).

Despite the prominence of wet soil and treading damage in Ireland, there have been few experiments that have evaluated the effect of treading damage under Irish grazing conditions. Mullen *et al.* (1974; 1978) investigated the effect of winter treading of a grass-only sward in Ireland by cattle stocked at 2.0 or 6.2 cattle ha^{-1} between December and April on subsequent herbage production (mown in June, August and October). It was found that annual herbage production was reduced by 2 to 15% but that herbage production at the first cut in June was reduced by 20 to 25%. In Chapter 6, the effect of treading under grazing by dairy cows in Ireland is investigated for a grass-clover sward on wet soil.

3. The Effects of Simulated Summer Grazing Management on White Clover Flowering and Herbage Production in a Grass-Clover Sward.

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Abstract

The development of growing points on white clover stolon into flowers rather than vegetative production has been associated with reduced herbage production. This experiment measured the effects of defoliation interval (21, 35 or 42 days) and defoliation height (3.6 cm, 4.5 cm or 6.0 cm) imposed during the summer on white clover flowering and herbage production until the following spring, in agrass-clover sward. Treatments were imposed between 11 May and 3 August. Thereafter, all treatments had a common defoliation regime imposed until the following May. Extending summer INT from 21 to 42 days resulted in a greater proportion of clover being produced as inflorescence. However, there were no relationships between flowering in summer (in terms of inflorescence per ha, proportion of clover harvested as inflorescence or g inflorescence per kg of stolon) and clover herbage production or stolon mass. However, INT and DH did affect herbage production. Increasing INT from 21 to 42 days resulted in higher grass production in summer (2.5 versus 2.1 t DM ha^{-1}; SEM = 0.08; P < 0.01) and higher annual (May to May) total herbage production (11.7 versus 10.9 t DM ha^{-1}; SEM = 0.23; P < 0.05). Lowering summer DH from 6.0 to 3.6

cm increased both grass and clover herbage production in summer, increased clover herbage production in autumn and resulted in higher total (May to May) clover herbage production (from 3.4 to 4.3 t DM ha^{-1}; SEM = 0.21; $P < 0.01$). Lowering DH also resulted in higher sward clover contents in summer and autumn, and higher stolon DM mass per ha at the end of the experiment.

3.1. Introduction

White clover (*Trifolium repens*) is the most important legume for grazing in temperate regions (Frame *et al.*, 1998; Peyraud *et al.*, 2009). It is most commonly grown in association with perennial ryegrass (*Lolium perenne*) where it can improve sward crude protein, organic matter digestibility, and herbage intake in ruminants (Dewhurst *et al.*, 2009; Kleen *et al.*, 2011). However, the main attribute of clover is that it facilitates biological N fixation (BNF) via associated *Rhizobia* bacteria and thereby reduces the fertilizer N requirements for agricultural production from grazed grassland (Gylfadóttir *et al.*, 2007).

Replacing fertilizer N use with BNF from clover has important environmental benefits such as increased biodiversity (Power and Stout, 2011), reduced fertilizer-N requirements and reduced green-house gas emissions (Ledgard *et al.*, 2009; Li *et al.*, 2011). These benefits can aid compliance with environmental regulations such as the EU Nitrates and Water Framework Directives (European Council, 1991; European Parliament and Council, 2000), and the EU 2020 strategy targets (European Commission, 2010b). Grass-clover swards can also improve profitability at farm level relative to grass-only swards (Doyle and Bevan, 1996; Falconer *et al.*, 2011;

Humphreys *et al.*, 2012). This has become increasingly so in recent years due to the substantial increase in fertilizer N price relative to farm product price since 1990 (World Bank, 1990-2012; Central Statistics Office (CSO), 2012b).One of the main obstacles to achieving the above benefits of clover in grazed grassland is difficulty in maintaining sward clover contents at effective levels (> 300 g kg^{-1} of herbage DM) from year to year (Frame and Newbould, 1986; Rochon *et al.*, 2004).

Maintaining persistency is a highly important target for farmers using white clover. Plants can persist in grassland through natural reseeding, vegetative persistence, or a mix of both. Chapman (1987) showed very low natural reseeding of white clover in grass-clover pastures grazed by sheep (approximately one new plant per 5.5 m^2 per year). In that study, over 90% of flowerheads were removed before they developed into seedheads, and only 4.4% of new seedlings survived to become mature plants with stolons. Therefore, natural reseeding is generally considered to make a relatively low contribution to clover persistence under grazing.

White clover flowering may be associated with lower stolon growth, herbage production and vegetative persistence (Gibson, 1957; Piano and Annicchiarico, 1995; Burggraaf *et al.*, 2006). This is believed to be due to the diversion of plant resources to growing flowers and developing seed (Kawanabe *et al.*, 1963), and to the development of lateral buds on a white clover stolon into flowers rather than new stolons (Thomas, 1987b).

Flowering characteristics therefore place a conflict of interest between seed and herbage production: high flowering and seed production is obviously a desirable trait for a seed producer but not for the livestock farmer. A solution would be a form of grazing management intervention that influenced flowering. Although flowering in white clover is primarily determined by genetic and environmental factors (such as day length), management factors can also influence flowering. For example, Thomas (1981) found that flowering in a seed-producing white clover monoculture could be encouraged by defoliation once in June as opposed to once in May. Under grazing conditions, grass-clover swards are defoliated much more frequently but there is still flexibility for management in terms of defoliation frequency (grazing rotation length) and defoliation intensity (post-grazing height). The effects of these factors on flowering and herbage production in white clover have not been investigated. The objectives of this experiment were to investigate the effect of defoliation interval and defoliation height during the main clover flowering period (May to August) on white clover flowering and herbage production into the following year, in a grass-clover pasture.

3.2. Materials and Methods

3.2.1. Experimental area

The experiment was conducted between May 2009 and May 2010 at Solohead Research Farm in Ireland (52˚51'N, 08˚21'W, 95 m above sea level). The soils were poorly drained Gleys (90%) and Grey Brown Podzolics (10%) overlaying Devonian sandstone with a depth ranging from 5 to 10 m. The soil had a clay-loam texture of 36% sand and 28% clay in the A1 horizon. Soil organic matter content was 13% and soil pH was 6.6 (O'Connell, 2005). The land was reseeded with perennial ryegrass (*Lolium perenne*) between 1985 and 1995 and over sown with clover (*Trifolium repens*) since 2000. Prior to the experiment the area was primarily used for grazing by dairy cows and to a lesser extent for silage production. It had received no fertilizer N since April 2007 and did not receive any during the experiment. Fertilizer P and K were applied at rates to replace that removed in herbage. Soil temperature (ºC at 10 cm depth) and rainfall amounts (mm) were measured every 30 minutes at an automatic meteorological station on the farm (Campbell Scientific Ltd, Loughborough, U.K.).

3.2.2. Experimental design

The experiment had a randomised complete block design with two experimental treatments: (i) summer defoliation interval (INT: 21, 28 or 42-day) and (ii) summer defoliation height (DH: 3.6, 4.5 or 6.0 cm above ground level). The plots (5 m × 1.5 m) were randomly distributed in each of five replicate blocks. The INT and DH treatments were imposed between 11 May and 3 August 2009 (summer). Thereafter, all treatments were defoliated on the same dates at the same DH (4.5 cm) between 4 August 2009 and 11 May 2010. A HRH-536 rotary blade lawn-mower (Honda®, Georgia, U.S.A.) was used to defoliate the plots at the prescribed cutting heights on the prescribed dates. The distribution of defoliation dates in summer is shown in Table 3.1. The common defoliation dates following that period were: 7 September 2009, 19 October 2009, 30 November 2009, 9 April 2010 and 10 May 2010.

Table 3.1. Summer defoliation dates for each treatment in 2009. Plots were harvested at defoliation heights of 3.6, 4.5 and 6.0 cm above ground on these dates.

Summer defoliation interval (INT)		
21-day	28-day	42-day
11 May†	11 May†	11 May†
1 June		
	8 June	
22 June		22 June
	6 July	
13 July		
3 August	3 August	3 August

† Initial harvest not included in herbage yield results.

3.2.3. Measurements

Herbage production

Herbage dry matter (DM) yield was measured from each plot on each defoliation date after the initial defoliation on the 11 May 2009: a strip (5 m × 0.55 m) was mown along the centre of each plot at the prescribed DH using the lawn-mower described above with fresh herbage harvested in a bag attachment. The remaining herbage on the borders was mown at the treatment DH and discarded. Harvested herbage was weighed and a 100g subsample taken for dry matter (DM) analysis as determined by drying in a forced-draught oven at 95°C for 16 hours.

Ash content and organic matter digestibility (OMD)

From the 7th September onwards, a second 100g subsample was taken of the above harvested herbage from each plots and analysed for *in vitro* organic matter digestibility (OMD) and ash content. These samples were dried at 40 °C for 48 hours and milled through a 2 mm screen. Samples were bulked for the sampling periods of (i) 7 September to 30 November 2009 and (ii) 15 February to 11 May 2010. Bulked samples for each plot were then analysed for ash content by placing in a 550ºC muffle furnace for 12 hours and OMD as described by Morgan *et al.* (1989).

Botanical composition

Botanical composition was measured throughout the experiment by randomly taking four snips (each 10 cm × 30 cm) from each plot with an electric hand shears (Accu-shears Gardena®, Ulm, Germany). This was done at the prescribed cutting height before each defoliation/harvest. The samples were sorted by hand into the following (i) grass, (iii) clover leaves (including petioles), (iv) clover inflorescences (including peduncles) and (iv) unsown broadleaf species. These samples were then dried for DM as described above. Unsown species were primarily *Taraxacum officinale* (dandelion), *Ranunculus repens* (creeping buttercup) and *Plantago lanceolata* (ribwort plantain) which in total, accounted for less than 0.05 of herbage production, were not affected ($P > 0.05$) by any of the treatment factors and were therefore omitted from the presented results. Sward clover content was defined as the proportion of herbage DM produced as clover (both leaves and inflorescence).

White clover flowering

The number of inflorescence per m^2 was counted from two quadrats (0.25m × 0.25m) randomly placed on each plot. This was conducted prior to each defoliation between 11 May and 7 September 2009. No flowering was observed outside these dates. Flowering intensity was defined as number of inflorescence produced (million ha^{-1}), weight per inflorescence (g DM), proportion of clover herbage produced as inflorescence (g kg^{-1} DM) and weight of inflorescence per unit stolon mass (g kg^{-1} DM).

Clover stolon mass

Clover stolon mass was sampled by cutting four sods (each measuring 10 cm × 10 cm to a depth of approximately 8 cm) were randomly from the border areas of each plot (avoiding the central strip used for measuring yield). The stolons and attached roots were manually separated from each sod, washed and dried for DM as described for herbage above. Stolon mass was measured on the 11 May 2009, 7 September 2009, 15 Mar 2010 and 10 May 2010.

3.2.4. Statistical analyses

All results were subjected to analyses of variance (ANOVA) using the proc mixed procedure in SAS (SAS, 2006). The following model was used: $X_{jkl} = \mu + I_j + H_l + T_k + IH_{jl} + IT_{jk} + HT_{lk} + IHT_{jkl} + e_{jkl}$ where X_{jkl} = dependent variable treatment mean, μ = overall mean, I_j = the fixed effect of the j^{th} INT, H_l = the fixed effect of the l^{th} DH, T_k = the fixed effect of the k^{th} season/sampling date and e_{jkl} = residual error term. A Tukey test was performed to identify differences between means. Sampling periods (for herbage production and herbage quality) or sampling dates (for sward clover content and flowering measurements) were entered as repeat measures. Sward clover content was averaged between 1 June and 13 July and allocated the 22 June as a sampling date in order to make comparison possible. The significance of any correlations within the results were analyzed with simple regression using proc glm in SAS (SAS, 2006).

3.3. Results

3.3.1. Meteorological data

Meteorological data are presented in Figure 3.1. The months of the experimental period generally had lower mean soil temperatures and higher annual rainfall than the same period during any of the previous ten years. Mean annual soil temperature was 9.2 ºC between May 2009 and April 2010, compared to the ten-year mean of 10.7 ºC (range: 9.6 to 12.0 ºC) for the same months. Total rainfall was 1,189 mm between May 2009 and April 2010, compared to the ten-year mean of 1,029 mm (range 793 to 1,205 mm) for the same months.

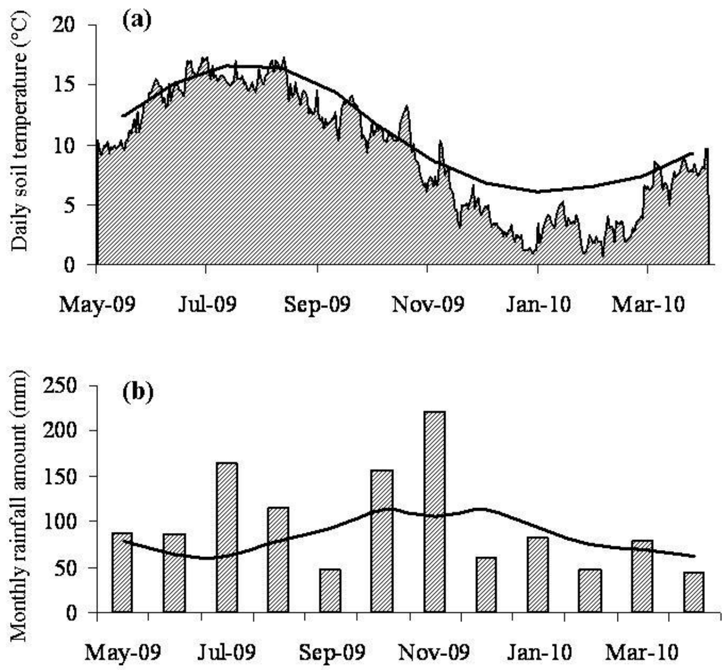


Figure 3.1. Daily soil temperature (a) and monthly rainfall (b) recorded at Solohead Research Farm during the experimental period. Grey columns show the levels during the experiment and black lines show the means of the previous ten years.

3.3.2. Flowering

The number of inflorescences per ha over time are shown in Figure 3.2. There was an interaction (P < 0.001) between INT and sampling date: flower numbers per ha were lower with the 42-day INT than the 21- or 28-day INT in June but this trend was reversed in August (Figure 3.2). There was no interaction between sampling date and DH. The average number of flowers per ha tended to be higher with the 3.6 cm DH (Figure 3.2).

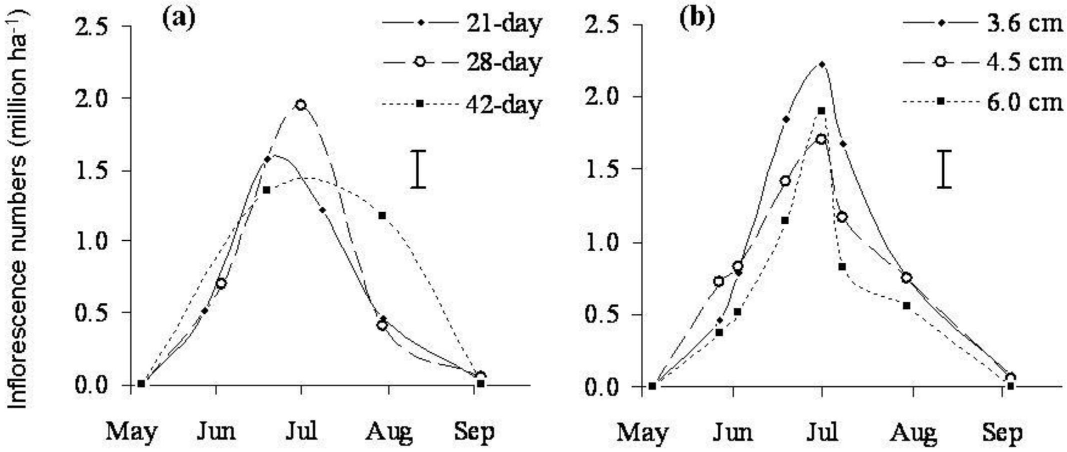


Figure 3.2. The number of inflorescences (million ha^{-1}), as affected by interactions between sampling date and (a) summer defoliation interval (INT; P < 0.001) and (b) summer defoliation height (DH; P > 0.05).

The total number of inflorescence produced per ha over the flowering period was lower with the 42-day INT than with the 28- or 21-day INT ($P < 0.001$; Table 3.2) and was lower with the 6 cm DH than with the 3.6 cm DH ($P < 0.001$). However, this trend was reversed for the weight per inflorescence head, with the combination of 6 cm DH and 42-day INT having heavier inflorescence heads than all other treatments ($P < 0.01$, Table 3.2) due to the more advanced development of seedheads in that treatment. The proportion of clover herbage DM produced as inflorescence and the amount of inflorescence per kg of stolon mass both tended to increase with increasing INT length, but were unaffected by DH (Table 3.2).

Table 3.2. Flowering parameters between 11 May and 3 August, as affected by summer defoliation interval (INT) and summer defoliation height (DH).

INT:	21-day			28-day			42-day		
DH:	3.6 cm	4.5 cm	6.0 cm	3.6 cm	4.5 cm	6.0 cm	3.6 cm	4.5 cm	6.0 cm
Total number of inflorescence produced (million ha^{-1})	4.54	3.98	2.89	4.19	2.94	2.73	3.03	2.65	1.90
Weight per inflorescence (g DM)	0.057	0.050	0.061	0.088	0.090	0.089	0.124	0.145	0.309
Proportion of clover herbage produced as inflorescence (g kg^{-1} DM)	131	140	128	162	178	169	178	200	291

SE of the means	INT	DH	INT × DH
Total number of inflorescence produced	0.262 **	0.262 **	0.454 NS
Weight per inflorescence	0.0159 ***	0.0159 *	0.0275 (P=0.05)
Proportion of clover herbage produced as inflorescence	12.6 ***	12.6 NS	21.8 *

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant.

3.3.4. Herbage production

Herbage production results are shown in Table 3.3. There were no interactions between INT and DH so only the main treatment effects are shown. In the summer period when experimental factors were imposed, the 42-day INT had higher grass ($P < 0.01$) and total herbage production ($P < 0.01$) than the 21-day but clover herbage production was not affected. In the same period, the 3.6 cm DH had higher grass ($P < 0.05$), clover ($P < 0.001$) and total herbage ($P < 0.001$) production than the 6.0 cm.

In the following autumn period, there was no effect of INT on any of the herbage production. Grass herbage production was lower following the 3.6 cm DH than the 6.0 cm DH ($P < 0.001$). However, the effect on clover herbage production was the opposite, with higher ($P < 0.05$) values following the 3.6 cm DH than the 6.0 cm. As a result, total herbage production in autumn was not significantly affected by DH. In the following spring, there was no carry-over effect of INT on grass, clover or total herbage production.

When the summed annual herbage production were compared, neither INT nor DH had a significant effect on annual grass herbage production (Table 3.3). Annual clover herbage production was not affected by INT but was significantly higher with the 3.6 cm DH than with the 6.0 cm ($P < 0.01$). Total annual herbage production was not significantly affected by DH but was higher ($P < 0.01$) with the 42-day than the 21-day INT.

Table 3.3. Grass, clover and total herbage production for three sampling periods: Summer (11 May to 3 August 2009), autumn (4 August to 30 November 2009) and spring (9 April to 10 May 2010) as affected by summer defoliation interval (INT) and summer defoliation height (DH).

	INT			DH			SEM	Significance		
	21-day	28-day	42-day	3.6 cm	4.5 cm	6.0 cm		INT	DH	DH×INT
Summer (May to August)										
Grass	2.08	2.16	2.45	2.51	2.12	2.06	0.083	**	*	NS
Clover	1.60	1.71	1.90	2.06	1.57	1.58	0.133	NS	***	NS
Total	3.67	3.87	4.35	4.57	3.68	3.63	0.139	***	***	NS
Autumn (August to November)										NS
Grass	3.07	3.25	3.29	2.88	3.25	3.48	0.105	NS	***	NS
Clover	1.50	1.32	1.33	1.51	1.43	1.20	0.072	NS	*	NS
Total	4.57	4.57	4.62	4.39	4.69	4.68	0.098	NS	NS	NS
Spring (April to May)										NS
Grass	1.96	2.03	2.09	1.95	2.11	2.03	0.063	NS	NS	NS
Clover	0.67	0.55	0.60	0.66	0.61	0.55	0.061	NS	NS	NS
Total	2.64	2.59	2.69	2.61	2.72	2.58	0.073	NS	NS	NS
Annual total (May to May)										
Grass	7.10	7.23	7.58	7.08	7.34	7.49	0.161	NS	NS	NS
Clover	3.75	3.79	3.83	4.33	3.66	3.37	0.212	NS	**	NS
Total	10.88	11.02	11.66	11.57	11.10	10.89	0.231	*	NS	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant.

Table 3.4. Pre-defoliation herbage mass (HM; kg DM ha^{-1}), herbage ash content (g kg^{-1} DM) and *in-vitro* organic matter digestibility (OMD; g kg^{-1} DM) over two sampling periods after treatments had been imposed: Autumn (4 August to 30 November 2009) and spring (9 April to 10 May 2010) as affected by summer defoliation interval (INT) and summer defoliation height (DH).

	INT			DH			SEM	Significance		
	21-day	28-day	42-day	3.6 cm	4.5 cm	6.0 cm		INT	DH	INT×DH
Autumn (August to November)										
HM	1523	1523	1541	1464	1563	1559	32.5	NS	0.06	NS
Ash	118	117	118	116	115	121	1.2	NS	**	NS
OMD	810	809	808	815	808	803	2.4	NS	**	NS
Spring (April to May)										
HM	1318	1294	1345	1304	1362	1291	36.4	NS	NS	NS
Ash	89	89	87	89	87	88	1.7	NS	NS	NS
OMD	843	845	848	848	843	846	3.5	NS	NS	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant.

3.3.5. Herbage mass (HM), ash and OMD

Pre-defoliation herbage mass, ash and OMD for autumn and spring are shown in Table 3.4. There was no effect of either INT or DH treatment on herbage mass, ash content, OMD content or CP content in autumn or the following spring. There were significant differences between the two periods with higher ash content, higher herbage mass and lower OMD in autumn than in the following spring ($P < 0.001$).

3.3.6. Sward clover content

Sward clover content during the study are shown in Table 3.5. There were no significant INT × DH or INT × sampling date interactions. However, there was a DH × sampling date interaction ($P < 0.001$), with the 3.6 cm having higher values than the 6.0 cm in June, September and October only. Mean annual sward clover content was higher ($P < 0.05$) with the 21-day INT than with the 28 or 42-day. Mean annual sward clover content was also higher with the 3.6 cm DH than with the 4.5 or 6.0 cm. At the end of the experiment in May 2010, there were no significant differences between any of the treatments.

3.3.7. White clover stolon mass

White clover stolon masses during the study are shown in Table 3.6. There were no two- or three-way interactions between INT, DH and sampling date. Mean stolon mass was higher ($P < 0.05$) with the 42-day INT than with the 21 or 28-day. Mean stolon mass was also higher ($P < 0.001$) with the 3.6 cm DH than with the 4.5 or 6.0 cm. At the end of the experiment in May 2010, there was no difference between INT treatments. However, stolon mass was still higher (P < 0.05) following the 3.6 cm DH than with the 4.5 or 6.0 cm.

Table 3.5. Sward clover content (g kg^{-1} DM) as affected by summer defoliation interval (INT) and summer defoliation height (DH) over time between June 2009 and May 2010.

	INT			DH				Significance		
	21-day	28-day	42-day	3.6 cm	4.5 cm	6.0 cm	SEM	INT	DH	INT×DH
Sampling date (D)										
22 Jun	371	379	411	433	354	373	30.1	NS	*	NS
3 Aug	511	513	466	477	511	501	19.8	NS	NS	NS
10 Sep	396	370	368	439	375	320	20.5	NS	**	NS
19 Oct	264	225	220	270	241	198	12.4	*	**	NS
30 Nov	134	82	72	105	107	77	13.3	*	NS	NS
9 Apr	122	141	122	150	114	121	15.3	NS	NS	NS
10 May	264	216	230	264	226	220	21.1	NS	NS	NS
Mean	294	275	270	305	275	259	11.8	NS	*	NS

* P < 0.05, ** P < 0.01, *** P < 0.001, NS = non-significant.

Table 3.6. White clover stolon mass (kg DM ha^{-1}) as affected by summer defoliation interval (INT) and summer defoliation height (DH) over time between June 2009 and May 2010.

	INT			DH				Significance		
	21-day	28-day	42-day	3.6 cm	4.5 cm	6.0 cm	SEM	INT	DH	INT×DH
Sampling date (D)										
7 Sep	967	977	1224	1154	1048	966	81.1	*	NS	NS
30 Nov	724	785	929	884	802	751	92.8	NS	NS	NS
15 Mar	334	347	358	401	303	334	38.9	NS	NS	NS
10 May	1003	974	1000	1167	902	909	81.9	NS	*	NS
Mean	757	771	878	902	764	740	55.5	NS	NS	NS

* P < 0.05, ** P < 0.01, *** P < 0.001, NS = non-significant.

Table 3.7. Correlation parameters for leaf herbage production and stolon mass as affected by flowering intensity in white clover. Sampling periods are shown for leaf herbage production: summer = 11 May to 3 August 2009, autumn = 4 August to 30 November 2009 and spring = 9 April to 10 May 2010. Sampling dates are shown for clover stolon mass.

	Number of inflorescences (million ha^{-1})				Proportion of inflorescence in clover herbage (g kg^{-1} DM)				Inflorescence production per unit stolon mass (g DM kg^{-1})			
	Intercept	Multiplier	R^2		Intercept	Multiplier	R^2		Intercept	Multiplier	R^2	
Clover leaf herbage production (kg DM ha^{-1})												
Summer	856.7	168.4	0.23	***	1797.1	-2.1	0.08	0.06	1433.3	0.00	0.00	NS
Autumn	1025.0	108.0	0.17	**	754.5	-0.8	0.05	NS	1480.8	-0.34	0.02	NS
Spring	499.0	31.2	0.17	NS	1588.3	-1.2	0.05	NS	656.7	-0.16	0.02	NS
Clover stolon mass (kg DM ha^{-1})												
7 September	944.1	28.5	0.01	NS	1249.4	-1.1	0.05	NS	1214.7	-0.53	0.05	NS
30 November	778.6	1.7	0.00	NS	1003.6	-1.1	0.04	NS	990.2	-0.59	0.05	NS
15 March	289.6	14.0	0.00	NS	375.3	-0.2	0.04	NS	380.2	-0.11	0.05	NS
10 May	756.3	67.8	0.08	NS	1065.0	-0.4	0.01	NS	942.3	0.17	0.00	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant.

3.3.8. Effect of flowering on clover leaf production and stolon mass

There were no significant negative relationships found between any of the flowering measurements and either clover leaf herbage production or stolon mass throughout the experiment (Table 3.7). The number of inflorescence per ha was significantly positively correlated with clover leaf herbage production per ha in summer (Table 3.7).

3.4. Discussion

3.4.1. Clover flowering

The number of inflorescences per unit area is commonly used in flowering and seed production studies because it has important implications for seed production (Thomas, 1996). The current experiment is in general agreement with previous work where defoliation increased the number of inflorescence per ha (Zaleski, 1961; Thomas, 1981; Connolly, 1990; Marshall *et al.*, 1993). However, a problem with using this parameter as a measure of flowering intensity is that it may simply be a result of higher sward clover content per ha or higher clover herbage production. This was observed in the current experiment in the positive correlation between inflorescence per ha and clover leaf herbage production (Table 3.7). Furthermore, the number of inflorescence per ha doesn't take into account the weight of individual inflorescence, which can change rapidly as seedheads develop (Kawanabe *et al.*, 1963).

The current experiment found that frequent defoliation at low cutting heights resulted in higher numbers of inflorescence per ha (Figure 3.2 and Table 3.2) but that the individual inflorescences were lighter. This is in agreement with the findings of Connolly (1990) where spring defoliation of a clover monoculture increased inflorescence per ha but reduced all other aspects of seed yield (florets per head, seeds per floret and seed weight). Clover seed weight increases rapidly as ripening commences, and can triple within ten days (Harris, 1987), which could explain the dramatic increase in inflorescence DM weight in June with the 42-day INT and 6.0 cm DH. This resulted in that treatment having the greatest proportion of DM allocated to inflorescence and seedhead production as opposed to leaf or stolon production.

3.4.2. Clover flowering and herbage production

Previous experiments have suggested that profuse flowering characteristics are associated with lower herbage production in white clover. Kawanabe *et al.* (1963) showed evidence of competition between inflorescence and stolons for resources within individual clover plants. Piano and Anniccharico (1995) extended this work to an evaluation of 16 landraces of Ladino white clover in Italy and reported that the persistence (yield after five years relative to yield after three years) was negatively correlated with the seed production characteristics (seed yield and seed weight). Burggraaf *et al.* (2006) compared a white clover cultivar with higher rates of inflorescence production with a control cultivar Huia and found that the high-flowering cultivar had poor agronomic performance (low DM production and weed ingress), which was attributed to the negative effect of flower production. The range of differences between treatments for flowering (both as numbers per ha and as a proportion of clover herbage DM) in the current experiment are similar to those reported by Burggraaf *et al.* (2006). Despite this, the current experiment found no association between flowering and clover herbage production.

The main difference between the current experiment and the previous experiments discussed above is that the previous experiments were all done with white clover monocultures whereas the current was a mixed grass-clover sward. Many reviews have concluded that competition from grass is the primary factor affecting sward clover content, herbage production and persistence in grass-clover swards (Frame and Newbould, 1986; Brock and Hay, 1996; Davies, 2001; Rattray, 2005). Therefore, while flowering may have had a relatively important influence on clover herbage production in monocultures, it appears that in the current experiment, the variation in simulated grazing management (INT and DH) had a greater effect on clover herbage production through other factors (as discussed below) than through flowering.

3.4.3. Herbage production, clover content and stolon mass

Increasing the INT from 21 to 42 days in summer increased grass production as might be expected for this time of year, with less interruption to the growth cycle and greater production of grass seedheads (Holliday and Wilman, 1965; Binnie *et al.*, 1997).

Clover herbage production was not significantly affected by INT. The experiment in Chapter 4 shows increased clover herbage production between July and December when INT was extended from 21 to 42 days (Table 4.2). In the current experiment, a similar trend was observed but the differences were smaller (0.30 instead of 0.42 t DM ha^{-1}) and the variation higher (0.133 SEM instead of 0.099). The fact that the treatments were in place for a shorter time period in the current experiment, coupled with the time of year, may explain the differences between the two experiments.

Increasing summer INT from 21 to 42 days resulted in 30% reductions in sward clover content in the following October-November, but did not significantly affect sward clover content in the following spring. Again, this contrasts with the results in Chapter 4, where the 21-day INT between July and December resulted in lower stolon mass and lower sward clover contents at the end of the experiment. The greater benefit of longer defoliation intervals in the autumn and early winter period is most likely due to the plant's requirement to accumulate storage reserves at that time of year (Hay *et al.*, 1989; Svenning *et al.*, 1997; Goulas *et al.*, 2001; Lüscher *et al.*, 2001). The results of the current experiment show that 21-day grazing intervals in summer are appropriate management for maintaining clover content in grass-clover swards.

Lowering DH had a positive effect on clover herbage production, clover content and stolon mass. This is in accordance with previous experiments (Briseno De La Hoz and Wilman, 1981; Frame and Boyd, 1987; Acuña and Wilman, 1993; Seresinhe *et al.*, 1994; Schils and Sikkema, 2001; Menneer *et al.*, 2003). This is generally attributed to increased light penetration of the sward and the difference in leaf morphology of grass and clover plants; the umbrella-like leaf structure of clover exposes it to higher leaf loss at a high DH than the blade-like leaf structure of grass (Woledge *et al.*, 1992; Thompson, 1993; Héraut-Bron *et al.*, 2001; Christophe *et al.*, 2006).

3.4.4. Wider implications

The current experiment shows the benefits of low defoliation heights in summer on subsequent clover herbage production, sward clover content and stolon mass. The 21-day defoliation interval resulted in slightly higher sward clover contents in October and November which may be beneficial to clover's year to year competitiveness, although there were no significant differences between treatments in the following May. These results suggest that tight grazing and approximately 21-day grazing intervals are appropriate management for grass-clover swards. A grazing interval of approximately 21 days is already recommended for perennial ryegrass swards during spring and summer in Ireland (Dillon *et al.*, 1999).

It is generally assumed that vegetative clonal growth is the main mechanism through which clover can persist in grass-clover swards and that natural reseeding is relatively unimportant. However, each inflorescence can supply between 0.024 and 0.112 g of clover seed under Irish conditions (Connolly, 1990). In the current experiment, the only treatment that was undefoliated long enough to enable some seedhead development was the 42-day INT, which held 1.35 million inflorescences per ha on the 22 June (Figure 3.2). Therefore, this treatment had the potential to supply 33 to 158 kg of clover seed per ha, depending on pollination, weather conditions, stage of development and the removal of seed at grazing/harvesting. However, given that current recommendations are to over-sow only 5 kg clover seed per ha to establish/maintain white clover in grassland (Humphreys and Lawless, 2008), only 3 to 15% of that potential seed yield would need to be realised.

The ability of such seeds to set and develop into mature plants needs to be assessed in Ireland. Chapman (1987) found that only 4.4% of naturally-set clover seedlings survived to maturity on a New Zealand hill farm continuously grazed by sheep. However, this has not been assessed under rotational cattle grazing or silage cutting. Current recommendations are to oversow after a silage cut, when the sward is more open (Humphreys and Lawless, 2008). As clover seedheads are more likely to develop in a silage/hay interval, natural reseeding in grass-clover pastures may be more important than previously assumed. This possibility is supported by the findings of Harris *et al.* (1999) in New Zealand where summer grazing deferrals of 25, 50, 75 and 100 days resulted in 0.33, 0.53, 1.47 and 2.27 million seedlings per ha, respectively. However, the agronomic potential of these naturally-sown plants would also need to be evaluated in comparison to commercially available cultivar seeds.

The alternative benefits of clover flowering also need to be considered. Unlike wind-pollinated grasses, clover flowers provide foraging sites for pollinating insects such as honeybees (*Apis* spp.) and bumblebees (*Bombus* spp.). Pollination is a vitally important ecosystem service for the production of fruit, vegetable, seed, biofuel, floral and other agricultural and horticultural products. Pollinating insects have annual economic values estimated at €153 billion worldwide (Gallai *et al.*, 2009), €15 billion for the European Union (European Parliament and Council, 2012), €210 million for the UK (Carreck and Williams, 1998) and €54 million for Ireland (Bullock *et al.*, 2008).

Abundance and diversity of pollinating insects have declined dramatically over the last three decades and this decline negatively impacts wild plant diversity, wider ecosystem stability, crop production, food security and human welfare (Aizen *et al.*, 2008; Potts *et* *al.*, 2010). Habitat loss is a major cause for the decline of many pollinators, particularly in the case of bumblebees (*Bombus* spp.) (Goulson *et al.*, 2006; Potts *et al.*, 2010) which are particularly important pollinators in temperate regions such as Ireland.

Grass-clover swards have the ability to provide habitat for important pollinating insects and thereby contribute to the EU 2020 strategy target to “halt biodiversity loss and degradation of ecosystem services by 2020” (European Parliament and Council, 2012) The current experiment found no link between flowering and subsequent herbage production in white clover but did find that flower density increased with clover content and that the 42-day defoliation interval resulted in a more even spread of flowering over the summer period, which may be beneficial to certain pollinator species.

3.5. Conclusions

Simulated summer grazing INT and DH affected clover flowering but this change in flowering did not have a measured effect on subsequent clover herbage production. Increasing INT from 21 to 42 days resulted in a higher proportion of clover being produced as inflorescence, due to more advanced seedhead development. However, there were no relationships found between any summer flowering parameters (inflorescence per ha, proportion of clover harvested as inflorescence or g inflorescence per kg of stolon) and any clover herbage production or stolon mass measurements. Increasing INT from 21 to 42 days resulted in higher grass production in summer and higher annual (May to May) total herbage production but did not significantly affect clover herbage production. Lowering summer DH from 6.0 to 3.6 cm increased grass production in summer and increased clover herbage production in summer and autumn.

Lowering DH also resulted in higher sward clover contents in summer and autumn, and higher stolon DM mass per ha at the end of the experiment. Low summer defoliation heights are recommended to maintain clover content in grass-clover swards.

4. The Effects of Simulated Summer-to-Winter Grazing Management on Herbage Production in a Grass-Clover Sward.

Phelan, P. Casey, I.A. and Humphreys, J. (2013) The effects of simulated summer-to-winter grazing management on herbage production and white clover persistence in a grass-clover sward. *Accepted to Grass and Forage Science, 21 January 2013 (in press).*

Abstract

The effects of summer-to-winter simulated grazing management factors, namely defoliation interval (INT: 21, 42, 56 or 84-days), defoliation height (DH: 2.7, 3.6, 5.3 or 6.0 cm) and final defoliation date (FIN: 23 September, 4 November or 16 December) on herbage production in a grass-clover sward were studied. Treatments were only imposed between July and December 2008, with all plots under common management in the following March to June 2009.

The 42-day INT achieved the highest (P < 0.001) total herbage production at 11.00 t DM ha^{-1}.Both shorter (21-day) and longer (56 to 84-day) intervals reduced annual clover herbage production and BNF estimates. Lowering DH from 6.0 to 2.7 cm in the summer-to-winter period increased sward clover content and clover herbage production through to the following June, six months after treatments ended. Delaying FIN from 23 September to 16 December had no significant effect on annual clover, grass or total herbage production. Spring-summer clover herbage production was positively correlated with spring-summer clover stolon mass (R^2 = 0.54, P < 0.001) and, to a lesser

extent, light penetration of the sward in the previous winter (R^2 = 0.16, P < 0.05). A 42-day INT with low DH (2.7 to 3.5 cm) is therefore recommended for grass-clover swards.

4.1. Introduction

White clover (*Trifolium repens*) is the most important legume for grazing in temperate regions (Frame *et al.*, 1998; Abberton and Marshall, 2005; Peyraud *et al.*, 2009). It is most commonly grown in association with perennial ryegrass (*Lolium perenne*) where it can improve sward crude protein, organic matter digestibility and herbage intake in ruminants (Bax and Schils, 1993; Søegaard, 1993; Wilman and Riley, 1993; Dewhurst *et al.*, 2009; Kleen *et al.*, 2011). However, the main attribute of clover is that it facilitates biological nitrogen fixation (BNF) via associated *Rhizobia* bacteria and thereby reduces the fertilizer nitrogen (N) requirements of agriculturally productive grassland (Rochon *et al.*, 2004; Gylfadóttir *et al.*, 2007; Humphreys *et al.*, 2009b; Del Prado *et al.*, 2011).

Replacing fertilizer N use with BNF from clover can improve profitability at farm level (Doyle and Bevan, 1996; Andrews *et al.*, 2007; Falconer *et al.*, 2011; Humphreys *et al.*, 2012). This has become increasingly so in recent years due to the substantial increase in fertilizer N price relative to farm product price since 1990 (World Bank, 1990-2012; Central Statistics Office (CSO), 2012b). Grass-clover swards also have important environmental benefits such as increased biodiversity (Power and Stout, 2011) and reduced green-house gas emissions (Ledgard *et al.*, 2009; Li *et al.*, 2011) relative to N-fertilized grass-only swards. This can aid compliance with environmental regulations such as the EU Nitrates and Water Framework Directives (European Council, 1991; European Parliament and Council, 2000), and the EU 2020 strategy targets (European Commission, 2010b).

One of the main obstacles to achieving the benefits of white clover in grazed grassland is difficulty in maintaining it in the sward at agronomically desirable levels (> 300 g kg^{-1} of herbage dry matter (DM) from year to year (Frame and Newbould, 1986; Thomas, 1992; Frame and Laidlaw, 1998; Rochon *et al.*, 2004). Autumn, winter and early spring are critical times for clover as it has lower growth rates and is generally less competitive for light than perennial ryegrass over that period (Frame and Newbould, 1986; Hart, 1987; Brock *et al.*, 1989; Woledge *et al.*, 1989; Woledge *et al.*, 1990; Davies, 2001). Lowering sward defoliation height has been shown to have a positive effect on clover content and herbage production in grass-clover swards during spring and summer (Frame and Boyd, 1987; Acuña and Wilman, 1993; Wilman and Acuña, 1993). However, the effect of defoliation height in the summer-to-winter period on subsequent clover content and herbage production in the following spring has not been investigated.

Rotational grazing in temperate climates often involves longer grazing intervals in autumn, as reserves of herbage are accumulated in order to extend the grazing season into early winter when growth rates fall below demand (Dillon *et al.*, 1999; Hennessy and Kennedy, 2009). Another important aspect of grazing management during autumn-winter is the final grazing (closing) date. This is the primary determinant of pre-grazing herbage mass in early spring (Roche *et al.*, 1996; Hennessy *et al.*, 2006; Ryan *et al.*, 2010). However, carrying high sward herbage masses for long periods over winter has been found to increase shading of clover leaves and stolon growing points which can result in lower sward clover contents in early spring (Laidlaw and Stewart, 1987; Laidlaw *et al.*, 1992).

The objective of this experiment was to investigate the effect of simulated key grazing management parameters in the summer-to-winter period (namely grazing interval, post-grazing height, and final grazing date) on sward light penetration in winter, sward clover content, clover stolon mass, BNF estimates, herbage production and herbage nutritive value in a grass-clover sward through to the following spring-summer.

4.2. Materials and Methods

4.2.1. Experimental area

The experiment was conducted between 1 July 2008 and 30 June 2009 on mown plots (each measuring 2 m × 8 m) at Solohead Research Farm in Ireland (52˚51'N, 08˚21'W, 95 m above sea level). The soils of the site are comprised of 90% poorly drained Gleys and 10% Grey Brown Podzolics overlaying Devonian sandstone at a depth ranging from 5 to 10 m. Drainage is impeded, and this contributes to waterlogged conditions under high rainfall. There is a perched water table, the surface of which ranges from 0 to 2.2 m below ground level. The soil has a clay-loam texture of 36% sand and 28% clay in the A1 horizon. Soil organic matter content was 13% and soil pH was 6.6 (O'Connell, 2005). The land has been under permanent grassland for over 50 years and was reseeded with perennial ryegrass (*Lolium perenne*) between 1985 and 1995. The particular site where this experiment was conducted was over-sown with clover (*Trifolium repens*) cultivars Crusader and Chieftain in May 2004 and again in May 2007. Prior to the experiment the area was primarily used for grazing by dairy cows and to a lesser extent for silage production. It had received no fertilizer N since April 2007 and did not receive any during the experiment. Fertilizer P and K were applied at rates to replace that removed in herbage. Soil temperature (ºC at 10 cm depth), rainfall amounts (mm) were measured at a meteorological station on the farm (Campbell Scientific Ltd, Loughborough, U.K.).

4.2.2. Experimental design

There were two experiments, with all treatments from both experiments distributed together to mown plots (each measuring 8m × 2m) in a randomised complete block design with five replications. Experiment (i) had the experimental factor of summer-to-winter defoliation interval (INT) and experiment (ii) had the experimental factor of final defoliation date (FIN). Both experiments also had the experimental factor of defoliation height (DH). The INT factor had four treatments: 21, 42, 56 or 84-day defoliation intervals. The FIN factor had three treatments: 23 September (Early), 4 November (Mid) or 16 December (Late). The DH factor had four treatments: 2.7, 3.6, 5.3 or 6.0 cm (as measured with a Filips rising plate meter (www.grasstec.ie)). Therefore, possible factorial interactions were: INT × DH and FIN × DH but not INT × FIN.

All treatments were applied by defoliation a HRH-536 rotary blade lawn-mower (Honda®, Georgia, U.S.A.). All experimental factors were only imposed between 2 July and 16 December 2008 (summer-to-winter). The defoliation dates in this period are shown in Table 4.1. Between the following 3 March and 30 June 2009 (spring-summer), all plots received common management of defoliating approximately every 30 days at a common cutting height of 4.5 cm in order to measure the carry-over effect from the previous summer-to-winter treatments. All plots were initially defoliated to their prescribed DHs on the 1 July and the herbage removed and discarded.

Twenty additional non-clover (NC) plots, where clover was eliminated with herbicide (Duplosan®: active ingredient = mecoprop-P (48% w/w), application rate = 2 litre ha^{-1}) in June 2008, were also included in the randomised block design to enable BNF estimates. These plots received the same management as the 42-day INT/Late FIN treatments with all four DHs described above (Table 4.1).

Table 4.1. Summer-to-winter defoliation dates for each treatment. Plots were harvested at defoliation heights (DH) of 2.7, 3.6, 5.3 and 6.0 cm above ground between 2 July and 16 December 2008 within a factorial arrangement between DH and defoliation interval (INT) and between DH and final defoliation date (FIN), NC = non-clover plots. In the following spring-summer (3 March to 30 June) all plots were harvested every 30 days at a cutting height of 4.5 cm.

Defoliation interval (INT)				Final defoliation date (FIN)			Non-clover (NC)
21 day	42 day†	56 day	84 day	Early	Mid	Late†	
1 Jul‡	1 Jul‡	1 Jul‡	1 Jul‡	1 Jul‡	1 Jul‡	1 Jul‡	1 Jul‡
22 Jul							
12 Aug	12 Aug			12 Aug	12 Aug	12 Aug	12 Aug
2 Sep		26 Aug					
23 Sep	23 Sep		23 Sep	23 Sep	23 Sep	23 Sep	23 Sep
14 Oct		21 Oct					
4 Nov	4 Nov				4 Nov	4 Nov	4 Nov
25 Nov							
16 Dec	16 Dec	16 Dec	16 Dec			16 Dec	16 Dec

† Same plots, ‡ Initial defoliation, herbage yield not included in results.

4.2.3. Measurements

Herbage production and nutritive quality

Herbage dry matter (DM) yield was measured from each plot on each defoliation date after the 1 July 2008 by mowing a strip (8 m × 0.55 m) along the centre of each plot at the prescribed DH using the lawn-mower described above. Fresh herbage was harvested in a bag attachment. The remaining herbage on the borders was mown and discarded.

Freshly harvested herbage was weighed and two subsamples of 100 g each were used for determination of dry matter (DM) and herbage nutritive value. The DM was determined by drying in a forced-draught oven at 95°C for 16 hours. The other sub-sample was dried at 40°C for 48 hours and milled through a 2 mm screen before analyses for ash content (550ºC muffle furnace for 12 hours), crude protein (CP; N content × 6.25; LECO 528 auto-analyser, LECO Corporation, ST. Joseph, MI USA), and *in vitro* organic matter digestibility (OMD) as described by Morgan *et al.* (1989).

4.2.4. Botanical composition

At the start of the experiment (27 June 2008) the botanical composition of the experimental area was measured by taking 30 snips (each 10 cm × 30 cm) randomly from the entire area with an electric hand shears (Accu-shears Gardena®, Ulm, Germany). The samples were bulked and sorted by hand into grass, clover and unsown broadleaf species before DM determination as described above. Throughout the experiment, the botanical composition was measured by taking six snips randomly from each plot at the prescribed cutting height before harvesting on each of the following dates: 23 September 2008, 16 December 2008, 3 March 2008, 28 April 2008 and 29 June 2008. Unsown species were primarily *Taraxacum officinale* (dandelion), *Ranunculus repens* (creeping buttercup), *Bellis perennis* (daisy) and *Plantago lanceolata* (ribwort plantain) which in total, accounted for less than 0.05 of herbage production and were not affected ($P > 0.05$) by any of the treatment factors and are therefore omitted from the presented results.

4.2.5. Clover stolon mass

Clover stolon mass was sampled immediately prior to the experiment by cutting 30 sods (each measuring 10 cm × 10 cm to a depth of approximately 8 cm) randomly from the entire area on 27 June 2008. The stolons and attached roots were manually separated from each sod, washed and dried for DM as described for herbage above. Throughout the experiment, six sods were randomly taken from each plot (avoiding the central strip used for measuring production) on the 23 September 2008, 16 December 2008, 3 March 2009, 28 April 2009 and 29 June 2009.

4.2.6. Biological nitrogen fixation (BNF)

Biological nitrogen fixation was calculated using the difference method (Hardarson and Danso, 1993; Haystead, 1993): $BNF = N_{GC} - N_{NC,}$ where N_{GC} was the total N yield from each grass-clover plot and N_{NC} was the mean total N yield from the non-clover plots.

4.2.7. Compressed sward height and light penetration

The proportion of light penetrating the sward to ground level (LP) was measured using an Accupar® LP-80 ceptometer (Decagon Devices, Pullman, Washington, USA). The device consisted of photosensors that measured photosynthetically active radiation (PAR; µmol m^{-2} s^{-1} in the 400-700 nm wavebands). Eighty photosensors were located 1 cm apart along a linear probe that was inserted into base of the sward canopy to measure below-canopy PAR. One external photosensor was connected to the device to give simultaneous measurement of above canopy PAR. These measurements were made immediately before and after each defoliation. Light penetration of the sward during the undefoliated period in winter (17 December to 3 March) was measured on 7 January and 18 February. Compressed sward height was measured using a Filips rising plate meter (www.grasstec.ie) with ten drops in every plot whenever LP was measured.

4.2.8. Net herbage accumulation (NHA)

Daily net herbage accumulation (NHA) rates were calculated by dividing the change in herbage mass (above defoliation height) since the last measurement by the number of intervening days without defoliation. Herbage mass for each plot was calculated from compressed sward height. Herbage mass estimates from compressed sward height were calibrated throughout the experiment by linear regression with the herbage yields from the lawnmower cuts.

4.2.9. Statistical analyses

All results were subjected to analyses of variance (ANOVA) for each sampling period using the mixed procedure in SAS (SAS, 2006). The first factorial experiment (INT × DH) was analyzed with the following model: $X_{jkl} = \mu + I_j + H_l + IH_{jl} + e_{jkl}$ where X_{jkl}

= dependent variable, I_j = the j[th] INT, H_l = the l[th] DH and e_{jkl} = residual error term. The second factorial experiment (FIN × DH) was analyzed with the above model but with F_j (the j[th] FIN) replacing I_j. No interactions between any of the main factors were found and the effect of DH on all results was similar in both models. Therefore, the two datasets were combined and a third ANOVA conducted using the above model with FIN and INT categorised as one factor to determine the effect of DH across both. Sampling dates (for clover content and clover stolon mass) were included as repeated measures within the above models using the compound symmetry (cs) covariance structure in SAS. The relationships between spring-summer sward clover content, clover herbage production, clover stolon mass and sward LP over winter were analyzed with simple linear regression using the GLM procedure in SAS (SAS, 2006).

4.3. Results

4.3.1. Meteorological data

The mean daily soil temperature and total monthly rainfall amounts are shown in Figure 4.1. Soil temperatures tended to be lower, and rainfall amounts higher, than the ten-year averages. The overall mean soil temperature was 9.5 ºC in the experimental period, and the total rainfall amount was 1205 mm. In comparison the ten-year means for the same months (1 July to 30 June) were 10.8 ºC and 1010 mm.

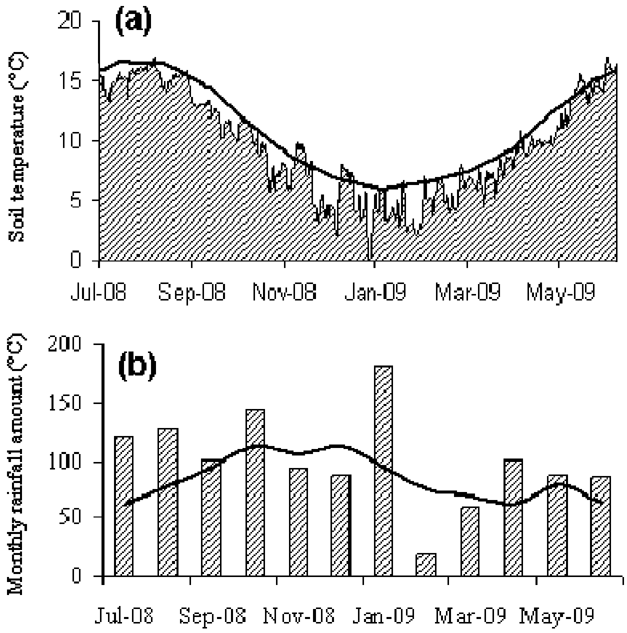


Figure 4.1. Daily soil temperature (a) and monthly rainfall (b) recorded at Solohead Research Farm during the experimental period. Shaded area/columns show the levels during the experiment and the black lines show the means of the previous ten years.

4.3.2. Herbage production and biological nitrogen fixation

Grass, clover and total herbage production are shown in Table 4.2. Clover herbage production in the summer-to-winter period was highest with the 42-day INT and lowest with the 84-day INT ($P < 0.001$). Grass herbage production in this period was not significantly affected by INT. However, in the following spring-summer, grass herbage production was highest following the 84-day INT and lowest following the 21-day INT ($P < 0.001$) whereas clover herbage production was not significantly affected (Table 4.2). As a result, total (grass + clover) herbage production in the summer-to-winter period was highest with 42-day and lowest with 84-day INT ($P < 0.001$). However, in the following spring-summer, herbage production increased in response to longer INT length ($P < 0.001$, Table 4.2). When the total annual herbage production between 2 July 2008 and 30 June 2009 is considered, the 42-day INT produced the highest amount of total herbage ($P < 0.001$).

Table 4.2. Mean grass, clover, and total herbage production (t DM ha^{-1}) as affected by summer-to-winter defoliation interval (INT; days), cutting height (DH; cm) and final defoliation date (FIN; Early = 23 September, Mid = 4 November and Late = 16 December).

Sampling period	INT					DH					FIN			
	21-day	42-day	56-day	84-day	SEM	2.7 cm	3.6 cm	5.3 cm	6.0 cm	SEM	Early	Mid	Late	SEM
2 July to 16 December 2008 (experimental management imposed during summer-to-winter)														
Grass	2.96	3.12	2.88	3.13	0.112	3.06	3.01	2.74	2.8	0.086	2.33^{a}	2.98^{b}	3.12^{b}	0.085 ***
Clover	2.29^{a}	2.71^{b}	1.57^{c}	0.91^{d}	0.099 ***	2.49^{a}	2.30^{a}	1.92^{b}	1.64^{b}	0.080 ***	2.51	2.55	2.71	0.094
Total	5.25^{a}	5.83^{b}	4.45^{c}	4.04^{d}	0.073 ***	5.55^{a}	5.31^{b}	4.67^{c}	4.44^{d}	0.061 ***	4.84^{a}	5.54^{b}	5.83^{c}	0.079 ***
3 March to 30 June 2009 (common management imposed during spring-summer)														
Grass	3.78^{a}	4.40^{b}	4.56^{b}	4.72^{b}	0.095 ***	4.37^{a}	4.39^{a}	4.56^{a}	4.88^{b}	0.078 ***	5.18^{a}	4.66^{b}	4.40^{b}	0.088 ***
Clover	0.57	0.76	0.88	0.9	0.102	1.03^{a}	0.81^{ab}	0.62^{bc}	0.42^{c}	0.077 ***	0.65	0.57	0.76	0.094
Total	4.36^{a}	5.17^{b}	5.44^{bc}	5.62^{c}	0.095 ***	5.41	5.20	5.18	5.31	0.082	5.82^{a}	5.23^{b}	5.17^{b}	0.097 ***
Annual (2 July 2008 to 30 June 2009)														
Grass	6.74^{a}	7.53^{b}	7.45^{b}	7.85^{b}	0.156 ***	7.43	7.40	7.30	7.68	0.126	7.50	7.65	7.53	0.142
Clover	2.86^{a}	3.47^{b}	2.45^{a}	1.81^{c}	0.161 ***	3.52^{a}	3.11^{a}	2.54^{b}	2.06^{c}	0.125 ***	3.16	3.12	3.47	0.139
Total	9.60^{a}	11.00^{b}	9.89^{a}	9.66^{a}	0.122 ***	10.96^{a}	10.51^{b}	9.85^{c}	9.75^{c}	0.109 ***	10.66	10.77	11.00	0.145

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Lowering DH from 6.0 to 2.7 cm resulted in higher herbage production of both grass ($P < 0.05$) and clover ($P < 0.001$) in the summer-to-winter period. In the following spring-summer this trend was reversed for grass but not for clover ($P < 0.001$, Table 4.2). As a result, total herbage production in spring-summer was not significantly affected by DH. Total annual herbage production tended to increase with lower DH ($P < 0.001$).

Clover herbage production was not significantly affected by FIN in either summer-to-winter or spring-summer. Grass herbage production in the summer-to winter period increased as FIN was delayed from Early to Late ($P < 0.001$). However, this trend was reversed in the following spring-summer ($P < 0.001$). As clover was not significantly affected by FIN in either time period, total herbage production followed similar trends to grass herbage production with the extension of FIN date from Early to Late resulting in higher herbage production in summer-to-winter and lower herbage production in spring-summer ($P < 0.001$, Table 4.2). Annual herbage production was not significantly affected by FIN.

Annual BNF estimates are shown in Figure 4.2. Annual BNF was significantly affected by INT with the highest values obtained with the 42-day INT and the lowest values obtained with the 84-day ($P < 0.001$, Figure 4.2a). Annual BNF also tended to increase as DH was lowered from 6.0 to 2.7 cm ($P < 0.001$, Figure 4.2b). Final defoliation date did not significantly affect BNF (Figure 4.2c).

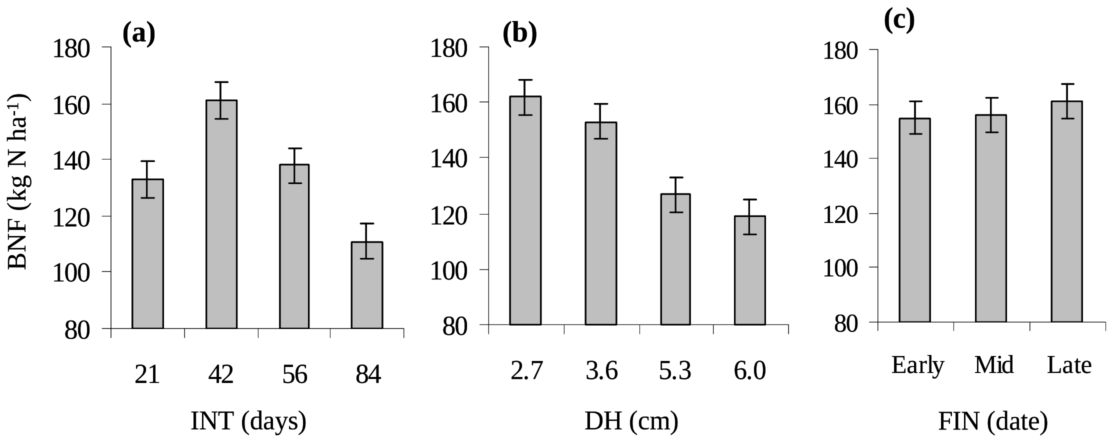


Figure 4.2. Biological nitrogen fixation (BNF; kg ha^{-1}; calculated using the total N difference method between 2 July 2008 and 29 June 2009) as affected by summer-to-winter defoliation interval (a; INT), defoliation height (b; DH) and autumn defoliation date (c; FIN). Error bars show the treatment SEM.

4.3.3. Net herbage accumulation rates (NHA) over time

Net herbage accumulation rates were affected by interactions between management factor and sampling period for all treatments (P < 0.001, Table 4.3). The 42-day INT had the highest NHA between July and September (P < 0.001) whereas the 56-day had the highest NHA between September and December (P < 0.001). In the following spring-summer NHA tended to increase with previous INT length and this effect lasted into the final months of the experiment in April and June (P < 0.001, Table 4.3).

The two lower DHs (2.7 and 3.6 cm) had higher NHA between July and December but this trend was reversed between the following December and March (P < 0.05). There was no significant difference between DH treatments in the following March to April period, but between April and June the lowest DH again resulted in the highest herbage accumulation rates (P < 0.01, Table 4.3).

The Early FIN resulted in the lowest NHA ($P < 0.001$) between September and the following March with negative values recorded at the end of that period. This resulted in the Early FIN accumulating approximately 0.42 t ha^{-1} less herbage DM than the Late FIN over winter ($P < 0.01$). However, this trend was reversed between the following March and April, with higher NHA following the Early FIN ($P < 0.05$; Table 4.3). In the final months of the experiment (April and June), there was no significant difference between FIN treatments (Table 4.3).

4.3.4. Herbage mass (HM), OMD and CP

Herbage mass increased with INT length throughout the experiment ($P < 0.001$, Table 4.4). Lowering DH increased HM between July and December but did not significantly affect it in the following March to June period (Table 4.4). *In vitro* organic matter digestibility (OMD) and crude protein concentration (CP) of herbage DM were both affected by an interaction between INT and time period ($P < 0.001$), with a trend of decreasing OMD and CP with increasing INT in summer-to-winter and the opposite trend in the following spring-summer (Table 4.4). The DH and FIN factors had no significant effect on CP or OMD.

Table 4.3. Mean net herbage accumulation rates (kg DM ha^{-1} day^{-1}) as affected by summer-to-winter defoliation interval (INT; days), defoliation height (DH; cm) and final defoliation date (FIN; Early = 23 September, Mid = 4 November, and Late = 16 December).

	INT					DH					FIN			
Sampling period	21-day	42-day	56-day	84-day	SEM	2.7 cm	3.6 cm	5.3 cm	6.0 cm	SEM	Early	Mid	Late	SEM
2 Jul to 23 Sep	55.2^{a}	59.4^{b}	45.1^{c}	40.1^{d}	0.80 ***	56.9^{a}	54.0^{a}	48.1^{b}	45.8^{c}	0.64 ***	57.7	58.3	59.4	0.91
24 Sep to 16 Dec	4.4^{a}	10.0^{b}	17.2^{c}	8.0^{d}	0.27 ***	12.0^{a}	10.9^{b}	9.5^{c}	8.0^{d}	0.25 ***	8.0^{a}	10.4^{b}	10.0^{b}	0.313 ***
17 Dec to 3 Mar	1.6^{a}	1.9^{a}	1.7^{a}	3.1^{b}	0.16 ***	-0.3^{a}	0.4^{b}	2.3^{c}	3.3^{d}	0.17 ***	-1.5^{a}	1.1^{b}	1.9^{b}	0.284 ***
4 Mar to 28 Apr	22.2^{a}	29.7^{b}	34.3^{c}	33.8^{c}	0.86 ***	28.6	28.1	30.2	29.4	0.73	33.2^{a}	30.6^{ab}	29.7^{b}	1.02 *
29 Apr to 30 Jun	45.0^{a}	51.2^{b}	56.3^{c}	54.9^{bc}	1.01 ***	52.4^{a}	50.7^{ab}	49.5^{ab}	49.2^{b}	0.83 *	53.4	50.9	51.2	1.16

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4.4. Mean pre-cutting herbage mass (above cutting height), ash content, crude protein content (CP) and *in vitro* organic matter digestibility (OMD) of harvested herbage as affected by summer-to-winter defoliation interval (INT; days), cutting height (DH; cm) and final defoliation date (FIN; Early = 23 September, Mid = 4 November and Late = 16 December), NC = non-clover plots with 42 day INT/Late FIN.

	INT					DH					FIN			
	21-day	42-day	56-day	84-day	SEM	2.7 cm	3.6 cm	5.3 cm	6.0 cm	SEM	Early	Mid	Late	SEM
2 July to 16 December 2008 (experimental management imposed during summer-to-winter)														
HM (t DM ha^{-1})	656^{a}	1458^{b}	1483^{b}	2018^{c}	25.6 ***	1845^{a}	1741^{b}	1545^{c}	1459^{c}	22.8 ***	2421^{a}	1847^{b}	1458^{c}	28.7 ***
Ash (g kg^{-1})	106^{a}	106^{a}	113^{b}	108^{ab}	1.2 ***	109^{a}	108^{a}	104^{b}	106^{ab}	0.9 ***	106^{a}	101^{b}	106^{a}	1.0 *
CP (g kg^{-1})	277^{a}	259^{b}	246^{b}	230^{c}	3.9 ***	247	248	250	250	3.4	231^{a}	250^{b}	259^{b}	4.1 ***
OMD (g kg^{-1})	804^{a}	794^{a}	758^{b}	754^{b}	3.8 ***	786	782	785	782	2.7	790^{a}	803^{b}	794^{a}	2.5 **
3 March to 30 June 2009 (common management imposed during spring-summer)														
HM (t DM ha^{-1})	870^{a}	1033^{b}	1089^{bc}	1124^{c}	19.0 ***	1081	1039	1036	1062	0.16	1165^{a}	1045^{b}	1033^{b}	19.4 ***
Ash (g kg^{-1})	104	104	104	106	0.95	104	104	105	105	0.90	104	104	104	1.2
CP (g kg^{-1})	199^{a}	208^{ab}	221^{b}	218^{b}	4.2 ***	209	214	214	211	3.50	216	211	208	4.5
OMD (g kg^{-1})	802^{a}	809^{ab}	815^{b}	810^{ab}	4.6 *	814	809	806	807	3.74	809	797	801	5.1

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

4.3.5. Sward clover content over time

The 84-day INT severely reduced sward clover content in September (*P* < 0.001, Figure 4.3a) but did not significantly affect it in December, March or April. However, by the end of the experiment, clover content was slightly higher (*P* < 0.05) following the 56 and 84 day INTs (mean 226 g kg^{-1}) than the 21-day INT (171 g kg^{-1}, SEM = 18.2, Figure 4.3a).

Lowering DH from 6.0 to 2.7 cm increased sward clover contents in September (*P* < 0.001). There was no significant difference between DH treatments in December and March but in the following April and June, sward clover content was again highest following the 2.7 cm DH and lowest following the 6.0 cm DH (*P* < 0.001, Figure 4.3b). At the end of the experiment, clover contents following the 2.7, 3.6, 5.3 and 6.0 cm DHs were 257, 219, 169 and 121 g kg^{-1}, respectively (SEM = 14.1, *P* < 0.001). Final defoliation date had no significant effect on sward clover content throughout the experiment (Figure 4.3c).

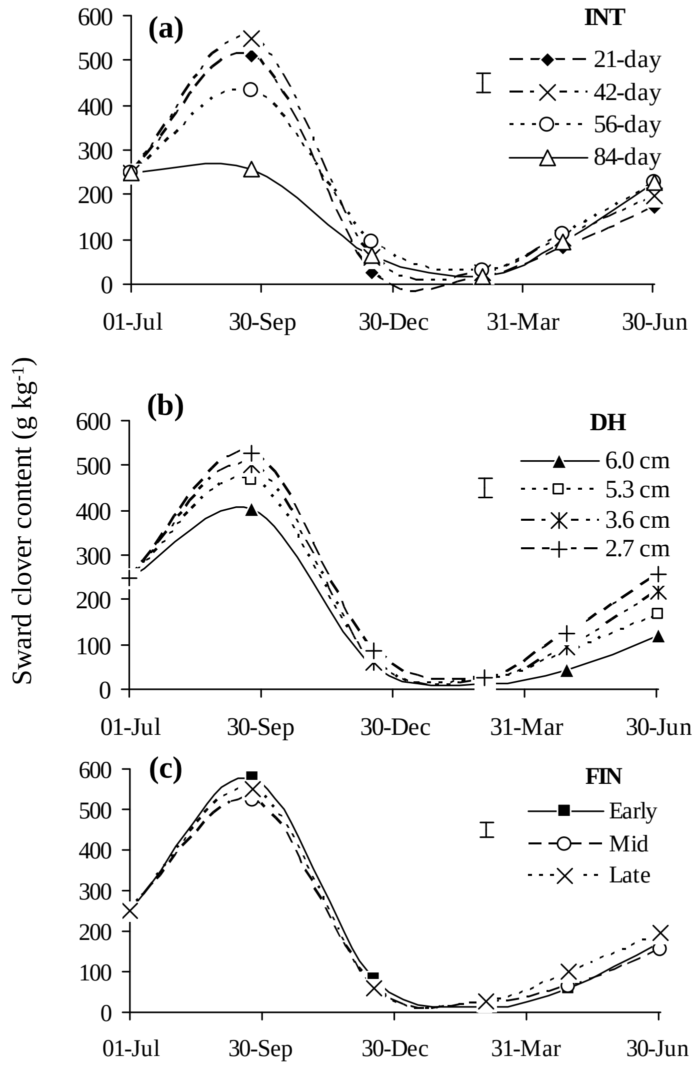


Figure 4.3. Sward white clover content (g kg^{-1} DM) as affected by interactions between sampling date and: summer-to-winter defoliation interval (INT; a; $P < 0.001$), summer-to-winter defoliation height (DH; b; $P < 0.01$), final defoliation date (FIN; c; $P > 0.05$) between 2 July 2008 and 30 June 2009). Error bars show the treatment SEM.

4.3.6. Clover stolon mass over time

The 21-day INT had lower stolon mass throughout the experiment ($P < 0.001$). The other INTs were affected by an interaction ($P < 0.05$) with sampling date where the 84-day had higher stolon mass than the 42-day in March only (Figure 4.4a). The 6 cm DH had lower stolon mass than the other three DH treatments in September and June (P < 0.01; Figure 4.4b).

There was an interaction (P < 0.05) between FIN and sampling date with the early FIN having the highest stolon mass in March and with the late FIN having the highest values at the end of the experiment in June (Figure 4.4b). Clover herbage production in spring-summer was significantly correlated with mean stolon mass for the same time period (Figure 4.5) across all plots.

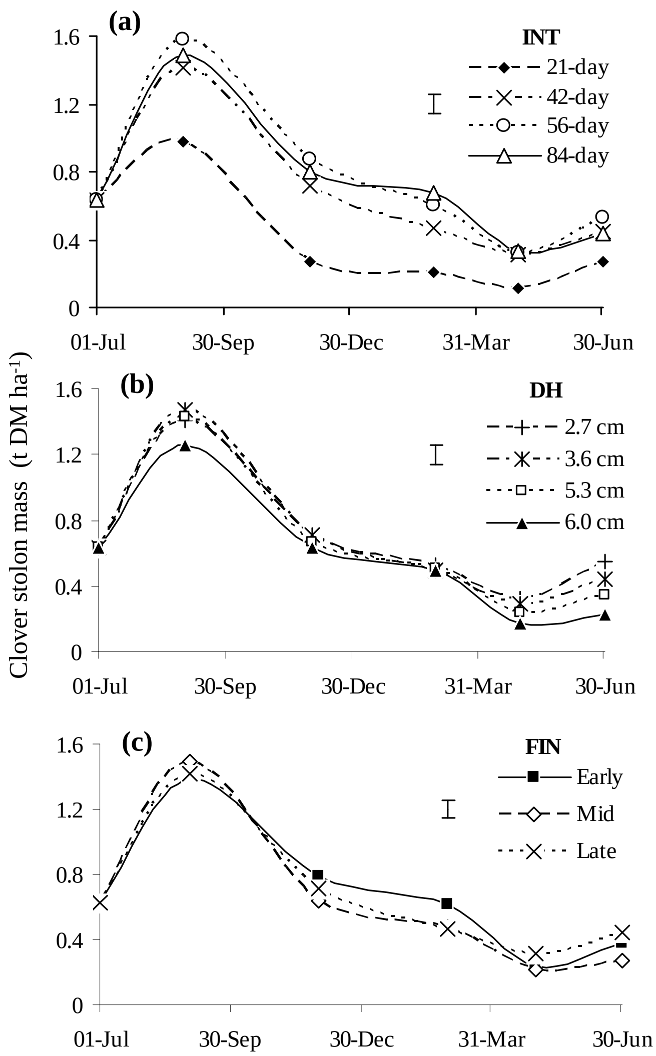


Figure 4.4. White clover stolon mass (t DM ha^{-1}) as affected by interactions between sampling date and: (a) summer-to-winter defoliation interval (INT, P < 0.05), (b) summer-to-winter defoliation height (DH, P < 0.01) and (c) final defoliation date (FIN, P < 0.05) between 2

July 2008 and 30 June 2009. Error bars show the treatment SEM.

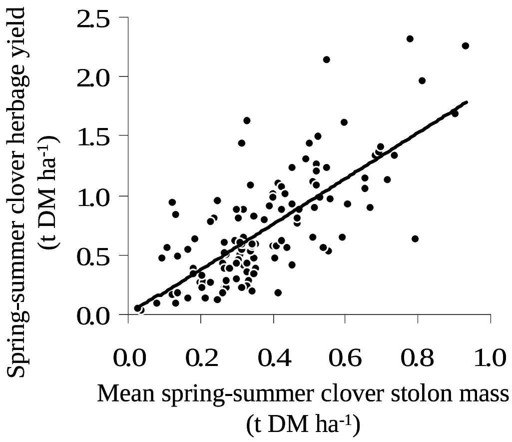


Figure 4.5. Relationship between mean white clover stolon mass in spring-summer (3 March to 30 June 2009) and white clover herbage production for the same period (fitted line: y = 1.91x – 0.00, R^2 = 0.54, *P* < 0.001).

4.3.7. Sward height and light penetration (LP)

Sward light penetration was negatively correlated with sward height and increased exponentially when compressed sward fell below 10 cm (y = $1.73e^{-0.27x}$, R^2 = 0.83, *P* < 0.001, not shown). Reducing INT tended to reduce sward height and increase LP between July and April (*P* < 0.001; Table 4.5). Lowering DH had a similar effect in reducing sward height and increasing LP between July and April (*P* < 0.001; Table 4.5). Delaying FIN reduced sward height and increased LP between October and April only (*P* < 0.001; Table 4.5).

Table 4.5. Mean pre-cutting sward height (SH) and light penetration (LP, proportion of PAR reaching the base of the sward) as affected by of summer-to-winter defoliation interval (INT; days), cutting height (DH; cm) and final defoliation date (FIN; Early = 23 September, Mid = 4^{th} November and Late = 16 December).

	INT					DH					FIN			
	21-day	42-day	56-day	84-day	SEM	2.7 cm	3.6 cm	5.3 cm	6.0 cm	SEM	Early	Mid	Late	SEM
Sward height (cm)														
2 Jul to 23 Sep	9.4^{a}	13.6^{b}	15.2^{c}	15.7^{d}	0.26 ***	12.7^{a}	12.9^{b}	13.6^{c}	14.2^{d}	0.19 ***	13.6	13.6	13.6	0.24
24 Sep to 16 Dec	5.0^{a}	5.8^{b}	8.6^{c}	7.4^{d}	0.17 ***	5.4^{a}	6.0^{b}	7.2^{c}	7.8^{d}	0.14 ***	7.0^{a}	5.8^{b}	5.8^{b}	0.18 ***
17 Dec to 3 Mar	4.6^{a}	4.7^{a}	4.4^{a}	5.6^{b}	0.10 ***	4.3^{a}	4.8^{b}	6.0^{c}	6.7^{d}	0.09 ***	7.5^{a}	5.9^{b}	4.7^{c}	0.15 ***
4 Mar to 28 Apr	6.9^{a}	8.1^{b}	8.9^{c}	8.9^{c}	0.19 ***	8.3	8.3	8.5	8.6	0.14	9.0^{a}	8.7^{b}	8.1^{c}	0.17 ***
29 Apr to 30 Jun	11.0^{a}	11.6^{b}	11.6^{b}	12.0^{c}	0.18 ***	12.0	11.6	11.2	11.4	0.14	11.6	11.5	11.6	0.16
Mean	7.4^{a}	8.8^{b}	9.7^{c}	9.7^{c}	0.11 ***	8.5^{a}	8.7^{a}	9.3^{b}	9.8^{b}	0.09 ***	9.7^{a}	9.1^{b}	8.7^{c}	0.11 ***
Light penetration (LP; proportion)														
2 Jul to 23 Sep	0.13^{a}	0.05^{b}	0.03^{c}	0.01^{d}	0.004 ***	0.07^{a}	0.06^{b}	0.05^{c}	0.04^{c}	0.003 ***	0.05	0.05	0.05	0.003
24 Sep to 16 Dec	0.52^{a}	0.42^{b}	0.26^{c}	0.17^{d}	0.015 ***	0.41^{a}	0.39^{a}	0.29^{b}	0.23^{c}	0.013 ***	0.23^{a}	0.40^{a}	0.42^{b}	0.018 ***
17 Dec to 3 Mar	0.75^{a}	0.73^{a}	0.75^{a}	0.66^{b}	0.014 ***	0.72^{a}	0.68^{a}	0.57^{c}	0.48^{d}	0.011 ***	0.26^{a}	0.53^{b}	0.73^{c}	0.016 ***
4 Mar to 28 Apr	0.33^{a}	0.24^{b}	0.23^{b}	0.22^{b}	0.009 ***	0.27	0.25	0.22	0.21	0.006 ***	0.18^{a}	0.22^{b}	0.24^{b}	0.009 ***
29 Apr to 30 Jun	0.10^{a}	0.09^{b}	0.09^{b}	0.09^{b}	0.003 ***	0.09	0.09	0.09	0.09	0.002	0.09	0.09	0.09	0.002
Mean	0.37^{a}	0.30^{b}	0.27^{c}	0.22^{d}	0.005 ***	0.32	0.29	0.25	0.21	0.005	0.18^{a}	0.27^{b}	0.30^{c}	0.006 ***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Clover herbage production in spring-summer (3 March to 30 June 2009) was correlated with LP over the non defoliated winter period (17 December to 3 March, Figure 4.6). However, there was no relationship between LP and clover stolon mass. Final defoliation date had the largest effect on LP over the non defoliated winter period, with the Early FIN allowing approximately half the amount of light to reach the base of the sward as that of the Late (Table 4.5).

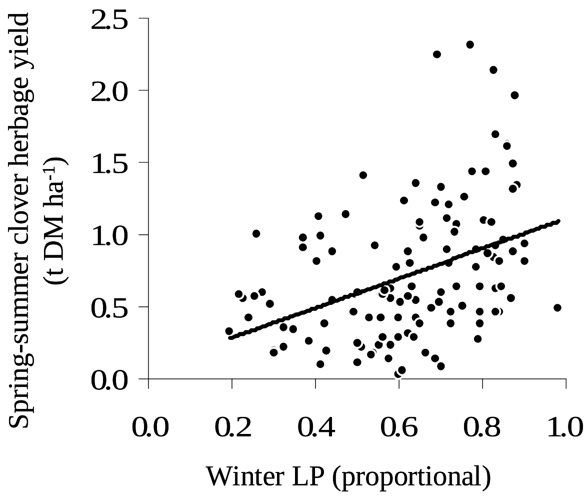


Figure 4.6. Relationship between the proportion of photosynthetically active light penetrating the sward in winter (LP; 17 December to 3 March) and subsequent clover herbage production in spring-summer (3 March to 30 June 2009; fitted line: y = 1.03 + 0.08, R^2 = 0.16, P < 0.05).

4.4. Discussion

4.4.1. Herbage production

Previous experiments have found increased clover production over time when the interval between defoliation was extended from 28 to 56 days (Wilman and Asiegbu, 1982b; Patterson *et al.*, 1995). However, the herbage masses attained in both those experiments were very low, approximately 1.5 to 3.7 times lower than in the present experiment. In contrast, Schils and Kraak (1994) reported lower clover herbage production when defoliation interval was extended (cutting four to five times per year instead of six to seven times). The range of treatments in the current experiment showed a curvilinear response of clover herbage production to defoliation interval in summer-to-winter, decreasing when defoliation interval was too short or too long (Table 4.2). The lack of an effect of INT on grass herbage production in the same period may have been due to lower competition from clover in the longer regrowth and/or to released N from the declining clover herbage mass.

In the following spring-summer, the trend of decreasing herbage production (of both grass and clover) following the shorter INT lengths indicated the depletion of plant reserves by frequent autumn-winter defoliation. This has previously been found in grass (Davies and Simons, 1979) and clover (Simon *et al.*, 2004) monocultures and can be attributed to the mobilisation of stored C and N to replace defoliated leaves (Vinther, 2006). The 56 and 84 day INTs increased NHA through to the end of the experiment in June, six months after treatments ended (Table 4.3).

Previous simulated grazing experiments have found that lowering defoliation heights during the main growing season (spring, summer and early autumn) on grass-clover swards can increase clover herbage with little or no effect on grass herbage production (Frame and Boyd, 1987; Acuña and Wilman, 1993; Schils and Sikkema, 2001). Across these previous experiments, each cm reduction in cutting height increased clover production by approximately 0.17 to 0.53 t DM ha^{-1} and total herbage production by approximately 0.26 to 0.51 t DM ha^{-1} with generally linear responses to cutting height. In comparison, the current experiment showed mean responses 0.21 to 0.40 t DM ha^{-1} per cm reduction in cutting height in the summer-to-winter period but this had large positive carry over effect in spring with 0.11 to 0.29 t DM ha^{-1} extra clover grown in spring-summer for every cm reduction in cutting height in the preceding summer-to-winter. This resulted in an annual response of 0.34 to 0.70 t ha^{-1} increased clover production and 0.14 to 0.54 t ha^{-1} increased total herbage production for every cm reduction of DH. These are relatively high responses compared to those cited above, considering that treatments were only imposed in the first half of this experiment. This shows that low DH of grass-clover swards in autumn-winter can be important in maintaining clover herbage production from year to year.

Final defoliation date did not significantly affect annual herbage production because the majority of clover herbage was produced before September (Figure 4.2 and Table 4.3). Delaying closing date after that month resulted in more grass being harvested in autumn/winter and not carried over to the following spring. The Early FIN had approximately 0.43 t DM ha^{-1} less net herbage accumulation between the final defoliation in September and the first defoliation in the following March, when compared to the Late FIN (Table 4.3). This is most likely due to higher senescence rates in the undefoliated treatment over this time period. Higher losses of herbage due to senescence (0.41 to 0.71 t DM ha^{-1}) were previously recorded between November and February from grass-only swards with a Early (20 September) FIN in Ireland (Hennessy *et al.*, 2006). The current experiment had relatively low herbage masses over winter which could explain the lower amounts of senescence (Carton *et al.*, 1988).

4.4.2. Herbage quality

Organic matter digestibility and CP values typically decline with increasing regrowth interval in perennial ryegrass swards due to increasing stem and dead material (Binnie *et al.*, 1997; Dillon *et al.*, 1998; Hennessy *et al.*, 2006). Binnie *et al.* (1997) found that OMD and CP declined at rates of 3.3 and 2.7 g kg^{-1} per day, respectively, in a grass-only sward when cutting interval was extended from 21 to 42 days in autumn. In comparison, the current experiment showed much slower rates of decline of 0.48 g kg^{-1} day^{-1} for OMD and 0.86 g kg^{-1} day^{-1} for CP when defoliation interval was extended from 21 to 42 days. This could be due to the later sampling dates, lower herbage masses and presence of clover in the current experiment. White clover can maintain higher stability of nutritive value during extended growth periods than perennial ryegrass (Dewhurst *et al.*, 2009). However, the rate of OMD decline increased to 2.57 g kg^{-1} day^{-1} after 56 days, which is when clover content in the sward also declined (Figure 4.3). Herbage intake is correlated with OMD (Baudracco *et al.*, 2010) and this experiment suggests the potential for clover to maintain nutritive value and intake rates for longer regrowth intervals in summer-to-winter than grass-only swards, which would enable greater management flexibility in extending the grazing season. However, this is only applicable to swards with high clover contents (Figure 4.3). Crude protein was relatively high and maintained above the 160 to 180 g kg^{-1} required for a grazing dairy cow (Chamberlain and Wilkinson, 1996) in all treatments.

4.4.3. Clover persistence into the following summer

The 21-day INT reduced clover stolon mass and clover herbage production in the following spring. Clover stolons function as major storage organs that accumulate proteins and carbohydrates in autumn that are important for cold-hardening, winter survival and subsequent spring regrowth (Hay *et al.*, 1989; Svenning *et al.*, 1997; Goulas *et al.*, 2001). Previous experiments have shown that frequent defoliation can deplete these reserves by reducing stolon mass and/or its concentrations of vegetative storage proteins and water soluble carbohydrate (Patterson *et al.*, 1995; Lüscher *et al.*, 2001; Vinther, 2006).

Lowering the DH in summer-to-winter had a strong positive effect on stolon mass, clover content and clover herbage production. Lower defoliation heights in spring and summer have previously been found to increase sward clover contents (Acuña and Wilman, 1993; Wilman and Acuña, 1993). This is generally explained as a response to increased light penetration of the sward and the difference in leaf morphology of grass and clover plants; the umbrella-like leaf structure of clover exposes it to higher leaf loss at high DH than the blade-like leaf structure of grass (Woledge *et al.*, 1992; Thompson, 1993; Héraut-Bron *et al.*, 2001; Christophe *et al.*, 2006). In the current experiment the magnitude of the carry-over effect of summer-to-winter DH on subsequent clover content and stolon mass increased as the following year progressed (Figure 4.3). This demonstrates the large benefit of DH in summer-to-winter on clover persistence from one year to the next.

Final defoliation date had no effect on clover herbage production or content in the sward in the following spring-summer. In contrast, Laidlaw and Stewart (1987) and Laidlaw *et al.* (1992) found that not defoliating over winter had a large negative effect on clover content in the following July. However, both those experiments finished defoliation in September and did not recommence defoliation until the following May whereas the current experiment had a much earlier commencement of spring defoliation in March, which may explain the differences between the experiments.

Sward clover content typically declines in winter and clover leaves that are present tend to be positioned lower in the sward than grass leaves (Woledge *et al.*, 1989; Woledge *et al.*, 1990). As a result clover is less competitive, particularly for light, than perennial ryegrass during the winter and early spring (Harris *et al.*, 1983; Woledge *et al.*, 1989; Woledge *et al.*, 1990). The current experiment found a positive relationship between the proportion of light (LP) reaching the base of the sward in winter and subsequent spring-summer clover herbage production which had not been previously reported. However, the low R^2 value of 0.16 shows that a low proportion of the variation was explained by LP. This was also reflected by the fact that the FIN treatments, which had the largest effect on LP over winter, did not significantly affect clover content or clover herbage production in the following year (Table 4.2 and Figure 4.3). This suggests that other factors such as plant storage reserves are also important for maintaining clover in grassland swards over winter.

4.4.4. Implications for grazing management

Grazing interval is often extended in autumn in order to increase the pre-grazing herbage mass and thereby extend grazing into early winter, when herbage growth rates fall below demand (Hennessy and Kennedy, 2009; MacDonald *et al.*, 2010). In Ireland recommended grazing intervals for perennial ryegrass swards are approximately 21 days in spring and early summer and increase to approximately 35 days in autumn (Dillon *et al.*, 1999). The current experiment shows that clover content, stolon mass and herbage production in grassland can benefit from such extension of inter-grazing intervals in autumn and frequent (21-day) defoliations in autumn should be avoided. Clover is conducive to extending the grazing interval in autumn due to its capacity to maintain high nutritive value during extended intervals of regrowth. However, this is dependant on keeping the interval of regrowth below the point at which clover content within the sward declines, which was greater than 42-days in the current experiment.

Tight defoliation clearly has potential to improve both herbage production and clover persistence in grazed grassland. However, this needs to be evaluated in actual grazing systems. There are large differences in sward dynamics between grazing and cutting experiments due to animal behaviour, selective grazing, excretion, treading, greater sward heterogeneity and management interactions in the former (Frame, 1993; Hodgson, 1993; Edwards *et al.*, 1996). Furthermore, previous experiments on grass-only swards have associated lower post-grazing heights with lower daily herbage intakes and milk yield per cow (Le Du *et al.*, 1979; Mayne *et al.*, 1987; McGilloway *et* *al.*, 1999; Maher *et al.*, 2003). Therefore, there is currently a need to measure the effect of post-grazing height on clover herbage production, sward clover content and animal production from grass-clover swards.

The average grazing season on Irish dairy farms ends on the 12 November and starts again on 26 February (Creighton *et al.*, 2011). The current experiment did not find reduced clover contents when swards were left undefoliated for this period of time over winter. However, in rotational grazing systems, individual paddocks are sequentially grazed. Therefore, the date of final grazing in winter strongly influences the start of grazing in spring on a per paddock basis. Under these conditions the first paddock grazed in February would have been last grazed in early to mid October (Hennessy and Kennedy, 2009; Ryan *et al.*, 2010). Furthermore, excessively wet soil conditions limit both first and final grazing dates on most Irish dairy farms (Creighton *et al.*, 2011). Therefore individual paddocks on wet areas of farms may be avoided and remain ungrazed for longer periods over the winter and early spring. This prolonged period without defoliation could have a more pronounced negative effect on clover content, as observed by Laidlaw and Stewart (1987) and Laidlaw *et al*. (1992) but needs to be compared to the possible risk of treading damage on subsequent herbage production and clover content (Menneer *et al.*, 2005a). This needs to be assessed under actual grazing conditions.

4.5. Conclusions

A 42-day interval between defoliations in summer-to-winter gave the highest annual white clover herbage production, BNF estimates and total herbage production. Both shorter (21-day) and longer (56 to 84-day) intervals reduced annual clover herbage production and BNF estimates. Lowering defoliation height from 6.0 to 2.7 cm in the summer-to-winter period increased sward clover content and clover herbage production through to the following June, six months after treatments ended. Delaying final defoliation date from 23 September to 16 December had no significant effect on annual clover, grass or total herbage production. Spring-summer clover herbage production was correlated with spring-summer clover stolon mass ($R^2 = 0.54$, $P < 0.001$) and, to a lesser extent, light penetration of the sward in the previous winter ($R^2 = 0.16$, $P < 0.05$). These results suggest that grazing management of grass-clover swards in late summer, autumn and winter, should practice approximately 42-day grazing intervals and low (< 5 cm) post-grazing herbage residuals in order to maximise clover herbage production and BNF. However, these treatments need to be evaluated in actual grazing systems.

5. Meta-analysis of the relationship between biological nitrogen fixation and clover herbage production in grass-white clover swards.

Abstract

The objective of this experiment was to explore the relationship between biological nitrogen fixation (BNF) and white clover herbage production in the available literature on grass-clover swards using mixed-model meta-analyses. In total, 29 peer-reviewed studies were used that had observations for both BNF and annual white clover herbage yield. The results were split into two datasets depending on whether BNF was measured using isotopic determination (ID) or the total nitrogen difference (ND) method. The ID dataset consisted of 20 studies and 102 observations. The ND dataset consisted of 9 studies and 91 observations. The relationship for the ID dataset was y = 36.1x + 2.2 (where y is BNF in kg ha^{-1} and x is clover herbage yield in t ha^{-1}). The corrosponding relationship for the ND dataset was y = 33.7 +33.1. When forced through the origin, the relationships suggested 36.6 and 45.0 kg N per tonne of white clover produced in the ND and ID datasets respectively. The higher BNF rates in the ND dataset were most likely a result of the assimilation of fixed N by co-habiting grass, which is generally not measured in ID studies.

5.1. Introduction

White clover (*Trifolium repens*) facilitates biological nitrogen fixation (BNF) and is often grown in grassland to reduce or replace fertiliser N requirements. For research purposes, this BNF is usually measured using either N_{15} isotopic determination (ID) or the total N difference method (ND). Isotopic determination is generally expensive and assumes no transfer of fixed-N to co-habiting grass whereas the total N difference is less expensive and includes uptake of fixed-N by cohabiting grass but can be more variable (Haystead, 1993). In recent years, the ID method has become the more common.

Measurements of BNF through ND, and particularly ID are not feasible on commercial farms because of the time, equipment and expertise required. However, an estimate of BNF is still desirable in order to assist sward N-budgeting. Herbage production is much easier to quantify than BNF and many Irish dairy farmers have reasonable estimates of herbage production on their farms. Teagasc also have an online calculator that farmers can use to calculate herbage production based on animal production and feed inputs (www.agresearch.teagasc.ie/moorepark/). Annual clover herbage production can therefore be calculated if an estimate of the annual average sward clover content is provided. If clover herbage production or sward clover content can be used to predict BNF, it can assist N-budgeting of grass-clover swards, as well as enabling calculation of the value of BNF to the farmer.

Other experiments have identified relationships between clover herbage production and BNF. Carlsson and Huss-Danell (2003) reviewed the literature and concluded from simple linear regression that white clover fixed approximately 31 kg of N per tonne of herbage produced in herbage alone. In another review, Hogh-Jensen *et al.* (2004) similarly concluded that white clover fixed between 30 and 43 kg N per tonne of herbage produced (not including fixed-N transferred to grass or soil, or retained in clover stolon, stubble and roots). However, simple regression across studies is not recommended in most research areas because the relationship tends to be influenced by the differences between studies rather than just the relationships between observations within each study (St-Pierre, 2001). This can lead to large errors. Meta-analyses using mixed-model methodology improves the accuracy of global relationships across a range of studies by blocking for the effect of study, and thereby deriving the true relationship across studies (St-Pierre, 2001). The objective of this experiment was to explore the relationship between clover herbage production and BNF measured by ID and ND in grass-clover swards using mixed-model meta-analyses of the available literature with an aim to deriving estimates of BNF from clover herbage production.

5.2. Materials and methods

A meta-analysis was conducted on 29 international peer-reviewed publications of experiments that presented, or enabled calculation of, (i) annual BNF (kg ha^{-1}), (ii) annual average white clover content of the sward (% of herbage DM), (iii) annual harvested herbage production (yield; t DM ha^{-1}). The results were split into two datasets depending on whether BNF was measured using isotopic determination (ID) or the total nitrogen difference (ND) method as described by (Haystead, 1993). The ID dataset consisted of 20 studies with a combined total of 102 data lines. The ND dataset consisted of 9 studies with a combined total of 91 data lines.

The relationships between annual BNF and clover herbage production were investigated within each dataset using the meta-analysis methodology described by St. Pierre (2001). The statistical model in the analysis was: $Y_{ijk} = \mu + S_i + \tau_j + S_{tij} + e_{ijk}$ where: Y_{ijk}= the dependent variable, μ = overall mean, S_i = the random effect of the ith study, S_{tj} = the fixed effect of the jth level of factor τ, Stij = the random interaction between the ith study and the jth level of factor τ, assumed and eijk = the residual errors. The effect of study was entered as a random effect using the RANDOM statement in PROC MIXED SAS (SAS, 2006). Regressions were compared with each other using ANCOVA with PROC MIXED in SAS (SAS, 2006).

5.3. Results and discussion

The summary statistics for the two datasets are presented in Table 5.1 Both datasets had similar annual total herbage yield, grass herbage yield and fertilizer N inputs. However, the ND dataset had significantly higher sward clover content, clover herbage yield and BNF.

Table 5.1. Summary statistics of the two datasets used in the meta-analysis: ID = isotopic determination of BNF, ND = natural difference method of determining BNF. All values are annual.

	ID	ND	SEM	*P*-value
Number of studies	20	9		
Total number of entries	102	91		
Fertilizer N input	46	32	8.8	0.237
Total herbage yield	9.8	9.9	0.38	0.857
Grass herbage yield	6.4	5.8	0.34	0.179
Clover herbage yield	3.2	4.1	0.22	0.007
Sward clover content	35%	42%	1.7%	0.007
Biological nitrogen fixation (BNF)	112	209	10.45	<.0001

The relationships between BNF and clover herbage yield are presented in figure 5.1 and the relationships between BNF and sward clover content yield are presented in figure 5.2. In both cases, there were highly significant positive correlations (P>0.001). When the regressions of the relationships were compared using ANCOVA there were no significant differences in slope between the ID and ND datasets in both figures. However the ID dataset had a significantly lower intercept in both figures ($P < 0.001$).

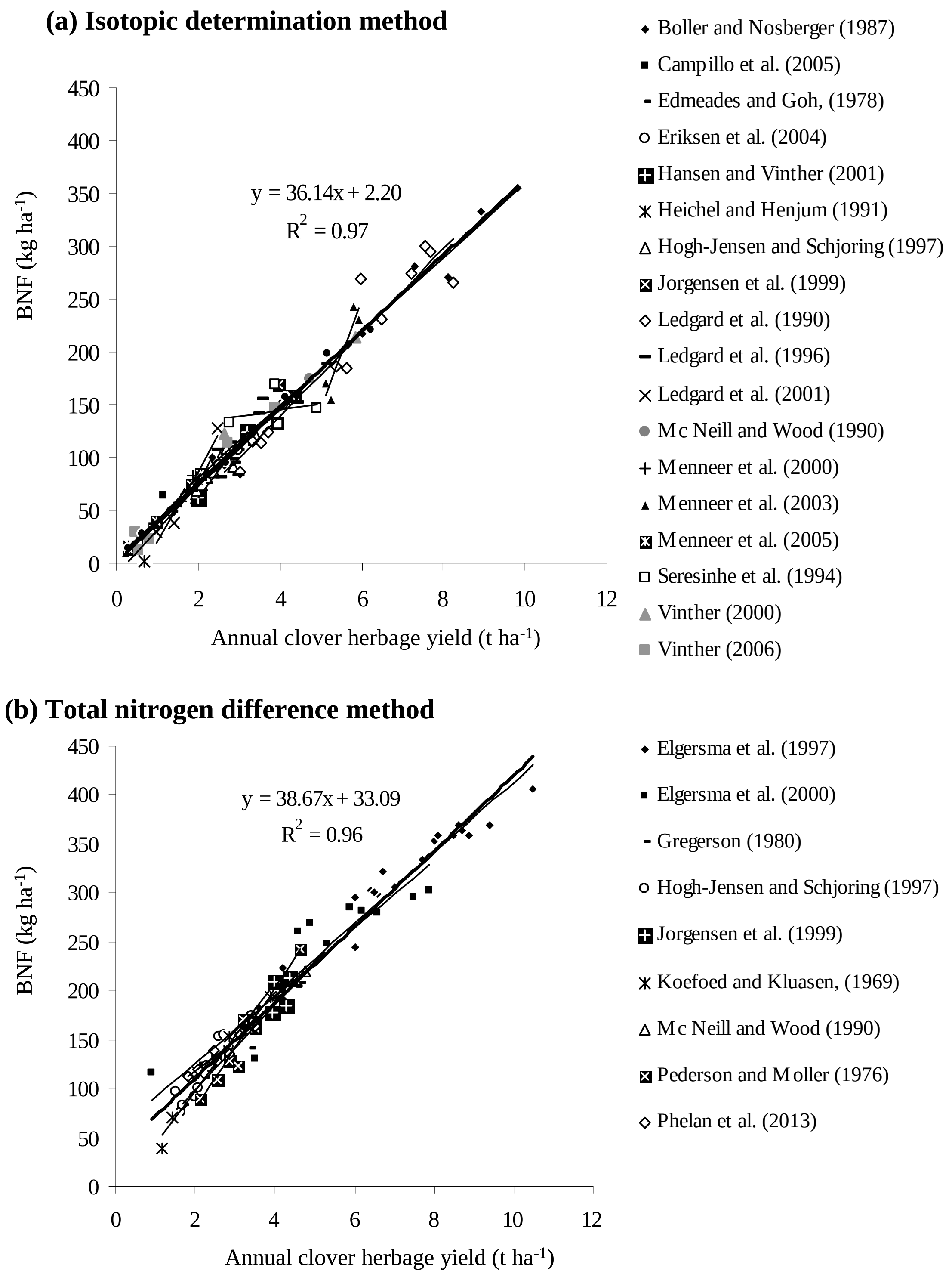


Figure 5.1. Meta-analysis of the relationship between annual clover herbage yield and biological nitrogen fixation (BNF) as measured by (a) isotopic determination (ID) and (b) the total nitrogen difference (ND) method for grass + white clover swards. Results for BNF adjusted for the effect of study according to the methodology of St. Pierre (2001).

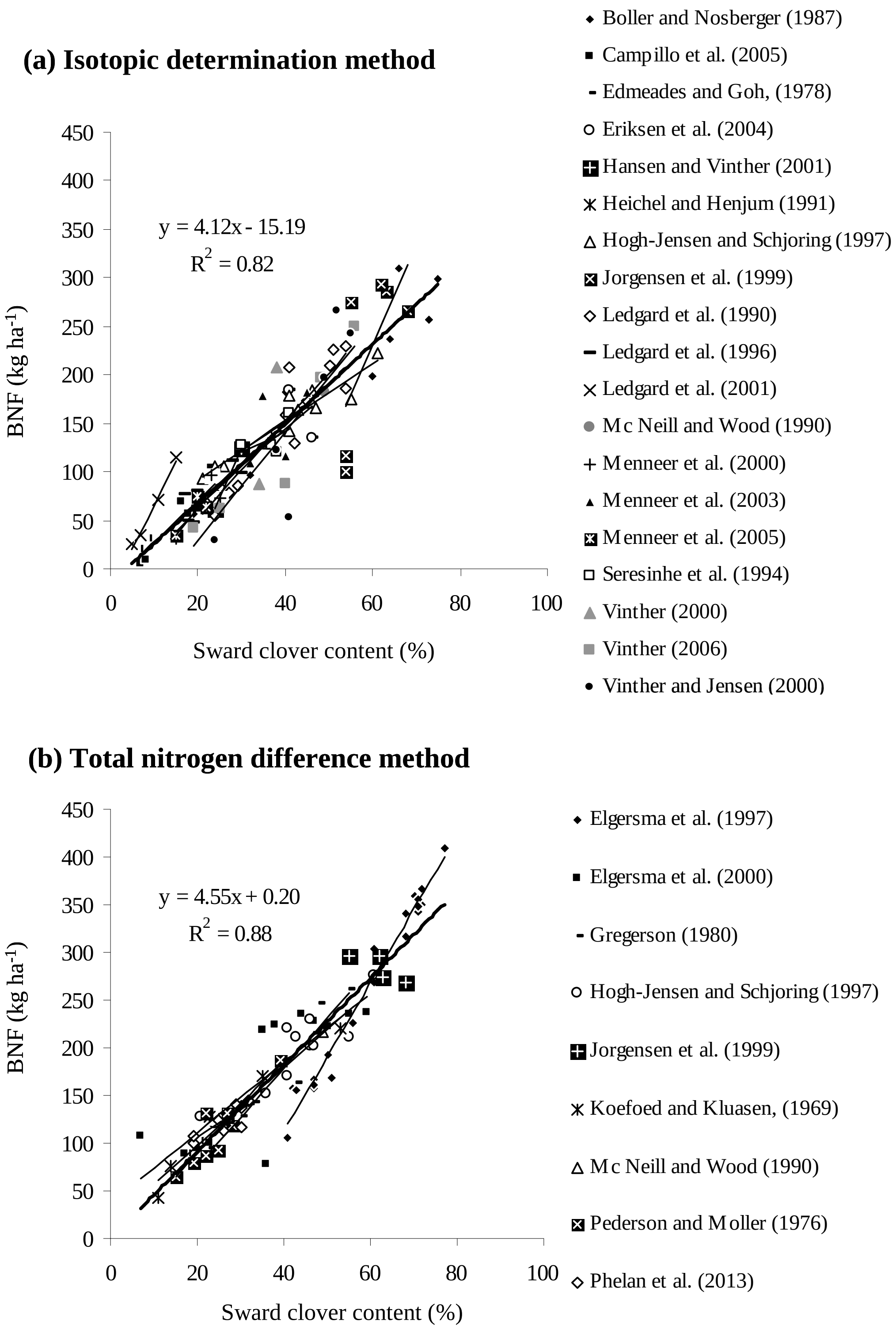


Figure 5.2. Meta-analysis of the relationship between annual average sward clover content and biological nitrogen fixation (BNF) as measured by (a) isotopic determination (ID) and (b) the total nitrogen difference (ND) method for grass + white clover swards. Results for BNF adjusted for the effect of study according to the methodology of St. Pierre (2001).

The higher intercept found in the ND dataset is most likely due to the transfer of fixed-N from clover to grass in the mixed swards, which is generally not measured in ID studies. However, the fact that this was evidenced by a higher intercept in the ND dataset, rather than an increased slope is surprising. It should also be noted that the ND dataset had higher mean values for clover herbage yields, sward clover contents and BNF (Table 5.1). While the meta-analysis methods used here account for variation between studies within each dataset, it is still limited by the range of values present. Therefore, the fact that the ND dataset had higher maximum and minimum values for clover herbage yield (see Figure 5.1) may have some influence on the difference between the regressions. However, removing data rows so that the datasets had similar ranges (by removing the highest 5 values from the ND dataset and the lowest 19 values from the ID dataset) did not result in large changes to the slopes or intercepts ($y = 35.3x + 5.5$ for ID and $y = 40.2x + 27.9$ for ND; compare with Figure 5.1).

Three studies had BNF that could be calculated for both ID and ND and were therefore included in both datasets: Jorgensen et al. (1999), Hogh-Jensen et al. (1997) and McNeill and Wood (1990). Isolating these studies revealed a significant difference in slopes between the two methodologies for the relationship between BNF and clover herbage yield, whereas the intercepts were not significantly different from each other, or zero (Figure 5.3). This difference in slopes suggests that the ND method resulted in 1.32 times higher kg BNF per tonne of clover than the ID method within these three experiments. Forcing the regression through the origin in Figure 5.1 gives slopes of 36.6 and 45.0 for the ID and ND datasets, respectively and suggests that the ND method had

1.23 times higher kg BNF per tonne of clover than the ID. Forcing the slope through the intercept may be suitable in this analysis as BNF would be expected to be close to zero in most grass-only swards.

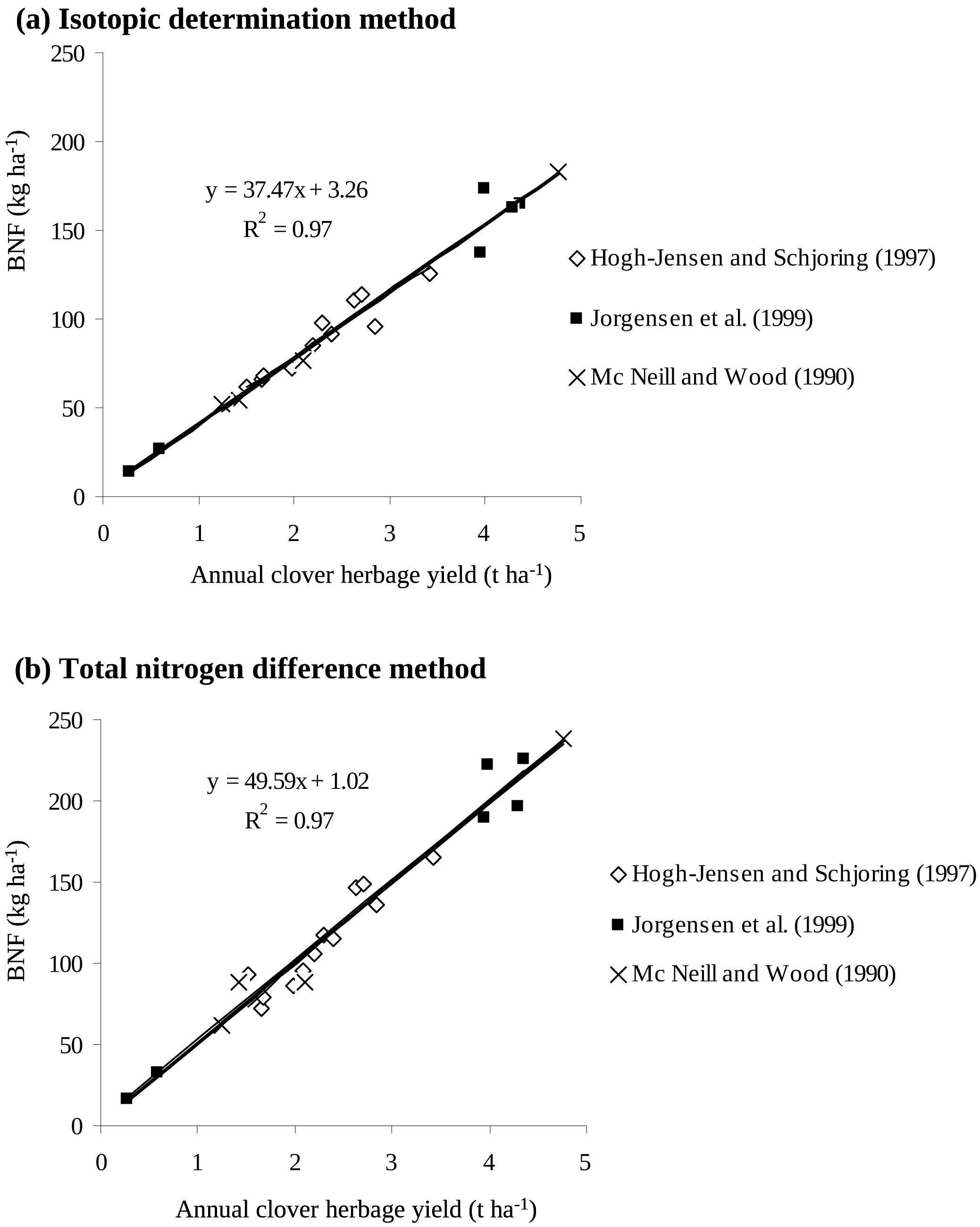


Figure 5.3. Meta-analysis of the relationship between clover herbage yield and biological N fixation (BNF) when calculated using either (a) isotopic determination (ID) or (b) total N difference (ND) within the same three studies.

These results therefore suggest that the ND method measured approximately 20 to 30% greater BNF per tonne of clover DM than the ID method. As stated above, this is most likely caused by the transfer of fixed N to the co-habiting grass and the fact that this is measured in the ND method, but not the ID method. It is widely recognized that fixed-N recovered in clover herbage is only a proportion of the total BNF, and that some fixed-N is transferred to soil and assimilated by the companion grass. Previous studies have shown that a large proportion of fixed-N can accumulate in unharvested clover material (stubble, stolons and roots) and is transferred to the soil or air from decomposing clover tissue or through the animal excretion pathway (Table 5.2). Some of this N is recovered by the grass component of the sward and can account for the higher BNF generally measured with the ND method.

Table 5.2. Summary of studies that investigated the percentage of fixed-N not recovered in harvestable clover herbage.

Source	System	Sward age	Soil type	Percentage fixed-N not recovered in clover herbage
Vinther (2006)	Cut	< 2 yrs	Sandy	28 % *
Jorgensen and Ledgard (1997)	Grazed	> 2 yrs	Sandy	72 %
Høgh-Jensen *et al.* (2004)	Cut	> 2 yrs	Clayey	83 %
Høgh-Jensen *et al.* (2004)	Cut	> 8 yrs	Clayey	103 %
Sturite *et al.* (2007)	Undefoliated	< 2 yrs	Clayey	150 % **

* Only based on fixed root:shoot ratios. All others include tissue turnover.
** Clover monocultures, all others grass-clover mixtures.

Elgersma *et al.* (1997; 2000) recorded a mean transfer rate of fixed-N to grass of 28% across various treatments (cultivars, fertilizer N, cutting frequency) but also found that it ranged from 11 to 77%, tending to be higher where fertilizer N was used and higher with smaller-leaved clover cultivars. Hogh-Jensen and Schorring (1997) recorded N-transfer rates of 3, 17 and 22% in a grass-clover sward in the first, second and third years after sowing, respectively. Laidlaw *et al.* (1996) found that, on average, 15 to 34%

of clover's fixed-N was transferred to grass and that it tended to be higher with small-leaved clover cultivars. When compared with the above studies, an apparent average transfer rate of approximately 20-30% could account for the difference between the ID and ND methods in the current study.

The relationship in Figures 5.1 and 5.2 suggest that a large amount of variation in BNF could be explained by either clover herbage yield or sward clover content. However the meta-analyses removed the effect of study by treating each study as a randomised block within the regression. While this is useful for determining the overall trend across studies, it does remove a large amount of between-study variation. Therefore it should be noted that the R^2 for the unblocked data was 0.87 and 0.91 for BNF vs. clover herbage in the ID and ND datasets, respectively whereas it was only 0.46 and 0.65 for BNF vs. sward clover content. Within each study, there also tended to be higher R^2 for BNF vs. clover herbage yield than for BNF vs. sward clover content. The N content in clover herbage tissue tends to be similar across a wide range of conditions (Dewhurst *et al.*, 2009) and the proportion of that N that is atmospherically-derived tends to vary less in grass-clover swards than in clover monocultures, due to the greater grass competition for soil-N (Høgh-Jensen *et al.*, 2004). Therefore, clover herbage production is a relatively useful parameter to estimate BNF from in grass-clover swards. However, growth rates can vary widely across climates, soil and management regimes which makes clover content less useful for estimating BNF.

5.4. Conclusions

Meta-analysis of the ID dataset revealed 36.6 kg of fixed N per tonne of clover herbage when assigned a fixed intercept of 0. Meta-analysis of the ND dataset (that includes fixed N transferred to grass) revealed approximately 45.0 kg of fixed N per tonne of clover herbage when assigned a fixed intercept of 0. The latter higher value is most likely caused by assimilation of fixed N by the cohabiting grass, suggesting that BNF estimates were 20 to 30% higher when fixed-N in grass herbage was included.

6. The Effects of Treading by Dairy Cows on Soil Properties and Herbage Production in Three White Clover Based Grazing Systems on a Clay Loam Soil.

Phelan P., Keogh, B., Casey, I.A., Necpalova, M. and Humphreys, J. (2012) The effects of treading by dairy cows on soil properties and herbage production in white clover based grazing systems on a clay loam soil. *Grass and Forage Science, DOI: 10.1111/gfs.12014.* *http://onlinelibrary.wiley.com/doi/10.1111/gfs.12014/full (accessed 29 January 2013).*

Abstract

White clover is a legume that can reduce fertilizer-N requirements, improve sward nutritive value and increase environmental sustainability of grazed grasslands. Previous experiments in glasshouse and mown plots have suggested that white clover may be more susceptible to treading damage in wet soils than perennial ryegrass. However, this phenomenon has not been investigated under actual grazing conditions. This experiment examined the effects of treading on clover content, herbage production and soil properties within three clover-based grazing systems on a wet soil in Ireland. Treading resulted in soil compaction, as evidenced by increased soil bulk density ($P < 0.001$) and reductions in the proportion of large (air-filled) soil pores ($P < 0.001$). Treading reduced annual herbage production of both grass and clover by similar amounts: 0.59 and 0.45 t ha^{-1}, respectively (P < 0.001). Treading did reduce sward clover content in June ($P < 0.01$) but had no effect on annual clover content, clover stolon mass or clover content at the end of the experiment. Therefore, there was little evidence that clover is more susceptible to treading damage than perennial ryegrass under grazing conditions on wet

soils.

6.1. Introduction

White clover (*Trifolium repens*) is an important legume for grazed grassland (Frame *et al.*, 1998; Abberton and Marshall, 2005; Peyraud *et al.*, 2009). Clover plays a key role in facilitating biological N fixation (BNF) and can thereby reduce fertilizer-N requirements for sward herbage production and nutritive value (Rochon *et al.*, 2004). Therefore, white clover is essential for organic grazing systems (Baars, 2002) but is also increasingly important for conventional (non-organic) systems because of the increasing price of fertilizer N, which has tripled in the last decade (World Bank, 1990-2010). Grazing systems based on mixed white clover and perennial ryegrass swards (hereafter grass-clover swards) can result in higher farm-level profitability than perennial ryegrass-only swards (Doyle and Bevan, 1996; Andrews *et al.*, 2007; Falconer *et al.*, 2011; Humphreys *et al.*, 2012). Replacing fertilizer-N use with higher clover use also has important environmental benefits such as increased biodiversity (Power and Stout, 2011), reduced nitrate leaching (Andrews *et al.*, 2007) and reduced greenhouse gas emissions (Li *et al.*, 2011). However, previous experiments on small mown plots (Menneer *et al.*, 2005a) or in pots in glasshouses (Grant *et al.*, 1991) have suggested that white clover is more susceptible to treading damage than perennial ryegrass. This greater susceptibility may compromise clover's suitability for farms on wet soils but has not been investigated under actual grazing conditions.

Treading (or treading) damage to plants and soils occurs under the hooves of grazing animals and can reduce subsequent herbage production from grassland (Drewry *et al.*, 2008; McDowell, 2008). This reduction is a result of the dislodgement, damage and burial of plants, and the compaction or consolidation of soil particles (Greenwood and McKenzie, 2001). Soil moisture reduces soil resistance to deformation and increases susceptibility to treading (Mapfumo and Chanasyk, 1998). However, intensive food production from grazed grassland is often centred in regions of high rainfall (Smit *et al.*, 2008). Ireland, in particular, has a high proportion of grazed grassland (0.90 of the utilized agricultural area) and receives high levels of rainfall, typically 750 to 1,200 mm year^{-1} (Central Statistics Office (CSO), 2012b). Coupled with geomorphic attributes, this high rainfall has resulted in 0.60 of the land area being classified as significantly wet with impeded drainage (Collins and Cummins, 1996) and 0.46 of Irish farms being on land classified as limited in agricultural use because of land wetness (National Farm Survey (NFS), 2007, 2008, 2009). In a recent survey, 0.60 of Irish dairy farmers identified wet soil as the limiting factor to grazing season length (Creighton *et al.*, 2011).

There is a lack of data on the effects of treading damage on subsequent herbage production from grazed grass-clover swards. The majority of experiments on treading damage are from grass-only swards and/or single, severe treading events on small plots (Curll and Wilkins, 1983; Davies and Armstrong, 1986; Vertes, 1989; Zegwaard *et al.*, 2000; Di *et al.*, 2001; Nie *et al.*, 2001; Menneer *et al.*, 2005a). However, treading under actual grazing conditions is repeated numerous times throughout the year (at each grazing) but is generally less severe, because most farmers attempt to avoid grazing when soil is too wet (Creighton *et al.*, 2011). Hence there is a need to investigate treading damage under actual grazing conditions, particularly in grazing systems based on grass-clover swards, given the possibility that clover is more susceptible to treading damage. Therefore, the objective of this experiment was to investigate the effects of treading by grazing dairy cows on soil properties and herbage production within three white clover-based grazing systems for dairy production on a soil with impeded drainage in Ireland.

6.2. Materials and Methods

6.2.1. Site characteristics

This experiment was conducted between February 2008 and February 2010 at Solohead Research Farm in Ireland (52˚51'N, 08˚21'W, 95 m above sea level). The soils are composed of 90% poorly drained gleys and 10% grey-brown podzolics overlaying Devonian sandstone. Soil depth ranged from 5 to 10 m. There is a perched water table ranging between 0 to 2.2 m below ground level. Drainage is impeded, which contributes to waterlogged conditions under high rainfall. The soil has a clay-loam texture of 36% sand and 28% clay in the A1 horizon. Soil organic matter content was 13% and soil pH was 6.2 in the top 20 cm of soil (O'Connell, 2005). The land has been under permanent grassland for over 50 years. It was reseeded with perennial ryegrass (*Lolium perenne*) between 1985 and 1995. Between 2001 and 2006 the grassland was oversown with white clover (*Trifolium repens*) as described by Healy *et al.* (2006). This method involved overcasting clover seed into the existing grass swards at the rate of 5 kg of clover seed per ha^{-1}, using a fertilizer spreader after grazing or silage cutting in spring. The grassland is used primarily for grazing by dairy cows and to a lesser extent for silage production. Soil temperature (ºC at 10 cm depth), rainfall amounts (mm) and dielectric soil moisture (v v^{-1} to 5 cm depth) were measured every 30 minutes at an automatic meteorological station on the farm (Campbell scientific Ltd, Loughborough, U.K.).

6.2.2. Experimental design

Three grazing management systems for dairy production from grass-clover swards in Ireland were established in 2008 in a complete randomised block design. Treading subplots were included in 2009 as a split-plot design with grazing system as the main plot and treading level as the subplot. The grazing systems and treading level subplots are described below and in Table 6.1.

6.2.3. Grazing systems

1. ES-100N: Early-Spring turnout with annual fertilizer input of 100 kg N ha^{-1}. This grazing system was typical of that recommended for conventional (non-organic) dairy production from cows grazing grass-clover swards in Ireland (Humphreys *et al.*, 2009a). The mean calving date was the 17 February and cows were primarily at pasture from February to November. Overall stocking rate was 2.1 cows ha^{-1}.

2. ES-0N: Early-Spring turnout with no fertilizer-N input. This grazing system was an example of organic dairy production from cows grazing grass-clover swards in Ireland. The mean calving date, turnout date and housing date were similar to the ES-100N system but the overall stocking rate was lower (1.6 cows ha^{-1}) as is typical to account for the lack of fertilizer-N input in organic systems (Humphreys *et al.*,

2009a).

3. LS-0N: Late-Spring turnout with no fertilizer-N input. This grazing system was an alternative example for organic dairy production from cows grazing grass-clover swards in Ireland. This system had a later calving date (12 April) to account for the lower supply of herbage in early spring that occurs in the absence of fertilizer-N applications in organic grazing systems. The overall stocking rate was 1.7 cows ha^{-1} between April and September. However, this was reduced to 1.3 cows ha^{-1} between October and January because extra land was included in the grazing area in order to extend the length of the grazing period and reflect the movement of calves/heifers off the main grazing block in such a system (see text below and Table 6.1).

Each grazing system was established in January 2008 and had 18 Holstein-Friesian dairy cows randomly assigned from groups based on lactation number (1, 2, 3 and ≥ 4) with a mean body weight throughout the experiment of 585 kg (SD = 58 kg). The land area used in the experiment had been divided into six blocks, each of which contained three paddocks. One paddock from each block was randomly assigned to each grazing system, and paddock sizes were adjusted to achieve the land areas that determined the stocking rates (Table 6.1). Mean paddock sizes were 1.42, 1.88 and 1.76 ha^{-1} (SD = 0.30) in the ES-100N, ES-0N and LS-0N, respectively. The land area of each system is shown in Table 6.1 and the overall stocking rates were calculated by dividing the number of cows by the respective land area. However, immediate stocking rates within each system changed over time as land was removed for silage production (Table 6.1). The stocking rate in the LS-0N was further complicated by the fact that an extra three paddocks (totalling 3.7 ha) were added between November and January (see footnotes at Table 6.1). These paddocks were managed similarly to the other paddocks in that system and were not included in any analyses or results.

Table 6.1. Details of the three white clover-based grazing systems investigated for treading damage in 2009. Each system had 18 Holstein-Friesian dairy cows grazing grass-clover swards.

Year		**2008**			**2009**	
Grazing system	**ES-100N**	**ES-0N**	**LS-0N**	**ES-100N**	**ES-0N**	**LS-0N**
Start of grazing year	21 Feb	19 Feb	16 Apr	20 Feb	22 Feb	15 April
End of grazing year	12 Nov	12 Nov	14 Jan	18 Nov	18 Nov	26 Jan
Mean days* grazing cow^{-1}	221	234	231	225	234	235
Total land area (ha)	8.5	11.3	10.5/14.2†	8.5	11.3	10.5/14.2†
Overall stocking rate (cows ha^{-1})	2.1	1.6	1.7/1.3†	2.1	1.6	1.7/1.3†
Monthly stocking rates when removal of silage areas were accounted for (cows ha^{-1})						
Feb-Mar	2.1	1.6		2.1	1.6	
Apr-May	4.2	2.3	3.4	3.4	2.0	3.2
Jun-Jul	2.1	2.8	2.3	2.4	2.4	3.3
Aug-Sep	2.1	1.6	1.7	2.1	1.6	1.7
Oct-Nov	2.1	1.6	1.3†	2.1	1.6	1.3†
Dec-Jan			1.3†			1.3†
Mean	2.5	2.0	2.0	2.4	1.8	2.2
Proportion of area harvested for silage						
1st cut (Apr – May)	0.5	0.3	0.5	0.4	0.2	0.5
2nd cut (Jun – Jul)	0.0	0.4	0.2	0.1	0.3	0.5
N-inputs (kg ha^{-1})‡						
Fertilizer	105	-	-	96	-	-
Slurry	90	65	65	93	69	65
Total	195	65	65	189	69	65

* 24 hr periods, a value of 0.5 was used when cows were kept in at night. † From September to January each year, an extra 3.7 ha was included in the LS-0N system. During the rest of the year this area was used for grazing heifers/calves and the area was not included in any analyses or results. ‡ Does not include biological N-fixation (see Table 6.3).

The cows were turned out to graze after calving in spring and generally remained outside until drying off in the following winter. However, cows were housed by night when ground conditions were too wet (surface water visible in large parts of the grazing area) or when herbage supply was too low (available pre-grazing herbage masses < 500 kg DM ha^{-1}). These practices were observed in order to mimic typical grazing management practices on farms. The total annual amount of time spent at pasture in each system is shown in Table 6.1. Strip grazing with temporary fencing was practiced in all three systems with each group of 18 cows allocated approximately 48 hours of grazing at each time. Each herd was moved to the next grazing strip when a post-grazing height of approximately 5 cm was reached, as measured twice per day with 50 drops of a Filips rising plate meter (www.grasstec.ie). A back-fence was used to stop cows returning to previously grazed areas. Excess herbage production was removed as baled silage (Table 6.1). Any slurry that was produced when the cows were housed was stored together and applied to the grassland during the following year on a proportional basis to stocking rates (see Table 6.1) as described by Humphreys *et al.* (2008). In the ES-100N system, fertilizer N was applied in the form of urea between February and April and calcium ammonium nitrate (CAN) during May.

6.2.4. Treading level subplots

Exclusion plots were installed in five paddocks from each of the three systems at the end of the first grazing year for each system (see Table 6.1). Treading levels were not implemented in the first year in order to establish the different grazing systems, and the herbage and soil conditions they represent. Each rectangular exclusion plot measured 1 m × 20 m, consisted of a wooden post at each corner and was surrounded by a single electrified wire at 1 m height above ground. Having one strand of wire at this height prohibited cows from walking on the exclusion plots but allowed them to fully graze the area within by enabling them to put their heads under the wire. Each exclusion plot was positioned perpendicular to, and 10 m distance from, the nearest paddock boundary. At initial set-up, it was observed that a greater amount of treading occurred in the immediate vicinity of the exclusion plots as the cows circumvented the exclusion plot fence. Therefore three levels of treading were investigated as subplots within each paddock in 2009:

1. The non-treaded, but grazed area within the exclusion plots (NT).

2. The highly treaded area within 2 m of each exclusion plot (HT).

3. The area further (> 2 m) from the exclusion plot with treading levels representative of normal grazing conditions in the paddock (T).

Exclusion plot fences were temporarily removed for all machinery operations. Areas that were visibly soiled with dung after grazing were marked with wooden stakes throughout the experiment and avoided when sampling herbage.

6.2.5. Experimental measurements

6.2.6. Soil properties

Soil bulk density (SBD; the mass of soil per unit volume of a soil core), volumetric water content (VWC; the mass of water per unit volume of a soil core), gravimetric water content (GWC; the mass of water per mass of soil) and air-filled porosity (AFP; the proportion of total porosity occupied by air) were calculated using the equations of Carter and Gregorich (2008) on the 28 July 2009 and the 20 February 2010. Cylindrical stainless steel cores (8 cm diameter × 5 cm) were used to extract soil samples from the layer 5 to 10 cm below the surface as this depth has been found to be the most appropriate for measuring changes to soil properties under treading by cows on grassland (Singleton and Addison, 1999). Ten such samples were randomly taken from each treading sub-plot in each paddock on each date. Samples were saturated and allowed to drain at 10 to 15°C for 5 hours. The wet weights and volumes were then recorded and samples were dried in a forced-draught oven for 72 hrs at 105 ºC. Any stones > 0.2 cm diameter were sieved out, weighed and volume determined by graduated cylinder water displacement. Air-filled porosity (AFP) is closely related to large soil pores (macropores) and was calculated using the following equation from Carter and Gregorich (2008): $AFP = (1 - \frac{SBD}{2.65} \times 100) - (GWC \times SBD)$ where SBD = soil bulk density (g cm^{-3}), VWC = volumetric water content (g cm^{-3}), GWC = gravimetric water content (g g^{-1}).

6.2.7. Soil deformation

Soil surface deformation (cm m^{-1}) was recorded after each grazing/treading event in 2009 by measuring the reduction in chain length when fitted to the contours of the soil surface (Saleh, 1994). Two such measurements were taken from random locations within each subplot after each grazing event using a chain attached to a wooden strut, both 2 m in length. Hoof print depth (cm) was also measured with a ruler from 10 random hoof prints within each subplot after each grazing/treading event. Cow hours per ha and subsequent length of recovery period (the time between successive grazing events for each paddock) were recorded throughout the experiment.

6.2.8. Water table depth

Shallow groundwater table depth (m below soil surface) was measured every 14 days from sampling wells in close proximity (< 5m) to each exclusion plot. These wells consisted of plastic pipes (0.42 cm diameter) inserted into shallow boreholes up to 2.4 m deep. The pipes extended 0.5 m above the soil surface and any space between the pipes and the surrounding soil profile was filled with sand and bentonite. Measurements were conducted with a Geosense electric water level meter with acoustic signal (Marton Geotechnical Services Ltd, Suffolk, U.K.).

6.2.9. Herbage production

Pre-grazing herbage mass in each paddock was measured throughout 2008 by cutting four strips (each 5 m × 0.55 m wide) using a HRH-536 lawn-mower (Honda®, Alpharetta, Georgia, U.S.A.) at a cutting height of 5 cm above ground level. Prior to silage-cutting, an Agria auto-scythe (Etesia U.K. Ltd., Warwick, U.K.) was used to cut three 5 m × 1.1 m strips. In both cases, cut herbage was bulked and a 100 g dried in a force-draught oven for 16 hours at 100 ºC for dry matter (DM) determination. Annual herbage production was calculated as the summed pre-grazing and pre-silage herbage DM masses for each paddock.

After the exclusion plots were installed at the end of 2008, herbage mass was also measured by cutting four quadrats (each 0.5 m × 0.5 m) at a height of 5 cm above ground from all sub-plots with an electric hand shears (Accu-shears Gardena®, Ulm, Germany) prior to each grazing and silage harvest. All cut herbage was bulked, dried for

DM and herbage production calculated as described above.

Post-grazing height was measured from each subplot with 30 drops of a Filips rising plate meter (www.grasstec.ie). The proportional reduction in pre-grazing herbage mass > 5 cm (PRHM) following each treading event was calculated in the T and HT subplots relative to the NT subplots as: $PRHM = \left(\frac{HMtr}{HMnt}\right) - 1$ where PRHM was the proportional difference, HMtr was the herbage mass (kg DM ha^{-1}) in the treaded (T and HT) subplots and HMnt was the herbage mass in the non-treaded (NT) subplot. This calculation was conducted on all pre-grazing herbage masses following a previous grazing/treading event.

6.2.10. White clover content in herbage, stolon and root mass, and BNF

White clover content of herbage in each paddock was measured from 30 herbage snips (each 10 cm × 30 cm) randomly taken with an electric hand shears (Accu-shears Gardena®, Ulm, Germany). This measurement was taken in April, June, August and November of each year. In 2009, ten such snips were also taken from each of the treading sub-plots in each of these months. All samples were manually separated into white clover or grass species and DM analysed as described for herbage production above. White clover stolon and root mass was measured in February, May, August and November of each year by cutting 30 random sods (each measuring 10 cm × 10 cm) to a depth of approximately 8 cm from each paddock. In 2009, ten such sods were also taken from each of the treading sub-plots on each sampling date. Stolons and attached roots were manually separated from the sods, washed and analysed for DM as described above. Biological N fixation (BNF) was estimated from annual clover herbage production using the model by Hogh-Jensen *et al.* (2004).

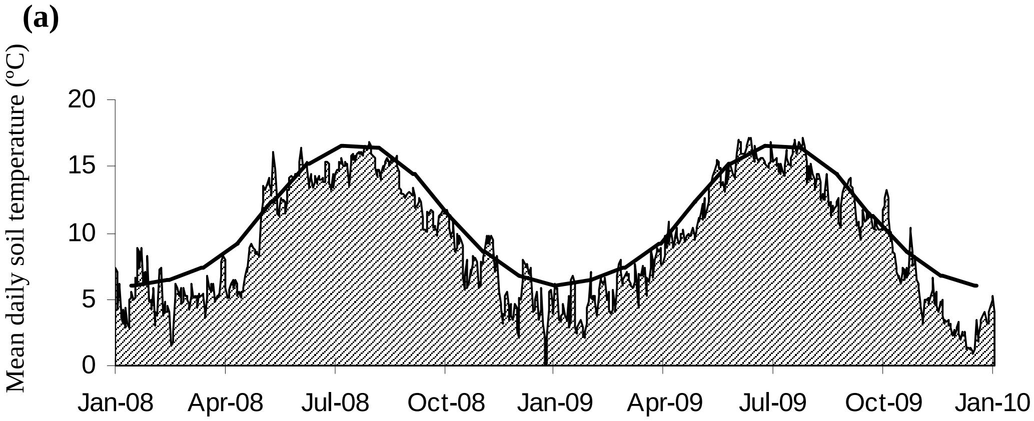
6.2.11. Statistical analysis

Two separate analyses of variance (ANOVAs) were conducted with the proc mixed procedure in SAS (SAS, 2006) to establish the effects of (i) grazing system and (ii) treading damage on all results. The effect of grazing system was analyzed using the following model: $X_{jkl} = \mu + S_j + Y_k + M_l + SY_{jk} + SM_{jl} + YM_{kl} + STM_{jkl} + e_{jkl}$ where X_{jkl} = the mean dependent variable, μ = the overall mean, S_j = the fixed effect of the jth grazing system, Y_k = the fixed effect of the kth year, M_l = the fixed effect of the lth month and e_{jkl} = residual error term. Year and month were entered as repeat measures using the un@cs covariance structure as recommended by Moser (2004). The effect of treading damage was analyzed using the following model: $X_{jkl} = \mu + S_j + T_l + M_k + SM_{jk} + TM_{lk} + e_{jkl}$ where T_l = the fixed effect of the lth treading level and all other abbreviations are the same as for the first model above. To account for the split-plot design, treading level was entered as a subplot (repeated measure) within each paddock (Singer, 1998). All herbage production results from the 'normal' treading level (T) sub-plots were numerically and statistically similar to the paddock scale results in 2009 (P > 0.15). Therefore the paddock-scale results in 2008 are representative of treading level T for that year and are labelled as such for simplicity in presenting of the results. The significance of any correlations within the results were analyzed with linear, log-linear or polynomial regression through analyses of covariance in proc mixed MIXED with the variable in question included as a covariate and a random intercept defined for month in the case of repeat measures (Singer, 1998; Moser, 2004).

6.3. Results

6.3.1. Meteorological data

Mean daily soil temperature and monthly rainfall amounts for the experimental period and the previous ten-year means are shown in Figure 6.1. Both years of the experiment had lower mean soil temperatures and higher rainfall amounts than any of the previous ten years. Mean annual soil temperature was 9.5 ºC in 2008 and 9.6 ºC in 2009, whereas it ranged from 10.0 to 11.8 ºC (mean = 10.9 ºC) in the previous ten-year period. Total rainfall was 1,228 mm in 2008 and 1,296 mm in 2009, whereas it ranged from 797 to 1,150 mm (mean = 1,009 mm) in the previous ten-year period. Monthly rainfall amounts were particularly higher than the ten year means in the summer months of both years (Figure 6.1a). However, some months, such as February and December in both years had lower rainfall amounts than the previous ten year means (Figure 6.1b).



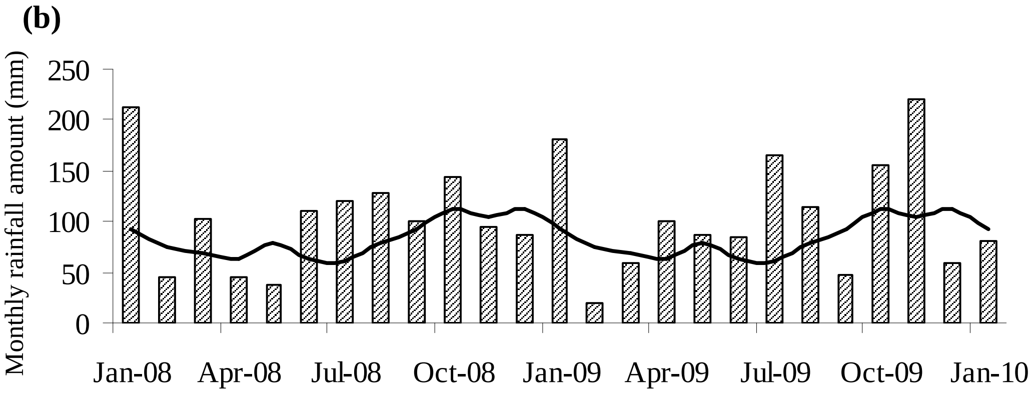


Figure 6.1. Mean daily soil temperature (a: ºC at 10 cm below soil surface) and monthly rainfall amounts (b: mm month-1) recorded throughout 2008 and 2009 at a meteorological station at Solohead Research farm. The solid black lines show the previous 10-year mean values.

6.3.2. Soil properties

Soil in both T and HT had higher bulk density, lower air-filled porosity and lower gravimetric water content than in NT ($P < 0.001$, Figure 6.2). Volumetric water content was not significantly affected by treading level. Samples taken in February 2010 had higher bulk density, higher air-filled porosity, lower gravimetric water content and lower volumetric water content than samples taken in the previous June 2009 ($P < 0.05$, Figure 6.3). The soil displayed strong shrink-swell characteristics across treatments with soil gravimetric water content being negatively correlated with soil bulk density (y =

1.21x - 1.68, R^2 = 0.94, P < 0.001, not shown). There were no differences between grazing systems for any of the soil properties (P > 0.05).

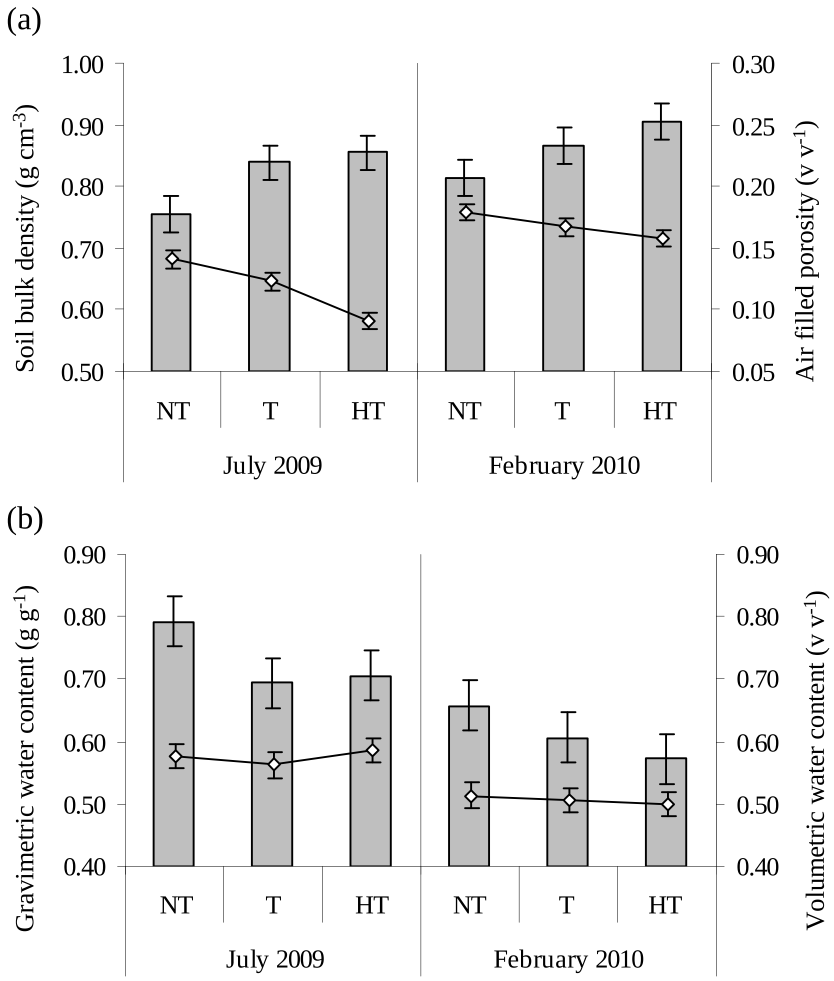


Figure 6.2. Effect of treading level on soil bulk density (columns in a, P < 0.001), air-filled porosity (lines in a, P < 0.001), soil gravimetric water content (columns in b, P < 0.001) and volumetric soil water content (lines in b, P > 0.05) for July 2009 and February 2010. Error bars show the treading level SEM. Treading levels: non-treaded (NT), treaded (T) and highly treaded (HT).

6.3.3. Soil deformation

Soil surface deformation was correlated with hoof print depth (y = 1.41x + 2.45, R^2 = 0.58, P < 0.001, not shown) and neither were significantly affected by grazing system or treading level. However, both were affected by month (P < 0.001, Figure 6.3). Hoof print depth followed the seasonal pattern of dielectric soil moisture as measured at the meteorological station, with the highest monthly means in spring (February to May), and in the following winter (November to January) periods, when soil moisture was generally greater than 0.60 v v^{-1} (Figure 6.3). Soil surface deformation followed a similar trend but tended to be higher in spring than in winter, despite similar soil moisture levels (Figure 6.3).

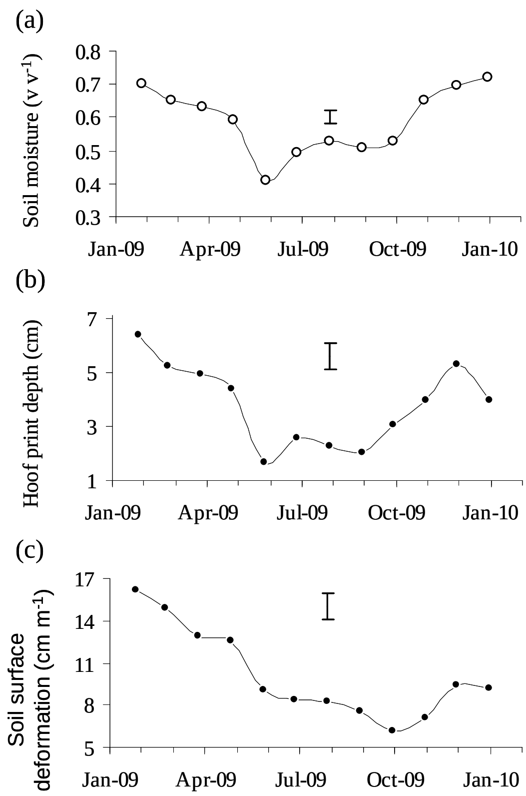


Figure 6.3. Changes over time in dielectrically measured soil moisture (a, P < 0.001), hoof print depth (b, P < 0.001) and soil surface deformation (c, P < 0.001). Values are monthly means and error bars show the SEM of month.

6.3.4. Water table depth

Water table depth fluctuated throughout the year with significant differences between months, but no clear seasonal effect (Figure 6.4). Annual mean values for soil bulk density, gravimetric water content, hoof print depth, and soil surface deformation were all significantly correlated with the mean water table depth in each paddock (Figure 6.5 and 6.6). The relationships were improved with the addition of a quadratic function (F-test: $P < 0.05$), indicating a reduced influence of water table depth when it was more than 1 m below the soil surface.

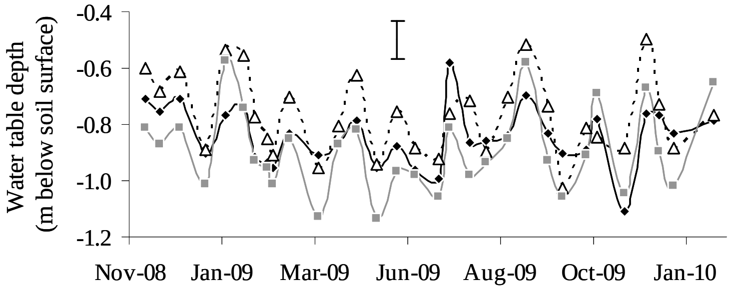


Figure 6.4. Sampling date and mean water table depth within each of the three grazing systems, ES-100N (◆), ES-0N (■) and LS-0N (△). See Table 6.1 for description of the systems. Error bars show the sampling date SEM ($P > 0.001$). Grazing system had no effect ($P < 0.05$).

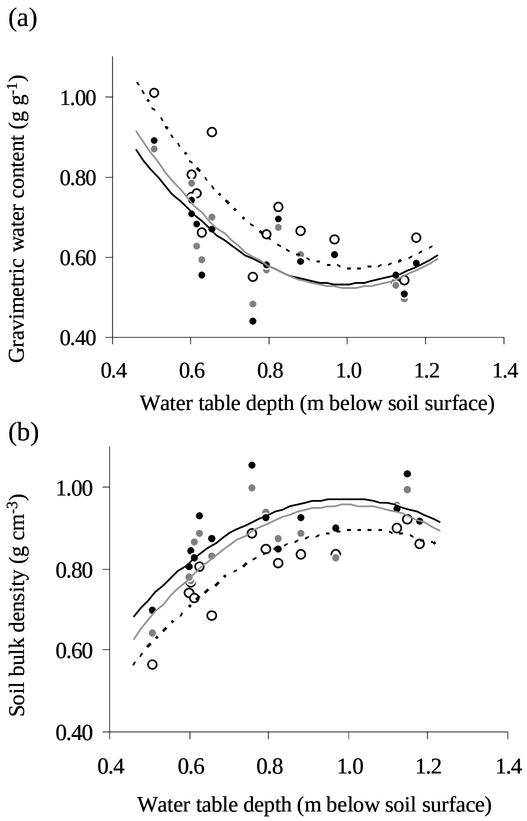


Figure 6.5. The relationships between mean annual water table depth and: (a) mean gravimetric water content for non-treaded (NT, ○, $y = -1.46x^2 + 2.99x - 2.10$, $R^2 = 0.72$, $P < 0.001$) treaded (T, ●, $y = -1.33x^2 + 2.67x - 1.86$, $R^2 = 0.70$, $P < 0.01$) and highly treaded (HT, ●, $y = -1.19x^2 + 2.35x - 1.70$, $R^2 = 0.61$, $P < 0.05$); (b) mean soil bulk density for NT ($y = -1.00x^2 + 2.07x – 0.18$, $R^2 = 0.81$, $P < 0.001$) T ($y = -1.14x^2 + 2.27x - 0.17$, $R^2 = 0.68$, $P < 0.01$) and HT ($y = -1.01x^2 + 2.01 – 0.03$, $R^2 = 0.62$, $P < 0.05$).

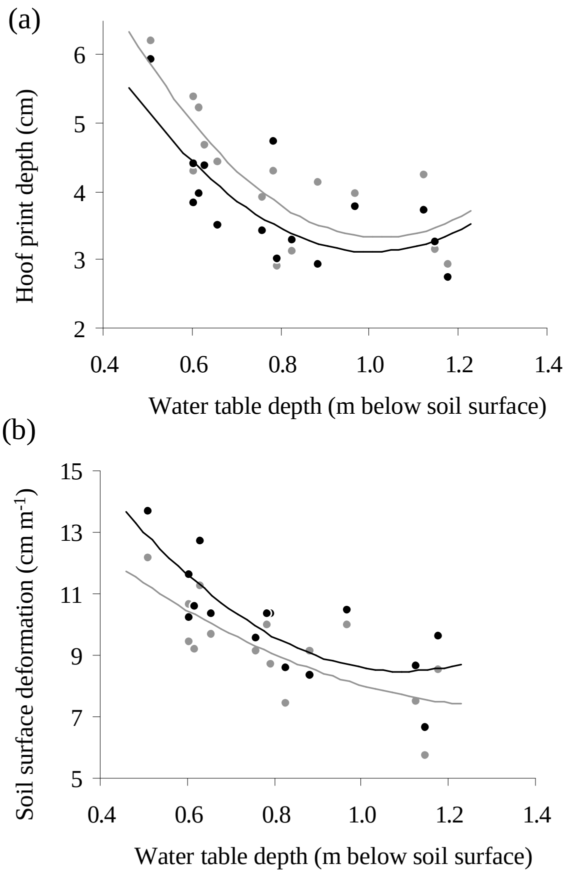


Figure 6.6. The relationships between mean annual water table depth and: (a) mean annual hoof print depth for treaded (T, ●, $y = 9.46x^2 – 19.37 + 13.2$, $R^2 = 0.66$, $P < 0.01$) and highly treaded (HT, ●, $y = 8.08x^2 – 16.25 + 11.29$, $R^2 = 0.54$, $P < 0.05$); (b) mean annual soil surface deformation for T ($y = 5.30x^2 – 14.56x +17.29$, $R^2 = 0.56$, $P < 0.05$) and HT ($y = 12.77 – 27.98x + 23.81$, $R^2 = 0.63$, $P < 0.01$).

6.3.5. Clover content, and stolon and root mass

White clover content of herbage was affected by an interaction between treading level and month, being lower ($P < 0.01$) in T and HT than NT for June but not for any of the other sampling dates (Table 6.2). Treading level had no effect on clover stolon mass or mean annual clover content.

Clover content was affected by an interaction between grazing system, month and year (P < 0.001, Table 6.2). In 2008 ES-100N had lower clover content than the other two systems in June whereas in 2009 ES-100N had lower clover contents in both June and August (P < 0.01, Table 6.2). The ES-0N and LS-0N had similar clover contents throughout 2008 but LS-0N had higher clover content in August 2009 (P < 0.01, Table 6.2). Clover stolon mass was also affected by an interaction between grazing system and year, being similar across systems in 2008, but significantly lower in ES-100N in 2009 (P < 0.01, Table 6.2).

Table 6.2. The effect of grazing system and treading level on white clover content of herbage dry matter (DM) and mass of white clover stolon and root DM ha-1. See Table 6.1 for description of grazing systems. Treading levels: non-treaded (NT), treaded (T) and highly treaded (HT).

Grazing system:		ES-100N			ES-0N			LS-0N		
Treading level:		**NT**	**T**	**HT**	**NT**	**T**	**HT**	**NT**	**T**	**HT**
		Mean clover content of herbage DM (g kg^{-1})								
2008	April		109			233			161	
	June		285			390			393	
	August		301			326			258	
	November		115			152			117	
	Mean		203			275			232	
2009	April	64	58	33	101	62	93	164	119	123
	June	257	165	186	360	322	300	364	294	305
	August	348	321	323	474	421	509	531	557	565
	November	122	112	129	148	161	197	152	156	155
	Mean	198	164	168	271	242	275	303	282	287
		Mean clover stolon and root mass (kg DM ha^{-1})								
2008	February		769			904			705	
	March		737			969			888	
	August		599			785			778	
	November		632			934			694	
	Mean		684			898			766	
2009	February	468	339	474	470	527	451	993	608	573
	March	425	344	260	651	710	628	798	797	781
	August	661	581	661	954	807	966	1083	1066	1252
	November	537	610	693	971	885	977	977	1052	1180
	Mean	523	469	522	762	732	756	963	881	947

S.E. of the means

	Clover content	**Stolon /root mass**		**Clover content**	**Stolon/root mass**
System	25.8 *	43.3 ***	Year × Month	23.4 ***	69.6 **
Year	16.8	34.8	System × Year × Month	40.6 ***	120.5
Month	19.9 ***	50.6	Treading	16.6	42.2
System × Year	29.1 *	60.3 **	Treading × Month	24.4 **	85.1
System × Month	34.5	86.7			

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

6.3.6. Effect of grazing system on herbage production and BNF

Annual herbage DM production was lower in ES-0N than the other two systems in both years (P < 0.05, Table 6.3). Annual grass herbage DM production was higher (P < 0.001) in ES-100N than in the other two systems in both years. Clover herbage production was affected by a significant interaction between grazing system and year, being similar across all three systems in 2008 (P > 0.05) and with lower (P < 0.001) clover production in ES-100N in 2009 (Table 6.3). The BNF estimates were also lower in ES-100N in 2009 (P < 0.05, Table 6.3). Post-grazing sward height was not affected by grazing system (mean = 4.97 cm, SEM = 0.042, P > 0.05).

Table 6.3. The effect of grazing system and treading level on herbage dry matter (DM) production (t ha-1) and estimated biological N fixation (BNF, kg ha-1). See Table 6.1 for description of grazing systems. Treading levels: non-treaded (NT), treaded (T) and highly treaded (HT).

Grazing system:		**ES-100N**			**ES-0N**			**LS-0N**		
Treading level:		**NT**	**T**	**HT**	**NT**	**T**	**HT**	**NT**	**T**	**HT**
					Mean herbage production (t ha^{-1})					
2008	Grass		8.22			6.41			7.11	
	Clover		2.13			2.47			2.07	
	Total		10.35			8.88			9.18	
2009	Grass	9.18	8.18	8.24	6.40	5.83	5.66	6.82	6.73	6.60
	Clover	2.12	1.69	1.71	3.16	2.80	2.74	4.04	3.58	3.42
	Total	11.30	9.88	9.94	9.56	8.63	8.39	10.86	10.31	10.02
					Mean BNF (kg N ha^{-1})					
2008			113			131			110	
2009		112	90	91	168	149	145	215	191	182

S.E. of the means

	Grass	**Clover**	**Total herbage**	**BNF**
System	0.293 ***	0.262 *	0.389 *	16.6 *
Year	0.239	0.137	0.319	10.8 *
System × Year	0.414	0.373 *	0.553	18.8 **
Treading	0.216 ***	0.230 ***	0.320 *	11.9 ***

* P < 0.05, ** P < 0.01, *** P < 0.001

6.3.7. Effect of treading on annual herbage production and BNF

Total herbage production in 2009 was lower ($P < 0.001$) in the T and HT subplots than in the NT subplots in the two early calving systems (ES-100N and ES-0N) but not in the late calving system (LS-0N, Table 6.3). Grass herbage DM production was affected by a similarly affected, with less grass grown in the treaded subplots (T and HT) in ES-100N and ES-0N but with no effect of treading in LS-0N (Table 6.3). Clover herbage production and BNF were reduced (P < 0.001) by treading in all three systems (Table 6.3).

The proportional reduction in herbage DM mass (PRHM) after each treading event was significantly affected ($P < 0.001$) by month with the greatest reduction occurring after treading in February, March and April (Figure 6.7). Analysis of covariance revealed that PRHM was not significantly affected by the number of cow hours per ha at each grazing but was negatively related ($P < 0.001$) to soil surface deformation (Figure 6.8a) and positively related ($P < 0.05$) to the recovery period (interval between successive grazing/treading events for each paddock, Figure 6.8b). The latter was non-linear and required log transformation. Recovery periods increased throughout the year and were therefore shorter in spring (March to May) than in autumn/winter (September to January). LS-0N had significantly longer recovery periods (61 days) throughout the experiment than ES-100N (36 days) and ES-0N (34 days, SEM = 8.9). Post-grazing sward height was not affected by treading level (mean = 4.97 cm, SEM = 0.042, $P > 0.05$).

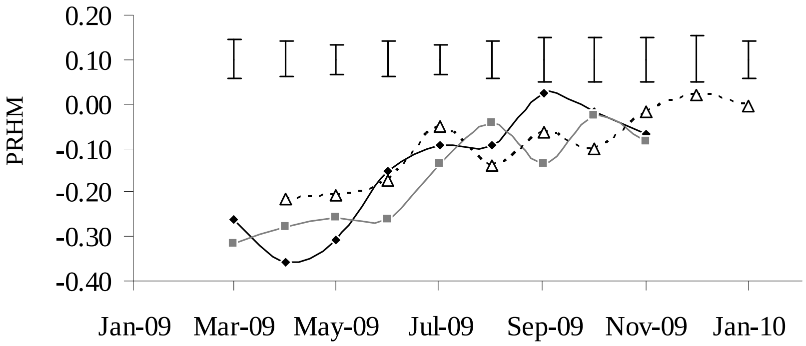


Figure 6.7. Effect of month ($P < 0.001$) and grazing system ($P > 0.05$) on the proportional reduction of pre-grazing herbage DM mass (PRHM) in treaded plots relative to non treaded plots following each grazing event: ES-100N (◆), ES-0N (■) and LS-0N (△). See Table 6.1 for description of grazing systems. Error bars show the SEM of sampling period (month).

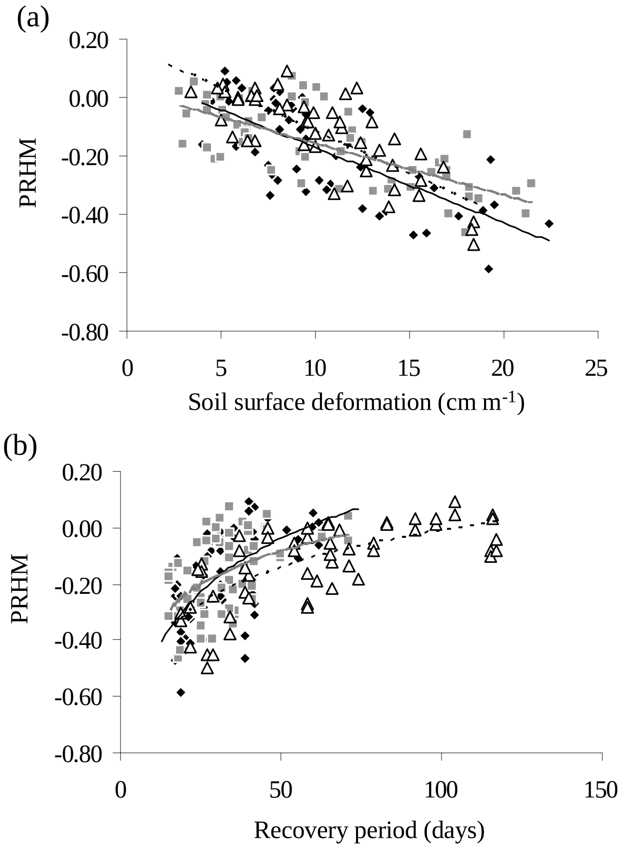


Figure 6.8. Relationships between the proportional reduction in pre-grazing herbage DM mass (PRHM) following each treading event (relative to non treaded subplots) and: (a) soil surface deformation for ES-100N (◆, y = -2.58 + 8.55, R^2 = 0.49, $P < 0.001$), ES-0N (■, y = -1.78x + 2.56, R^2 = 0.51, $P < 0.001$ and LS-0N (△, y = -2.92x + 17.56, R^2 = 0.62, $P < 0.001$); (b) recovery period after treading: ES-100N (y = 27(Ln)x - 110, R^2 = 0.40, $P < 0.01$), ES-0N (y = 17(Ln)x - 75, R^2 = 0.23, $P < 0.05$) and LS-0N (y = 19(Ln)x - 88, R^2 = 0.50, $P < 0.01$). See Table 6.1 for description of grazing systems.

6.4. Discussion

6.4.1. Effect of treading on soil properties

Treading by dairy cows under all three grazing systems caused compaction of the soil, as evidenced by the increase in bulk density. Total soil porosity is a simple inverse function of soil bulk density for a given soil (Carter and Gregorich, 2008) and therefore, was not presented here. However, air-filled porosity is the proportion of total soil porosity that is occupied by air and is particularly important for soil drainage because it is closely associated with large soil pore spaces that have diameters > 30 μm (macropores). These macropores are essential conduits for air and water to plant roots, as well as for drainage through the soil layers (Pietola *et al.*, 2005; Drewry *et al.*, 2008). In contrast smaller pores (micropores) tend to withhold water and slow down soil drainage through capillary action. Therefore, the large reduction in air-filled porosity in HT in July 2009 has clear negative implications for grazing management on wet soils, where maintaining soil drainage is essential.

Optimum air-filled porosity for pasture production is generally between 0.15 to 0.20 (Drewry *et al.*, 2008) and a critical minimum value is 0.10, below which diffusion of air to plant roots can become limiting (Lipiec and Hakansson, 2000). In the present experiment, mean air-filled porosity was lower than 0.10 when sampled in July 2009, which would have restricted grass and clover herbage production. However, by the following February (2010), mean air-filled porosity had recovered to within the above optimum values in all treading treatments and the effect of treading on the other soil properties was less pronounced (Figure 6.3), indicating some natural recovery of the soil from the treading damage accrued during the main grazing season (Wheeler *et al.*, 2002).

The strong shrink-swell characteristics displayed by the soil in the current experiment does suggest large potential for soil recovery during wetting-drying cycles (Boivin *et al.*, 2004; O'Keefe, 2009). This shrink-swell soil capacity and lack of dry conditions or high bulk density values (compared with those outlined in the review by Drewry *et al.* (2008)) also indicated low mechanical impedance of root growth or earthworm movement, which would have helped to ameliorate the negative effects of treading. The shrink-swell capacity of soil is associated with high clay and organic matter concentrations and can increase a soil’s ability to recover from compaction (Boivin *et al.*, 2004; Zhang *et al.*, 2005). The absence of cultivation of soil under permanent grassland results in high accumulations of soil organic matter, particularly in areas with high rainfall, cool temperatures and heavy textured soils (Haynes, 1986). This experiment demonstrates that such soil is likely to become compacted under rotational grazing but can also recover substantially by the start of the following grazing season. Maintaining the organic matter content of such soils is therefore important for their ability to recover from treading damage.

6.4.2. Effect of water table and soil moisture on susceptibility to treading

Soil resistance to penetration increases with soil bulk density (Carter, 1990; Vaz *et al.*, 2001). As the water table approached the surface in the current experiment, bulk density decreased and gravimetric water content increased, resulting in lower resistance to penetration and higher susceptibility to treading, as evidenced in the relationships between water table depth and (a) hoof print depth and (b) soil surface deformation (Figure 6.7). These relationships highlight the importance of water table depth and land drainage on susceptibility to treading damage in these soils. Historically, Ireland has had a low level of artificial drainage relative to its European counterparts. Approximately 0.20 of the utilized agricultural area in Ireland has undergone field drainage, compared with 0.65 in the U.K and 0.74 in the Netherlands (Collins and Cummins, 1996). The results of this experiment show that increased drainage and lowering of the water table has potential to reduce localised susceptibility to treading damage. The relationships in Figure 6.7 indicate that a water table depth of 1 m below the soil surface was optimal to reduce susceptibility to treading damage. Further research is required to assess these relationships and their mechanisms over different soil types and in different years. The effect that such drainage would have on soil organic matter and plant growth also needs to be investigated.

Hoof print depth followed the same monthly trend as soil moisture measured at the meteorological station reflecting the effect of soil moisture on soil resistance to penetration (Vaz *et al.*, 2001). Soil surface deformation did not follow the same trend and was lower in autumn than in spring, despite similar soil moistures/water table depths. The reasons for this difference need further research and may be due to seasonal changes in animal behaviour (Linnane *et al.*, 2001), sward structure (Brock *et al.*, 1996), root mass (Tisdall and Oades, 1979) or soil properties (Wheeler *et al.*, 2002).

6.4.3. Herbage production and clover content in the grazing systems

Herbage production in ES-100N was lower in the current experiment than in previous years at this site. In comparison, Humphreys *et al*. (2009) recorded a mean annual herbage DM production of 11.5 t ha^{-1} with an ES-100N grazing system between 2003 and 2006. The lower values (9.8 to 10.3 t ha^{-1}) in the current experiment are reflective of the lower soil temperatures and higher rainfall experienced in 2008 and 2009.

Although there were differences in grass and clover herbage production between the grazing systems, attributing these differences to specific factors such as N-fertilizer use or winter grazing is problematic because the design was not factorial and stocking rate also differed between the systems. However, a large amount of previous research has already shown that fertilizer-N applications increase grass production and reduce clover contents in grass-clover swards, leading to lower clover herbage production in subsequent years (Frame, 1987; Davies and Evans, 1990; Ledgard *et al.*, 1996; Laidlaw and Withers, 1998; Griffith *et al.*, 2000; Davies, 2001; Loiseau *et al.*, 2001). Similarly, a large amount of research has also found that winter grazing can reduce competition from grass and thereby increase clover contents and herbage production in subsequent years (Davidson and Robson, 1986; Laidlaw and Stewart, 1987; Holmes *et al.*, 1992; Laidlaw *et al.*, 1992). Therefore, it is most likely that the lower clover production in the ES-100N and the higher clover production in the LS-0N were due to N-fertilizer use and winter grazing, respectively and it can be concluded that an LS-0N type system is most likely to promote sward clover content, BNF and herbage production when compared to an ES-100N type system.

6.4.4. Effect of treading on white clover competitiveness and BNF

In this experiment, treading reduced clover content by 0.21 in June but did not affect it at other times of the year or result in significant annual differences in clover content. White clover stolon and root mass were also similar across treading treatments throughout the experiment. Menneer *et al.* (2005a) recorded much larger changes in clover content (reduced by 0.70 for over 100 days) following a single treading event in spring. This can be explained by the much more severe treading damage in the experiment by Menneer *et al.* (2005a) which involved treading by the equivalent of 450 cows ha^{-1} for 2.5 hrs. In comparison, each individual treading/grazing event in the present experiment had a mean temporary stocking rate of approximately 25 cows per ha for 44 hours, which is more representative of typical farm practice. When at pasture full-time (22 hours per day), dairy cows only actively graze for approximately 9 hours (Kennedy *et al.*, 2011). In comparison, the cows in the experiment of Menneer *et al*. (2005a) were manually herded up and down the plots for the duration of treatments and pressure exerted under the hooves of a walking dairy cow can be more than double that of the same cow when standing (Scholefield *et al.*, 1985). Therefore, the lower effect of treading on clover content and stolon mass in the current experiment can be attributed to the lower amount of treading damage done under typical grazing conditions. Under these conditions, the current experiment found that treading reduced both clover and grass annual herbage production by similar amounts (0.45 and 0.59 t ha^{-1}, respectively).

Another experiment that suggested susceptibility of white clover to treading was that of Grant *et al.* (1991), which found that the combination of defoliation and stolon burial (mimicking grazing with treading) of clover plants grown in glasshouse pots reduced plant growth relative to defoliation-only plants (mimicking grazing-only). However, the results of Grant *et al.* (1991) were found in single clover plants grown in individual glasshouse pots in the absence of plant competition. Competition in productive grassland such as agricultural grass-clover swards is extremely high and competition with the grass portion of the sward is the primary determinant of clover growth and persistence (Davies, 2001). Under such high competitive stress, the effect of treading can actually benefit some individual plants by reducing competition, as evidenced by Menneer *et al.* (2005a) where individual clover plants were significantly larger in treaded treatments one year after the treading event.

In the present experiment, BNF was calculated from clover herbage production according to the model of Hogh-Jensen *et al.* (2004) and not directly measured. Therefore, the proportion of N derived from the atmosphere in clover herbage is assumed to be constant across treading treatments. Menneer *et al.* (2005b) found that the reduction in annual BNF (measured using the ^{15}N isotope determination technique) following treading was similar to the reduction in annual clover herbage production as assumed in the present experiment. While there were significant reductions in annual clover production and BNF due to treading under grazing conditions in the present experiment, the concurrent reduction in annual grass production resulted in no evidence of a large loss of competitiveness of white clover relative to grass.

6.4.5. Effect of treading on herbage production

The 0.05 to 0.13 proportional reduction in mean annual herbage production caused by treading of grass-white clover swards in the present experiment is comparable to reductions of 0.02 to 0.15 previously recorded for N fertilized grass-only swards under grazing conditions (Mullen *et al.*, 1974; Mullen *et al.*, 1978; Drewry and Paton, 2000). In contrast, much higher reductions in herbage production of both grass-clover and grass swards have been recorded in plot-based treading experiments, which generally entailed more deliberate applications of treading damage and/or shorter time scales (Curll and Wilkins, 1983; Davies and Armstrong, 1986; Vertes, 1989; Zegwaard *et al.*, 2000; Di *et al.*, 2001; Nie *et al.*, 2001; Menneer *et al.*, 2005a).

In the present experiment, a large range in the reduction of pre-grazing herbage mass (PRHM) were recorded following individual treading events (+ 0.09 to -0.58, Figure 6.8). The PRHM was negatively related to the amount of soil surface deformation and positively related the intervening recovery period length (Figure 6.8). Previous experiments have also found that the negative effects of treading on herbage production were greatest where soil deformation was high and generally declined with longer periods of recovery, however no such relationship has previously been described (Pande *et al.*, 2000; Zegwaard *et al.*, 2000; Nie *et al.*, 2001; Pande, 2002; Menneer *et al.*, 2005a).

6.4.6. Implications for grazing management

The results show that reductions in subsequent herbage mass following a treading event were highest in spring in all three grazing systems (Figure 6.7). However, spring grazing is important for reducing feed costs (Clark *et al.*, 2007; Dillon *et al.*, 2008). Therefore, there is a need for decision support tools and management options that reduce treading damage at this time of the year. In the present experiment, it was found that the reduction in pre-grazing herbage mass following a treading event was related to soil surface deformation. However, routinely measuring soil surface deformation on commercial farms is probably impractical. Furthermore, it is conducted after the treading damage has already been done. Soil strength, measured by penetration resistance before grazing may be a more suitable guidance tool (Scholefield and Hall, 1985) and further work in developing this method for farm use is required.

Management strategies such as restricting access to paddocks may reduce treading damage and most farmers already house by night when soil conditions are considered too wet (Creighton *et al.*, 2011; Läpple *et al.*, 2012). The current experiment used this practice this but still recorded considerable losses of herbage production, particularly in spring. Therefore, more severe restriction of access to pasture may be required for grazing on wet soils. Kennedy *et al.* (2011) has shown that restricting cows access to pasture in early spring to 3.0 or 4.5 hour periods ("on-off grazing") after milking can result in similar pasture utilization and animal performance as unrestricted access. This on-off grazing has considerable potential to reduce the amount of treading damage. However, the suitability of this strategy for swards with high clover content needs to be investigated due to the possible increased risk of tympanites (bloat) when animals are fasted (Clarke and Reid, 1974; Majak *et al.*, 1995).

Another option might be to select lighter cows for grazing on wet soils. Breeding research has revealed the economic advantages of using Jersey × Holstein-Friesian crossbred cows over Holstein-Friesian cows for dairy production (Clark *et al.*, 2007; Buckley *et al.*, 2008; Prendiville *et al.*, 2009). These crossbreds are also considerably smaller, lighter and exert lower pressure per cm^2 on soil (Herbin *et al.*, 2011). Therefore the research of Jersey × Holstein-Friesian cows currently needs to be extended to the effects on treading damage and herbage production under grazing conditions for farms on wet soils.

6.5. Conclusion

Treading under grazing conditions compacted the soil and reduced annual grass and clover herbage production, but did not result in large reductions in the competitiveness of clover relative to grass. Therefore, treading damage under typical grazing management does not compromise the usefulness of white clover for reducing fertilizer N use on farms in areas with high rainfall and wet soil conditions. However treading did have negative effects on herbage production and soil functioning. The results show that soil susceptibility to treading was negatively related to water table depth until water table depth reached approximately 1.0 m below the soil surface.

Treading in spring resulted in a greater proportional reduction (0.20 to 0.35) of subsequent pre-grazing herbage mass than treading in winter (0 to 0.08) across the three grazing systems. However, early turnout to grazing in spring is an important component of efficient grassland-based dairy production. Therefore, there is a need for greater development of decision support tools and management strategies that minimise the negative effects of treading on herbage production at this time of the year.

7. The Effect of Target Post-Grazing Height on Sward Clover Content, Herbage Production and Dairy Production from a Grass-White Clover Pasture.

Phelan P., Casey, I.A. and Humphreys, J. (2013) The effects of target post-grazing height treatment on sward clover content, herbage yield and dairy production from a grass-white clover sward. *Journal of Dairy Science*, *DOI: 10.3168/jds.2012-5936, http://www.journalofdairyscience.org/article/S0022-0302(13)00044-1/fulltext (accessed 30 January 2013).*

Abstract

White clover (*Trifolium repens*) is an important legume for grazed grassland that can increase the profitability and environmental sustainability of milk production. Previous experiments on mown grass-clover plots suggest that low post-grazing heights (PGH) can increase sward clover content and herbage production. However, this has not been tested in actual strip/rotational grazing systems with dairy cows. Furthermore, lowering PGH in grass-only swards (typically perennial ryegrass without white clover) has previously been associated with reduced milk yields per cow. The objective of this experiment was to investigate the effect of PGH by dairy cows on clover content, herbage production and milk production from strip-grazed grass-white clover swards in Ireland. There were three target PGH treatments of 4, 5 and 6 cm in place for entire grazing seasons (February to November) for three consecutive years (2007 to 2009). Each treatment had a mean of 21 Holstein-Friesian dairy cows that strip-grazed a mean annual area of 10.2 ha. Post-grazing height was measured twice a day with a rising plate meter and cows were moved to the next strip once the target PGH was reached.

Annual fertilizer nitrogen (N) input was 90 kg N ha^{-1} for each treatment. The PGH treatment did not significantly affect annual milk yield (6,202 kg cow^{-1}), solids-corrected milk yield (6,148 kg cow^{-1}), fat, protein or lactose yields (265, 222 and 289 kg cow^{-1}, respectively), cow liveweight (592 kg) or body condition score (3.01). The PGH treatment also had no significant effect on sward white clover content (196 g kg^{-1}). However, herbage production of both grass and clover were significantly higher with the 4 cm PGH treatment compared to the 6cm. Mean annual herbage production was 11.1, 10.2 and 9.1 t OM ha^{-1} for the 4, 5 and 6 cm treatments, respectively. The lower herbage production in the 6cm PGH treatment resulted in lower annual silage production, greater housing requirements and a substantially higher net silage deficit (-1,917 kg OM cow^{-1}) when compared to the 5 cm or 4 cm treatments (-868 kg and -192 kg OM cow^{-1}, respectively). Grazing to a PGH of 4 cm is therefore recommended for grass-white clover swards.

7.1. Introduction

White clover (*Trifolium repens*) facilitates biological nitrogen (N) fixation (BNF) through it's association with *Rhizobium* bacteria and can thereby reduce fertilizer N requirements of grassland for dairy production (Gylfadóttir *et al.*, 2007; Humphreys *et al.*, 2009a; Del Prado *et al.*, 2011). White clover is most commonly grown in association with perennial ryegrass (*Lolium perenne*) and increasing it's content in herbage can improve sward nutritive value, herbage intake rates and milk production per cow (Harris *et al.*, 1998; Dewhurst *et al.*, 2009; Kleen *et al.*, 2011). Replacing fertilizer N use with white clover-based BNF can also improve economic performance at farm level (Doyle and Bevan, 1996; Falconer *et al.*, 2011; Humphreys *et al.*, 2012). This is particularly so in recent years due to the large increase in fertilizer N price relative to milk price (World Bank, 1990-2012). White clover can also have important environmental benefits such as increased biodiversity (Power and Stout, 2011) and reduced green-house gas emissions (Li *et al.*, 2011) for dairy production from grazed grassland.

One of the main challenges to achieving the above benefits from white clover is maintaining effective sward clover content (> 300 g kg^{-1}) within the sward from year to year (Frame and Laidlaw, 1998; Rochon *et al.*, 2004). Previous experiments on mown, small-scale grass-clover plots have shown that lowering defoliation height can increase clover content and clover herbage production (Frame and Boyd, 1987; Acuña and Wilman, 1993; Phelan *et al.*, 2009). The beneficial effect of lowering defoliation height on sward clover content is generally attributed to reduced shading of the clover growing points by grass (Thompson, 1993; Héraut- Bron *et al.*, 2001; Christophe *et al.*, 2006). As a result, low post-grazing heights (PGH) are often recommended for grass-clover swards. However, the effect of PGH on clover content in grass-clover swards has not been investigated in actual strip or rotational grazing systems for dairy production over entire grazing seasons. Furthermore, daily herbage allowance (DHA) experiments on grass-only swards have found that lower PGH can be associated with lower milk yields per cow and lower milk fat, protein or lactose concentrations (Le Du *et al.*, 1979; Maher *et al.*, 2003; Curran *et al.*, 2010).

The objectives of this experiment were to measure the effects of imposing a target PGH of either 4, 5 or 6 cm on sward white clover content, herbage production and milk production from grass-clover swards in a strip grazing system with dairy cows over a number of grazing seasons. It was hypothesised that (i) lowering PGH would increase sward clover content, which would increase BNF and herbage production, but that (ii) lowering PGH would also reduce milk yield per cow.

7.2. Materials and Methods

7.2.1. Experimental area

This experiment was conducted from January 2007 to December 2009 at Solohead Research Farm in Ireland (52˚51’N, 08˚21’W, 95 m above sea level). The soils of the farm are 90% poorly-drained gleys and 10% grey-brown podzolics with a depth ranging from 5 to 10 m, overlaying Devonian sandstone. Drainage is impeded, and this contributes to waterlogged conditions under high rainfall. The soil has a clay-loam texture of 36% sand and 28% clay in the A1 horizon. Soil organic matter content was 13% and soil pH was 6.6 before the experiment. The land has been under permanent grassland for over 50 years but was reseeded with perennial ryegrass between 1985 and 1995 and oversown with white clover between 2001 and 2006 as described by Humphreys *et al*. (2009).

The botanical composition of the swards (in g kg^{-1} of herbage dry matter (DM), sampled in September 2008) was found to be predominantly perennial ryegrass (approximately 750 g kg^{-1}) and white clover (approximately 200 g kg^{-1}). Unsown species were primarily *Taraxacum officinale* (dandelion), *Ranunculus repens* (creeping buttercup), *Bellis perennis* (daisy) and *Plantago lanceolata* (ribwort plantain) which in total, accounted for less than 50 g kg^{-1}.

Soil temperature (ºC at 10 cm depth) and rainfall amounts (mm) were measured every 30 minutes at an automatic meteorological station on the farm (Campbell scientific Ltd, Loughborough, U.K.). The experimental area was 40.8 ha in 2007 and 25.5 ha in both 2008 and 2009.

7.2.2. Experimental design and grazing management

The experiment was a complete randomized block design consisting of three treatments that were target post-grazing sward heights (PGH) of 4, 5 or 6 cm imposed for entire grazing seasons (February to November) over three consecutive years (2007 to 2009).

The experimental area was divided into six sections according to soil type and drainage status in January 2007. One paddock from each section was randomly assigned to PGH treatment and remained under that treatment until the end of the experiment in December 2009. Paddock size ranged from 1.46 to 3.30 ha^2 in 2007 (mean =

2.27) and ranged from 0.94 to 1.97 ha^2 in both 2008 and 2009 (mean = 1.42). The total stocking rate in each treatment was 1.99 cows per ha in 2007 and 2.12 cows per ha in 2008 and 2009.

Cows were turned out to graze approximately three days after calving in mid-February and remained at pasture until they were dried off and housed full-time at the end of November. Exceptions were made when ground conditions were too wet (soil moisture > 60%) and/or when herbage supply was too low, which generally occurred when herbage growth rates were below demand and pre-grazing herbage mass was lower than 500 kg DM per ha (above PGH). On such occasions, cows were housed by night and fed grass-clover silage *ad libitum*.

Each treatment was under strip-grazing management with approximately 0.25 to 0.50 of each paddock allocated each time. The PGH (cm above the soil surface) was measured twice per day from 50 drops with a Filips rising plate-meter (www.grasstec.ie). Cows were moved to the next pasture area once the target PGH was achieved and a back-fence was used to stop animals returning to previously grazed areas.

Slurry produced during housing was stored together and reapplied to each treatment equally during the following grazing season using regulatory protocols (European Communities Good Agricultural Practice for Protection of Water Regulations, 2009, S.I. No. 101) and an umbilical system with downward facing splash plate. Each treatment received annual mineral fertilizer N input of 90 kg ha^{-1}, applied in the form of Urea between February and April, and Calcium Ammonium Nitrate in May of each year. These were the only forms of synthetic fertilizer N used in this study and no fertilizer

N was applied during the remainder of the growing season.

Excess herbage production was identified throughout the experiment and removed for silage production. These areas were selected when herbage growth rates exceeded demand resulting in pre-grazing herbage mass greater than 2000 kg of DM herbage (above PGH) per ha. Such areas were generally closed from grazing between 2 April and 28 May (1st cut silage) or between 23 May and 10 July (2nd cut silage) of each year. The proportion of the grazing area that was removed for silage production are presented in the results.

7.2.3. Animals

Each February, all cows on the farm were divided into four main groups on the basis of lactation number (1, 2, 3 & ≥4) and then sub-divided into sub-groups of three on the basis of calving date. From within each sub-group one cow was randomly assigned to each PGH herd. This procedure was repeated each spring.

The experiment consisted of 81 Holstein-Friesian dairy cows in 2007 (21 primiparous and 60 multiparous) and 54 (12 primiparous and 42 multiparous) in 2008 and 2009. Mean calving date was 20 February (SD = 22 days).

Cows received concentrate feed supplementation (approximately 26% barley, 26% corn gluten, 35% beet pulp and 12% soybean meal) at rates of approximately 3 to 5 kg cow^{-1} between February and April and 0 to 4 kg cow^{-1} between April and November, depending on herbage availability and quality (see results). When housed over winter, the groups were fed silage *ad libitum*. The mean composition of fed silage (± SD) throughout the experiment was: 78 g kg^{-1} ash (± 13.3), 784 g kg^{-1} OMD (± 6.3) and 122 g kg^{-1} CP (± 18.9).

7.2.4. Measurements

7.2.5. Sward measurements

7.2.6. Herbage production and feeding quality

Before each grazing event, herbage DM production was sampled by cutting two to four random strips, each 5 m long and 0.55 m wide, using a HRH-536 lawn-mower (Honda®, Georgia, U.S.A.) set to the target PGH. Before harvesting grass-clover for silage, herbage DM production was measured from four strips (5.0 m × 1.1 m) using an Agria auto-scythe. On each occasion the harvested herbage was bulked and weighed to determine herbage mass and a 100 g sub-sample dried for 16 hours in a forced-draught oven at 95 °C for DM content. Annual herbage production (kg DM ha^{-1}) was calculated as the sum of herbage removed as pre-grazing and pre-silage cuts. Growth rates for each pre-grazing and pre-silage cut were calculated by dividing the herbage mass by the regrowth interval.

A second 100 g sub-sample of each herbage production sample was freeze-dried and milled through a 0.2 mm sieve before analyses for ash content (550ºC muffle furnace for 12 hours), crude protein (CP; N content × 6.25; LECO 528 auto-analyser, LECO Corporation, ST. Joseph, MI USA), and *in vitro* organic matter digestibility (OMD) as described by Morgan *et al.* (1989). Herbage production and CP results are presented as dry organic matter (OM) as recommended by Elgersma and Schlepers (1996). Daily herbage allowance (DHA; kg OM above target PGH) was calculated retrospectively by dividing the herbage production from pre-grazing cuts by the number of days at pasture. The net energy (NE) content of pre-grazing herbage was calculated from the ash content, OMD and CP (Jarrige *et al.*, 1986; Jarrige, 1989; O'Mara, 1996).

Silage fed to housed animals was randomly sampled (n = 88) throughout the experiment by taking a grab sample of approximately 100 g before feeding. This was analysed for ash, OMD and CP using near infra-red spectroscopy (NIRS, Model 6500, FOSS-NIR System, 3400 Hillerød, Denmark).

7.2.7. White clover content in herbage, stolon mass, and biological nitrogen fixation

White clover content of herbage in each paddock was measured from 30 randomly-distributed herbage snips (each 10 cm × 30 cm) taken to the target PGH of each treatment with electric hand shears (Accu-shears Gardena®, Ulm, Germany). This was done in April, June, and September of each year. All samples were manually separated to determine the white clover content of herbage DM.

White clover stolon mass was measured in February, May, August and November of each year by cutting 30 random sods (each measuring 10 cm × 10 cm) to a depth of approximately 8 cm from each paddock. White clover stolons with attached roots were manually separated from the sods, washed and analysed for DM as described above. Annual grass and clover herbage production was calculated from the mean annual clover content of each paddock and its respective annual herbage production. The annual BNF was estimated from annual clover herbage production, sward age and soil type using the model by Hogh-Jensen *et al.* (2004).

7.2.8. Animal production measurements

7.2.9. Milk production, liveweight and body condition score

Milking was conducted at 07:30 h each morning and 15:30 h each evening. Individual cow milk yield (kg) was recorded at each milking and the fat, protein and lactose concentrations of milk from each cow was measured for a successive morning and evening milking once per week using a Milkoscan 203 analyzer (Foss Electric DK-3400, Hillerod, Denmark). Solids-corrected milk yield (SCM) was calculated using the equation of Tyrell and Reid (1965). The liveweight (LW) of each cow was recorded once per week using weighing scales and the Winweigh software package (Tru-test Limited, Auckland, New Zealand). Body condition score (BCS) of each cow was recorded once every two weeks using the methodology of Edmonson *et al*,(1989).

7.2.10. Days at pasture and intake estimates

Days at pasture were recorded for each cow with a value of one ascribed to each 24 hr period and a value of 0.5 if the animal was at pasture by day only. The amount of concentrate fed per cow was recorded at each milking (Dairymaster; Causeway, Co. Kerry, Ireland) and silage intake was estimated as silage fed to cows when housed.

Intake of grazed pasture OM by each cow was estimated from the difference between NE provided from silage and concentrate and that needed to meet the NE requirements for milk production, maintenance and pregnancy (Jarrige *et al.*, 1986; Jarrige, 1989;

O'Mara, 1996). The NE content of concentrate was calculated from the constituent ingredients and the NE content of silage was calculated from *in vitro* DM digestibility (DMD) (O'Mara, 1996). The NE content of grazed herbage was calculated from the OMD, CP and Ash content (Jarrige *et al.*, 1986; Jarrige, 1989; O'Mara, 1996). Cow NE requirements were calculated from milk yield, fat, protein and lactose content and liveweight (Jarrige *et al.*, 1986; Jarrige, 1989; O'Mara, 1996). Daily herbage intake (DHI; kg OM) was estimated for each cow by dividing the annual amount of herbage intake by the number of days at pasture.

7.2.11. Statistical analyses

All results were subjected to analyses of variance using the mixed procedure in SAS (SAS, 2006) with the following model: $X_{jkl} = \mu + P_j + Y_l + M_k + PY_{jl} + PM_{jk} + YM_{lk} + PYM_{jkl} + e_{jkl}$ where X_{jkl} = dependent variable treatment mean, μ = overall mean, P_j = the fixed effect of the j^{th} PGH, Y_l = the fixed effect of the l^{th} year, M_k = the fixed effect of the k^{th} sampling date where applicable and e_{jkl} = residual error term. Year and sampling date (for sward clover content and clover stolon mass) were entered as a repeat measures using the un@cs covariance structure as recommended by Moser (2004). Paddock was replicate for sward measurements and cow was replicate for animal measurements. Year was used as replicate for sward/animal measurements that were calculated on a herd basis for each treatment: the proportional land area removed for silage, amount of herbage produced as silage, silage surplus and DHA.

7.3. Results

7.3.1 Meteorological data

Mean daily soil temperature and monthly rainfall amounts for the experimental period and the previous ten-year means are shown in Figure 7.1. Mean soil temperature was 10.2, 9.5 and 9.6 ºC in 2007, 2008 and 2009 respectively, whereas the previous ten year mean was 10.9 ºC (range: 10.0 to 11.8 ºC). Total rainfall was 990, 1,228 and 1,296 mm in 2007, 2008 and 2009 compared to the previous ten-year mean of 1,009 mm (range 797 to 1,150 mm). Therefore 2007 showed values within the range of previous years but 2008 and 2009 were both cooler and wetter (Figure 7.1).

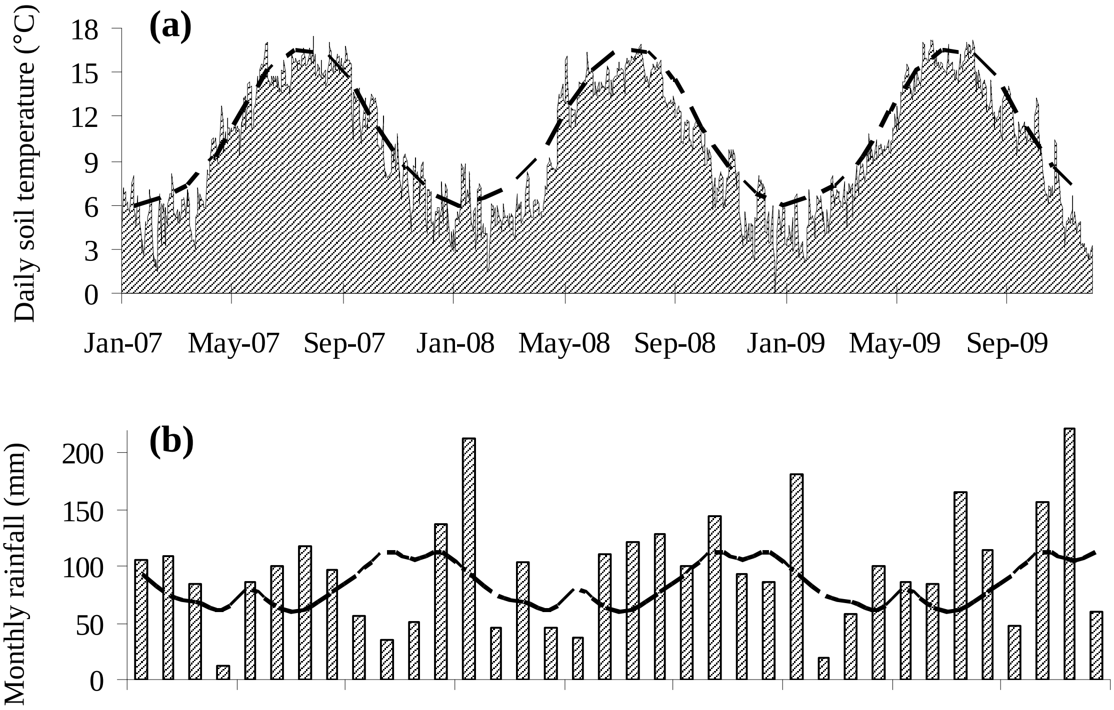


Figure 7.1. Mean daily soil temperature (a) and monthly rainfall amounts (b) recorded at the meteorological station at Solohead Research farm between January 2007 and December 2009. The x-axis shows the month and year. The shaded areas show the recorded values during the experimental period and the broken black lines show the previous ten-year mean values (1996-2006).

7.3.2. Measured post-grazing heights

The mean monthly PGH measured throughout the experiment are shown in Figure 7.2. The overall mean PGH for over the course of the experiment were 4.1, 5.1 and 5.9 cm for the 4, 5 and 6 cm treatments respectively ($P < 0.001$, SEM = 0.02, Figure 7.2).

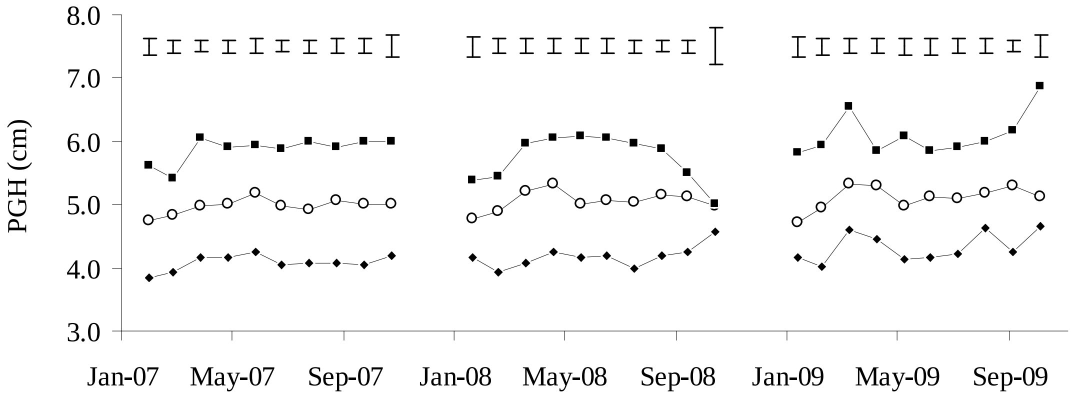


Figure 7.2. Effect of target post-grazing height (PGH) treatment on actual PGH measured throughout the experiment. Target PGH treatments were: 4 cm (◆), 5 cm (○) and 6 cm (■). Error bars show the SEM of PGH treatment; $P < 0.001$.

7.3.3. Clover persistence and annual grass and clover herbage production

Sward clover content and clover stolon mass per ha are shown in Table 7.1. Target PGH treatment had no significant effect on sward clover content or clover stolon mass ($P > 0.05$). However, both were affected ($P < 0.05$) by year: 2009 had higher clover content but lower stolon mass than 2007 (Table 7.1). Clover content and stolon mass were also affected by interactions ($P < 0.001$) between year and sampling date: both tended to increase throughout the year in 2007 and 2009, but not in 2008.

Table 7.1. Effect of target post-grazing height (PGH) treatment on mean annual sward clover content, clover stolon mass and annual herbage production of grass and clover.

Year	2007			2008			2009			SEM		
PGH	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	PGH	Year	PGH × Year
Sward white clover content (g kg^{-1})	169	143	165	211	219	198	253	196	213	17.7 NS	15.2 **	26.4 NS
White clover stolon mass (kg DM ha^{-1})	605	544	555	624	616	582	464	482	404	35.7 NS	41.4 *	71.6 NS
Herbage production (t OM ha^{-1}												
Grass	10.7	10.2	8.6	8.4	7.6	7.5	7.2	7.2	6.0	0.31 **	0.31 ***	0.63 NS
Clover	2.2	1.7	1.7	2.3	2.0	1.8	2.4	1.8	1.7	0.18 *	0.18 NS	0.94 NS
Total herbage	12.8	11.9	10.3	10.7	9.6	9.3	9.7	9.0	7.6	0.35 ***	0.35 ***	0.60 NS
Harvested as (t OM ha^{-1} year^{-1}):												
Pre-grazing cuts:	8.3	8.3	7.4	8.2	7.4	8.1	7.4	7.1	6.0	0.29 NS	0.29 *	
Pre-silage cuts:	4.5	3.6	2.9	2.5	2.3	1.2	2.3	1.9	1.6	0.18 *	0.18 *	

* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, NS = non-significant (*P* > 0.05).

Annual grass, clover and total herbage production is shown in Table 7.1. The 4 cm PGH treatment increased annual grass (P < 0.05), clover (P < 0.05) and total herbage OM production (P < 0.001) in comparison to the 6 cm in all three years. Grass and total herbage OM production was lower in 2008 and 2009 than 2007, across all treatments (P < 0.001). BNF estimates were higher with the 4 cm PGH than the 6 cm (P < 0.05, Figure 7.3) and were not affected by year. The daily herbage growth rates throughout the experiment are shown in Figure 7.4. The 4 cm treatment had higher growth rates than the 6 cm (P < 0.05) and 2007 had higher growth rates than 2009 (P < 0.001).

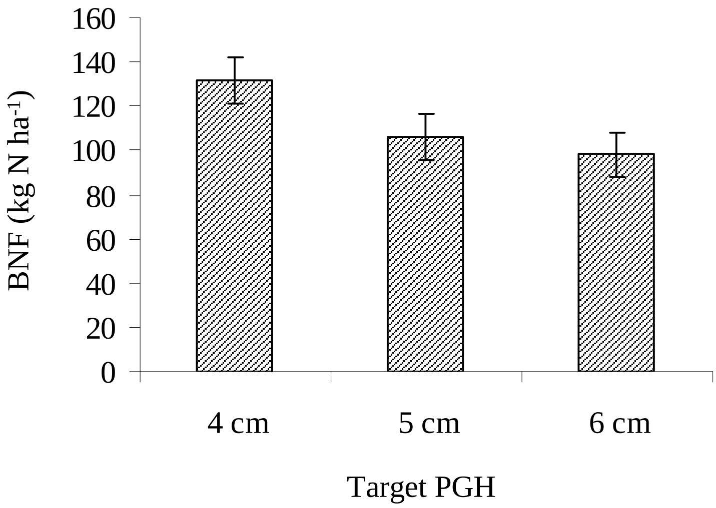


Figure 7.3. Effect of target post-grazing height (PGH) treatment on annual biological nitrogen fixation (BNF) estimates (P < 0.05). Year had no significant effect (P > 0.05). Error bars show the SEM of PGH treatment.

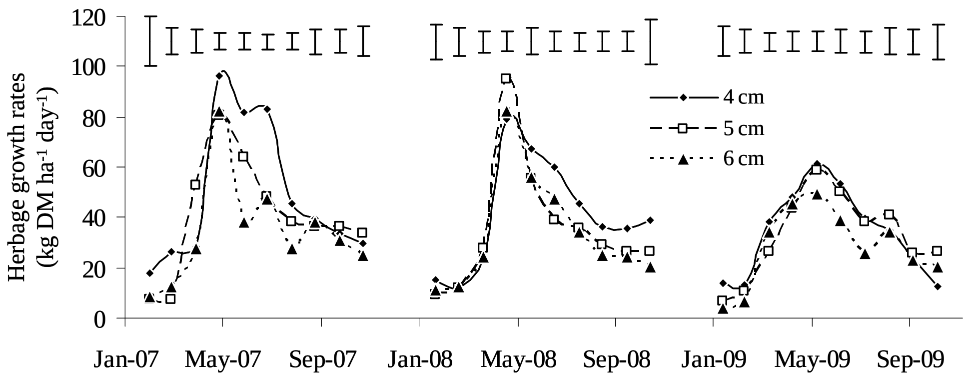


Figure 7.4. Effect of target post-grazing height (PGH) treatment on daily herbage growth rates throughout the experiment (PGH treatment effect: $P < 0.001$).

7.3.4. Pre-grazing herbage quality

The mean ash content, OMD and CP of pre-grazing herbage are shown in Table 7.2. Mean OMD of pre-grazing herbage was not affected by PGH. However, there was a significant interaction between PGH and year ($P < 0.01$) where the 6 cm had higher OMD than the 4 cm in 2007 only. CP was lower ($P < 0.05$) and ash content higher ($P < 0.001$) with the 4 cm PGH (Table 7.2).

The time interval between successive grazings for each paddock (rotation length) was longer with the 4 cm than the 6 cm ($P < 0.05$, Table 7.2). However, there was an interaction with year, where the 5 cm had the longest intervals in 2008 only. Pre-grazing herbage mass (above the target PGH) followed a similar trend and was greater with the 4 cm than the 6 cm. The differences between the 4 and 5 cm were affected by an interaction with year ($P < 0.01$), where the 4 cm had longer intervals and higher herbage masses in 2007 and 2009 but not in 2008, when the 5 cm had the highest pre-grazing herbage mass (Table 7.2).

Table 7.2. Effect of target post-grazing height (PGH) treatment on herbage ash content, organic matter digestibility (OMD) and crude protein (CP), interval length between grazings and pre-grazing herbage mass above PGH.

Year	2007			2008			2009			SEM		
PGH	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	4 cm	5 cm	6	PGH	Year	PGH × Year
Ash content (g kg^{-1} DM)	120	107	100	122	95	98	125	110	102	2.86 ***	2.86 NS	4.94 NS
OMD (g kg^{-1} DM)	811	819	829	821	816	817	819	829	816	2.4 NS	2.4 NS	4.2 **
CP (g kg^{-1} OM)	206	221	232	195	195	204	202	206	201	3.3 *	3.3 ***	5.8 NS
Interval between grazings (days)	25.9	21.3	21.6	27.3	33.5	28.2	34.0	31.3	29.9	0.80 *	0.80 ***	1.38 **
Pre-grazing herbage mass (kg OM ha^{-1})	1169	848	796	1058	1211	991	1045	945	835	32.2***	32.1**	55.5**

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant ($P > 0.05$).

Table 7.3. Effect of target post-grazing height (PGH) treatment on grazed pasture, silage and concentrate intake per cow.

Year	2007			2008			2009			SEM		
PGH	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	PGH	Year	PGH × Year
Days at pasture	251	249	251	222	221	221	230	225	189	1.76 ***	1.7 ***	3.0 ***
DHA (kg OM cow^{-1})†	16.7	16.8	14.8	17.4	15.7	17.2	15.1	15.0	15.0	0.53 NS	0.53 NS	
<u>Intake per cow:</u>												
Grazed pasture (kg OM)	3212	3195	3244	2951	2991	3025	2833	2790	2639	58.6 NS	58.4 ***	101.1 NS
Silage (kg OM)	1054	1068	1056	1324	1333	1335	1242	1288	1622	16.5 ***	16.4 ***	28.4 ***
Concentrate (kg OM)	312	307	310	478	477	476	616	615	615	10.8 NS	10.7 ***	18.5 NS
DHI (kg OM)‡	12.8	12.9	13.0	13.3	13.5	13.7	12.3	12.4	13.9	0.27 NS	0.27 NS	0.47 NS

*** $P < 0.001$, NS = non-significant ($P > 0.05$).

† Daily herbage allowance above target PGH, estimated from pre-grazing cuts.
‡ Daily herbage intake at pasture, estimated from energy balances.

7.3.5. Days at pasture and feed intake per cow

Post-grazing height had no effect on estimated intake of grazed pasture or on the amount of concentrate fed (Table 7.3). Daily herbage allowance and DHI estimates were also not affected by PGH (Table 7.3). The amount of silage fed to cows was also similar across treatments in 2007 and 2008. The weather conditions in 2008 and 2009 resulted in less days at pasture, less grazed grass in the cow's diet and greater demand for silage and concentrate across treatments in comparison to 2007 ($P < 0.001$). In 2009, the 6 cm PGH had less days at pasture and consequently required a higher amount of silage to be fed than the other two treatments. This was due to management decisions to house the 6 cm PGH cows by night in April, August and October because the lower herbage production in that system gave less options of dry paddocks to graze than the other treatments.

7.3.6. Milk production, LW and BCS

Annual milk, SCM, fat, protein and lactose yields were not significantly affected by PGH (Table 7.4). Milk fat, protein and lactose contents were also not significantly affected (not shown). There was no difference in LW or BCS during, or at the end of, each lactation (Table 7.4).

There were differences between years with lower SCM ($P < 0.01$), fat ($P < 0.01$) protien ($P < 0.05$) and lactose yields ($P < 0.01$) in 2007 compared with 2008 (Table 7.4). Liveweight was higher in 2007 and 2008 than 2009 ($P < 0.001$).

Table 7.4. Effect of target post-grazing height (PGH) treatment on mean annual milk yields, component yields, cow liveweight (LW) and body-condition score (BCS).

Year	2007			2008			2009			SEM		
PGH	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	PGH	Year	PGH × Year
Milk yield (kg cow^{-1})	5896	6008	6183	6375	6371	6452	6140	6174	6218	118.2 NS	117.7 NS	203.9 NS
SCM (kg cow^{-1})	5746	5790	6032	6313	6377	6487	6196	6148	6242	111.3 NS	110.9 **	192.0 NS
Fat (g kg^{-1})	247	247	257	271	274	280	271	266	273	5.2 NS	5.1 **	8.9 NS
Protein (g kg^{-1})	210	216	225	227	230	235	220	218	220	4.2 NS	4.1 *	7.2 NS
Lactose (g kg^{-1})	270	272	283	300	301	303	288	291	291	5.4 NS	5.4 **	9.3 NS
Mean LW (kg cow^{-1})	595	608	612	599	602	600	555	574	586	8.1 NS	8.2 **	14.1 NS
LW at end of lactation (kg cow^{-1})	627	638	622	652	656	644	584	609	610	8.9 NS	8.9 **	15.4 NS
Mean BCS	2.94	3.00	3.01	3.00	3.00	3.02	2.99	3.08	3.05	0.023 NS	0.023 NS	0.040 NS
BCS at end of lactation	2.89	2.98	2.94	3.00	3.03	3.01	2.93	3.06	3.01	0.032 NS	0.031 NS	0.054 NS
Days in Milk	288	286	287	299	295	297	286	289	288	2.7 NS	2.7*	4.7 NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant.

Table 7.5. Effect of target post-grazing height (PGH) on the proportion of the grazing area removed for silage production, the amounts of herbage produced either as pre-grazing or pre-silage cuts, the amounts of silage harvested, ensiled and the end-of year silage surplus/deficit.

Year	2007			2008			2009				
PGH	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	4 cm	5 cm	6 cm	PGH	Year
Proportional area removed from each grazing systems for:											
1st cut silage (May)	0.56	0.43	0.40	0.42	0.51	0.25	0.29	0.37	0.12	0.042 NS	0.042 NS
2nd cut silage (July)	0.38	0.22	0.09	0.14	0.00	0.00	0.20	0.11	0.17	0.039 NS	0.039 NS
Mean	0.47	0.33	0.25	0.28	0.26	0.13	0.25	0.24	0.15	0.023 *	0.023 *
Silage (kg OM ha^{-1}):											
Ensiled†	3.73	2.94	2.36	2.08	1.89	1.01	1.86	1.50	1.30	0.145 *	0.145 **
Fed	2.09	2.12	2.09	2.80	2.82	2.83	2.63	2.73	3.43	0.146 NS	0.146 *
Surplus or deficit‡	1.32	0.56	0.07	-0.91	-1.10	-1.91	-0.91	-1.34	-2.22	0.090 **	0.090 **

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = non-significant.
† Proportional loss during ensilage assumed to be 0.25 (McGechan, 1989; McGechan, 1990).
‡ Silage ensiled minus silage fed.

7.3.7. Silage surplus/deficit

The higher herbage production with the 4 cm PGH treatment resulted in a greater proportion of the grazing area being taken out for silage making ($P < 0.001$, Table 7.5). As a result, there were differences in the amount of silage harvested between all treatments, with the 4 cm producing the most and 6 cm the least ($P < 0.05$, Table 7.5). When combined with the greater demand for silage in the 6 cm (Table 7.4) there were large differences in silage surplus/deficit between treatments ($P < 0.001$, Table 7.5). Year also had a large effect ($P < 0.001$), with a surplus of silage for all three treatments in 2007, and a deficit in all three treatments in 2008 and 2009. Combined over the three years of the experiment, the 4, 5 and 6 cm PGH resulted in net silage deficits of –192, -868 and -1,917 kg OM cow^{-1}, respectively.

7.4. Discussion

7.4.1. Grazing management

PGH is not an independent management variable in actual grazing systems because, in order to remove more herbage and achieve a lower PGH (from a given pre-grazing herbage mass), it either takes a higher immediate stocking rate (as in DHA experiments) or the same immediate stocking rate grazing for a slightly longer time period (as in the current experiment). Sward herbage mass was similar across treatments at the start of the experiment, and after silage was removed. As a result, it took longer to initially graze swards down to 4 cm than it did to 6 cm at these times. Furthermore, the increased herbage production with the 4 cm PGH resulted in more area being removed for silage in that system (Table 7.5). Therefore, the results from this experiment should be interpreted as being due to the system-level effects of achieving a target PGH rather than the independent effects of PGH/defoliation height. As this is representative of on-farm conditions where the interrelatedness of grazing management factors is unavoidable, the results and outcomes are relevant for commercial farms.

7.4.2. Clover content and herbage production

Previous experiments on mown-plots found that sward clover content, clover stolon mass, clover herbage production and BNF all increased in response to lowering defoliation height, with little or no effect on grass herbage production (Frame and Boyd, 1987; Acuña and Wilman, 1993; Schils and Sikkema, 2001). Across these previous experiments, each cm reduction in cutting height increased annual clover production by approximately 0.17 to 0.53 t DM ha^{-1} and annual total herbage production by approximately 0.26 to 0.51 t DM ha^{-1}, with generally linear responses to defoliation height. In comparison, each cm reduction PGH in the current experiment, increased annual clover production by 0.34 t DM ha^{-1} (0.28 t OM) and annual total herbage production by 1.23 t DM ha^{-1} (1.01 t OM). Unlike the previous mown experiments, grass production also increased, which accounts for the lack of a difference in the sward clover content in this experiment.

However, there are large differences between grazing and mowing experiments due to the interrelatedness of management variables discussed above, animal treading (Menneer *et al.*, 2005a), selective grazing (Rutter, 2006), and animal excretion (Bao *et al.*, 1998; Yayato *et al.*, 2000). Under grazing conditions, transfer of fixed-N from clover to grass can be higher (Hatch and Murray, 1994; Menneer *et al.*, 2005b; Auerswald *et al.*, 2010), and the competitive ability of clover lower (Edwards *et al.*, 1996; Davies, 2001) than in mown plots, which may have accounted for the lack of differences in clover content in the current experiment. This is supported by results from grass-clover swards under continuous (or set-stocked) grazing with cattle and sheep. For example, continuous grazing experiments with sheep and cattle have found that lowering grazing height can increase (Laidlaw *et al.*, 1995), reduce (Gibb and Baker, 1989) or have no effect (Del Pozo *et al.*, 1996) on sward clover content. However, the continuous grazing in the above examples allowed animal's unrestricted access to the entire grazing area. The current experiment expands this work to PGH in strip-grazing systems with dairy cows and shows that lowering target PGH from 6 to 4 cm can increase both grass and clover herbage production although the proportion of white clover in the sward did not change.

The total annual herbage production response to lowering PGH was large in comparison to the previous plot-based experiments. The BNF estimates increased by an average of 17 kg ha^{-1} year^{-1} in response to each cm reduction in PGH. This would indicate an extremely high response rate of 59 kg extra OM herbage per kg of fixed N, compared with the 10-20 kg reported for fertilizer N (Keating and O'Kiely, 2000; O'Donovan *et al.*, 2004; Sun *et al.*, 2008). However, the BNF estimates in this experiment are based solely on clover herbage production, and do not account for any changes in the proportion of fixed-N in clover tissue, N transfer rates or N accumulation rates that could have occurred under the different treatments (see Hogh-Jensen *et al*. (2004) for model description). Lowering defoliation height can also increase grass herbage production without any source of additional N through increased tillering, photosynthetic efficiency, increased light penetration of the sward and less respiration and senescence below the defoliation height (Binnie and Harrington, 1972; Parsons *et al.*, 1988; Lee *et al.*, 2008a; Lee *et al.*, 2009). Furthermore, the longer grazing intervals and greater amount of silage harvesting associated with the lower PGH's may have further increased herbage production by giving the plants more time in the high growth rate zone of the sigmoidal growth curve (Parsons and Penning, 1988; Binnie *et al.*, 1997).

7.4.3. Pre-grazing herbage mass, OMD and crude protein

Lowering target PGH in the current experiment reduced herbage OMD in 2007 and CP concentration throughout the experiment. Lowering PGH (by reducing DHA) has previously been associated with increases in herbage OMD and CP in grass-only swards (McEvoy *et al.*, 2009; Curran *et al.*, 2010; McEvoy *et al.*, 2010). However, in those experiments pre-grazing herbage mass had a larger effect on OMD and CP than PGH (both were lower at higher pre-grazing herbage masses). Lowering PGH in the current experiment increased the time it took for cows to rotate through the paddocks, which consequently increased pre-grazing herbage mass (Table 7.3). The lower OMD and CP values of pre-grazing herbage grazed to 4 cm in the current experiment could therefore be attributed to the higher pre-grazing herbage mass associated with that treatment.

7.4.4. Milk yields

The current experiment found no effect of PGH on milk yield per cow or milk composition. This contrasts with previous experiments on grass-only swards (primarily perennial ryegrass with no clover) where herbage intake, milk yield, protein, fat or lactose concentrations per cow declined with PGH (Le Du *et al.*, 1979; Maher *et al.*, 2003; Curran *et al.*, 2010). However, the current experiment used grass-clover swards. White clover can increase herbage intake compared to grass-only herbage (Bax and Schils, 1993; Harris *et al.*, 1998; Ribeiro Filho *et al.*, 2003). Therefore it's presence in the current experiment may have mitigated the negative effect of lowering PGH on herbage intake/milk yield that was observed in those previous experiments on grass-only swards. Another difference is that the above experiments were all daily herbage allowance (DHA) experiments where pre-grazing herbage mass was either measured or controlled, and the PGH was a result of adjusting the immediate stocking rate. In such experiments, animal interactions at higher densities and overly restrictive DHA allocations may also be responsible for the lower herbage intakes and milk yields. This was shown by Lee *et al.* (2008b) who found that reducing PGH from 6 to 4 cm could actually increase milk yield per cow when DHA was kept similar, although the fact that there was no effect on milk solids yield (fat + protein) suggested a transitory effect.

In the current experiment DHA was not measured before grazing and PGH was implemented simply by not moving the cows to new pasture until the target PGH was achieved. This is similar to how target PGH are likely to be achieved on most commercial farms, given the time, labour, skill and infrastructure that is required to calculate and allocate DHA. When DHA and DHI estimates were calculated retrospectively in the current experiment, no significant difference between treatments was found, which is in accordance with the lack of a difference in milk yields per cow between treatments. However, in DHA experiments, DHA is usually measured to ground level (Peyraud *et al.*, 1996; Lee *et al.*, 2008b) or to a common sward height (Maher *et al.*, 2003; McEvoy *et al.*, 2009), which was not the case in the current experiment.

All of the above experiments also began later in the year (April at the earliest) and were conducted for shorter time periods (30 weeks at the most). In comparison, the current experiment was conducted from initial turnout in February to housing in November, which is similar to the grazing season length on most Irish dairy farms (Creighton *et al.*, 2011). The imposition of DHA/PGH treatments for short periods after animals had been housed indoors (Le Du *et al.*, 1979; Mayne *et al.*, 1987) or were already grazing to a common PGH (Maher *et al.*, 2003; McEvoy *et al.*, 2009; Curran *et al.*, 2010; McEvoy *et al.*, 2010) may have had a greater effect on behaviour, herbage intake (and consequently milk yield) than was observed in the current experiment. Measuring the effect of PGH throughout the entire year in the current experiment also resulted in the experimental period including periods when herbage growth rates were lower than demand and supplementary feeds (silage and concentrate) were fed to cows. This could have buffered the effect that PGH would have on milk yield when compared to experiments that excluded the use of supplementary feeds (Baudracco *et al.*, 2010). However, all dairy farms in the experimental region use supplementary feeds during the year and the levels of supplementation were low compared to the national average for dairy farms (636, 880 and 765 kg OM cow^{-1} for 2007, 2008 and 2009, respectively) (National Farm Survey (NFS), 2007, 2008, 2009).

7.4.5. Implications for grazing management

Achieving a target PGH of 4 cm increased BNF estimates and herbage production which, as stocking rate was constant across treatments, resulted in a greater proportion of the land being used for silage production when compared to achieving a target PGH of 6 cm. The weather conditions of the years in the current experiment highlight the implications of silage surplus/deficit on farms with impeded drainage. Extremely wet soil conditions can reduce herbage growth through anoxia (McFarlane *et al.*, 2003) and treading damage by grazing animals (Greenwood and McKenzie, 2001; Nie *et al.*, 2001; Drewry *et al.*, 2008). This not only results in lower silage production but also increases silage demand as animals are more likely to be housed.

All treatments required more housing at night during the wetter years of 2008 and 2009. However, the 6 cm PGH treatment was housed for a total of 41 and 36 days more than the 4 and 5 cm treatments, respectively. This occurred when the 6 cm PGH herd was housed at night during April, August and October. Figure 7.4 shows the growth rates for the PGH treatments throughout the experiment. The difference between treatments in 2009 was not much greater at these times of the year than in previous years. However, overall growth rates were lower across all treatments in 2009 and this exacerbated the problems of grazing on wet soil because it further limited the options of drier paddocks that could be grazed. Wet soils in this region require a flexible management approach where the drier paddocks are grazed when conditions are wettest. When herbage supply is lower than demand (approximately 36 kg OM ha^{-1} day^{-1}) for long periods, as it was with the 6 cm PGH in 2009, the ability to do this is curtailed.

Financial net margins have already been shown to be negatively correlated with annual rainfall at Solohead Research Farm (Humphreys *et al.*, 2012). In 2007 all the treatments had a net silage surplus but the weather conditions in 2008 and 2009 cancelled this surplus and resulted in net silage deficits in all treatments by the end of the experiment. However, the cost of producing and feeding on-farm silage is approximately 2.8 to 3.2 times higher than grazed pasture on a € per energy-unit-provided basis (O'Donovan *et al.*, 2011). Therefore, the increased herbage production with the 4 cm PGH might be more profitably exploited by increasing the SR. Recent meta-analysis shows that increasing stocking rate by 1 cow ha^{-1} (minimum = 1.3, maximum = 4.5) generally reduces milk yield per cow by 8.7% but increases milk yield per ha by 19.6% (McCarthy *et al.*, 2010).

As SR might be better defined by changes in feed availability/requirements (Holmes *et al.*, 2002; McCarthy *et al.*, 2010), increasing stocking rate in response to the greater herbage production that was achieved with the 4 cm treatment would be logical. However, increasing stocking rate would essentially reduce DHA, with the reductions in DHI and milk production per cow as discussed previously, more likely to occur. Furthermore, as mentioned previously in the discussion, it is not necessarily just lowering the PGH that resulted in increased herbage production, as longer regrowth intervals and a greater amount of silage cutting were also associated with that treatment. Increasing stocking rate could also increase the risk of treading damage which could negate any increase in herbage production on soils with impeded drainage. Management strategies such as on-off grazing (Kennedy *et al.*, 2011) and extending the grazing interval (Phelan *et al.*, 2011) need to be used and further developed in such a scenario.

7.5. Conclusions

The hypothesis that lowering PGH from 6 cm to 4 cm would increase sward clover content and herbage production, but reduce milk yield per cow in the grazing systems of this experiment was partially accepted as there was an increase in herbage production, but there was no increase in sward clover content or reduction in milk yield per cow. Lowering target PGH from 6 to 4 cm was associated with longer herbage regrowth intervals, higher herbage production of both grass and clover and higher silage production. Milk production, liveweight or body condition score in Holstein-Friesian dairy cows was not affected by PGH in this experiment.

A target PGH of 4 cm is therefore recommended for grass-clover swards under grazing systems similar to the ones described as it increased herbage production without negatively affecting animal performance. The effect of increasing stocking rate while lowering PGH on herbage and animal production from grass-clover swards needs to be investigated.

8. Discussion and conclusions

8.1. Defoliation Height/Post-Grazing Height

In both the simulated grazing experiments (Chapters 3 and 4), lowering DH increased clover content, clover herbage production and total annual herbage production. These results are in line with the results of previous experiments (Clark *et al.*, 1974; Briseno De La Hoz and Wilman, 1981; Frame and Boyd, 1987; Acuña and Wilman, 1993; Seresinhe *et al.*, 1994; Schils and Sikkema, 2001). However, the current research showed that these effects of DH can persist for some time after treatments have ended. Lowering DH in autumn (Chapter 4) was found to be particularly beneficial to sward clover content and herbage production in the following spring, with significant increases herbage production (Table 4.3.), sward clover content (Figure 4.3.) and clover stolon mass (Figure 4.4.) in the following May/June, six months after the DH treatments had ended.

Under actual grazing conditions with dairy cows (Chapter 7), there was no significant increase in sward clover content with lower DH/PGH. There were significant increases in clover herbage production when PGH was lowered, but unlike the results of the experiments on mown plots (both in this thesis and in previous work (Figure 2.16)), grass herbage production also increased substantially. Consequently, lowering PGH did not result in significant increases in sward clover content but did result in larger increases in total annual herbage production (1.0 t DM per cm reduction in PGH) than was expected from mown plots (0.4 t DM per ha (Figure 2.16)).

The above differences between the results of the DH experiments and that of the PGH experiments can be attributed to the general differences between grazing and mowing experiments. Under grazing conditions, animal treading (Menneer *et al.*, 2005a), selective grazing (Rutter, 2006), and animal excretion (Bao *et al.*, 1998; Yayato *et al.*, 2000) could all affect the competitive interaction between clover and grass, resulting in higher transfer of fixed-N from clover to grass (Hatch and Murray, 1994; Menneer *et al.*, 2005b; Auerswald *et al.*, 2010) and lower competitiveness of clover (Edwards *et al.*, 1996; Davies, 2001).

The fact that excess herbage was removed as silage may have also had a positive feedback effect on herbage production, as silage harvests have previously been found to increase clover and grass herbage production (Sheldrick *et al.*, 1987; Harris *et al.*, 1999). The results of Chapter 7 suggest that the recommendations of low PGHs for grazed grass-clover swards may not improve the competitiveness and persistence of clover as much as for mown plots. Nevertheless low target PGHs are still recommended for the benefit of increased herbage production in systems such as the ones described. The fact there was a net silage deficit in all three systems at the end of the three years highlights the difficult growing conditions in 2008 and 2009. However, the much larger silage deficit in the 6 cm (-1,917 kg OM cow^{-1}) when compared to the 5 cm or 4 cm treatments (-868 kg and -192 kg OM cow^{-1}, respectively) shows the importance of the increased herbage production with the lower PGH treatments.

Previous DHA experiments on have found that lowering PGH (by offering less daily herbage per animal above a certain height) reduced milk yield per cow (Le Du *et al.*, 1979; Mayne *et al.*, 1987; Maher *et al.*, 2003; Ribeiro Filho *et al.*, 2005; McEvoy *et al.*, 2009; Curran *et al.*, 2010; McEvoy *et al.*, 2010). The experiment in Chapter 7 found no significant effect of PGH on milk yield, milk composition, live weight or BCS. The main differences between the experiment in Chapter 7 and the above previous experiments were that the (i) current experiment was conducted on grass-clover pastures as opposed to grass-only pastures, (ii) PGH was implemented by moving cows once the target PGH had been reached, instead of adjusting stocking rate in a DHA experiment (iii) the current experiment was conducted over entire grazing seasons (February to November) as opposed to shorter (< 30 week periods) after animals had been housed indoors or were already grazing to a common PGH. The manner in which PGH was imposed in the current experiment is similar to how PGH is likely to be imposed on commercial farms in the region. Calculating and allocating specific DHAs requires time, labour, skill and infrastructure that are unlikely on most Irish farms.

The above results suggest that lowering target PGH from 6 to 4 cm can increase herbage production from grass-clover swards without significant negative effects on milk production. However, caution should be taken when dispensing this advice to farmers because grazing management advice is usually given in spring and summer, when the grazing season is already well underway. Lowering PGH in such systems is more similar to many of the DHA experiments described above, and is therefore more likely to negatively affect herbage intake and milk production.

8.2. Defoliation Interval

Summer (May to August) defoliation interval (INT) was investigated on mown plots in Chapter 3, and autumn (summer-to-winter: July to December) INT was investigated on mown plots in Chapter 4. Treatments were: 21, 28 and 42 days for summer INT and 21, 42, 56 and 84 days for autumn INT and in both experiments, the 42-day INT had the highest annual herbage production (Tables 3.2 and 4.2). However, the effect of increasing INT from 21 to 42 days on annual herbage production was greatest when it was conducted in autumn (15%) instead of summer (7%). Furthermore, increasing summer INT from 21 to 42 days significantly reduced subsequent sward clover content in autumn whereas increasing summer-to-winter INT in the same manner had no effect on sward clover content at any point in the experiment. In fact, the summer-to-winter INTs of 56 and 84 days actually resulted in higher sward clover contents in the following summer than the 21 day INT. Subsequent herbage production was also unaffected by summer INT whereas the 21 day summer-to-winter INT resulted in lower subsequent spring grass and total herbage production. All of the above suggests that grazing intervals of 21-days are appropriate management for grass-clover swards in summer, but that longer grazing intervals have beneficial effects in autumn and winter.

Current recommendations for grass-only swards in Ireland are to have grazing intervals of approximately 21 days in summer (Dillon *et al.*, 1999) and to increase grazing interval in autumn in order to build standing herbage masses that can be rationed out to livestock when growth falls below demand, thereby extending the grazing season (Hennessy and Kennedy, 2009). The current study shows that white clover is well suited to such a system and that extending the grazing interval in autumn did not negatively affect subsequent sward clover content or herbage production. In fact, white clover appears to be conducive to farmer's ability to increase grazing intervals in autumn because it has slower rates of decline in CP and OMD values than perennial ryegrass (Dewhurst *et al.*, 2009).

8.3. Fertilizer N Use

The comparison between the grazing systems in Chapter 6 shows that the ES-100N (which received 90 kg fertilizer N in spring) had lower sward clover contents than the ES-0N or the LS-0N (both which received no fertilizer N). Attributing this difference only to fertilizer N is difficult because the systems also differed in stocking rates and, in the case of LS-0N, turnout and housing dates. However, a large amount of previous research has clearly shown that fertilizer-N applications increase grass production and reduce clover contents and BNF in grass-clover swards (Frame, 1987; Davies and Evans, 1990; Ledgard *et al.*, 1996; Laidlaw and Withers, 1998; Griffith *et al.*, 2000; Davies, 2001; Loiseau *et al.*, 2001; Trott *et al.*, 2004).

Despite the negative effects of fertilizer N on sward clover content, BNF and year-to-year clover persistence, there are substantial benefits in terms of herbage production, particularly in early spring when clover growth rates are low and grass response rates to fertilizer N are high. The higher carrying capacity in the ES-100N than in the ES0N and LS0N was reflected in the higher stocking rate and animal production per ha (milk production per cow was similar across systems; Appendix 23).

Exploiting the benefits of fertilizer N without compromising clover persistence is an obvious research goal. The results of Chapter 6 suggest that clover content and herbage production can be improved by withholding N fertilizer use on swards that received fertilizer N in previous years, especially when winter grazing is included. This suggests that one way to maintain clover persistence would be to withhold fertilizer N input in a proportion of paddocks in a rotational fashion each year, in order to deplete soil N and regain sward clover content. This could be achieved in a rotational grazing system by only using fertilizer N on those paddocks that will be grazed earliest in the year (and will therefore heave been closed first in the previous autumn) and rotating this management through the paddocks each year.

8.4. Winter Grazing

The results from the mown plots (Chapter 4) and the grazing systems (Chapter 7) show different results in terms of the effects of autumn/winter closing date on subsequent sward clover content and herbage production. Delaying closing date from 23 September to 15 December in the mown plots had no effect on subsequent sward clover content or clover herbage production (although it did reduce grass yields in early spring). In contrast Chapter 6 found that the ES-0N (with a closing date of 12 November) had lower sward clover contents, clover herbage production and BNF estimates when compared to the LS-0N (with a closing date of 14 January). However, the lower stocking rates and greater silage removal in the LS-0N when compared to the ES-0N (Table 6.1) mean that a more factorial experiment may be needed to correctly define the effects of closing date in such rotationally grazed systems on white clover sward content.

Most previous studies concluded that maintaining high herbage masses for long periods over winter are detrimental to clover persistence (Davidson and Robson, 1986; Laidlaw and Stewart, 1987; Holmes *et al.*, 1992; Laidlaw *et al.*, 1992). The lack of an effect of closing date in the mown swards needs to be viewed in the context of the relatively lower winter herbage masses and relatively lower grass competition in that experiment in comparison to grazed swards. The results of Chapter 6 are in line with the aforementioned previous experiments where winter grazing was beneficial to sward clover content and herbage production.

8.5. Treading Damage

Unlike the majority of previous studies, the experiment in Chapter 6 investigated the effect of treading damage under actual grazing conditions (as opposed to imposed treading damage on small plots). These previous experiments generally imposed severe treading treatments and recorded large reductions in herbage yield of up to 88% in grass swards (Drewry *et al.*, 2008) and up to 70% reductions in clover content of grass-clover swards (Menneer *et al.*, 2005a). Chapter 6 recorded much lower reductions in total herbage production (5 to 14%) and sward clover content (21% in June only and no affect on annual clover content). However, it should be noted that reductions in herbage production of up to 58% were recorded following individual treading events in spring. The lower impact on annual herbage production in the present study is attributed to the lower levels of treading damage imposed over the course of the entire grazing season and the fact that cows were removed from paddocks to avoid excessive damage during adverse conditions, as is common practice on Irish dairy farms.

Drewry and Paton (2000) found that repeated treading by dairy cows under grazing conditions (70 to 90 cows ha^{-1} for 24 hrs) in New Zealand reduced a perennial ryegrass pasture production by 12 to 13% on well and poorly drained soils respectively, which is similar to the results in the current experiment. Likewise, Mullen *et al.* (1974) treaded grass swards on clay-loam soil in Ireland at annual stocking rates of 2.0 or 6.2 cattle ha^{-1} each month and found that treading reduced annual herbage production by only 2% to 7%. However, in a following paper Mullen *et al.* (1978) reported that the low effect of treading had been due to high levels of N-fertilizer (350 kg N ha^{-1}) and the reduction increased to 15% with no applied N. In contrast, the reduction in herbage production in this experiment was actually highest in the N fertilized treatment (ES-100N) which might is attributable to the higher stocking rate in that treatment.

The lack of a significant effect of treading on total herbage production in the LS0N system is surprising given the high soil moisture during the two winter periods. However, stocking rates were considerably lower during the winter periods in comparison to early spring due to additional ground for winter grazing and the absence of land allocated to silage production. The effects of treading damage on herbage production were greatest in spring in all three systems with mean reductions of approximately 30% for March, April and May (Figure 6.7). Such a large reduction in herbage production at this time of year has important negative consequences for Irish dairy farms, given the predominance of spring-calving systems and the relative importance of grass growth at this time.

The effect of treading on the herbage mass at individual grazing events (PRHM) varied greatly (+ 9% to -58%) and was found to be significantly related to the amount of soil surface deformation and recovery period thereafter (Figure 6.8). This is in line with previous observations (Cluzeau *et al.*, 1992; Zegwaard *et al.*, 2000; de Klein, 2001; Nie *et al.*, 2001; Pande, 2002; Menneer *et al.*, 2005a). Therefore, from a grazing management perspective, soil surface deformation should be avoided as much as possible but if it does occur, extending the recovery period (e.g. silage removal) can reduce the negative effect.

The negative effects of treading on soil drainage also need to be considered. The experiment in Chapter 6 found that treading under normal grazing conditions increased soil bulk density and reduced the proportion of larger, air-filled soil pores. This has negative implications for transfer of air to plant roots and drainage of these already poorly-drained soils which may increase susceptibility to treading even further. Although recovery was observed in the following February, it is clear that treading damage under grazing conditions can have negative effects on soil functioning within the grazing year, and may have longer-term effects at deeper soil levels or on soils with less ability to recover.

8.6. Overall conclusions

Low DH and PGH increased clover herbage production and/or sward clover content. Unlike some previous experiments, lowering PGH was not associated with any reduction in milk yield per cow. Therefore, low (4 to 5 cm) PGHs should be practiced on grass-clover swards but care should be taken maintain intake by ensuring that unsupplemented animals are not suddenly forced to graze tight in swards that have previously been grazed lax. This was done in the current study by imposing target PGH from initial turnout to the final grazing date in winter.

Simulated grazing intervals of 21 days in summer and 42 days in autumn gave the best results in terms of balancing herbage production, herbage quality (CP and OMD) and sward clover content into the following year. These are similar to current recommendations for grass-only swards in Ireland and white clover may actually assist these aspects of grazing management by maintaining greater herbage quality (and intake rates) for longer periods of regrowth than grass-only swards.

Fertilizer-N application reduces clover’s ability to compete with grass but is generally useful for increasing early spring herbage (grass) production and stocking rate, both of which are important drivers of profitability on Irish dairy farms. Grass-clover swards require flexible, strategic fertilizer N management that only applies fertilizer N when and where it is needed most. Early spring has previously been identified as the best time to apply fertilizer on grass-clover swards clovers contribution (in terms of BNF and herbage production) is low, and fertilizer N response of grass is high. However, subsequent sward clover content is likely to be lower and other management techniques (as identified here) are needed to offset the decline. Ultimately, some form of lowering the soil N-inputs may be needed. This might be achieved by rotating silage/hay removal through the paddocks or not applying fertilizer-N/slurry to a proportion of the paddocks each year (on a rotational basis).

Winter grazing was associated with subsequent increases in sward clover content and herbage production. However, achieving this on farms with early spring turnout and higher stocking rates can be difficult. However, within a rotationally grazed farm, there is considerable variation in the final grazing date of each individual paddock. There is also usually variation in sward clover content between paddocks. Therefore, grass-clover paddocks in which the farmer wants to increase sward clover content should be selected as the last paddocks to graze, where possible.

Treading damage was associated with reductions in herbage production of both grass and clover. The effect of treading damage was greatest in spring, with reductions of herbage production by up to 58% in spring and reductions of sward clover content in June. Particular care should be taken at this time of year, when silage ground has been removed and stocking rates are higher. More research is needed to find practical solutions that enable farmers to reduce treading damage of pasture (both grass and grass-clover) on Irish farms.

8.7. Overall implications

Irish agriculture is heavily dependent on grazed grass, with approximately 90% of the UAA as grassland. Therefore grazing management is central to Irish agriculture. Many scientific publications have established the major economic and environmental benefits of white clover for grazed-grassland farming systems (Andrews *et al.*, 2009; Ledgard *et al.*, 2009; Peyraud *et al.*, 2009; Woodfield and Clark, 2009; Li *et al.*, 2011). Previous research at Solohead has shown that white clover can increase profitability of Irish dairy farming, especially if the two-decade upward trend in fertilizer prices continues

(Humphreys *et al.*, 2012).

Despite these known benefits at research level, the use of white clover on Irish farms is generally believed to be very low. Many Irish farmers view white clover as being difficult to manage correctly, with unstable year-to-year productivity and persistence. The experiments in this thesis have addressed some of these concerns by finding simple management techniques that improved clover herbage production and/or sward clover content into the following year. Appropriate grazing management of white clover should target summer grazing intervals of approximately 21-days, autumn grazing intervals of approximately 42-days, extended winter grazing where possible, avoidance of treading damage (particularly in spring) and the minimization of fertilizer-N use.

These findings contribute to the overall understanding of the interaction between grazing management, sward performance and animal production. The findings can assist in on-farm achievement of the established economic and environmental benefits of white clover.

8.9. Recommendations for Future Work

8.9.1. Meta-analysis of grazing management parameters.

There have been a huge number of experiments on grazing management over the last 50 years. The availability of the results of these experiments has increased dramatically in the last decade through online sources, along with guidelines for meta-analyses and modelling (Singer, 1998; St-Pierre, 2001; Sauvant *et al.*, 2008). At relatively low monetary cost in comparison to field trials, the findings of these previous experiments could be reviewed and compiled and used to test global hypotheses on grazing management. Relationships can be explored using meta-analysis (as in Figures 2.4, 2.15 and 2.16) and used to build mechanistic models of the management-plant-animal interaction. This approach would also enable more quantitative identification of knowledge gaps.

8.9.2. Pre-grazing herbage mass.

The experiments in Chapters 3 and 4 defined grazing interval in terms of days as this is the way grazing interval is generally perceived by farmers and therefore, recommendations should be communicated as such. Pre-grazing herbage mass is a more technical definition of grazing interval that more adequately describes the effect of grazing interval on the sward but is not generally measured by farmers.

In grass swards, OMD and CP generally decline as pre-grazing herbage mass increases and this decline can negatively affect intake by grazing livestock (Binnie *et al.*, 1997; Dillon *et al.*, 1998; Hennessy *et al.*, 2006; Baudracco *et al.*, 2010). White clover maintains OMD, CP and intake rates for longer periods of regrowth than perennial ryegrass and therefore has the potential to offset the declines of these parameters in grass swards. Therefore, there is a need to determine the effects of pre-grazing herbage mass on herbage production, sward clover content and animal performance from grass-clover swards. Carruthers and Henderson (1994) found that bloat occurrence was negatively associated with pre-grazing sward height in New Zealand. Therefore, higher pre-grazing sward heights may actually be more appropriate for grass-clover swards in order to avoid bloat.

8.9.3. Reasons for the low use of white clover on Irish farms.

Approximately 70% of Irish dairy farmers are stocked at less than 2.1 cows ha^{-1} (National Farm Survey (NFS), 2011) and could increase profitability with white clover (Humphreys *et al.*, 2012). However, a questionnaire survey in 2008 found that only 5% of Irish dairy farmers actively used white clover (P. Creighton, personal communication). Therefore, there needs to be more research done to quantify (i) the actual sward clover contents on Irish farms, (ii) the opinions, experiences and concerns that farmers have about white clover and (iii) the current use of management practices that promote/inhibit sward clover contents. This would enable bettor tailoring of research to address farmers concerns.

8.9.4. Macro-economic and macro-environmental analysis of white clover use in Ireland

The economic and environmental benefits/costs associated with white clover in Ireland need to be quantified. As stated above, it is expected (but not fully quantified) that sward white clover contents in Irish grassland are quite low. If this was to be increased, it could have large-scale implications for fertilizer use and seed-sales in Ireland. For example, Caradus *et al*. (1996) estimated that white clover contributed NZ$ 3 billion per year to NZ's economy in the early 1990's (see Chapter 2.3.6). A similar quantification of the current and potential contribution from white clover to the Irish economy needs to be performed along with the environmental benefits such as reduced green-house gas emissions and increased pollinator abundance (which have their own monetary values). Quantifying the net value of increased clover use in Irish agriculture could identify the cost-benefits of initiatives such as environmental subsidisation for clover sowing.

8.9.5. Wet soil management in Ireland

Wet soil is the primary limitation to grazing on Irish dairy farms (Creighton *et al.*, 2011). It limits the length of the grazing season and increases costs associated with housing and feeding livestock (Shalloo *et al.*, 2004). It also makes grassland management much more difficult because paddocks that are immediately due for grazing, fertilizer/slurry applications, silage removal, etc. can become inaccessible for months. As Chapter 6 shows, grazing on wet soil can substantially reduce herbage production, particularly in spring.

Given the predominance of wet soil conditions in Ireland, it is surprising that more studies have not been conducted into measures that can alleviate the problem of wet soils in grazed grassland in Ireland. Kennedy *et al*. (2009) have shown that on-off grazing can be used reduce treading without reducing herbage intake. Other factors need to be explored. For example, a comparison of different sward types and cultivars for resistance to treading damage under bovines needs to be performed for Ireland as was done with sheep in New Zealand (Edmond, 1964). Pre-grazing herbage mass has also been found to reduce the effects of treading damage to swards (Brown, 1968; Pande, 2002). Whether this could be beneficial in ranges that do not affect herbage production or animal performance has not been ascertained. Land drainage is another solution but full cost-benefit analyses (both financial and environmental) of the various existing drainage options needs to be performed, along with investigation of novel drainage techniques.

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Appendix

Appendix 1. Table 3.2 ANOVA results

Total number of inflorescence produced (million ha^{-1})					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
INT	2	12.31979751	6.15989876	5.97	0.0058
DH	2	15.04884391	7.52442196	7.29	0.0022
INT*DH	4	1.58403129	0.39600782	0.38	0.8189
Weight per inflorescence (g DM)					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
INT	2	0.15272804	0.07636402	20.17	<.0001
DH	2	0.03662524	0.01831262	4.84	0.0138
INT*DH	4	0.06616622	0.01654156	4.37	0.0056
Proportion of clover herbage produced as inflorescence (g kg^{-1} DM)					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
INT	2	61239.00578	30619.50289	12.86	<.0001
DH	2	11571.77644	5785.88822	2.43	0.1023
INT*DH	4	25416.36756	6354.09189	2.67	0.0477

Appendix 2. Table 3.3 ANOVA results.

		Grass		**Clover**		**Total**	
Effect	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
INT	2	5.93	0.0059	0.62	0.5421	5.14	0.0109
DH	2	0.64	0.5347	8.13	0.0005	3.55	0.0393
INT*DH	4	0.93	0.4564	1.19	0.3212	1.16	0.3425
Period	2	162.26	<.0001	112.39	<.0001	262.2	<.0001
INT*Period	4	0.84	0.5073	1.82	0.1302	2.96	0.0254
DH*Period	4	10.44	<.0001	2.16	0.0789	11.96	<.0001
INT*DH*Period	8	0.6	0.778	0.44	0.8946	0.45	0.8885

Appendix 3. Table 3.4 ANOVA results.

		Ash		**OMD**		**HM**	
Effect	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
INT	2	0.33	0.7188	0.12	0.8888	0.51	0.6002
DH	2	2.32	0.1132	2.92	0.0604	2.58	0.0828
INT*DH	4	0.69	0.6053	3.38	0.0138	1.40	0.2410
Period	1	598.08	<.0001	227.32	<.0001	55.65	<.0001
INT*Period	2	0.53	0.5955	0.69	0.5055	0.12	0.8872
DH*Period	2	2.59	0.0890	1.55	0.2190	1.24	0.2948
INT*DH*Period	4	0.34	0.8482	0.89	0.4768	0.70	0.5918

Appendix 4. Table 3.5 and Table 3.6 ANOVA results.

Clover content				Stolon mass			
Source	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
INT	2	1.20	0.31	INT	2	1.42	0.2541
DH	2	4.06	0.03	DH	2	2.47	0.10
INT*DH	4	1.12	0.36	INT*DH	4	0.75	0.56
Period	6	150.49	<.0001	Period	3	52.72	<.0001
INT*Period	12	1.54	0.11	INT*Period	6	0.87	0.5158
DH*Period	12	1.6	0.0924	DH*Period	6	0.47	0.8282
INT*DH*Period	24	0.76	0.7848	INT*DH*Period	12	0.74	0.71

Appendix 5. Table 4.2 ANOVA results

Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
INT:				**DH:**				**FIN:**			
Grass											
INT	3	10.01	<.0001	DH	3	1.99	0.1171	FIN	2	0.34	0.7131
DH	3	1.01	0.3909	Fact	5	6.97	<.0001	DH	3	2.64	0.0537
INT*DH	9	0.89	0.537	Fact*DH	15	0.93	0.5268	FIN*DH	6	0.58	0.7421
Date	1	334.85	<.0001	Date	1	807.77	<.0001	Date	1	749.79	<.0001
INT*Date	3	6.96	0.0002	Fact*Date	3	9.84	<.0001	FIN*Date	2	44.69	<.0001
DH*Date	3	10.42	<.0001	DH*Date	5	22.28	<.0001	DH*Date	3	2.43	0.0699
INT*DH*Date	9	1.08	0.384	Fact*DH*Date	15	1.25	0.2371	FIN*DH*Date	6	1.00	0.428
Clover											
INT	3	24.14	<.0001	DH	3	33.13	<.0001	FIN	2	2.38	0.0975
DH	3	21.9	<.0001	Fact	5	19.35	<.0001	DH	3	20.45	<.0001
INT*DH	9	0.53	0.8532	Fact*DH	15	0.61	0.8666	FIN*DH	6	0.51	0.7971
Date	1	234.85	<.0001	Date	1	604.97	<.0001	Date	1	733.09	<.0001
INT*Date	3	40.44	<.0001	Fact*Date	5	36.5	<.0001	FIN*Date	2	0.22	0.7996
DH*Date	3	0.21	0.8896	DH*Date	3	1.38	0.2511	DH*Date	3	2.68	0.051
INT*DH*Date	9	0.54	0.8426	Fact*DH*Date	15	0.74	0.7391	FIN*DH*Date	6	0.8	0.5703
Total											
INT	3	29.89	<.0001	DH	3	30.95	<.0001	FIN	2	1.85	0.1631
DH	3	32.05	<.0001	Fact	5	24.23	<.0001	DH	3	10.53	<.0001
INT*DH	9	1.12	0.3553	Fact*DH	15	1.34	0.1842	FIN*DH	6	1.07	0.3889
Date	1	18.4	<.0001	Date	1	30.35	<.0001	Date	1	0	0.9991
INT*Date	3	104.62	<.0001	Fact*Date	5	67.85	<.0001	FIN*Date	2	47.9	<.0001
DH*Date	3	19.74	<.0001	DH*Date	3	23.28	<.0001	DH*Date	3	9.74	<.0001
INT*DH*Date	9	1.55	0.1359	Fact*DH*Date	15	1.18	0.2899	FIN*DH*Date	6	0.61	0.72

Appendix 6. Figure 4.2 (BNF) ANOVA results.

Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
INT:				**DH:**				**FIN:**			
INT	3	10.55	<.0001	DH	3	13.98	<.0001	FIN	2	0.25	0.78
DH	3	13.22	<.0001	Fact (INT/FIN)	5	8.56	<.0001	DH	3	5.18	0.0035
INT*DH	9	0.92	0.5138	DH*Fact	15	1.07	0.3973	FIN*DH	6	1.33	0.2618

Appendix 7. Table 4.3 (NHA) ANOVA results.

Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
INT:				**DH:**				**FIN:**			
INT	3	57.84	<.0001	DH	3	20.46	<.0001	FIN	2	0.18	0.8379
DH	3	15.50	<.0001	Fact	5	63.91	<.0001	DH	3	6.02	0.0014
INT*DH	9	1.29	0.2610	Fact*DH	15	1.53	0.0920	FIN*DH	6	0.93	0.4804
Date	4	4100.37	<.0001	Date	4	6011.53	<.0001	Date	4	2859.51	<.0001
INT*Date	12	67.71	<.0001	Fact*Date	20	46.25	<.0001	FIN*Date	8	4.01	0.0002
DH*Date	12	16.18	<.0001	DH*Date	12	20.38	<.0001	DH*Date	12	5.48	<.0001
INT*DH*Date	36	0.80	0.7904	Fact*DH*Date	60	0.97	0.5602	FIN*DH*Date	24	0.95	0.5403

Appendix 8. Table 4.4 ANOVA results.

INT:				DH:				FIN:			
Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
HM:											
INT	3	427.80	<.0001	DH	3	42.49	<.0001	FIN	3	15.89	<.0001
DH	3	38.16	<.0001	Fact	5	404.36	<.0001	DH	2	255.75	<.0001
INT*DH	9	3.88	0.0002	Fact*DH	15	2.19	0.0080	FIN*DH	6	0.16	0.9867
Date	1	570.17	<.0001	Date	1	1802.68	<.0001	Date	1	1714.46	<.0001
INT*Date	3	194.99	<.0001	Fact*Date	3	35.26	<.0001	FIN*Date	2	144.80	<.0001
DH*Date	3	27.15	<.0001	DH*Date	5	212.45	<.0001	DH*Date	3	15.17	<.0001
INT*DH*Date	9	4.40	<.0001	Fact*DH*Date	15	2.57	0.0016	FIN*DH*Date	6	0.83	0.5459
Ash:											
INT	3	5.59	0.0012	DH	3	2.13	0.1010	FIN	3	1.69	0.1735
DH	3	2.14	0.0980	Fact	5	7.29	<.0001	DH	2	3.80	0.0258
INT*DH	9	0.44	0.9084	Fact*DH	15	0.82	0.6537	FIN*DH	6	0.92	0.4861
Date	1	23.82	<.0001	Date	1	13.70	0.0004	Date	1	0.04	0.8499
INT*Date	3	5.21	0.0020	Fact*Date	5	5.99	<.0001	FIN*Date	2	3.08	0.0508
DH*Date	3	6.84	0.0003	DH*Date	3	5.48	0.0016	DH*Date	3	0.78	0.5102
INT*DH*Date	9	0.65	0.7495	Fact*DH*Date	15	0.84	0.6293	FIN*DH*Date	6	0.70	0.6538
CP:											
INT	3	3.96	0.0100	DH	3	0.51	0.6784	FIN	3	0.07	0.9742
DH	3	2.35	0.0763	Fact	5	3.54	0.0045	DH	2	2.88	0.0618
INT*DH	9	0.44	0.9091	Fact*DH	15	0.94	0.5180	FIN*DH	6	1.31	0.2625
Date	1	207.99	<.0001	Date	1	224.98	<.0001	Date	1	96.73	<.0001
INT*Date	3	26.47	<.0001	Fact*Date	5	17.76	<.0001	FIN*Date	2	8.50	0.0004
DH*Date	3	0.42	0.7423	DH*Date	3	0.21	0.8880	DH*Date	3	0.10	0.9592
INT*DH*Date	9	1.34	0.2221	Fact*DH*Date	15	0.93	0.5354	FIN*DH*Date	6	0.56	0.7592
OMD											
INT	3	11.35	<.0001	DH	3	0.93	0.4278	FIN	3	0.94	0.4262
DH	3	0.50	0.6839	Fact	5	9.20	<.0001	DH	2	0.16	0.8486
INT*DH	9	0.58	0.8078	Fact*DH	15	0.96	0.4951	FIN*DH	6	1.37	0.2362
Date	1	103.09	<.0001	Date	1	92.61	<.0001	Date	1	4.10	0.0456
INT*Date	3	28.87	<.0001	Fact*Date	5	25.39	<.0001	FIN*Date	2	5.05	0.0082
DH*Date	3	1.26	0.2902	DH*Date	3	0.38	0.7651	DH*Date	3	1.30	0.2793
INT*DH*Date	9	1.21	0.2957	Fact*DH*Date	15	1.41	0.1476	FIN*DH*Date	6	1.59	0.1586

Appendix 9. Figure 4.3 (sward clover content) ANOVA results.

INT::				DH:				FIN:			
Effect	DF	F Value	Pr > F	Effect	DF	FValue	Pr > F	Effect	DF	FValue	Pr > F
INT	3	9.09	<.0001	DH	3	26.16	<.0001	FIN	2	2.85	0.0596
DH	3	17.25	<.0001	Fact	5	6.87	<.0001	DH	3	15.87	<.0001
INT*DH	9	0.7	0.7115	Fact*DH	15	0.87	0.5973	FIN*DH	6	0.65	0.6914
Date	4	345.63	<.0001	Date	4	695.71	<.0001	Date	4	625.16	<.0001
INT*Date	12	12.07	<.0001	Fact*Date	20	11.77	<.0001	FIN*Date	8	1.31	0.2394
DH*Date	12	2.46	0.0044	DH*Date	12	3.16	0.0002	DH*Date	12	2.12	0.0166
INT*DH*Date	36	0.5	0.9932	Fact*DH*Date	60	0.69	0.9601	FIN*DH*Date	24	0.96	0.5233

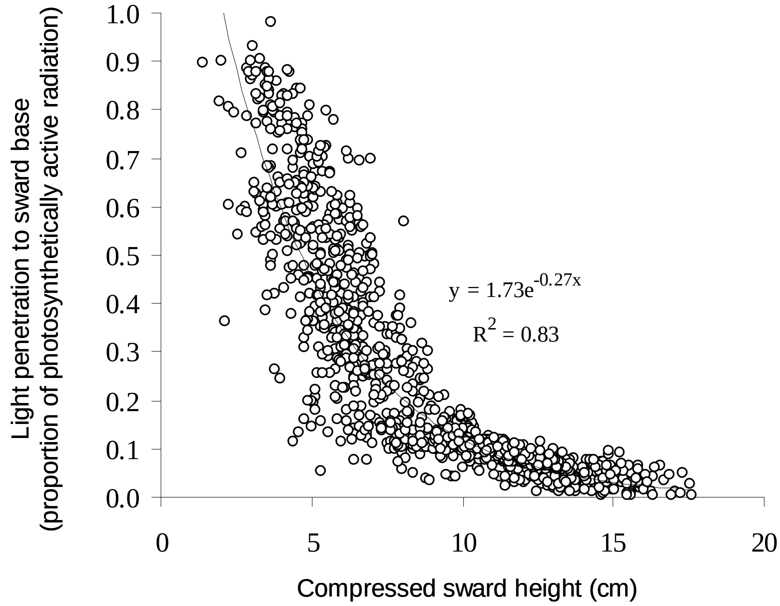
Appendix 10. Figure 4.4 (Stolon mass) ANOVA results.

INT:				DH:				FIN:			
Effect	DF	F Value	Pr > F	Effect	DF	FValue	Pr > F	Effect	DF	FValue	Pr > F
INT	3	25.78	<.0001	DH	3	10.84	<.0001	FIN	2	1.16	0.3232
DH	3	2.38	0.0779	Fact	5	38.63	<.0001	DH	3	3.62	0.0195
INT*DH	9	0.59	0.7995	Fact*DH	15	0.94	0.5152	FIN*DH	6	0.26	0.9546
Date	4	348.62	<.0001	Date	4	428.35	<.0001	Date	4	415.22	<.0001
INT*Date	12	3.8	<.0001	Fact*Date	20	2.7	0.0001	FIN*Date	8	3.31	0.0015
DH*Date	12	3.78	<.0001	DH*Date	12	1.73	0.0579	DH*Date	12	1.46	0.144
INT*DH*Date	36	0.88	0.6634	Fact*DH*Date	60	0.96	0.5543	FIN*DH*Date	24	2.12	0.0028

Appendix 11. Table 3.5 ANOVA results.

Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
LP:											
INT	3	180.74	<.0001	DH	3	125.07	<.0001	FIN	2	134.08	<.0001
DH	3	120.17	<.0001	Fact	5	168.82	<.0001	DH	3	46.21	<.0001
INT*DH	9	3.97	0.0005	Fact*DH	15	2.75	0.0004	FIN*DH	6	1.80	0.1002
Date	4	2619.49	<.0001	Date	4	2489.72	<.0001	Date	4	778.13	<.0001
INT*Date	12	36.61	<.0001	Fact*Date	12	27.57	<.0001	FIN*Date	8	45.66	<.0001
DH*Date	12	25.22	<.0001	DH*Date	20	51.42	<.0001	DH*Date	12	12.46	<.0001
INT*DH*Date	36	1.79	0.0054	Fact*DH*Date	60	1.42	0.0268	FIN*DH*Date	24	1.00	0.4608
SH:											
Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
INT	3	139.92	<.0001	DH	3	68.58	<.0001	FIN	2	40.74	<.0001
DH	3	29.01	<.0001	Fact	5	127.84	<.0001	DH	3	45.55	<.0001
INT*DH	9	1.61	0.1316	Fact*DH	15	1.65	0.057	FIN*DH	6	1.00	0.4229
Date	4	1453.53	<.0001	Date	4	1974.98	<.0001	Date	4	1084.56	<.0001
INT*Date	12	35.24	<.0001	Fact*Date	12	17.31	<.0001	FIN*Date	8	11.55	<.0001
DH*Date	12	14.36	<.0001	DH*Date	20	27.76	<.0001	DH*Date	12	8.34	<.0001
INT*DH*Date	36	0.99	0.4923	Fact*DH*Date	60	1.34	0.0529	FIN*DH*Date	24	1.77	0.0176

Appendix 12. Exponential relationship between compressed sward height (SH) and sward light penetration described in Chapter 4. Points are raw data from all individual measurements during the course of the experiment. Relationship derived using Microsoft Excel graph tools.



Appendix 13. Chapter 5. Results from ANCOVA comparing the correlations of annual clover herbage yield and BFN from (i) meta-analyses and (ii) the plots in Chapter 4.

	DF	Type III SS	Mean Square	F Value	Pr > F
DM	1	430333.4	430333.4	903.11	<.0001
Source (meta analysis or Solohead plot results)	1	4145.9	4145.9	8.7	0.0036
Slope	1	36.5	36.5	0.08	0.7822
Intercept	1	26791.4	26791.4	56.48	<.0001

Appendix 14. Figure 6.2 ANOVA results for the effect of grazing system (syst) and treading level (tread) on soil properties.

		GWC		VWC		SBD		AFP	
Effect	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
syst	2	0.40	0.67	1.31	0.28	0.32	0.73	1.94	0.15
tread	2	18.23	<.0001	1.78	0.18	45.02	<.0001	14.15	<.0001
Month	1	38.71	<.0001	89.75	<.0001	5.28	0.03	31.69	<.0001
syst*Month	2	0.07	0.93	0.59	0.56	0.15	0.86	1.14	0.33
tread*Month	2	1.97	0.15	2.94	0.06	1.02	0.37	2.19	0.12

Appendix 15. Table 6.2. ANOVA results for the effect of grazing system and treading level on sward clover content and stolon mass.

		Clover content		Stolon mass	
Effect	DF	F Value	Pr > F	F Value	Pr > F
System	2	5.17	0.0241	19.35	<.0001
Treading	2	1.93	0.1637	0.44	0.6453
Month	3	75.19	<.0001	12.86	<.0001
Treading*Month	6	3.24	0.0064	0.66	0.6855
Year	1	0.24	0.6257	2.59	0.1100
System*Month	6	0.90	0.4957	0.45	0.8457
Year*Month	3	24.57	<.0001	5.06	0.0024
System*Year	2	3.27	0.0428	4.18	0.0174
System*Year*Month	6	3.98	0.0015	0.04	0.9998

Appendix 16. Table 6.3. ANOVA results for the effect of grazing system and treading level on annual herbage production.

		Total		Grass		Clover		BNF	
Effect	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
System	2	7.59	0.0016	13.73	0.0008	5.62	0.0190	9.91	0.0029
Treading	2	3.63	0.0355	17.90	<.0001	18.61	<.0001	16.90	<.0001
Year	1	0.09	0.7633	0.95	0.3400	2.37	0.1365	6.34	0.0132
System*Year	2	1.24	0.3068	0.22	0.8041	3.50	0.0464	7.51	0.0179

Appendix 17. Table 7.1 and Figure 7.3. Effect of PGH and year on herbage production and BNF.

		Grass		Clover		Total		BNF	
Effect	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
PGH	2	5.88	0.0054	3.24	0.0485	8.47	0.0008	3.35	0.0439
Year	2	24.99	<.0001	0.27	0.7677	17.76	<.0001	0.33	0.7197
PGH*Year	4	0.66	0.6249	0.17	0.9531	0.37	0.8261	0.17	0.9512

Appendix 18. Effects of PGH on sward clover content and clover stolon mass (mean annual results in Table 7.1).

Clover content

Effect	DF	FValue	Pr > F
PGH	2	0.56	0.5840
Year (Y)	2	5.77	0.0076
PGH*Y	4	0.46	0.7659
Month (M)	2	87.41	<.0001
PGH*M	4	0.26	0.9018
Y*M	4	7.82	<.0001
PGH*Y*M	8	0.75	0.6482

Least square means

Effect	PGH	Year	Month	Mean	SD	t Value
PGH	4cm			211	17.66	11.95
PGH	5cm			186	17.66	10.52
PGH	6cm			192	17.66	10.87
Year		2007		159	9.67	16.43
Year		2008		209	18.36	11.40
Year		2009		221	17.62	12.51
PGH*Y	4cm	2007		169	16.75	10.11
PGH*Y	4cm	2008		211	31.81	6.64
PGH*Y	4cm	2009		253	30.52	8.28
PGH*Y	5cm	2007		143	16.75	8.52
PGH*Y	5cm	2008		219	31.81	6.87
PGH*Y	5cm	2009		196	30.52	6.42
PGH*Y	6cm	2007		165	16.75	9.83
PGH*Y	6cm	2008		198	31.81	6.24
PGH*Y	6cm	2009		213	30.52	6.97
Month			4	80	13.88	5.76
Month			6	214	14.27	14.97
Month			9	295	13.88	21.26
PGH*M	4cm		4	86	24.05	3.58
PGH*M	4cm		6	226	24.72	9.14
PGH*M	4cm		9	321	24.05	13.36
PGH*M	5cm		4	76	24.05	3.18
PGH*M	5cm		6	208	24.72	8.41
PGH*M	5cm		9	273	24.05	11.36
PGH*M	6cm		4	77	24.05	3.22
PGH*M	6cm		6	207	24.72	8.38
PGH*M	6cm		9	291	24.05	12.11
Y*M		2007	4	72	13.24	5.45
Y*M		2007	6	148	13.24	11.17
Y*M		2007	9	256	13.24	19.37
Y*M		2008	4	107	24.74	4.31
Y*M		2008	6	259	26.65	9.73
Y*M		2008	9	262	24.74	10.59
Y*M		2009	4	61	24.13	2.52
Y*M		2009	6	234	24.13	9.68
Y*M		2009	9	367	24.13	15.21
PGH*Y*M	4cm	2007	4	84	22.93	3.67
PGH*Y*M	4cm	2007	6	161	22.93	7.00
PGH*Y*M	4cm	2007	9	263	22.93	11.47
PGH*Y*M	4cm	2008	4	101	42.85	2.36
PGH*Y*M	4cm	2008	6	275	46.15	5.95
PGH*Y*M	4cm	2008	9	258	42.85	6.02
PGH*Y*M	4cm	2009	4	73	41.80	1.74
PGH*Y*M	4cm	2009	6	243	41.80	5.80
PGH*Y*M	4cm	2009	9	443	41.80	10.60
PGH*Y*M	5cm	2007	4	67	22.93	2.93
PGH*Y*M	5cm	2007	6	131	22.93	5.69
PGH*Y*M	5cm	2007	9	231	22.93	10.05
PGH*Y*M	5cm	2008	4	107	42.85	2.50
PGH*Y*M	5cm	2008	6	265	46.15	5.74
PGH*Y*M	5cm	2008	9	284	42.85	6.62
PGH*Y*M	5cm	2009	4	55	41.80	1.31

Stolon mass

Effect	DF	FValue	Pr > F
PGH	2	0.52	0.6032
Year (Y)	2	3.41	0.0463
PGH*Y	4	0.11	0.9782
Month (M)	3	69.13	<.0001
PGH*M	6	1.34	0.2605
Y*M	6	33.93	<.0001
PGH*Y*M	12	1.47	0.1523

Least square means

Effect	PGH	Year	Momth	Mean	SD	t Value
PGH	1			514	35.69	14.39
PGH	2			548	35.69	15.34
PGH	3			564	35.69	15.81
Year		2007		568	39.21	14.48
Year		2008		607	42.31	14.35
Year		2009		450	42.57	10.57
PGH*Y	4cm	2007		605	67.92	8.91
PGH*Y	4cm	2008		624	73.29	8.51
PGH*Y	4cm	2009		464	73.73	6.29
PGH*Y	5cm	2007		544	67.92	8.01
PGH*Y	5cm	2008		616	73.29	8.41
PGH*Y	5cm	2009		482	73.73	6.54
PGH*Y	6cm	2007		555	67.92	8.17
PGH*Y	6cm	2008		582	73.29	7.94
PGH*Y	6cm	2009		404	73.73	5.49
Month			1	356	27.39	12.99
Month			2	458	27.39	16.73
Month			3	598	27.39	21.84
Month			4	755	27.39	27.56
PGH*M	4cm		1	362	47.44	7.64
PGH*M	4cm		2	524	47.44	11.04
PGH*M	4cm		3	624	47.44	13.16
PGH*M	4cm		4	746	47.44	15.73
PGH*M	5cm		1	344	47.44	7.26
PGH*M	5cm		2	414	47.44	8.73
PGH*M	5cm		3	632	47.44	13.31
PGH*M	5cm		4	801	47.44	16.87
PGH*M	6cm		1	361	47.44	7.61
PGH*M	6cm		2	437	47.44	9.21
PGH*M	6cm		3	539	47.44	11.36
PGH*M	6cm		4	717	47.44	15.12
Y*M		2007	1	166	52.12	3.18
Y*M		2007	2	340	52.12	6.53
Y*M		2007	3	700	52.12	13.42
Y*M		2007	4	1066	52.12	20.45
Y*M		2008	1	646	56.24	11.48
Y*M		2008	2	764	56.24	13.58
Y*M		2008	3	541	56.24	9.62
Y*M		2008	4	479	56.24	8.51
Y*M		2009	1	256	56.58	4.53
Y*M		2009	2	271	56.58	4.79
Y*M		2009	3	554	56.58	9.79
Y*M		2009	4	719	56.58	12.71
PGH*Y*M	6cm	2007	1	207	90.28	2.29
PGH*Y*M	6cm	2007	2	336	90.28	3.73
PGH*Y*M	6cm	2007	3	648	90.28	7.18
PGH*Y*M	6cm	2007	4	1027	90.28	11.37
PGH*Y*M	6cm	2008	1	693	97.42	7.11
PGH*Y*M	6cm	2008	2	728	97.42	7.47
PGH*Y*M	6cm	2008	3	479	97.42	4.91
PGH*Y*M	6cm	2008	4	428	97.42	4.39
PGH*Y*M	6cm	2009	1	184	98.00	1.88

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Continued from previous (Appendix 21).

PGH*Y*M	5cm	2009	6	228	41.80	5.46
PGH*Y*M	5cm	2009	9	305	41.80	7.30
PGH*Y*M	6cm	2007	4	65	22.93	2.85
PGH*Y*M	6cm	2007	6	153	22.93	6.65
PGH*Y*M	6cm	2007	9	276	22.93	12.03
PGH*Y*M	6cm	2008	4	112	42.85	2.61
PGH*Y*M	6cm	2008	6	239	46.15	5.18
PGH*Y*M	6cm	2008	9	245	42.85	5.71
PGH*Y*M	6cm	2009	4	55	41.80	1.32
PGH*Y*M	6cm	2009	6	230	41.80	5.50
PGH*Y*M	6cm	2009	9	354	41.80	8.46
PGH*Y*M	6cm	2009	2	247	98.00	2.52
PGH*Y*M	6cm	2009	3	490	98.00	4.99
PGH*Y*M	6cm	2009	4	697	98.00	7.11
PGH*Y*M	5cm	2007	1	113	90.28	1.25
PGH*Y*M	5cm	2007	2	296	90.28	3.28
PGH*Y*M	5cm	2007	3	673	90.28	7.45
PGH*Y*M	5cm	2007	4	1094	90.28	12.12
PGH*Y*M	5cm	2008	1	630	97.42	6.47
PGH*Y*M	5cm	2008	2	604	97.42	6.20
PGH*Y*M	5cm	2008	3	599	97.42	6.15
PGH*Y*M	5cm	2008	4	632	97.42	6.49
PGH*Y*M	5cm	2009	1	290	98.00	2.96
PGH*Y*M	5cm	2009	2	342	98.00	3.49
PGH*Y*M	5cm	2009	3	622	98.00	6.35
PGH*Y*M	5cm	2009	4	675	98.00	6.89
PGH*Y*M	4cm	2007	1	177	90.28	1.96
PGH*Y*M	4cm	2007	2	388	90.28	4.30
PGH*Y*M	4cm	2007	3	778	90.28	8.61
PGH*Y*M	4cm	2007	4	1077	90.28	11.93
PGH*Y*M	4cm	2008	1	615	97.42	6.31
PGH*Y*M	4cm	2008	2	959	97.42	9.84
PGH*Y*M	4cm	2008	3	545	97.42	5.60
PGH*Y*M	4cm	2008	4	376	97.42	3.86
PGH*Y*M	4cm	2009	1	295	98.00	3.01
PGH*Y*M	4cm	2009	2	224	98.00	2.28
PGH*Y*M	4cm	2009	3	550	98.00	5.61
PGH*Y*M	4cm	2009	4	786	98.00	8.02

Appendix 19. Effect of PGH on pregrazing and presilage herbage production (Table 7.1).

Pregrazing cuts

Effect	DF	F Value	Pr > F		
PGH	2	1.78	0.2796		
Year	2	5.21	0.0769		
Least Squares Means					
Effect	PGH	Year	Mean	SD	t Value
PGH	4		7.9	0.29	27.42
PGH	5		7.6	0.29	26.3
PGH	6		7.1	0.29	24.76
Year		2007	8.0	0.29	27.84
Year		2008	7.9	0.29	27.09
Year		2009	6.8	0.29	23.56

Presilage cuts

Effect	DF	F Value	Pr > F		
PGH	2	11.86	0.0208		
Year	2	30.89	0.0037		
Least Squares Means					
Effect	PGH	Year	Mean	SD	t Value
PGH	4		3.1	0.18	17.69
PGH	5		2.6	0.18	14.6
PGH	6		1.9	0.18	10.82
Year		2007	3.7	0.18	20.78
Year		2008	2.0	0.18	11.43
Year		2009	1.9	0.18	10.91

Appendix 20. ANOVA results for table 7.2.

		Ash		OMD		CP		Grazing interval		Pre-grazing herbage mass	
Effect	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
PGH	2	18.64	<.0001	0.89	0.4124	4.45	0.0122	2.98	0.0516	8.03	0.0004
Year	2	1.67	0.1899	0.55	0.5769	12.49	<.0001	38.3	<.0001	7.35	0.0007
PGH*Year	4	0.64	0.6333	3.46	0.0085	1.52	0.1964	4.02	0.0072	4.41	0.0016

Appendix 21. ANOVA results for Table 7.3. Note that DHA used year as replicate, therefore no PGH*year interaction.

		Days at grass		**DHA**		**DHI**	
Effect	Num DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
PGH	2	18.79	<.0001	1.07	0.4252	1.51	0.2227
Year	2	128.78	<.0001	2.36	0.2104	1.56	0.2129
PGH*Year	4	17.40	<.0001			1.03	0.3954
		Grazed pasture intake		**Silage intake**		**Concentrate intake**	
Effect	Num DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
PGH	2	1.67	0.1907	24.21	<.0001	0.01	0.9913
Year	2	18.16	<.0001	143.41	<.0001	220.44	<.0001
PGH*Year	4	1.04	0.3884	22.10	<.0001	0.00	1.0000

Appendix 22. ANOVA results for Table 7.4.

		Milk		SCM		Fat		Protein		Lactose	
Effect	DF	FValue	Pr > F	FValue	Pr > F	FValue	Pr > F	FValue	Pr > F	F Value	Pr > F
PGH	2	0.40	0.6687	0.68	0.5056	0.68	0.5067	0.94	0.3944	0.34	0.7146
Year	2	2.62	0.0756	6.59	0.0017	7.21	0.001	2.99	0.0528	6.4	0.0021
PGH*Year	4	0.10	0.9813	0.12	0.9761	0.07	0.9918	0.3	0.8799	0.14	0.9657
		LW		**Mean LW**		**Mean BCS**		**BCS end lact**		**DIM**	
Effect	DF	FValue	Pr > F	FValue	Pr > F	FValue	Pr > F	FValue	Pr > F	FValue	Pr > F
PGH	2	0.57	0.5654	1.09	0.3373	1.54	0.2177	1.72	0.1821	0.03	0.9706
Year	2	6.72	0.0015	4.77	0.0095	1.73	0.1795	1.85	0.1596	4.49	0.0125
PGH*Year	4	0.36	0.8377	0.26	0.9049	0.32	0.8675	0.2	0.9404	0.11	0.9791

Appendix 23. Mean annual milk productions for the grazing systems in Chapter 6. Means were compared with ANOVA in SAS using the PROC GLM procedure.

Year	2007			2008			SEM		
System	ES100N	ES0N	LS0N	ES100N	ES0N	LS0N	System	Year	S*Y
Milk yield (kg cow^{-1})	6371	6511	6605	6174	6265	6082	162.8	132.9	230.3
SCM (kg cow^{-1})	6377	6541	6393	6148	6276	6041	154.2	125.9	218.1
Fat (kg cow^{-1})	274	282	273	266	270	262	7.2	5.8	10.1
Protein (kg cow^{-1})	230	236	228	218	226	221	5.5	4.5	7.8
Lactose (kg cow^{-1})	301	308	309	291	295	279	7.8	6.3*	11.0
Fat + Protein (kg cow^{-1})	504	518	502	484	497	483	12.2	9.9	17.2
Days in Milk	295	299	303	289	288	289	2.5	2.0**	3.5
Days at grass	221	234	231	225	234	235	3.6	3.0	5.1
Concentrate fed (kg DM cow^{-1})	502	502	720	647	577	624	15.1***	12.3***	21.3***
Stocking rate	2.12	1.59	1.53	2.12	1.59	1.53			
Milk yield (kg ha^{-1})	13507	10352	10106	13089	9961	9305			
Fat + Protein (kg ha^{-1})	1069	823	768	1026	790	739			
Concentrate fed (kg DM ha^{-1})	1064	798	1101	1372	917	955			

*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.