Aspects of low fertiliser nitrogen input seasonal pasture-based milk production

Thesis submitted as partial requirement for the degree of

Doctor of Philosophy

by

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List of Abbreviations

ANOVA	Analysis of variance
APC	Average pasture cover
BCS	Body condition score
BNF	Biological nitrogen fixation
BW	Bodyweight
C	Carbon
C:N	Carbon:nitrogen
CAN	Calcium ammonium nitrate
CAP	Common Agricultural Policy
CEC	Cation exchange capacity
CFR_{max}	Maximum curd firming rate
CP	Crude protein
CSO	Central Statistics Office
DAFM	Department of Agriculture, Food and the Marine
DM	Dry matter
DMI	Dry matter intake
DON	Dissolved organic nitrogen
EC	European Commission
ECE	Economic Commission for Europe
ES0N	Early spring calving without fertiliser nitrogen
ES100N	Early spring calving with annual fertiliser nitrogen input of 100 kg·ha^{-1}
EU	European Union
G′	Elastic shear modulus
GC	Grass-clover system
GLM	General linear model
GO	Grass-only system
GS	Gel strength
GT	Gelation time
h	Hour
ha	Hectare
IVOMD	*In vitro* organic matter digestibility

K	Potassium
kg	Kilogram
LS0N	Late spring calving without fertiliser nitrogen
LW	Liveweight
mm	Millimetre
N	Nitrogen
N_r	Reactive nitrogen
N_2	Di-nitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
NMA	National Milk Agency
NPN	Non-protein nitrogen
NS	Not significant
NUE	Nitrogen use efficiency
OCBs	Organic certification bodies
OMD	Organic matter digestibility
P	Phosphorus
Pa	Pascal
PCC	Peak clover content
PP	Pasture production
PGH	Post-grazing height
PGPM	Pre-grazing pasture mass
R^2	Coefficient of determination
SCC	Somatic cell count
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean
SMB	Soil microbial biomass
SMD	Soil moisture deficit
SOC	Soil organic carbon

SOM	Soil organic matter
t	Tonne
TN	Total nitrogen
UK	United Kingdom
VSMC	Volumetric soil moisture content
w/w	Weight for weight

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.

Katie Scully

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Abstract

Synthetic fertiliser nitrogen (N) is a significant contributor to the environmental footprints of dairy products from pasture-based systems. Biological N fixation (BNF) in association with white clover can reduce dependency on fertiliser N and consequently lower environmental footprints. This thesis investigated aspects of white clover management strategies in seasonal pasture-based dairy production. One experiment quantified the supply of background N in Irish permanent grasslands. Another investigated the productivity of white clover-based grassland for milk production based on several systems-scale studies. A third experiment examined the effect of fertiliser N input to grass-clover swards and calving date on the productivity of pasture-based dairy production. Mean background N was 141 kg ha^{-1} across Irish grasslands and varied both temporally and spatially during the growing season. The background N was influenced by soil physical and chemical properties along with meteorological factors. Dairy production based on low-input clover-based grassland receiving fertiliser N input of approximately 97 kg N ha^{-1} was similar to N-fertilised grass-only receiving 244 kg N ha^{-1} in several systems-scale studies. Mean annual and seasonal pasture production per ha and milk production per cow was similar between these two grassland systems. Sward white clover and BNF decreased with increasing annual N fertilisation. A mean calving date in mid-February is recommended for zero-fertiliser N input white clover-based systems. A later than typical calving date (in mid-April) resulted in inefficient use of pasture for milk production in a zero-fertiliser N white clover-based system. Extending lactation into the following winter resulted in lower concentrations of milk constituents and affected processing characteristics of late lactation milk. Nevertheless, maximising overwintering of stolon and root dry matter mass by tight winter grazing increased white clover productivity and persistence in grassland. There is considerable potential for milk production on clover-based swards receiving low or no input of fertiliser N.

Chapter 1. Introduction

1.1 Thesis Background

Prior the commercialisation of synthetic mineral fertiliser N during the late 1960's and 1970's pasture production was largely dependent on non-fertiliser N sources; N_2 fixed by legumes and the mineralisation of N in crop residues, animal manures and soil organic matter (Keeney, 1982; Whitehead, 1995; Jarvis et al., 2011). In Ireland, intensive pasture-based dairy systems are reliant on imports of fertiliser N to sustain high forage production (Mihailescu et al., 2014; Burchill et al., 2016). The excess of nutrients in the environment is a major source of air and water pollution, negatively impacting biodiversity and climate change (Delaby et al., 2020; Hoekstra et al., 2020). Hence, recent policy within the European Union "Farm to Fork Strategy" targets a 20% reduction in fertiliser N use relative to peak usage in 2018 (EC, 2020). Concerns with increasing cost and environmental impact of high fertiliser N inputs have led to a reappraisal of the role of legumes, particularly white clover (*Trifolium repens* L.) (Humphreys et al., 2017).

Biological N fixation (BNF) associated with white clover can compensate for reduced fertiliser N application and result in similar levels of pasture production on grass-clover swards to grass only-swards receiving 240 kg N ha^{-1} fertiliser N (Enriquez-Hidalgo et al., 2018). Grass-clover swards with an annual sward clover content of approximately 200 g DM kg^{-1} can biologically fix between 100 and 200 kg N ha^{-1} (Black et al., 2009; Burchill et al., 2014a; Enriquez-Hidalgo et al., 2016). In practice, clover abundance in grassland tend to be variable from year to year partly due to competition with the companion grass (Frame and Newbould, 1986; Frame and Laidlaw, 1998; Burchill et al., 2014a). At the same time, white clover is perceived as difficult to manage with few long-term studies that investigate management to promote clover productivity and persistence in grazed grassland. These barriers have made the uptake of white clover and subsequent conversion to organic production less attractive to farmers.

The Irish Government has targeted a fivefold increase in land area under organic production by 2030 (Government of Ireland, 2021). Ireland's temperate climate is advantageous to milk production from grass-clover swards over a long growing season (Humphreys et al., 2009a). The inclusion of white clover in pastures is a key component of organic and low fertiliser N systems of milk production. The wider adoption of white clover in Irish grassland has immense potential to lower the reliance for fertiliser N on farms that is not only considered economically unsustainable but also environmentally undesirable.

Knowledge of the capacity of soils under permanent grassland to supply plant-available N from soil organic matter (background N) during the growing season is an important prerequisite to improve N use efficiency (NUE; proportion of N imported recovered in agricultural products). Average pasture-based milk production systems have a low NUE ranging 23 to 37% (Mihailescu et al., 2014; Burchill et al., 2016). Further improvements in farm NUE and maximising supply of non-fertiliser N sources will be required to decouple production from reactive N emissions.

1.2 Thesis Objective

The objective of this thesis was to review N sources in grassland used in dairy production systems, identify knowledge gaps and investigate the various aspect of white clover management strategies and nitrogen inputs on seasonal pasture-based milk production. Consequently, nitrogen supply from sources of background N in Irish permanent grasslands were quantified, long term effects of white clover productivity were analysed and the effect of fertiliser N input to grass-clover swards and calving date on the productivity of pasture-based dairy production were investigated at grazing system scale.

1.3 Thesis Layout

This thesis contains six chapters. Following this introduction, Chapter 2 reviews literature associated with this thesis including an overview of the N cycle, requirements of N for grassland and aspects of white clover in pasture-based milk production in Ireland. An overview of the organic farming sector and regulations are also outlined. Chapter 3 investigates the spatial and temporal variation of the background release of N from soil organic matter in Irish grasslands. Chapter 4 carries out a retrospective analysis into the productivity of white clover-based grassland for milk production on a poorly drained clay loam soil over nineteen years. Chapter 5 examines the effect of fertiliser N input to grass-clover swards and calving date on the productivity of pasture-based dairy production. Finally, Chapter 6 includes a general discussion and synthesis of the findings in the thesis along with overall implications and potential avenues for future work.

Chapter 2. Literature Review

2.1 Introduction

Pasture production is largely dependent on natural N sources; N_2 fixed by legumes and the mineralisation of N in crop residues, animal manures and soil organic matter (Keeney, 1982; Whitehead, 1995; Jarvis et al., 2011) prior the commercialisation of synthetic fertiliser N (Galloway et al., 2004). Pasture-based systems can have a lower reliance on synthetic fertilisers, while also delivering environmental, biodiversity, and animal welfare benefits (Humphreys et al., 2017; Delaby et al., 2020). This literature review aims to examine research on aspects of low fertiliser nitrogen input seasonal pasture-based milk production to identify knowledge gaps. Soils and the terrestrial nitrogen cycle is investigated. Particular attention is paid to the requirement for N by pastures and management strategies to optimise white clover content within pastures. The effect of seasonal pasture-based dairy systems in terms of milk composition and processability characteristics is also reviewed. Finally, the organic agriculture sector will be discussed and perceptions of dairy exports explored.

2.2 Soil

2.2.1 Soil physical properties

Soil is biologically active, porous medium composed of solid minerals, organic matter, water and gas. It originates from the parent material that has formed from physical and chemical weathering of primary and secondary minerals (Birkeland, 1999). Soil texture refers to the relative proportions of mineral particles of a soil (< 2 mm), specifically the sand, silt and clay (USDA, 1951; Black, 1986; FAO, 2006). Sand is comprised of coarse to fine mineral particles within the size range of 50 µm to 2 mm (USDA, 1951). Silt consists of very fine particles intermediate in size between sand and clay with a range of 2 µm to 50 µm (USDA, 1951). Clay particles are the finest mineral particles at smaller than 2 µm (USDA, 1951).

The common field method of determining the textural class of an individual soil is feel of the constituents (Brady and Weil, 2008). Soil texture can be more accurately measured in the laboratory using laser diffraction (BS ISO 13320:2009), hydrometer (BS 1377-2:1990) or pipette method (BS 1377-2:1990). Once the relative proportions of mineral particles of a soil are known, its texture can be classified in accordance with a standardised classification method (USDA, 1951). The United States Department of Agriculture (USDA) classifies soil into one of 12 major textural classes. This classification system is based in order of increasing proportions of the finer separates < 2 mm: sand, loamy sand, sandy loam, silt-loam, silt, sandy clay loam, clay loam, silty clay loam, sandy clay, silty clay and clay (USDA, 1951). Predominantly, soils with higher clay content tend to have lower soil temperatures, lower macroporosity, higher soil moisture and higher nutrient retaining ability, whereas sandy soils are associated with higher soil temperatures, higher macroporosity, lower soil moisture and lower nutrient retaining ability (Gardiner and Radford, 1980).

Soil structure is formed by aggregation of primary soil particles into discreet soil units (FAO, 2006). The level of soil structural development upon the soil texture and organic matter content will influence a wide range of soil physical and chemical properties including soil fertility, porosity, drainage and water holding capacity (Tisdall and Oades, 1982; Murphy, 2015).

The soil profile refers to the vertical section of the soil comprising of the parallel soil horizons layers to the land surface, including the geological parent material (Gardiner and Radford, 1980). The characteristics of the profile are important in terms of assessing the true character for plant growth including root development, moisture storage and nutrient supply. Most soil profiles include the main horizons identified as A, B and C. The A horizon is the uppermost layer in mineral soils and resembles the surface soil. The surface soil is most abundant with the living matter e.g. fungi, bacteria, earthworms, small animals and plant roots. The B horizon lies immediately beneath the A and is referred to the sub-soil. Lying between the A and C horizons, it possesses properties of both. The quantity of living organisms is fewer than in the A horizon and there is a relatively high content of iron and aluminium oxidise, humus or clay due to leaching from the overlying horizon. The C horizon refers to the geological material below the A and B horizons. The geological material consists of loose and partly decayed rock such as glacial drift to that of the soil has been developed. This horizon contains less organic matter, less weathered and is usually lighter in colour than the overlying horizon.

2.2.2 Soil organic matter

Soil organic matter (SOM) is defined as the organic fraction of the soil predominantly made up of carbon, oxygen, hydrogen and nitrogen. These fractions consist of animal and plant residues and soil biota at various stages of decomposition (Oades, 1988; Weil, 2004). Soil organic carbon (SOC) is the main constituent of SOM in soil and plays a vital role in soil properties, processes and functions (Smith et al., 2015).

The SOM can be classified into three fractions based on turnover rates and availability to plants; (i) active fraction, (ii) slow fraction and (ii) passive fraction. The active fraction involves OM derived from plant and animal residues, which are mineralised rapidly. Soil microbes utilise the SOC of the active fraction as a source of energy and available N (Tisdall and Oades, 1982). The nutrients in the slow fraction can take many years to become available for plant uptake, while the passive fraction comprises stable organic compounds resistant to decomposition (humus) (Brady and Weil, 2008). The climate, soil type and microenvironment are important factors regulating the rate of decomposition and net mineralisation of the OM (Mary and Recous, 1994; Krull at al. 2003; Li et al., 2019b).

Mineral soils form most of the world's cultivated land and contains OM contents ranging from trace to 30% (Bot and Benites, 2005). Although SOM is a minor constituent of soil, it has a direct role in plant nutrient cycling and availability (Saljnikov et al., 2013). This

SOM reservoir accounts for 95% of the soil N, half of the phosphorus (P) and a significant proportion of the sulphur (S) and other macro- and micro-nutrients (Saljnikov et al., 2013). The high cation exchange capacity of OM enables it to adsorb and release cations to meet the requirements of the growing plant. Gardiner and Radford (1980) reported soils with high OM content to have a high cation exchange capacity (CEC; range 25 to 40 m.eq./100 g of soil). Soils with a low OM content also have a low CEC generally less than 12 m.eq./100 g and this is mainly contributed by the clay fraction (Gardiner and Radford, 1980). Additionally, Murphy (2015) reported that the CEC of SOM to be minimal below a pH of 5.5.

Sandy soils contain relative little OM or clay, with a very low cation exchange capacity (Hassink, 1995b). On the other hand, heavier textured soils have a strong binding capacity given high clay and OM contents. Soils with higher clay content increase the potential for aggregate formation. Macroaggregates physically protect OM modules from further degradation caused by microbial attack (Swangjang, 2015).

The ratio between SOC and total soil N; the C:N ratio of the SOM, is often taken as a proxy indicator of the decomposition of SOM (Troeh and Thompson, 2005; Swangjang, 2015). Reported C:N ratios range from very low (< 8) to very high (> 30). The C:N ratio of SOM active fraction typically exceeds 15, while the slow fraction ranges between 10 and 25 and the passive fraction ranges between 7 and 10 (Brady and Weil, 2008). Substrates with a narrow C:N ratio (< 25:1) such as green manure and leguminous cover residues, favour mineralisation. Substrates with a high C:N ratio and lignin (such as straw), have greater demand for N when incorporated into the soil and thus result in N immobilisation (Whitehead, 1995). McDonald et al. (2014) reported C:N ratio for 30 Irish soils was 10·3 :1 ± 1·13.

Soil OM decomposition potential is limited by soil temperature. The majority of soil microorganisms are mesophilic and prefer moderate temperatures (Haynes 1986; Herlihy 1979). Soil biological activity requires moisture and oxygen and thus optimal microbial activity occurs at field capacity (soil moisture content < 60%; Bot and Benites, 2005). The growth of many microorganisms is restricted by poor aeration within the soil matrix. In agricultural soils, changes in soil management practices affect SOM. Management practices that alter soil biological activity include repetitive tillage or burning of vegetation (Ogle et al., 2005). Archer (1988) reported a range between 3 and 10 t SOM-N ha^{-1} under grassland compared with 1 and 4 t ha^{-1} under arable cropping in the upper 15 cm of soils in the United Kingdom (UK). Irish permanent grassland soils have been reported to contain approximately 7 t N ha^{-1} in the upper 20 cm (Gardiner and Radford, 1980; McGrath and Zhang, 2003). The continual transformation of SOM in the soil mainly due to biological activity releasing a small proportion of this N as plant-available N in the soil solution see 2.4.4.

2.2.3 Soils of Ireland

Soil types in a specific location or site differ in the layering of soil horizons and are grouped according to soils with similar kind, degree of expansion of horizons and arrangement in the soil. They are also governed by similar temperatures, base status and soil moisture. There are 10 main great soil groups in Ireland; Podzols, Brown Podzolics, Brown Earths, Grey Brown Podzolics, Blanket Peats, Gleys, Basin Peats, Rendzinas, Regasols and Lithosols (Gardiner and Radford, 1980). Figure 2.1 depicts the soil texture and drainage status of soils in Ireland. Fine loamy soils are most frequent whereas sandy soils with high natural drainage are rare (Creamer et al., 2016). Total agricultural land area is comprised of 45% as moderately to poorly drained, whereas 35% are well to excessively drained (Figure 2.1).

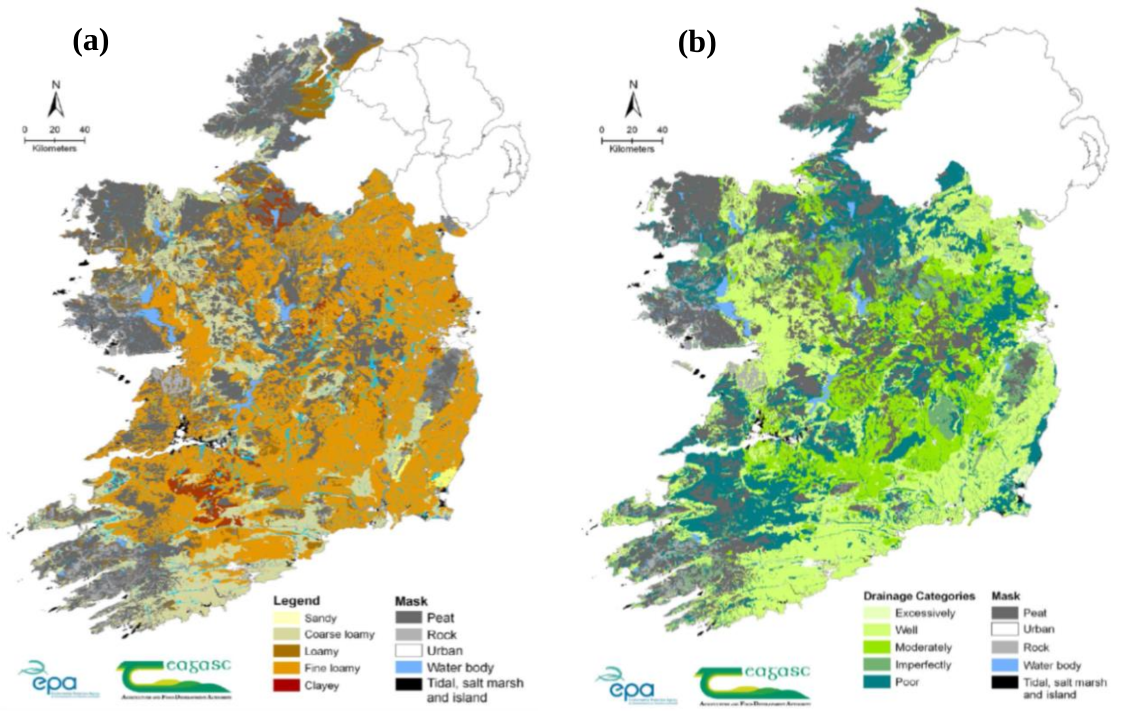

Figure 2.1. Indicative soil texture (a) and drainage status (b) of soils in Ireland (Creamer et al., 2016).

2.3 Terrestrial nitrogen cycle

2.3.1 Overview of reactive nitrogen and the nitrogen cycle

Nitrogen is an essential element for the development, growth and functioning of all living organisms (plants, animals and humans). It is a primary constituent of many biomolecules, including DNA, protein and chlorophyll (Erisman et al., 2013). Although N is abundant in the atmosphere (approximately 78%) as dinitrogen gas (N_2), it is generally inaccessible in this form to most organisms, leading to a scarcity of usable N resources and often limiting primary productivity in many ecosystems (Galloway et al., 2004; Jarvis et al., 2011).

Dinitrogen has a strong triple bond between the N atoms in the N_2 molecule, which makes it relatively inert. A process of 'N fixation' breaks this triple bond and reduces N_2 to reactive N (N_r) which is 'biologically available' for primary producers, such as plants. Following fixation, N_r can undergo several microbial, chemical and physical processes to alter the level of N available for crop uptake (immobilisation, mineralisation, plant assimilation, ammonification and nitrification) before being returned to atmospheric N_2 by denitrification to complete the N cycle. During these processes, N_r exists in many different N forms, including, organic (urea, amines, nucleic acids and proteins) and inorganic (ammonia (NH_3), ammonium (NH_4^+), nitrous acid (NO), nitrite (NO_2^-), nitrate (NO_3^-), nitric acid (HNO_3^-), nitrogen oxide (NO_x) and nitrous oxide (N_2O)). The N cycle is inherently leaky from the initial fixation step through to the final return of N_r to atmospheric N_2 (Whitehead, 1995). Nitrogen losses can negatively impact the air quality, drinking water, biodiversity loss, freshwater eutrophication or NO_3^- enrichment of water bodies, stratospheric ozone depletion and climate change which can be to the detriment of human health and the environment (Erisman et al., 2013).

2.3.2 Farm nitrogen cycle

As pointed out above N is a dynamic nutrient, which is continually recycled between the atmosphere, the soil solution, SOM, plant material and soil organisms (Murphy, 2015). Large reserves of N are held within (i) the soil (crop residue, SOM or mineral form), (ii) plants and animals, as nucleic and amino acids, and (iii) stored manure. The various inputs, outputs, losses and internal movements and transformations of N are outlined in Figure 2.2.

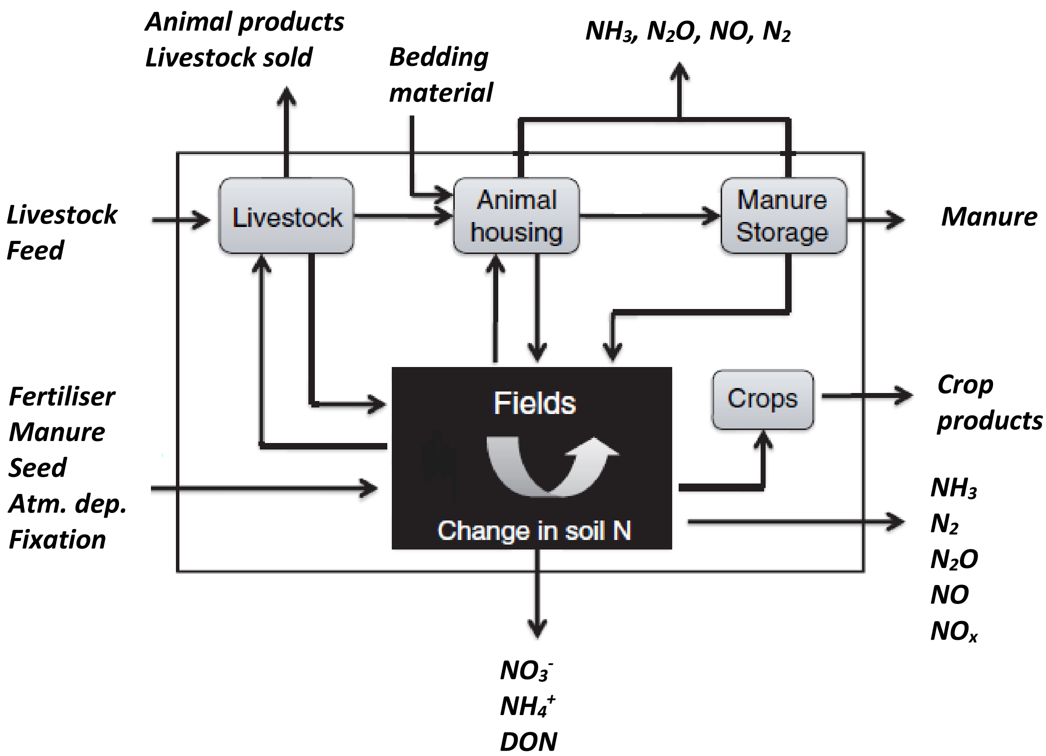

Figure 2.2. Nitrogen flows and transformations on farms. Atm. dep. = atmospheric deposition, DON = dissolved organic nitrogen. Adapted from Jarvis et al. (2011).

Livestock farms typically import N in the form of synthetic fertiliser, manures, seed, atmospheric deposition of N, bedding material (straw), N_2 fixation by legumes, animal feed and livestock purchases. There is an export of N from the farm through the sale of animal products such as milk and livestock or as crop products such as cereals and straw. Furthermore, some manure can be exported from the farm. The other N removals from the farm are environmentally damaging losses from the components of livestock production and cropped or grazed pastures. These can include losses to groundwater in surface runoff or leaching by the form of NO_3^-, NH_4^+ and dissolved organic N (DON) and to the atmosphere as N_2O, NH_3 gas and NO_x. There is also a major loss of N_2 from farms, although it is environmentally benign (Jarvis et al., 2011).

The farm N cycle also consists of internal transfer and transformation of N within farms. Crops and forages can take up N, which are directly consumed by livestock. Any excess consumed N not incorporated into milk and animal protein (Tamminga, 1992), is directly excreted in dung and urine by grazing livestock or indirectly through the application of animal manure to pastures after collection and storage in livestock housing and manure storage facilities. Agricultural soils contain a large pool of organically bound N that undergo a variety of microbial mediated transformations, which are associated with SOM turnover. This soil N result in either sequestration into relatively immobile forms or release and transformation into forms that are available for uptake by plants or further transfer into losses (Jarvis et al., 2011).

2.3.3 Nitrogen inputs and transformations

2.3.3.1 Mineralisation and immobilisation

Mineralisation of organic N and immobilisation of inorganic N are two opposing processes taking place simultaneously in soils. Mineralisation is the transformation process whereby NH_4^+ or NH_3 is released by soil microbial biomass (SMB) as they utilise organic N compounds combined in SOM as an energy source (Jansson and Persson 1982; Jarvis et al., 1996). The capacity of a soil to release inorganic N is dependent upon the equilibrium of soil processes with soil management and climatic condition. Mineralisation is always coupled with immobilisation i.e. reverse transformation of inorganic N into organic form. Much of the released NH_4^+ and NO_3^- are assimilated rapidly by the SMB and transformed into the organic N constituents of their cells during the oxidation of suitable C substrates (Jarvis et al., 1996). Immobilisation temporarily locks up N and the organic N contained in the SMB is re-mineralisation during the turnover phase.

2.3.3.2 Nitrification and denitrification

The loss of N by biological nitrification and denitrification is a two-step process. Nitrification is the microbial oxidation of NH_4^+ to NO_3^- by autotrophic bacteria (*Nitrosomonas* and *Nitrobacter*) under aerobic conditions (Whitehead, 1995). Nitrate is the substrate for denitrification, which is responsible for the stepwise reduction of soil

NO_3^- to N_2O and gaseous N_2 (Jarvis et al., 1996). The main factors regulating both N_2 and N_2O emissions from agricultural soils are soil NH_4^+ and NO_3^-, organic C, soil moisture (particularly water-filled pore space), soil temperature, soil pH along with management (stocking densities, N fertilisation rates and drainage) and climatic factors (temperature and rainfall) (Luo et al., 1999; Burchill et al., 2014b).

2.3.3.3 Plant nitrogen assimilation

Crop yields are directly related to soil N availability and supply through the root system to plants (see 2.4.3). Legumes are an exception to this where N is assimilated in the form NH_4^+ directly from the nodules. Plants assimilate N from the soil in the form of NO_3^- and NH_4^+ ions (Jackson et al., 2008). Ammonium ions in soil carry a positive electrical charge and are attracted to the negative charges of clay and organic particles in the soil. Once attached to the soil matrix, NH_4^+ is subject to the cation exchange process. Nitrate ions carry a negative electrical charge and repel to clay and organic particles (McNeill and Unkovich, 2007). Therefore, NO_3^- ions are free to move with the soil water, whereby they are brought to the soil surface via moisture transpiration and carried back down with rainfall or irrigation. As a consequence NO_3^- can become more susceptible to leaching with excess precipitation over evapotranspiration.

2.3.3.4 Nitrogen cycling in excreta

Livestock in pasture-based production systems play a major role in N cycling through their excretion of ingested N in urine and dung (Haynes and Williams, 1993; Selbie et al., 2015). Soil microorganisms decompose proteins in dung with the resulting mineralised N taking different pathways within the N cycle. Urinary N is mainly in an inorganic form and does not require microbial activity to be plant available. Grazing animals return high loads of N (200 to 2000 kg ha^{-1} $year^{-1}$) in urine and dung patches which exceed the use capacity of grasslands (Nyameasem et al. 2021). Approximately, 75 to 95% of the N ingested from herbage is excreted and returned to pasture either directly or indirectly

(Tamminga, 1992; Whitehead, 1995; Spek et al., 2013). Nitrogen excreted while indoors is stored and applied to land in a more uniform fashion as slurry or farmyard manure but N losses are observed during storage and land application (Pereira et al., 2011; Bourdin et al., 2014).

2.3.3.5 Nitrogen fixation

There are three major N fixation processes: lightning, biological fixation and ammonia fertiliser production (see below). Biological N fixation is the reduction of N_2 into (NH_3 or NH_4^+) a plant available N form, through the exploitation of the nitrogenase enzyme system (Crush, 1987; Unkovich et al., 2008). There is a wide range of N_2-fixing organisms; free-living N_2 fixers (Cyanobacteria, heterotrophs and autotrophs), while others fix N_2 in association with plant hosts such as the *Fabaceae* family (legumes). Heterotrophic free-living N_2 fixers have been found to contribute small amounts of N to agricultural systems (mostly < 5 kg N ha^{-1} yr^{-1}; Unkovich et al., 2008).

As a legume, white clover (*Trifolium repens* L.) develops root nodules that house symbiotic N–fixing, *Rhizobium leguminosarum biovar trifolii* L. bacteria (Crush, 1987). The relationship between the host plant and rhizobia is symbiotic; the plant provides the bacteria with C assimilated from photosynthesis in return for fixed N from the bacteria. This demanding process places a C burden on the host plant (Schulze, 2004) and this is estimated at 6 g C per g of fixed N (Vance and Heichel, 1991). There are various field methods for measuring BNF which have been extensively reviewed (Unkovich et al., 2008; Burchill et al., 2014a). In brief, there are three mains methods:

1. The N difference method involves the comparison of N yields from control (non-fixing) sward and the N_2 fixing sward sown in the same soil. A simple equation is then used to calculate BNF:

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

2. The ^{15}N isotopic determination method has been the most widely used which is based on the presence of lower ^{15}N:^{14}N ratios in N derived from the atmosphere. This measurement can be carried out from two approaches by using the natural abundance of ^{15}N usually found in the soil or the application of ^{15}N enriched fertiliser. The proportion of N derived from the atmosphere in white clover is calculates according to Unkovich et al. (2008).

3. Acetylene reduction method measures the rate of the nitrogenase enzyme activity by replacing the acetylene (C_2H_2) with ethylene (C_2H_4) instead of the usual N_2 reduction to NH_3 (Ledgard and Steele, 1992). This method involves the incubation of whole plants or plant parts in a sealed vessel containing pure C_2H_2 gas. The rate of reduction is measured with a gas chromatographer to calculate nitrogenase activity. Crush (1987) proposes that this process measures the short-term rates of N-fixation carried out in pot experiments under a controlled atmosphere which is difficult to scale up to annual measurements or field studies.

Most of the BNF values reported in the literature only take into account shoots grown above the cutting height. Relatively few studies account for fixed N accumulated in stolon, stubble and root, soil and grass and hence may often be underestimated (Korsaeth and Eltun, 2000). Complete plant sampling is laborious and requires long term measurement for quantification. Additionally, there is seasonal variation in the amount of N in the white clover stubble and below ground material (Høgh-Jensen et al., 2004). Effectively, Høgh-Jensen et al. (2004) has developed literature-derived equations for estimating the total BNF under different sward ages (< 2 years or > 2 years), management situations (cutting or grazing) and soil type (clayey or sandy) based on clover herbage production. This model is one of the few that includes the various non-harvested herbage sinks for fixed N. The BNF activity values reported in the literature are influenced by edaphoclimatic and management factors (see 2.5.3).

2.3.3.6 Nitrogen fertiliser

Since 1913, dinitrogen has been fixed chemically to NH_3 using the Haber-Bosch process by reacting atmospheric N_2 with hydrogen in the presence of an iron catalyst under high pressure and temperature (Galloway et al., 2004). This short circuit of the N cycle has led to greater proportions of ammonia fertiliser to be added to the soils compared to reliance on N fixed by legumes and the mineralisation of N in crop residues, animal manures and soil organic matter.

2.4 Grassland in Ireland

2.4.1 Agricultural land

Ireland comprises 4.4 million ha of agricultural land, with permanent grassland accounting for 81% of total utilised area (CSO, 2016). Permanent grassland which is defined as land that has not been cultivated within five years but can mean any grassland between this narrow definition and semi-natural grassland that has not been cultivated within living memory (Allen et al., 2011). Intensively managed grassland typically contains a diverse range of plant communities, including perennial ryegrass monocultures and white clover-ryegrass mixtures (Wilkins and Humphreys, 2003). There is a low proportion of reseeding annually of agricultural land (1.5 to 2% of the grassland area) primarily with perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens* L.) included in seed mixtures (Humphreys and Casey, 2002; Creighton et al., 2011; Shalloo et al., 2011).

2.4.2 Climate

The Irish climate is cool, humid and temperate maritime characterised given its' close proximity to the Atlantic Ocean and the Gulf Stream. These climatic conditions provide an environment suitable for pasture growth with evenly distributed rainfall and relatively narrow temperature range; averaging 15.5°C in summer and 4.5°C in winter (Walsh, 2012). Average annual rainfall ranges from 750 mm in areas of the east/northeast and 1,400 mm along the west of Ireland (Keane and Sheridan, 2004). Evapotranspiration ranges between 390 to 570 mm yr^{-1} (Mills, 2000). Precipitation exceeds evapotranspiration in all regions. In Ireland, October, November, December and January are the wettest months given the large-scale Atlantic depressions that bring strong polar and tropical westerly winds and considerable frontal rain. Frost typically occurs, approximately 25 days per year in coastal areas and 50 days inland of the country (Met Éireann, 2020).

2.4.3 Requirement for N by grassland

Plants require sufficient soil N supply for photosynthesis. The photosynthetic capacity of leaves is reduced if the organic N content of leaves is lower than 30 g kg^{-1} (Parsons and Chapman, 2000). Whitehead (1995) calculated that pasture harvested by cutting or grazing in a temperate environment and producing between 8 and 15 t DM ha^{-1} usually contains between 200 and 350 kg N ha^{-1}. Grassland swards producing close to maximum annual yields of between 14 and 16 t DM ha^{-1} needs to take up around 450 kg N ha^{-1} (Richards, 1977; Prins, 1983; Hopkins et al., 1990; Hassink, 1995). However, the amount needed as fertiliser N will be less than this as plant-available N from soil organic matter (background N) is a contributing source.

In a review of data from 108 sites in the UK, Richards (1977) reported a mean supply of background N of 101 kg N ha^{-1} ranging from some sites with very low values (< 50 kg N) to others releasing over 200 kg N ha^{-1}. At 16 sites in the UK, Hopkins et al. (1990) recorded an annual background N of 112 kg N ha^{-1} ranging between 40 kg N ha^{-1} from upland sites to over 150 kg N ha^{-1} from several of the more productive lowland sites. In another study also conducted in UK, annual background N was 135 kg ha^{-1} from an undrained silty clay loam soil receiving no previous or current fertiliser N (Gill et al., 1995). In the Netherlands, Hassink, (1995) found that annual background N ranged between 43 and 201 kg N ha^{-1} on 11 sandy soils (mean = 128 kg N ha^{-1}) and between 45 and 233 kg N ha^{-1} on 10 loam and clay soils (mean = 137 kg N ha^{-1}).

Background N in permanent pastures has not been well considered in many N fertilisation recommendations for grassland, which usually only considers total soil organic matter and cropping history as indicators of background N for the coming season (Broadbent, 1984; Carpenter-Boggs et al., 2000; Sharma and Bali 2018). The level of background N will vary between locations due to altitude, latitude, climate and soil characteristics (Hassink, 1995). Background N in permanent pastures can be substantial and therefore can affect the requirement for other sources of N (Hassink, 1995). As the background N quantity increases the requirement for fertiliser N declines.

2.4.4 Measurement and prediction of background N release of SOM

Background N is estimated in field or laboratory experiments as the release of inorganic N from organic residues of SOM. Since 1900, soil N supply has been estimated using biological and chemical methods or field trials (Bremner, 1965; Keeney, 1982; Griffin, 2008; Ros et al., 2011a). Chemical methods, such as hot water extraction, heating in 2 M KCl, or absorbance of $NaHCO_3$ soil extracts at 200 nm light wavelength offer rapid, but not universally accepted techniques for predicting background N due to mixed levels of success worldwide (Griffin, 2008; Nannipieri and Eldor, 2009; Ros et al., 2011a).

Biological methods include short- and long-term soil incubations that measure relative production of inorganic N produced by the mineralisation process over a defined period (Fox and Piekielek, 1984; Stanford and Smith, 1972; McDonald et al., 2014). Short-term incubations are conducted over a period of 1 to 6 weeks and long term studies lasting up to 30 weeks. Biological methods disrupt soil structure by mixing, sieving, drying, and/or rewetting (Jarvis et al., 1996; McDonald et al., 2014). Moreover, soils under these methods are incubated in filtration units in a variable temperature incubator that mimic field growing conditions (Stanford and Smith, 1972; Carpenter-Boggs et al., 2000; Wade et al., 2016; Risch et al., 2019). Nevertheless it is difficult to fully represent actual field conditions in the laboratory given that background N release is regulated by factors, such as temperature, rainfall, soil properties, microbial biomass and enzyme activity (Herlihy 1979; Jansson and Persson, 1982; Jarvis et al., 1996; Risch et al., 2019). The use of data from incubations studies to predict field mineralization rates has had variable success. Cabrera and Kissel (1988) found laboratory incubations methods significantly over predicted by 67 to 343% the amounts of background N in the field. In a recent study, Risch et al. (2019) reported that background N measured in the laboratory was weakly correlated (R^2 = 0.31) with background N measured under field conditions across 30 grassland sites worldwide.

One of the simplest methods to estimate the capacity of soils to supply background N is to use the crop as a sink for any plant-available N released by the soil; the plant-N-uptake method (Jarvis et al., 1996; Brye et al., 2003). This method assumes that N uptake by a crop equates to background N when the crop is grown in the absence of external inputs (fertilisers, excreta, fixation) (Hassink, 1995; Jarvis et al., 1996; Brye et al., 2003). This method accounts for several factors under growing conditions such as climate (temperature, soil moisture) along with abiotic and biotic soil characteristics. While many previous international studies have measured the annual quantity of background N (Gill et al., 1995; Hassink 1995a), few have reported availability of background N at different stages of the growing season. This plant-N-uptake method can determine background N throughout the growing season to provide information necessary for weekly estimates of pasture N requirement throughout the growing season. The spatial and temporal variation of the background release of nitrogen from soil organic matter in Irish grasslands will be investigated further in Chapter 3.

2.5 White clover in agriculture

2.5.1 Biology of white clover

Following germination, white clover goes through three stages of development including seedling, taproot and the clonal growth (Thomas, 1987b; Brock et al., 2000). Following germination and development of the root system, the first leaf that appears is a heart shaped unifoliate leaf. The germinated seedling develops into a rosette with a tap root, which typically survives for two years (Frame and Newbould, 1986; Brock et al., 2000). Early in the rosette stage there is the development of stoloniferous growth habit which branches off from the rosette (Jennings and Foster, 2020). At each node along the stolon there are two root primordia, leaf and an axillary bud. All leaves of white clover that follow leaf emergence have a trifoliate lamina (three leaflets) attached to the stolon by the petioles (Burdon, 1983).

In the early stages, rhizobia bacterial infection occurs on the lateral roots (Thomas, 1987b). The bacteria colonies start to proliferate at points on the rhizosphere of white clover root hairs. The plant reacts by curling a root hair that grows to encase the bacterial colony in a nodule (Crush, 1987). Finally, the taproot dies and the lower root primordia develops into a nodal root network. The plant has entered the clonal phase when the independent plantlets grow towards light, moisture and nutrients within the soil and canopy (Brock et al., 2000). The stolons of white clover plants are regarded as the structural unit of the mature plant. The structure of the mature white clover is depicted in Figure 2.3.

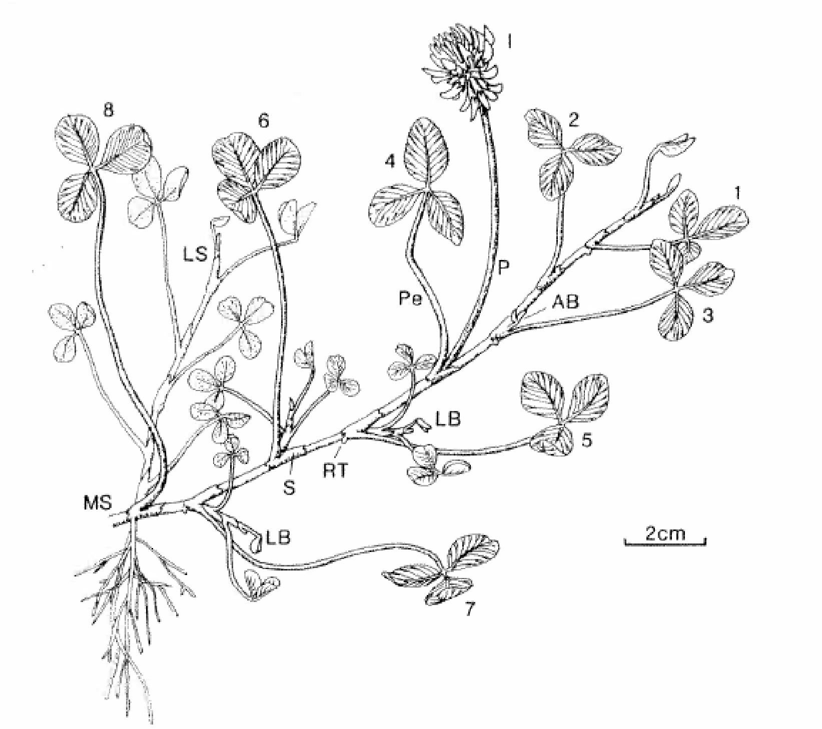

Figure 2.3. Structure of a mature white clover plant. MS = main stolon; AB = axillary buds; LB = lateral branches; LS = stolon; S = stipule; Pe = petiole; RT = nodal root primordium; I = inflorescence; P = peduncle. Emerged leaves on the main stolon are numbered 1 to 8, 1 being the youngest (Thomas,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

When older plants fragment or die the newer plant material can survive as ramets resulting in a complex population structure. These stolons grow horizontally along the ground in a *repens* (‘creeping’) effect on or just below the soil surface that consist of a series of nodes and internodes as the apical bud grows (Frame and Newbould, 1986; Thomas, 1987b). There are difficulties in measuring plant population density and many studies measure growing point density (quantity of active apical and axillary buds per unit area) as population parameter (Höglind and Frankow-Lindberg, 1998). In addition, stolon length or stolon mass per unit area are alternative measurements. Pinxterhuis (2000) stated that the age and season demonstrates the node’s budding differentiation ability to develop the plant according to the environmental conditions. The direction of growth is governed by the apical bud, which is positioned at the opposite extreme of the primary root.

White clover is regarded as a short-lived perennial plant, which possesses two complementary mechanisms of reproduction. The different survival strategies predominantly accord to climate and/or cultivar. In cool temperature regions, vegetative reproduction of white clover relies on stolon growth as opposed to seed reproduction under dry hot conditions (Harris, 1987; Williams, 1987). Stolon growth occurs through means of branching or elongation (Frame and Newbould, 1986). Branching refers to the generation of new stolons from axillary buds of a pre-existing stolon, which provides resilience against stress factors such as trampling, high defoliation and soil heterogeneity (Chapman et al., 1996; Pinxterhuis 2000). Burdon (1983) reported that a yearly extension rate of 18 cm could be expected under competitive field condition; extension rates varied from 0.7 to 2.5 cm week^{-1}. Stolon elongation is whereby the internode elongates through growth of its apical bud. The plant growth is commonly a mixture of both branching and elongation influenced by factors such as clover cultivar, weather, soil fertility and grazing management (Brock et al., 1988; Black et al., 2009).

2.5.2 White clover cultivars

The cultivars of white clover are classified according to leaf size into small, medium, large and very large (Frame and Boyd, 1987). Small-leaved cultivars are best suited to continuous grazing i.e. sheep grazing given the tolerant of close defoliation. Medium leaf sized cultivars are most appropriate to rotational grazing. Large and very large-leaved varieties are suited for lax cattle grazing or conservation as leaves are less tolerant to close defoliation and compete better with grass (Andrews et al., 2007; Black et al., 2009). Indeed, continuous defoliation from livestock induces all white clover cultivars to produce smaller leaves due to phenotypic plasticity (Pinxterhuis, 2000).

Notwithstanding the leaf size continuum that dominates white clover cultivar classification, there is prospect for novel traits to improve clover agronomic performance (Widdup and Barrett, 2011; Nölke et al., 2021). However, breeding objectives for perennial ryegrass and white clover species have progressed independently of each other. For example, the increased spring growth and pasture production attained by grass cultivar breeders has not been achieved by their clover breeding counterparts (Abberton and Marshall, 2005; Hoyos-Villegas et al., 2019). Indeed, overwintering by cold-tolerant plants from northern Europe have the morphology ability to reduce loss over winter. These plants are usually smaller leaved and more prostrate than the larger, frost sensitive Ladino types that typically originate from Mediterranean populations (Frame and Newbould, 1986; Collins et al., 1991). Nevertheless, medium leaved 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). Therefore, plant breeding aimed to increase the rate of stolon formation and decrease stolon death to improve persistency.

It may be prudent to use mixtures of cultivars to safeguard against unforeseen circumstances and variations in management practices. It has been observed at Solohead Research Farm that a 1:1 ratio of Chieftain and Crusader both medium-leaved varieties are appropriate to conditions. Humphreys and Lawless (2008) recommend over-sowing clover seed onto 20% of the farm each year at a rate of 5 kg ha^{-1}. Humphreys et al. (2008) demonstrated that clover could be introduced into farm scale systems through the method of over-sowing into first-cut (May) silage stubble at very little cost by mixing white clover seed with a P and K fertiliser using a fertiliser spreader. This method of establishment and maintenance of botanical composition is desirable in terms of management. Nevertheless, the temperature, soil moisture, sowing depth and soil fertility need consideration when sowing as these factors affect the seed germination and seedling emergence of white clover (Chu et al., 2022).

2.5.3 Effect of soil physical and chemical properties on white clover

White clover requires relatively high levels of macroporosity and low bulk density (mass of soil per unit volume of a soil core) for sufficient soil-air movement aimed at effective BNF process in the root nodules (Vidrih and Hopkins, 1996). The BNF process of rhizobia bacteria can produce excess H^+ in the soil solution (Crush, 1987). Therefore, lime application to ameliorate soil acidity is required to increase soil populations of rhizobia and enhance root nodulation, stimulate nodule nitrogenase activity and manage acidification of soil in grasslands (Evans et al., 1977; Brock et al., 1989; Hayes et al., 2019). A greenhouse pot study found that increasing soil pH from 5.4 to 6.1 resulted in a doubling of clover production (Bailey and Laidlaw, 1999). Furthermore, manganese and aluminium toxicity associated with acidic soils restrict root growth and reduce the volume of soil explored for moisture and nutrients (Brock et al., 1989; Bailey and Laidlaw, 1999; Hayes et al., 2019).

When compared to grasses, white clover is a poorer competitor for soil water and nutrients, which is attributed to the different root system characteristics (Frame and Newbould, 1986; Davies, 2001). Perennial ryegrass has a denser, longer and more finely branched root system than white clover (Evans, 1977). The three main macronutrients that affect grassland productivity are N, phosphorus (P) and potassium (K). The supply of P and K in the soil can limit white clover growth, with P being critical for shoot morphology during the establishment (Brock et al., 1989; Bailey and Laidlaw, 1999). Bailey and Laidlaw (1999) found that K deficiency lowered leaf expansion rates before impacting on stolon branch formation. Low BNF activity may be partly related to the low soil N demands associated with slow white clover growth.

White clover in late winter/early spring, relies on freely available soil mineral N to compete with the companion grass for nutrients (Nesheim and Boller, 1991). However, high levels of inorganic N in the soil, irrespective of its origin (from mineralisation or fertiliser N addition) cause the inhibition of root-hair infection, nodule growth and development, thus a reduction in the amount of N fixed. Nonetheless, this response differs according to the cultivar, form and amount of N, time and site of application, age of the host plant and environmental conditions (Frame and Newbould 1986).

2.5.4 Effect of weather characteristics on white clover

The minimum temperature for white clover growth is considered to be 5.8°C with the optimum around 25°C (Frame and Newbould, 1986; Davies, 1992). However, the temperature requirement for BNF ranges from 9ºC to 27°C (Frame and Newbould, 1986). Ryle et al. (1989) found that nitrogenase activity increased linearly from 5 to 23°C. White clover survival is reduced at temperatures below -10°C (Frame and Newbould, 1986). However, the level of survivability depends on the rate at which the temperature drops as clover displays cold hardening. Cold hardening is when the plant restricts water uptake and mobilises vegetative storage proteins and water soluble carbohydrates at the onset of cold weather to avoid damage from freezing (Hart, 1987; Frankow-Lindberg, 2001). These compounds typically accumulate in the plant over the autumn period (Frankow-Lindberg, 2001).

The shallower rooting system of white clover results in susceptibility to low soil moisture associated with hot summers (Burdon, 1983; Brock et al., 1989). Drought conditions have long term effects on the ability of white clover to fix N (Ledgard and Steele 1992). On the other extreme, Crush (1987) found that low soil oxygen levels at very high moisture content inhibited both root metabolism and BNF activity. Nevertheless, pot-grown plants of white clover subjected to repeated soil flooding over a nine-week period, showed the ability to adapt morphologically with increased aerenchyma production and development of larger cell vacuoles (Pugh et al., 1995). The BNF rate decreased when the control plants were exposed to sudden flooding event compared to repeated flooded plants.

2.5.5 Management of grass-clover swards

2.5.5.1 Nitrogen input

Biological N fixation in association with white clover can introduce an additional 50 to 250 kg N ha^{-1} yr^{-1} of plant available N to farming systems (Unkovich et al., 2008). In Ireland, Burchill et al. (2014a) found BNF ranged between 14 and 128 kg N ha^{-1} on grass-clover swards with fertiliser N ranging from 0 to 280 kg N ha^{-1}. Differences in BNF rates were attributed to variations in the proportion of N derived from the atmosphere and the sward clover content (Harris and Clark, 1996; Høgh-Jensen and Schjørring, 1997; Peoples et al., 2012; Burchill et al., 2014a). However, the effect of fertiliser N is generally short-lived (< six weeks), as reductions in BNF rates are consequently caused by lower sward clover contents (Høgh-Jensen and Schjørring, 1997; Schulze, 2004; Enriquez-Hidalgo et al., 2016). Ledgard et al. (2001) demonstrated that increasing fertiliser N application from 0 to either 200 or 400 kg N ha^{-1} reduced BNF from 154 to 99 and 39 kg N ha^{-1}, respectively, and sward clover content from 152 to 107 and 49 g DM kg^{-1}, respectively. Moreover, a five-year study in Kiel, Germany by Trott et al. (2004) found large reductions in sward clover content with increasing fertiliser N input under a range of grazing and cutting managements systems (Figure 2.4).

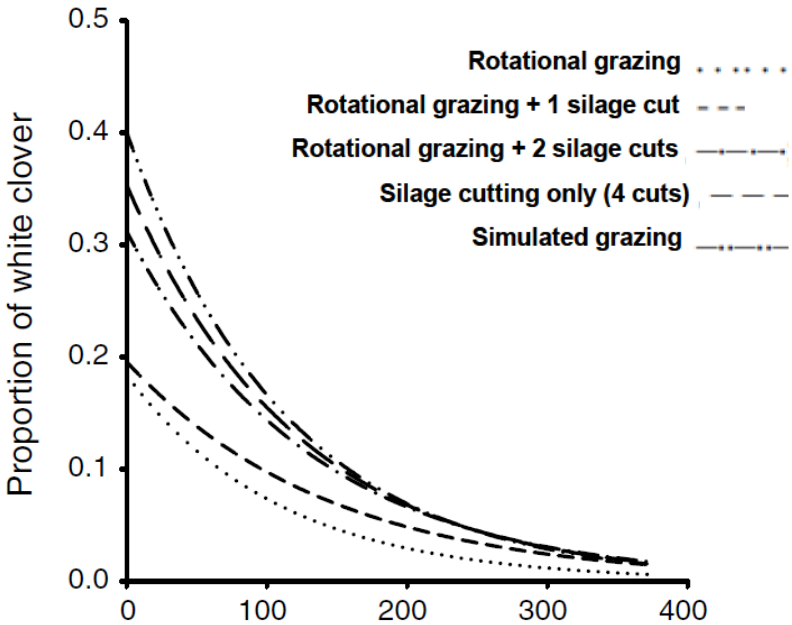

Figure 2.4. The effect of total fertiliser N on the annual proportion of white clover under different defoliation (cutting and grazing management) systems, averaged over the 5 years from 1997 to 2001 (Trott et al., 2004).

At three Northern European field sites, Rasmussen et al. (2013) found that N transfer from white clover to grass occurs usually late in the growing season compared to early and peak growing season. Therefore, applying fertiliser N later in the growing season when sward clover content is highest would be less efficient than applying it earlier in the year when clover contents are low. Enriquez-Hidalgo et al. (2018) found that increasing N rate reduced clover content, especially during the warmest months of the year. This study found that treatments receiving annual fertiliser N of 120, 196 and 240 kg N ha^{-1} had clover content of 270, 217 and 196 g DM kg^{-1}, respectively compared to 333 g DM kg^{-1} to swards that received no N (Enriquez-Hidalgo et al., 2018).

Grass-clover swards have been found to have lower pasture accumulation during early spring compared to N-fertilised grass swards due to differences in minimum temperature for growth of plant species (Frame and Newbould, 1986; Collins et al., 1991; Guy et al., 2021). As a result, some fertiliser N input may be required to increase herbage production in early spring (Whitehead, 1995). Previous studies have found that strategic application of fertiliser N in early spring at rates < 90 kg N ha^{-1} have been found to mitigate the reduced pasture performance in spring with no adverse effect on annual clover production (Laidlaw, 1980; Humphreys et al., 2009; Enriquez-Hidalgo et al., 2016). In the Netherlands, Schils et al. (2000a) found that grass-clover system receiving fertiliser N input of 17 kg N ha^{-1} in spring produced 95% of the annual pasture of a grass-only swards receiving annual fertiliser N input of 208 kg N ha^{-1}. Likewise, Humphreys et al. (2009) reported annual pasture production on grass-clover system receiving fertiliser N input of 90 kg N ha^{-1} in spring produced 92% of a grass-only swards receiving annual fertiliser N input of 226 kg N ha. In a recent four-year plot study, Enriquez-Hidalgo et al. (2018) showed that the mean cumulative pasture production from grass-clover swards was greater than N-fertilised grass swards receiving the same N rate. In contrast, Egan et al. (2018) observed similar pasture production between N-fertilised grass system receiving 250 kg N ha^{-1} and two grass-clover systems receiving 250 kg N ha^{-1} and 150 kg N ha^{-1}. As a result, the application of fertiliser N on the 150 kg N ha^{-1} on grass-clover swards was augmented by BNF to reach similar pasture production.

2.5.5.2 Grazing management

In temperate regions, white clover populations follow a 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 (Hay, 1983; Frame and Newbould, 1986). This is associated with temperature and radiation budgets (Nolan et al., 2001; Phelan et al., 2014). As a result, lower positioning of white clover can restrict light penetration through the canopy during the winter and early spring compared with taller companion grasses (Woledge et al., 1990; Wachendorf et al., 2001).

The defoliation regime of pasture has a large impact on the interaction between the grass and white clover components of the sward. Lowering defoliation height from 6 cm to 4 cm on grass-clover systems increased BNF and pasture DM production (Phelan et al., 2013a). Autumn management of pasture in temperate regions involves prolonging the defoliation frequency between grazing events as pasture growth rates fall below demand. As a result, accumulated pasture can be grazed during the winter period. Phelan et al. (2014) found increasing defoliation frequency to 42 days on grass-clover sward during the summer-to-winter period resulted in the highest annual clover DM production, BNF and total annual pasture DM production under simulated grazing. Moreover, reductions in annual clover herbage production and BNF were observed with both shorter (21 day) and longer (56 to 84 day) defoliation intervals (Phelan et al., 2014). Therefore, grassland management decisions made during the summer period of July and August will have knock-on consequences for the remainder of the grazing season and the subsequent year (Fenger et al., 2021).

Previous studies have demonstrated that carrying high pasture masses for extended periods over winter are unfavourable for clover persistence (Laidlaw and Stewart; 1987; Holmes et al., 1992; Laidlaw et al., 1992). Laidlaw and Stewart (1987) found that a single grazing by sheep in November improves clover content relative to no winter grazing.

Subsequent to that experiment, Laidlaw et al. (1992) carried out additional treatments of sheep grazing in (a) November (b) November, January and March, (c) March and (d) no grazing. It was concluded that treatment b contributed to greater white clover performance across the three years. In contrast, Lüscher et al. (2001) observed that more frequent defoliation in winter affected emergence of nodes (by 60%) and buds (by 67%). This had a negative impact on stolon mass in spring relative to competition from grass in ungrazed sward. The contrast in the experiments could be related to the milder winter in Northern Ireland (Laidlaw et al., 1992) compared with Switzerland (Lüscher et al., 2001).

Awareness of the cyclic nature of clover content, annual stolon death and renewal cycle can determine grazing management practices as to exploit white clover productivity via vegetative propagation (Brock and Hay, 2001). Williams et al. (2003) found no evidence of clover content decline for 9 years with no applied N and 100 kg N ha^{-1} fertiliser N compared to 200 kg N ha^{-1} under rotational grazing by sheep. However, specific effects of clover content under rotational grazing by dairy cows are still not fully understood. A retrospective analysis of the productivity of white clover on poorly drained clay loam within an Irish pasture-based system will be evaluated in Chapter 4.

2.5.6 Nutritional value and milk production

White clover has superior nutritional value compared to other forage species with high protein, low structural fibre values and relatively high organic dry matter (Wilman and Riley, 1993; Phelan et al., 2015). These characteristics contribute to higher digestibility and palatability throughout the grazing season, as there is relatively little stem development in the grazing horizon of the sward with increasing maturity in comparison to perennial ryegrass (Dewhurst et al., 2009; Johansen et al., 2017). Furthermore, white clover nutritive value allows for improved rumen degradation that contributes to higher DM intake (Thomson et al., 1985; Wilkins et al., 1994; Rutter et al., 2004; Steinshamn, 2010).

The proportion of white clover in the grazed swards must remain high for any potential benefit on herbage intake and milk yield (Harris et al., 1998; Ribeiro Filho et al., 2003). Ribeiro Filho et al. (2003) observed increased voluntary intakes by 16% and milk yield by 12% when average clover content was approximately 420 g kg^{-1}, compared with N-fertilised grass-only swards. Following on from this study, Ribeiro Filho et al. (2005) found that a clover content of 270 g kg^{-1} of herbage DM was not sufficient for increased intake or milk yield. Similarly, Dineen et al. (2018) reported a decrease in milk production with a mean sward white clover content of < 290 g kg^{-1}. In two short-term indoor feeding trials in New Zealand, Harris et al. (1998) found that the proportion of white clover in the pasture must remain high (600 to 700 g DM kg^{-1}) compared to 200 g DM kg^{-1}during mid and late lactation for any potential benefit of clover on herbage intake and milk production.

Results showed the optimum white clover DM content in the diet for milk production was 550 to 650 g DM kg^{-1} (Harris et al., 1997). However, such high contents are rare in permanent grassland except for short periods during the late summer and early autumn (Humphreys et al., 2017). Previous systems-scale studies have reported conflicting evidence of the effect of sward clover DM content on milk production. A four year whole farm study in Ireland found no differences between a grass only swards receiving 226 kg fertiliser N ha^{-1} and grass-clover swards (annual clover contents of 218 g kg^{-1}) receiving 90 kg fertiliser N ha^{-1} (Humphreys et al., 2009). In contrast, McClearn et al. (2019) reported cows grazing grass-clover systems receiving 250 kg fertiliser N ha^{-1} (mean annual clover contents of 230 g kg^{-1}) had significantly greater annual milk yields (+ 597 kg cow^{-1}) and milk solid (fat + protein) yields (+ 48 kg cow^{-1}) compared with cows grazing N-fertilised grass system receiving 250 kg fertiliser N ha^{-1}. However, white clover content reduced significantly over the four year period from approximately 360 g DM·kg^{-1} in 2014, 240 g DM·kg^{-1} in 2015, 180 g DM·kg^{-1} in 2016 and 140 g DM·kg^{-1} in 2017 (McClearn, 2019). This highlight in whole farm studies that long term milk production performance from white clover grazing systems are still imperfectly understood.

2.6 Dairy production systems

2.6.1 Seasonal pasture-based dairying systems

Pasture-based systems are common in temperate regions such as in Ireland, New Zealand, parts of Australia, the United States and Europe where pasture growth is abundant (Le Gall et al., 2009; Hofstetter et al., 2014; Roche et al., 2017; Joubran et al., 2021). Rates of pasture growth are highly seasonal in temperate latitudes (Hurtado-Uria et al., 2013). The period when pasture growth meets the demand by grazing dairy cows is limited. In Western Europe pasture deficits due to low growth rates typically occur in late autumn, winter and early spring. Traditional seasonal pasture-based dairy systems are designed to maximises the synchrony between the herd demand for feed to the production of home-grown feed (grazed and ensiled pasture) with minimal purchased concentrate feed (Ramsbottom et al., 2015; Ruelle et al., 2018). Hence, herds seasonally calve in the spring (January to April) matching calving date to the commencement of pasture growth. In Ireland, 85% of dairy cows were calved in the months of January to April with February accounting for 38% in 2021 (ICBF, 2021).

The absence of fertiliser N application to white clover systems results in poor spring growth and consequently lower pasture production in spring (see 2.5.5.1). Conversely, white clover tends to be prominent in the sward from mid-summer onwards and this can be rationed to grazing dairy cows in a controlled way during early winter. Hence, a later calving date in April compared to February might better align pasture supply with the requirement for feed by the spring-calving herd. The effect of fertiliser N input to grass-clover swards and calving date on the productivity of pasture-based dairy production will be investigated in Chapter 5.

2.6.2 Milk composition and factors affecting milk composition

Typically bovine milk contains lactose (4.8%), fat (3.7%), protein (3.4%), minerals (0.7%), vitamins and water (87%) (Fox et al., 2015) although there can be considerable variation in these constituents including genetics (Tyrisevä et al., 2004; Chen et al., 2016; Visentin et al., 2017), physiological (O'Brien et al., 2006; Auldist et al., 2010; Lin et al., 2017), nutritional composition of the diet (Reid et al., 2015; O'Callaghan et al., 2016; Gulati et al., 2018; Doran et al., 2021) and environmental factors (Auldist et al., 1998; Fox et al., 2017). Milk proteins are heterogeneous with 75 to 80% casein, 15 to 20% whey protein and 5% non-protein nitrogen (NPN) (Fox et al., 2015).

2.6.3 Effect of late lactation milk on milk processing characteristics

Seasonal pasture-based systems result in compositional fluctuations in milk associated with stage of lactation, level of feeding and diet quality compared to non-seasonal systems (O'Brien et al., 1999; O'Brien et al., 2006; Timlin et al., 2021). As a result, these seasonal production systems can have implications for the processability and functionality of milk, particularly in late lactation (Lucey and Fox, 1992; Auldist et al., 1998; Downey and Doyle, 2007; Timlin et al., 2021). As the majority of dairy cows enter late lactation simultaneously, changes in milk composition occur because of physiological changes that occur in mammary glands (Phelan et al., 1982). Milk volume and lactose concentrations decrease with the later stages of lactation with increases in total protein and fat levels (O'Brien et al., 1996; Auldist et al., 1998; Guinee et al., 2007). The reduced concentration of lactose in late lactation coincides with the increase in the concentrations of sodium and chloride to maintain the osmotic pressure of milk similar to that of blood (Auldist et al., 1998). Furthermore, late lactation milk can have elevated somatic cell count (SCC) (O'Connell et al., 2015), reduced casein number (Reid et al., 2015), increased NPN (Phelan et al., 1982; Mehra, et al., 1999; Auldist et al., 1998), extended coagulation times and weaker gel structures reducing cheese-yielding efficiency (Guinee et al., 1997; Auldist et al., 2010).

2.6.4 Rennet-induced gelation

Milk processing characteristics are indicators that describe the ability of raw milk to be transformed into different dairy products. Indicators of milk processability for cheese production include the rennet gelation properties (Guinee et al., 1997; Fox et al., 2017). Chymosin, a specific milk proteolytic enzyme found in the abomasum of young ruminants is the main rennet starter used for coagulation of milk in cheesemaking. Rennet-induced gelation of milk involves two phases, enzymatic hydrolysis of κ-casein followed by aggregation and gelation of the rennet-altered micelles forming a three-dimensional gel (Lucey, 2002; Corredig and Salvatore, 2016; Fox et al., 2017). The rennet-gelation of milk can be monitored by low-amplitude strain oscillation rheometry which records gel firmness referred to as elastic shear modulus (G') over time (Sandra et al., 2012; Fox et al., 2017).

The concentrations of proteins especially casein have a positive relationship with the rennet gel strength and firming rate (Guinee et al., 1997; Corredig and Salvatore, 2016). Milk intended for cheese production should coagulate within a short time interval resulting in a fast firming time to produce a firm curd. If the three criteria is fulfilled, then the amount of cheese yield is maximised (Guinee et al., 2006). Guinee et al. (2006) reported that cheddar cheese yield increases by approximately 0.25 to 0.30 kg/100 kg of milk for every 0.1 g/100 g increase in milk protein in the range 3.0 to 4.5 g/100 g.

2.6.5 Irish dairy industry

The National Milk Agency (NMA) reported in 2020 that the Irish domestic milk supply was comprised with 78% of milk across seven months; between March to September, and 22% between October to February, inclusive (NMA, 2021). 95% of domestic milk supplied was utilised for dairy products, which were exported, while 5% was used for liquid consumption on the domestic market (NMA, 2021). Ireland has built a strong track record of producing and exporting high quality and nutritious dairy products. For example, the earlier limits placed on milk production by the EU milk quota triggered; (i) gradual consolidation among co-operatives, (ii) processors expanded overseas through the acquisition of existing facilities and (iii) processors moved production onto higher value-added products and ingredients (Donnellan et al., 2015).

OECD-FAO (2019) stated that worldwide milk production is expected to grow at 1.7% per annum over the next decade and reach 981 Mt by 2028. This trend of growth is faster than most other main agricultural commodities with growing demand for milk and dairy products due to population growth, urbanisation, rising incomes and dietary changes. Consumption is expected to grow faster than domestic production, resulting in substantial increase in dairy trade across borders particularly for densely populated countries such as China, Indonesia, Vietnam, Africa and the Middle East (OECD-FAO, 2019).

International markets outside the EU and UK increased by 7% in 2020 and accounted for 49% of dairy and specialised ingredient exports amounting to €1 billion (NMA, 2021). China accounts for 16% of all dairy exports and is Ireland's second largest market followed by the UK (Bord Bia, 2021). Cui (2016) reported that China's imports of baby formula from Ireland was 24,436 tons, accounting for 13.9% of total imports of baby formula. Consequently, long term prospects for the dairy sector are positive and achievable with further demand for exports internationally given the Food Wise 2025 report (DAFM, 2015). Food Wise 2025 report outlines Ireland is in a strong position to capitalise on growing demand for food which meets a range of 'free from' requirements (DAFM, 2015). There is a gradual shift in consumer attitudes to health and wellbeing, with many discerning consumers looking for natural and organic food.

Currently demand internationally for infant formula produced to organic standards is increasing, particularly from China. This growth in demand is due to a number of food scares in China related to milk adulterated with melamine in 2008 (Li et al., 2019a).

Consequently, tighter regulation has curtailed domestic milk production in recent years, following the food safety problems. The two child policy liberalisation has also further increased growth opportunities and demand for the expanding populations. Cui (2016) identified that the affluent middle class in China is increasing per capita income, stimulating demand for imported organic infant formula, which is seen as a safer product. In addition, results from the survey indicated that 55% of people would prefer foreign brands imported from organic and more reputable brands compared to domestic, non-organic baby formula (Cui, 2016). However, as things stand the supply chain for the production of organic infant formula does not exist in Ireland. Läpple and Cullinan (2012) found that structural and economic factors had an impact on the high organic conversion uptake rate among dairy farmers. Moreover, the lack of organic dairy processing facilities across Ireland limits the growth for dairy processors in terms of sufficient raw material in close proximity, which will improve the competitiveness in the supply chain.

2.7 Organic agriculture

2.7.1 Organic farming sector

Organic agriculture has attracted greater attention in recent decades due to consumer preference. The environmental, biodiversity and animal welfare issues associated with intensive farming have given further societal demands for quality products produced in a natural environment (Ditlevsen et al., 2019). The latter is recognised by the proposals for the future Common Agricultural Policy (CAP) and in line with the Farm to Fork and Biodiversity Strategies as part of the European Green Deal. Both strategies target at least 25% of European agricultural land under organic farming by 2030 (EC, 2019). In 2019, organic farming area accounted for 8.5% of EU's agricultural area (Figure 2.5) and expected trends indicate that with the present growth rate, the EU will reach approximately 17% by 2030 (Eurostat, 2021). Organic farming area in Ireland accounted for 1.6% with 73,952 ha of utilisable agricultural area (Eurostat, 2021). The Irish

Government has targeted to increase the area farmed organically in Ireland from 73,952 ha to 350,000 ha by 2030 (Government of Ireland, 2021).

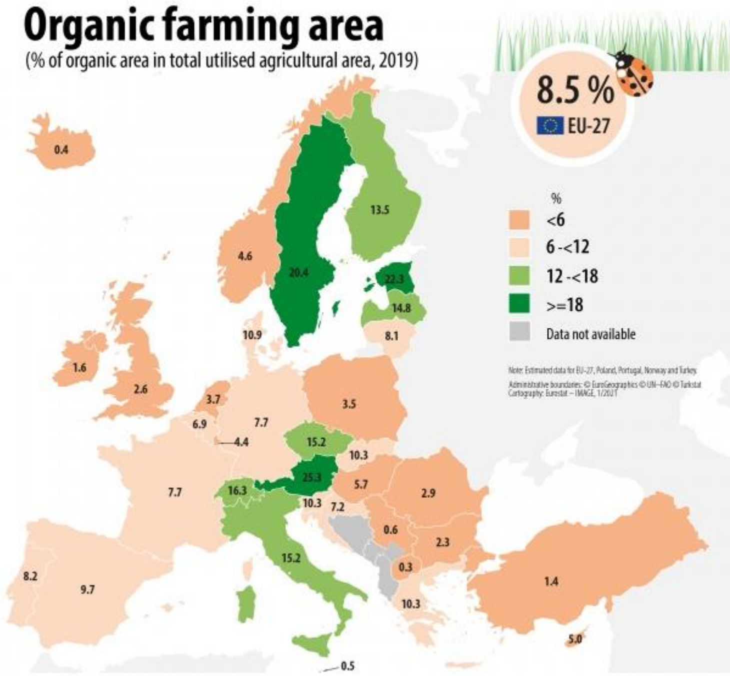

Figure 2.5. Organic farming area 2019 (Eurostat, 2021).

2.7.2 Organic agriculture in Ireland

Organic farms in Ireland are mainly located in the west and southwest where counties Cork and Kerry account for around 34% of producers (Läpple and Cullinan, 2012). Organic farms in Ireland are predominantly involved in drystock farming of beef cattle or sheep. DAFM (2019) reported that there is 62 organic dairy farmers which supply domestic organic milk production accounting for volumes of 0.11% of the national milk pool. The low cost seasonal pasture-based milk production system in Ireland (see 2.6.1) has potential to take advantage of growing worldwide markets (see 2.6.5). However, current supply of organic milk is failing to meet market requirement for added value products. The extensive nature of the drystock sector implies that farmers could easily switch to organic production with few amendments in farm management practices and very little entry costs (Läpple, 2010).

In Ireland, Läpple (2013) surveyed organic, former organic and conventional farmers to compare environmental attitude, risk attitude, profit orientation and information seeking attitude. It was found that conventional farmers were the most profit orientated and risk adverse group compared to former organic farmers. In addition, organic farmers were most environmentally aware and ranked highest for information gathering compared to conventional farmers. It was concluded that the impact of both economic and technical barriers inhibit the increase conversion to organic farming.

2.7.3 Institutional regulations and support

A key element of organic farming from other approaches to sustainable farming is the internationally acknowledged standards and certification. The standards for organic production along with labelling, processing, inspection and marketing within the European Union are defined and enshrined in law by Council Regulation EC 834/2007, which is backed up by Statutory Instruments Nos. 112 of 2004 and 698 of 2007 (EC, 2007). The Department of Agriculture, Food and the Marine (DAFM) are the main authority in Ireland responsible for ensuring that the organic farms adhere to European regulations. Nevertheless, EU legislation allows member states to use private inspection bodies to carry out the examination and licensing system of organic operators. The main competent organic certification bodies (OCBs) in Ireland include the Irish Organic Association (IOA) and Organic Trust Limited (DAFM, 2019). The duties of the OCBs are initial certification of the farm and annual inspections. Further duties include the promulgation of organic farming to improve marketing with the organic symbol increasing knowledge and awareness. This certification system stipulates best farming practices along with explicit standards, which is imperative to the integrity of organic systems.

2.7.4 Organic livestock standards and key principles

The International Federation of Organic Agriculture Movements (IFOAM) defines the four principles as health, ecology, fairness and care where disregarding any of these elements could jeopardise the aims of organic farming (Luttikholt, 2007).

The principle of health states that organic agriculture should support and enhance the health of the soil, plants, animals, humans and the planet unitedly. Ecological systems and cycles should be integral part of organic agriculture that enhance and promote biodiversity, biological cycles and soil biological activity to ensure potentially hazardous chemicals are prohibited from the food chain. The principle of care should succeed a precautionary and responsible manner to safeguard the health and well-being of current and future generations. Therefore, integrated management ameliorating the impacts of intensive agriculture on the environment will maintain high animal welfare and food quality standards (Rahmann et al., 2016).

Nevertheless, an overhaul of current organic regulations is changing given the rapidly growing sector and came into force on 1 January 2021. These new regulation were designed to improve precautionary measures, simplify rules for additional range of products and thereby ease certification for small farmers. The standards for livestock husbandry adhere to the Council Regulations (EC) 834/2007 and (EC) 889/2008 as amended relating to organic production. Elucidating the major concerns that arise for organic dairy production management under the EU regulations (EC, 2007) that need to be stipulated at farm level are;

1. Dairy cows are limited to a stocking rate of 170 kg N ha^{-1} yr^{-1}, this is equivalent to 2 LU ha^{-1}. Maintenance of soil fertility depends on the appropriate rotations, alternating silage and grazing ground with precise allocation of livestock manures and slurry. In addition, farms can be fertilised through resources of mineral fertiliser for trace elements from the following Table 2.1.

Table 2.1. Source of mineral fertilisers on organic farms (EC, 2007).

Phosphorus (P)	Ground Rock Phosphate (typically 11% P) works best in soils at < pH 6.5
Potassium (K)	A. Potassium sulphate (also known as SOP or sulphate of potash) 41% K 18% S B. Potassium sulphate with magnesium and sulphur: (e.g., Patentkali®) 26% K 6% Mg 17% S C. Potassium sulphate with sodium and sulphur (e.g., Magnesia-Kainit®) 9% K 3% Mg 20% Na 4% S
Other fertilisers	A. Basic Steelworks slag (variable content, check with supplier) Contains some P, K, and other trace elements. B. Seaweed fertilisers - potassium (K) and other trace elements

Note: The OCBs should be consulted to ensure that the fertiliser product is permitted for use.

2. Organic livestock must originate and be sourced from certified farms for grazing purposes, however, if unavailable, non-organic animals must be validated to OCBs. The producer is allowed to use artificial insemination and natural service for reproduction with appropriate breeds. Furthermore, dairy cows must be under organic management for at least the final six months of the conversion period (i.e. all concentrate supplementation must be organic sourced from the final six months of conversion) prior to land been awarded organic status and milk produced organically.

3. Livestock housing requires the need to increase cubicle size for animal welfare that supports the developmental, physiological and ethological needs of livestock. Each dairy cow requires 6 m^2 $head^{-1}$ and outdoor areas are set to 4.5 m^2 $head^{-1}$. Up to 50 % of housing area must be of solid floor construction where ample dry bedding (straw, sawdust, wood chips) ensures animals remain dry and clean at all times. There is a requirement for 3 m^2 per individual animal within cubicles for an adequate resting area that must also have a layer of bedding. Calves up to 100 kg require 1.5m^2 of housing and other animals require 1m^2 of housing area per 100 kg of live weight. The breeding bull requires 10 m^2 of housing with an open-air exercise area.

4. Feed supply of organic livestock must consist of at least 60% roughage, fresh or dried fodder, or silage. This percentage may be reduced to 50% for a maximum period of three months in early lactation for animals in dairy production. Therefore, establishment and management of clover-based swards are crucial to the success of organic dairy farms. Concentrate supplementation must be organically sourced with maximum daily intake of less than 40% of the animal's daily diet. In addition, specific trace elements and mineral compounds should be provided.

5. Animal health on organic livestock farms aims to reduce the dependency on preventative veterinary medicines and build herd health. A herd health plan is drawn up in consultation with a veterinary surgeon prior to conversion to outline a protocol and maintained by the farmer to operate a livestock system, which conforms to standards. Where treatment is essential, chemically synthesised medicinal products or antibiotics should be used under the responsibility of a prescribing veterinarian. The number of treatments must comply with organic certification requirements and legal withdrawal periods should adhere under specific guidelines. Vaccination is permitted, under derogation, in cases where there is a known disease risk. For mastitis control, two courses of treatment are restricted within a twelve month period. However, where treatment is exceeded, the animal should be sold conventionally or undergo a further fifteen-month conversion period. Appropriate husbandry management practices should ensure hygienic conditions for animals. The maintenance of good animal health is important to the economic viability of organic dairy farms to deliver milk with the functionality for a wide range of processing applications, which is safe for human consumption.

2.8 Summary and research objectives

Background N in permanent pastures has not been well considered in many N fertilisation recommendations for grassland. Quantifying background N under Irish condition will allow for better integration of background N with clover fixed N and with fertiliser N. Such an approach offers substantial opportunity for lowering reliance on fertiliser N for pasture-based dairy production. Moreover, white clover has long been promoted as a sustainable alternative to synthetic fertiliser N. This thesis develops a further understanding of the impact of white clover on the whole farm system in terms of its effect on milk and pasture DM production.

Therefore the specific objectives of this thesis are:

1. Investigate the spatial and temporal variation of the background release of plant-available N from soil organic matter in Irish grasslands
2. Carry out a retrospective analysis of the productivity of white clover-based grassland for milk production; identifying management practices such as N fertilisation and grassland grazing management for optimising white clover content and BNF
3. Examine the effect of fertiliser nitrogen input to grass-clover swards and calving date on the productivity of pasture-based dairy production

Chapter 3. Spatial and temporal variation of the background release of nitrogen from soil organic matter in Irish grasslands

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Abstract

Knowledge of the capacity of soils under permanent grassland to supply plant-available nitrogen (N) from soil organic matter (background N) is an important prerequisite to improve N use efficiency and hence mitigate N loss to the environment. Background N was quantified by measuring N uptake in grass in the absence of external N inputs at 14 grassland sites in Ireland. Background N was measured weekly at 9 sites and monthly at 5 sites for between 2 and 4 years. Soil physical and chemical properties along with meteorological factors were analysed for associations with background N. Mean annual background N during the main growing season (February to October) ranged from 67 kg ha^{-1} (poorly drained clay soil) to 218 kg ha^{-1} (imperfectly drained loam soil) with a mean of 141 kg ha^{-1}. Soil pH, air temperature and depth of A1 horizon were found to explain 51% of annual variation of background N. Monthly background N was low in February, increased to reach a maximum during May, declined in June and July, increased during August and then declined during the remainder of the growing season. The mean daily background N ranged from 0.30 kg ha^{-1} in February to 0.79 kg ha^{-1} in May and 0.29 kg ha^{-1} in November. Background N varied both temporally and spatially in Irish permanent grasslands. Better integration of background N into fertilisation strategies across the growing season can improve N use efficiency in permanent grassland.

3.1 Introduction

Before synthetic mineral fertiliser N became widely available and cost effective during the late 1960's and 1970's grass production was largely dependent on N_2 fixed by legumes and the mineralisation of N in crop residues, animal manures and soil organic matter (SOM) (Jarvis et al., 2011; Keeney, 1982). Since then the negative environmental impacts of synthetic mineral N have grown to the extent that they are now of global concern (ECE, 2020). The European Commission aims to mitigate its use for agricultural purposes (ECE, 2020) and current policy targets a 20% reduction in fertiliser N use within the European Union by 2030 (EC, 2019).

Soils under permanent pastures in Ireland contain an average of 7 t ha^{-1} of N in the A1 horizon (Gardiner and Radford, 1980; McGrath and Zhang, 2003). Most of this N is contained in SOM. The continual transformation of SOM in the soil mainly due to biological activity releases a small proportion of this N as plant-available N into the soil solution. This source of N is known as background N. One of the simplest methods to estimate the capacity of soils to supply background N is to use the crop as a sink for any plant-available N released by the soil; the plant-N-uptake method (Brye et al., 2003; Jarvis et al., 1996). For this method, N uptake by a crop is assumed to equate with background N when the crop is grown in the absence of external inputs such as artificial fertiliser N, excreta and N fixation by legumes. Denitrification and atmospheric deposition is difficult to exclude under field conditions and, hence, it is typically included with background N. The average atmospheric deposition of N ranges between 8 and 12 kg ha^{-1} in Ireland (Henry and Aherne, 2014). Additionally, background N made available to plants by heterotrophic free-living N_2 fixers in the soil is usually relatively small (< 5 kg ha^{-1}; Unkovich et al., 2008).

Background N has not been well considered in many N fertilisation recommendations for grassland, which usually only consider total soil organic matter and cropping history as indicators of background N for the coming season (Broadbent, 1984; Carpenter-Boggs et al., 2000; Sharma and Bali, 2018). Hence, there is a risk of inefficient application of fertiliser N, which entails the risk of N loss either as nitrate to waters or as NH_3, N_2O and NO_x to the atmosphere (Burchill et al., 2016). These N losses pose a threat to the environment with potentially important implications for human health (Erisman et al., 2013). Asynchrony between N supply and crop demand for N plays a central role in determining the proportion of N lost (Campbell et al., 1995; Crews and Peoples, 2005). It is therefore important to synchronise fertiliser N supply with the varying N requirements by crops throughout the growing season. In temperate regions, pasture growth increases markedly during the spring, from early March onwards in the Northern hemisphere, and greatly increases the requirement for N inputs (Corral and Fenlon, 1978). Nitrogen supplied by background N can meet some of this requirement of grassland (Hassink, 1995a; Hopkins et al., 1990; Humphreys et al., 2003; Prins, 1983).

A number of methods exist that aim to predict the supply of background N. Most studies of background N have entailed laboratory techniques involving chemical extraction or laboratory incubations (Carpenter-Boggs et al., 2000; McDonald et al., 2014). These methods use small volumes of disturbed soil. The soils are incubated in filtration units in a variable temperature incubator that mimic field growing conditions (Carpenter-Boggs et al., 2000; Risch et al., 2019). Nevertheless it is difficult to fully represent actual field conditions in the laboratory given that background N release is regulated by factors, such as exposure to oxygen, ambient temperature, soil moisture content, microbial biomass and enzyme activity (Herlihy, 1979; Jansson and Persson, 1982; Jarvis et al., 1996; Risch et al., 2019). In a recent study, Risch et al. (2019) reported that background N measured in the laboratory was weakly correlated (R^2 = 0.31) with background N measured under field conditions across 30 grassland sites worldwide.

At present, information from plant-N-uptake studies provide the most extensive datasets for estimating background N under grassland (Jarvis et al., 1996; Keeney, 1982; Willigen, 1991) and it has been proposed that background N measured using the plant-N-uptake method could be used to guide N fertilisation of grassland (Hart et al., 1986; Jenkinson et al., 1985). While previous studies have measured the annual quantity of background N (Gill et al., 1995; Hassink, 1995a), few have reported availability of background N at different stages of the growing season; at monthly or weekly intervals over multiple years and site locations.

The objective of this study was threefold: (i) to quantify the capacity of a range of soil types at different locations in Ireland to supply background N under permanent grassland on an annual basis; (ii) to evaluate the effect of physical and chemical properties of these soils along with meteorological factors for associations with the capacity of soils to supply background N; (iii), and to determine the contribution that background N can make to pasture N requirements on a weekly basis throughout the growing season.

3.2 Materials and Methods

3.2.1 Description of sites

This study was conducted at 14 sites in Ireland between 2001 and 2004. These sites were under long term permanent grassland predominantly used for grazing and silage production and receiving annual artificial fertiliser N input of between 100 and 200 kg ha^{-1}. There were substantial differences between the sites in terms of parent material, soil classification, soil texture, drainage status and depth of A1 horizon (Table 3.1). The botanical composition of swards at each site was predominantly perennial ryegrass (*Lolium perenne*). Each autumn prior to making measurements of background N during the following year, a selective herbicide (CMPP.P, mecoprop–p, 600g l^{-1}) was applied to swards to remove any clover. Prior to the commencement of the experiment, the soil at each site was sampled and tested to determine capacity to supply plant-available P and K following extraction using Morgan's solution (Na acetate + acetic acid, pH 4.8). Soil pH was also determined. In the spring of each experimental year, all sites received a basal dressing of granular superphosphate (8% P + 12% Sulphur) at a rate of 30 kg P ha^{-1}. Potassium sulphate (42% K) was applied to the plots every four weeks or so (after harvest; see below) throughout the growing season to provide the equivalent of 248 kg K ha^{-1} yr^{-1}. All fertiliser was applied to the plots by hand.

Table 3.1. Site location and description of soil types at the 14 sites.

Site	County	Location	Parent material[1]	Soil classification[1]	Surface texture	Drainage status[1]	Depth of A1 horizon[1] (cm)
Athenry	Galway	53°17'26"N, 8°45’36"W	Limestone diamicton (shallow)	Shallow brown earth	Loam	Well to excessively	20
Blue Ball	Offaly	53°13'34"N, 7°38’57"W	Carboniferous limestone diamicton	Brown earth/ Grey brown podzolic	Sandy loam	Well to excessively	17
Ballyhaise	Cavan	54°03'00"N, 7°19’00"W	Sandstone diamicton	Pseudo gley	Clay Loam	Imperfectly drained	22
Ballinamore	Leitrim	54°03'20"N, 7°47’30"W	Calcareous shale diamicton	Gley	Clay loam	Poorly drained	8
Clonakilty	Cork	51°37'37"N, 8°51’10"W	Sandstone shale diamicton	Brown podzolic	Sandy loam	Well drained	41
Clonroche	Wexford	52°26'56"N, 6°43’05"W	Ordovician shale diamicton	Acid brown earth	Loam	Well drained	36
Gurteen	Tipperary	53°02'18"N, 8°00’26"W	Limestone diamicton	Grey brown podzolic	Clay loam	Well drained	23
Johnstown Castle	Wexford	52°17'57"N, 6°30’40"W	Cambrian shale diamicton	Grey brown podzolic	Loam	Imperfectly drained	36
Kildalton	Kilkenny	52°21'10"N, 7°18’26"W	Sandstone limestone diamicton	Acid brown earth	Loam	Well drained	33
Kilmaley	Clare	52°49'48"N, 9°06’49"W	Glacial drift of Namurian shale origin	Gley	Clay loam	Poorly drained	14
Moorepark	Cork	52°10'00"N, 8°15’00"W	Sandstone limestone diamicton	Acid brown earth	Loam	Well drained	33
Oak Park	Carlow	52°51'39"N, 6°54’49"W	Calcareous limestone of Weichsel age	Grey brown podzolic	Loam	Well drained	25
Pallaskenry	Limerick	52°39'17"N, 8°51’49"W	Limestone diamicton	Grey brown podzolic	Loam	Well drained	38
Solohead	Tipperary	52°30'30"N, 8°12’20"W	Limestone diamicton	Gley/ Grey brown podzolic	Clay loam	Poorly drained	25

[1]Source: Gardiner and Radford (1980).

3.2.2 Experimental layout and design

Plots were laid down at each site during the autumn preceding the first year of harvest (Table 3.2). Plots were arranged in a randomised complete block design with three replications at each site. All replicates were harvested on each harvest date at each site. Plot size was 1 m × 5 m with a 0.55 m buffer area around each plot. There were two sets of replicated plots at each site. One set (A) received no fertiliser N input (as described above) and was used for measurement of background N in the first year. The other set (B) received 150 kg ha^{-1} of fertiliser N during the first year. Herbage was harvested from these set B plots at the same intervals as the set A plots. Herbage from the B plots was discarded after harvest. During the second year plots in set B received no fertiliser N input and were used for measurement of background N. Plots in set A received 150 kg ha^{-1} of fertiliser throughout the growing season and harvested herbage was discarded as described above. Following this schedule measurement of background N was alternated between plots in set A and set B every second year. This approach was taken to avoid the excessive depletion of soil N reserves.

There were two harvesting regimes (Table 3.2). At all sites all plots were harvested at four-week inter-harvest intervals throughout the growing season. However, for sites Ballyhaise, Ballinamore, Clonroche, Johnstown Castle, Moorepark, Oak Park, Kildalton, Kilmaley and Solohead there were a series of overlapping sets of plots with one set of plots harvested each week, at four-week inter-harvest intervals, using the methodology described by Corrall and Fenlon (1978). Hence, at these sites a set of plots was harvested each week throughout the entire growing season. At the remaining sites; Athenry, Blue Ball, Clonakilty, Gurteen and Pallaskenry there was only one set of plots per site, which were harvested at four-week inter-harvest intervals throughout the growing season.

Table 3.2. Harvesting regime, harvesting interval and experimental years at each site.

Site	Harvesting regime[1]	Harvesting interval	Experimental period
Ballinamore	Weekly	4 weeks	2002, 2003
Ballyhaise	Weekly	4 weeks	2003, 2004
Clonroche	Weekly	4 weeks	2003, 2004
Johnstown Castle	Weekly	4 weeks	2003, 2004
Kildalton	Weekly	4 weeks	2003, 2004
Kilmaley	Weekly	4 weeks	2002, 2003, 2004
Moorepark	Weekly	4 weeks	2001, 2002, 2003, 2004
Oak Park	Weekly	4 weeks	2003, 2004
Solohead	Weekly	4 weeks	2001, 2002, 2003, 2004
Athenry	Every 4 weeks	4 weeks	2003, 2004
Blue Ball	Every 4 weeks	4 weeks	2003, 2004
Clonakilty	Every 4 weeks	4 weeks	2003, 2004
Gurteen	Every 4 weeks	4 weeks	2003, 2004
Pallaskenry	Every 4 weeks	4 weeks	2003, 2004

[1] Mean initial weekly harvesting commenced from 1 March to 15 November and mean initial 4 weeks harvest date commenced from 8 March to the 8 November.

3.2.3 Meteorological data

Rainfall, air temperature, wind speed and sunshine hours were recorded at automated weather stations (Campbell Scientific Ltd. Loughborough, UK) at Athenry, Ballyhaise, Ballinamore, Clonakilty, Clonroche, Johnstown Castle, Kildalton, Kilmaley, Moorepark, Oak Park and Solohead. Where meteorological data was not measured on site, values were calculated from the corresponding values recorded at synoptic weather stations according to the procedures described by Hamilton et al. (1988) and Keane (2001). Location of weather stations were Boora, Birr, Springfield Castle, Shannon airport, Dublin airport, Knock airport, Cork airport and Mullingar. The hybrid grassland model of Schulte et al. (2005) assuming appropriate soil drainage criterion for each site was used for estimation of soil moisture deficit (SMD).

3.2.4 Soil physical and chemical properties

Twenty-five soil samples were collected at random using a hand gouge augur to depths of 0 to 10 cm and 10 to 20 cm across each site in spring 2004. Samples were bulked at each depth resulting in one composite sample per depth at each site. Each sample was passed through a 6.3 mm sieve to remove root mass and large rocks. Each soil sample was then dried at 40°C, crushed, sieved (2 mm) and analysed in triplicate for total N (TN) by dry combustion using a LECO CN-2000 elemental analyzer (LECO Corp., St. Joseph, MI). Soil organic C was determined by pre-acidification of the soils sample using 10% HCl and 1 drop concentrated HCl prior to dry combustion using a LECO CN-2000 elemental analyzer (LECO Corp., St. Joseph, MI). The ratio of carbon to nitrogen was calculated for each site and depth layer. Soil organic matter was determined by loss-on-ignition at 500°C for 16 h (Storer, 1984). Soil texture was determined by particle size analysis using the pipette method (Avery and Bascomb, 1974) and United States Department of Agriculture (USDA) classification of sand, silt, and clay (Brady and Weil, 2008). Soil pH (1:2) was measured in water using a Mettler Toledo electrode pH meter (Mettler-Toledo, Zurich, Switzerland) for composite soil samples at depth of 0 to 20 cm.

3.2.5 Herbage harvesting and analyses

At each harvest an area of 0.55 m x 5 m was cut along the middle of each plot using a Honda HRH 536 lawnmower to a stubble height of 5 cm. The harvested herbage was weighed and sampled for analysis. A 100 g sub-sample was dried for 16 h in a forced-draft oven at 95°C to determine dry matter (DM) content. A second 100 g sub-sample was dried at 40°C for 48 hours, milled through a 0.2 mm sieve and the N concentration in herbage DM was determined using a Leco N analyzer FP-528 (Leco Corporation, St. Joseph, MI, USA). Herbage DM yield (kg DM ha^{-1}) was calculated by multiplying the DM content by the fresh yield of the herbage. The remaining herbage on each plot and surrounding buffer area was harvested at each harvest date and discarded.

Table 3.3. Soil physical and chemical properties (mean values of three replicates) of each depth interval (0 to 10 cm and 10 to 20 cm) across the 14 sites. Note soil pH measured at depth of 0 to 20 cm.

Site	Soil depth	Soil texture			Total nitrogen	Organic carbon	Organic matter	C/N ratio	Soil pH
		Sand	Silt	Clay					
		% w/w			%				
Athenry	0-10 cm	38	37	25	0.41	3.99	10.60	9.73	6.0
	10-20 cm	41	35	24	0.29	2.75	8.10	9.48	
Blue Ball	0-10 cm	55	25	20	0.53	5.44	16.45	10.28	6.8
	10-20 cm	60	23	17	0.47	4.52	11.88	9.63	
Ballyhaise	0-10 cm	37	33	30	0.50	4.81	14.23	9.68	5.9
	10-20 cm	36	33	31	0.41	3.93	12.07	9.58	
Ballinamore	0-10 cm	21	43	36	0.67	6.89	17.73	10.26	5.3
	10-20 cm	23	45	32	0.39	3.76	11.67	9.62	
Clonakilty	0-10 cm	54	27	19	0.35	3.80	10.18	10.86	6.4
	10-20 cm	54	28	18	0.26	2.90	8.40	11.15	
Clonroche	0-10 cm	39	33	28	0.43	4.68	14.43	10.88	6.1
	10-20 cm	45	34	21	0.31	2.84	10.85	9.16	
Gurteen	0-10 cm	31	41	28	0.57	5.55	14.80	9.74	6.5
	10-20 cm	35	38	27	0.46	4.36	11.90	9.48	
Johnstown Castle	0-10 cm	42	37	21	0.32	3.30	10.00	10.31	6.5
	10-20 cm	42	39	19	0.22	1.94	6.13	8.82	
Kildalton	0-10 cm	44	32	24	0.39	4.04	11.10	10.36	6.3
	10-20 cm	43	36	21	0.27	2.40	8.33	8.89	
Kilmaley	0-10 cm	26	47	27	0.56	6.21	15.67	11.16	5.4
	10-20 cm	25	48	27	0.32	3.39	8.83	10.72	
Moorepark	0-10 cm	50	31	19	0.33	3.27	8.05	10.06	6.4
	10-20 cm	47	33	20	0.29	2.85	7.20	10.00	
Oak Park	0-10 cm	48	29	23	0.29	2.97	8.05	10.24	6.4
	10-20 cm	49	27	24	0.24	2.14	7.30	8.92	
Pallaskenry	0-10 cm	42	34	24	0.46	4.51	12.20	9.80	6.4
	10-20 cm	44	34	22	0.34	3.03	9.40	8.91	
Solohead	0-10 cm	35	36	29	0.57	4.91	15.07	8.66	6.7
	10-20 cm	37	35	28	0.47	4.13	12.97	8.79	

3.2.6 Estimation of background N

In the present study, N uptake by herbage was calculated by multiplying N concentration in herbage DM by herbage DM yield for each harvest. This was regarded as background N during the preceding four-week inter-harvest interval similar to Jarvis et al. (1996). Nitrogen uptake within each plot during each inter-harvest interval was divided by the number of days within the inter-harvest interval to give the average daily uptake of background N during the inter-harvest interval. Average daily uptake across overlapping plots, arranged according to Corral and Fenlon (1978), was used to calculate average daily uptake on a weekly basis. Likewise mean daily uptake on a four-week basis was also calculated in such a way as to approximately coincide with calendar months throughout each year (Table 3.2). Annual background N for each site was calculated from the total N uptake in herbage DM throughout each year.

3.2.7 Pasture N requirement during the growing season

In order to evaluate the capacity of background N to contribute to pasture N requirements on a weekly basis throughout the growing season it was compared with the herbage N uptake following high fertiliser N input at two sites. Replicated plots at Moorepark and Solohead received 350 kg fertiliser N ha^{-1} yr^{-1} applied in ten applications (calcium ammonium nitrate, 27.5% N, 5% S) at three-week intervals from February to September (Table 3.4) during 2001 and 2002 growing seasons. Plots were arranged in a randomised complete block design with three replications at each site. Similar to above, there were a series of overlapping sets of plots with one set of plots harvested each week using the methodology described by Corrall and Fenlon (1978). The weekly N uptake of these swards was determined using methodology similar to that described above.

Table 3.4. Timing and rates (kg ha^{-1}) of application of fertiliser N to the 350N treatments during 2001 and 2002.

Year	2001			2002		
N-input treatment	350N			350N		
Plot	1	2	3	1	2	3
	Fertiliser N (kg ha^{-1})					
11-Feb	25			25		
18-Feb		25			25	
25-Feb			25			25
04-Mar	25			25	25	
11-Mar		25				25
18-Mar			25			
25-Mar	60					
01-Apr		60				
08-Apr			60	60		
15-Apr					60	
22-Apr	60					60
29-Apr		60		60		
06-May			60		60	
13-May	50					60
20-May		50		50		
27-May			50		50	
03-Jun	30					50
10-Jun		30		30		
17-Jun			30		30	
24-Jun	30					30
01-Jul		30		30		
08-Jul			30		30	
15-Jul	25					30
22-Jul		25		25		
29-Jul			25		25	
05-Aug	25					25
12-Aug		25		25		
19-Aug			25		25	
26-Aug	20					25
02-Sep		20		20		
9-Sep			20		20	
16-Sep						20

3.2.8 Statistical analysis

Background N for each harvest date at sites between 2 to 4 years were subjected to analysis of variance (ANOVA) and analysed as a two factor (site x year) using GLM in SAS (SAS 9.4). Mean daily herbage N requirement per week were analysed using ANOVA including week and site as factors. Annual background N at sites were subject to ANOVA using PROC MIXED and analysed with weather data (air temperature, rainfall and SMD) to examine main effects of each variable and interactions with weather x year interactions. Data was subjected to multiple regression analyses. The dependent variables tested were annual background N, monthly and weekly background N. Independent variables tested were soil clay content, depth of A1 horizon, soil pH, C:N ratio, rainfall, air temperature and SMD. Year was not included as an independent variable in this analysis in order to capture across year effects. Before analysis of each model a test for multicollinearity between independent variables was conducted using PROC CORR and PROC REG in SAS. Variables with a Pearson's correlation coefficients $r > |0.8|$ or a variance inflation factor greater than 10 were removed from the model. Quadratic terms and interactions of second order between independent variables were considered in the model. A stepwise selection process was applied with a 5 % significance level for inclusion and exclusion of variables into the model. Partial R^2 values shown refer to the part of the model R^2 explained by each additional variable in the model when all previous variables were controlled. Therefore, model variables are shown in the order of selection by the GLMSELECT procedure. Results are presented as mean ± standard error.

3.3 Results

3.3.1 Meteorological data

The lowest mean annual air temperature of 9.5 °C was recorded at Ballinamore. Highest mean annual air temperature of 10.7 °C was at Kildalton and Johnstown Castle (Table 3.5). Highest annual rainfall of 1442 mm was at Kilmaley (Table 3.6). Mean air temperature and SMD increased in the month of June through to September. Highest mean monthly rainfall across sites was in October 2002 and amounted to 177 mm (Table 3.6). There was no ($P > 0.05$) effect of SMD on annual background N and no interaction between SMD and year. Annual background N was lowered (P < 0.01) by high rainfall amounts and was higher (P < 0.001) with higher air temperature.

Table 3.5. Average monthly air temperature (°C) at 14 sites averaged over four years (2001 to 2004). SEM is the standard error of the mean of the interaction between month and year at each site.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	SEM
						Air temperature (°C)							
Athenry	5.5	6.0	7.3	9.3	11.8	14.0	15.0	15.8	14.0	10.3	9.0	6.0	0.44
Ballinamore	4.8	5.0	6.3	8.5	11.0	13.8	14.8	15.3	13.0	9.5	7.8	5.0	0.50
Ballyhaise	4.8	5.0	6.3	8.3	11.5	13.8	14.8	15.3	13.3	9.3	8.0	5.3	0.44
Blue Ball	5.3	5.5	7.3	9.0	11.5	14.0	15.5	16.0	13.8	10.0	8.3	5.8	0.47
Clonakilty	6.0	6.3	7.3	8.5	11.0	13.0	14.5	15.3	14.0	10.3	8.8	6.5	0.41
Clonroche	5.5	5.5	7.0	9.0	11.3	13.8	14.8	16.0	13.8	10.0	8.3	6.3	0.47
Gurteen	5.3	5.5	7.0	8.8	11.3	13.8	14.8	15.5	13.5	9.8	8.3	5.8	0.48
Johnstown Castle	6.3	6.5	7.3	9.0	11.5	14.0	15.5	16.3	14.5	11.3	9.3	7.3	0.45
Kildalton	6.3	6.3	7.5	9.5	11.8	14.5	15.5	16.5	14.3	10.8	9.3	6.8	0.46
Kilmaley	5.5	6.0	7.0	8.8	11.3	13.5	14.5	15.0	13.5	10.0	8.5	6.3	0.44
Moorepark	5.5	6.0	7.3	8.8	11.3	14.0	15.3	16.0	13.5	10.0	8.5	6.3	0.48
Oak Park	5.0	5.5	6.8	9.3	11.8	14.3	15.8	16.3	13.5	10.0	7.8	5.5	0.48
Pallaskenry	5.8	6.0	7.3	9.0	11.3	13.8	15.0	15.8	13.5	10.0	8.8	6.0	0.49
Solohead	5.5	6.0	7.3	8.8	11.3	14.0	15.3	16.0	13.5	10.0	8.5	6.3	0.48
Year													Mean
2001	4.0	5.1	5.8	8.0	11.9	13.3	15.2	14.9	13.4	12.0	8.4	4.9	9.7
2002	7.1	6.8	7.6	8.9	10.9	12.9	14.7	15.6	13.5	9.7	8.6	6.3	10.2
2003	5.3	5.7	7.7	9.6	11.1	14.1	15.9	16.6	13.8	9.5	8.1	6.1	10.3
2004	5.5	5.4	7.0	8.9	11.6	14.8	14.6	15.9	14.2	9.3	8.5	6.8	10.2

Table 3.6. Average monthly rainfall and soil moisture deficit (SMD) at 14 sites averaged over four years (2001 to 2004). SEM is the standard error of the mean of the interaction between month and year at each site.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	SEM
						Rainfall (mm $month^{-1}$)							
Athenry	102	93	75	77	85	91	94	83	70	124	121	93	18.7
Ballinamore	130	112	98	83	109	105	85	77	75	147	118	116	20.5
Ballyhaise	81	82	66	72	81	83	69	71	56	110	96	75	18.3
Blue Ball	69	65	57	61	71	74	61	62	44	108	79	62	16.1
Clonakilty	163	122	113	98	95	103	70	86	95	121	131	118	28.4
Clonroche	119	91	83	114	84	88	85	74	63	173	110	86	24.7
Gurteen	67	56	50	65	70	68	56	56	47	98	72	60	14.9
Johnstown Castle	102	97	71	91	74	72	89	71	65	164	110	95	25.4
Kildalton	112	85	69	88	82	75	58	84	68	168	93	75	24.1
Kilmaley	146	133	104	91	92	124	99	81	93	158	179	143	27.7
Moorepark	105	81	84	81	75	80	58	83	54	122	90	83	21.7
Oak Park	73	63	55	65	67	63	53	73	41	110	81	68	16.8
Pallaskenry	120	96	88	87	78	83	67	73	53	136	104	89	22.1
Solohead	91	86	68	71	77	69	66	83	51	113	91	82	19.7
Year													Annual
2001	68	76	95	87	34	61	77	99	69	134	62	62	924
2002	163	161	62	95	133	97	63	62	30	177	169	110	1322
2003	76	70	62	79	113	103	96	17	52	57	129	92	945
2004	114	53	90	65	45	75	51	124	99	161	61	92	1029
SMD						SMD (mm $month^{-1}$)							
Athenry	1.5	4.3	7.5	14.8	16.5	20.3	12.8	17.3	20.0	11.8	1.3	3.0	4.85
Ballinamore	-8.3	-4.3	-0.5	8.5	12.5	21.0	15.8	18.0	21.5	-1.5	-7.0	-6.3	6.22
Ballyhaise	-4.5	0.3	-1.3	11.3	8.8	6.5	12.0	14.5	29.5	10.3	-3.3	-6.5	6.70
Blue Ball	2.8	6.0	9.0	21.8	21.5	31.3	30.3	31.3	41.0	22.5	5.8	3.3	6.91
Clonakilty	0.5	2.8	4.3	6.3	17.3	24.8	21.8	24.8	24.0	11.5	2.0	2.3	4.69
Clonroche	1.3	4.8	5.8	10.0	21.5	30.3	23.5	24.5	37.8	14.0	4.5	3.0	6.07
Gurteen	2.5	5.5	9.8	19.5	20.5	31.0	31.8	34.3	41.8	22.3	8.5	3.8	7.03
Johnstown Castle	1.5	4.8	6.0	10.0	21.8	31.3	24.5	27.0	37.0	12.5	4.5	2.5	6.35
Kildalton	2.3	5.8	8.8	15.5	22.0	35.5	38.8	32.8	45.3	19.8	9.5	4.3	7.23
Kilmaley	-8.0	-5.0	-1.5	5.8	11.0	17.3	2.5	10.0	17.3	5.5	-8.8	-6.5	6.22
Moorepark	1.5	4.5	7.0	12.0	22.8	32.0	31.3	28.8	41.8	23.8	8.3	4.0	7.39
Oak Park	2.3	4.5	9.0	20.3	23.5	36.0	40.5	31.8	48.8	22.8	6.5	3.0	7.04
Pallaskenry	1.5	4.5	7.8	12.3	17.8	30.8	26.0	28.5	39.8	22.8	3.5	2.8	6.92
Solohead	-7.5	-4.5	-1.0	9.3	13.3	30.8	30.5	25.5	38.8	20.0	-3.3	-5.5	8.62
Year													Mean
2001	0.2	2.0	0.9	1.0	30.3	42.7	33.5	18.4	33.0	0.9	3.0	3.9	14.2
2002	-2.1	-1.3	3.0	12.2	4.3	4.9	19.4	21.8	44.6	17.0	-2.4	-0.9	10.1
2003	-0.5	2.2	10.0	30.5	6.8	15.2	14.7	40.0	51.0	43.4	6.7	-0.5	18.3
2004	-0.9	6.7	6.4	6.7	30.3	45.5	29.8	19.5	9.8	0.9	1.9	-0.7	13.0

3.3.2 Spatial pattern of background N

There was a difference (P < 0.001) in mean (of two to four years) annual background N between sites. Mean annual background N ranged between 67 and 218 kg ha^{-1} with a mean ± SEM of 141 ± 7.5 kg ha^{-1} across sites (Figure 3.1). Johnstown Castle and Pallaskenry were the sites with highest mean annual background N. Mean annual background N at Ballinamore and Kilmaley were lower ($P < 0.001$) than at the other sites (Figure 3.1). Background N at some sites differed ($P < 0.05$) between years. Background N at Kilmaley was 87, 56 and 57 kg ha^{-1} in 2002, 2003 and 2004 respectively, with a mean of 67 ± 4.0 kg ha^{-1}. Mean annual background N at Johnstown Castle was 236 kg ha^{-1} in 2003 and 195 kg ha^{-1} in 2004, with an average of 216 ± 8.2 kg ha^{-1}. Relationships were not significant between annual background N and TN content ($R^2 = 0.09$, $P > 0.05$) and between annual background N and SOM content ($R^2 = 0.09$, $P > 0.05$). Three factors (soil pH, air temperature and depth of the A1 horizon) were found to have significant ($P < 0.001$) associations with, and accounted for 51% of the observed variation, in annual background N (Table 3.7). For each unit increase in soil pH within the range 5.3 to 6.9, there was an increase in mean annual background N of 43 ± 7.7 kg ha^{-1} ($P < 0.001$, partial $R^2 = 0.30$). Likewise for each increase of 1°C in annual air temperature there was an increase in background N of 56 ± 11.3 kg ha^{-1} ($P < 0.001$, partial $R^2 = 0.14$). The depth of the A1 horizon of the soils in this study was positively associated with an increase in annual background N with an increase of 1.4 ± 0.37 kg ha^{-1} per each 1 cm increase in depth of the A1 horizon ($P < 0.01$, partial $R^2 = 0.07$).

Table 3.7. Summary of the stepwise selection process from the multiple regression analysis of factors associated with annual background N.

Dependent variable	Step	Independent variable	Estimate (S.E.)	Partial R^2	Model
Annual background N (kg ha^{-1})	0	Intercept	-735.6 (114.85)***		$R^2 = 0.51$ RMSE = 33.25 n = 99
	1	Soil pH	42.6 (7.65)***	0.30	
	2	Air temperature (°C)	55.9 (11.30)**	0.14	
	3	A1 depth (cm)	1.4 (0.37)**	0.07	

S.E. = Standard error; RMSE, root mean square error.

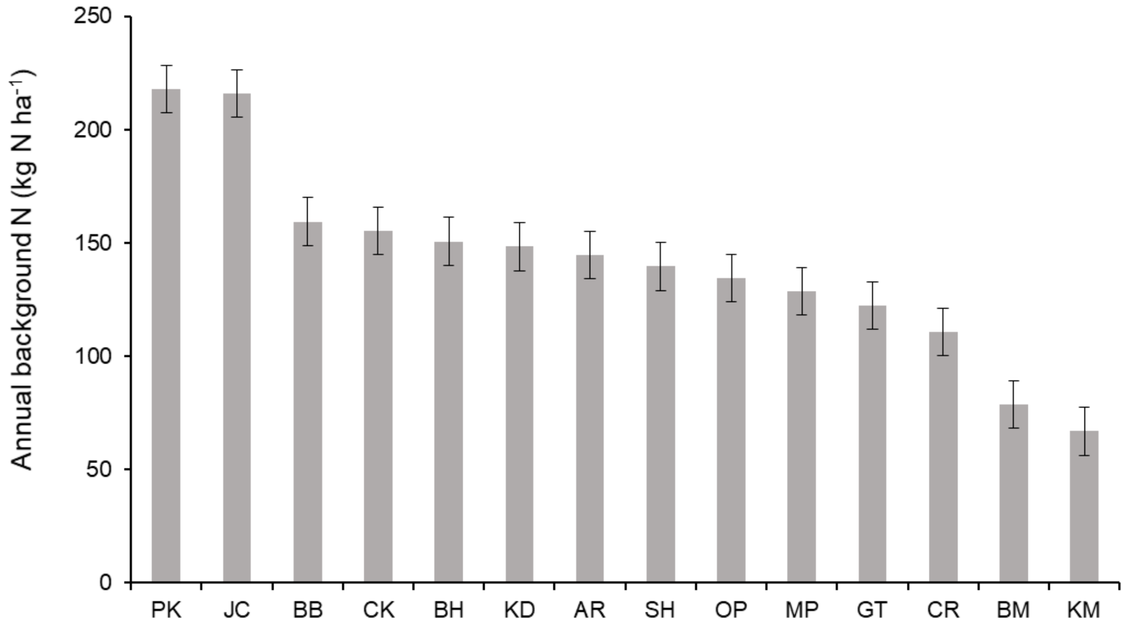

Figure 3.1. Annual background N at the 14 sites (Error bars represent standard error for site x year interaction; $P < 0.001$). PK, Pallaskenry; JC, Johnstown Castle; BB, Blue Ball; CK, Clonakilty; BH, Ballyhaise; KD, Kildalton; AR, Athenry; SH, Solohead; OP, Oak Park; MP, Moorepark; GT, Gurteen; CR, Clonroche; BM, Ballinamore; KM, Kilmaley.

3.3.3 Temporal pattern of background N

Daily background N varied ($P < 0.001$) across the year (Figure 3.2). Across all 14 sites, background N was found to be the lowest in February (with an average of 0.30 ± 0.042 kg ha^{-1}), and it increased to a peak of 0.79 ± 0.042 kg ha^{-1} in May. Background N declined during June and July but increased again and reached another peak during August, after which it continued to decline for the remainder of the growing season to 0.29 ± 0.049 kg ha^{-1} in November (Figure 3.2b). Multiple regression analyses for both monthly and weekly background N showed poor relationships ($r^2 < 0.21$) with independent variables (data not shown). Stepwise regression of daily background N found that soil pH, organic carbon, sand content and previous week air temperature explained 21% of the variation in background N between weeks. Likewise stepwise regression found soil pH, air temperature and SMD explained 15% of variation between months.

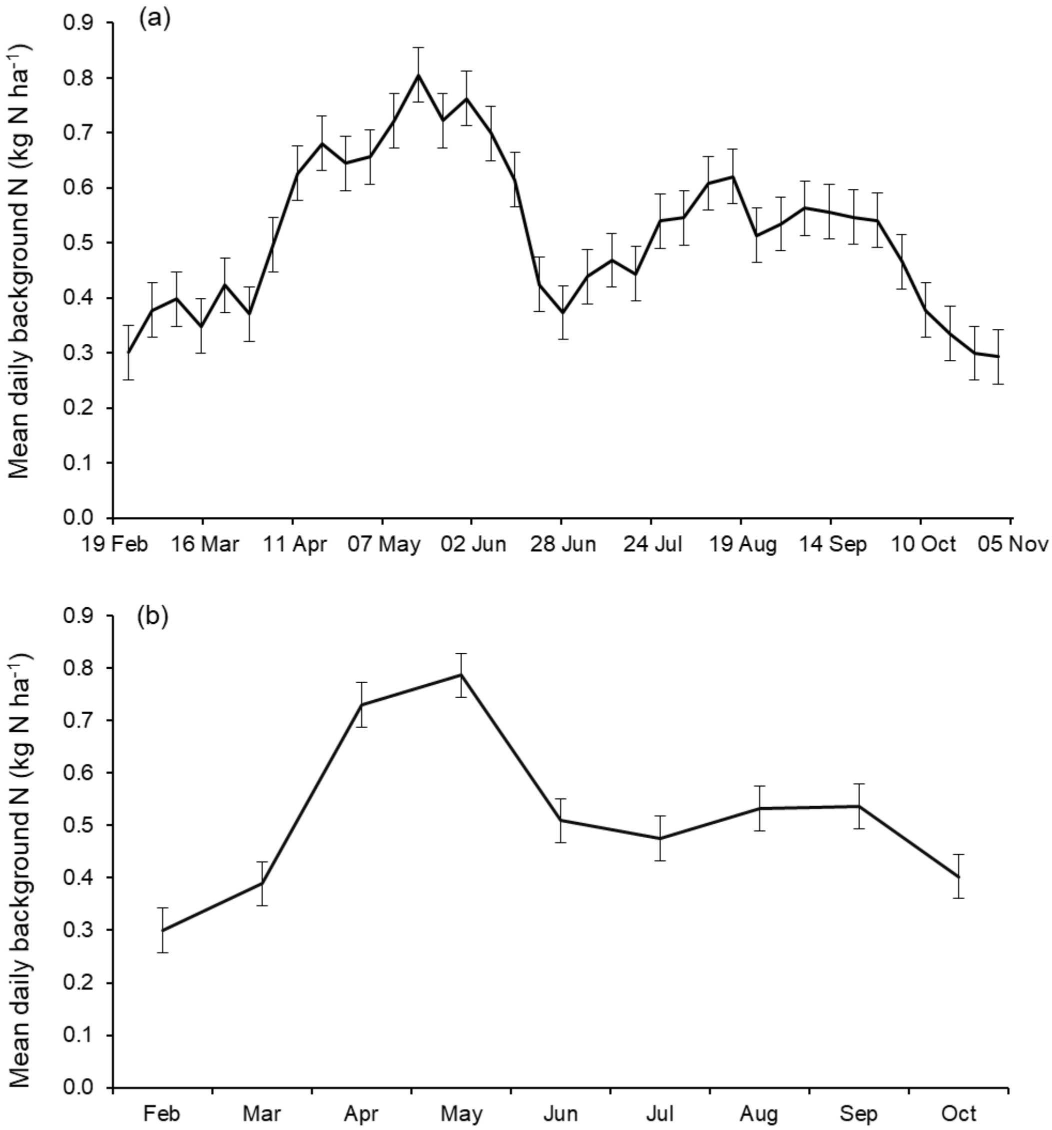

Figure 3.2. Mean daily background N variation measured (a) at weekly intervals at 9 sites and (b) monthly at 14 sites denoted in Table 3.2, error bars represent standard error of the mean for site x date interaction *(P* < 0.001).

3.3.4 Pasture N requirement during the growing season

Mean annual background N for two years was 114 kg ha^{-1} at Moorepark and 132 kg ha^{-1} at Solohead (Figure 3.3). For 350N treatment, mean annual herbage DM production averaged over two years at Moorepark was 10.01 t ha^{-1} with total annual N uptake of 411 kg ha^{-1} (Figure 3.3a). Likewise at Solohead herbage DM production was 10.65 t ha^{-1} with total annual N uptake of 406 kg ha^{-1} (Figure 3.3b). The mean annual background N amounted to approximately 0.28 and 0.33 of the annual uptake of plant-available soil N for Moorepark and Solohead, respectively. Background N at Moorepark represented 0.48, 0.40, 0.22, 0.21, 0.19, 0.23, 0.35 and 0.49 of the 350N requirement for March, April, May, June, July, August, September and October, respectively (Figure 3.3a). At Solohead, the corresponding proportions were 0.44, 0.39, 0.28, 0.24, 0.23, 0.29, 0.44 and 0.67 (Figure 3.3b).

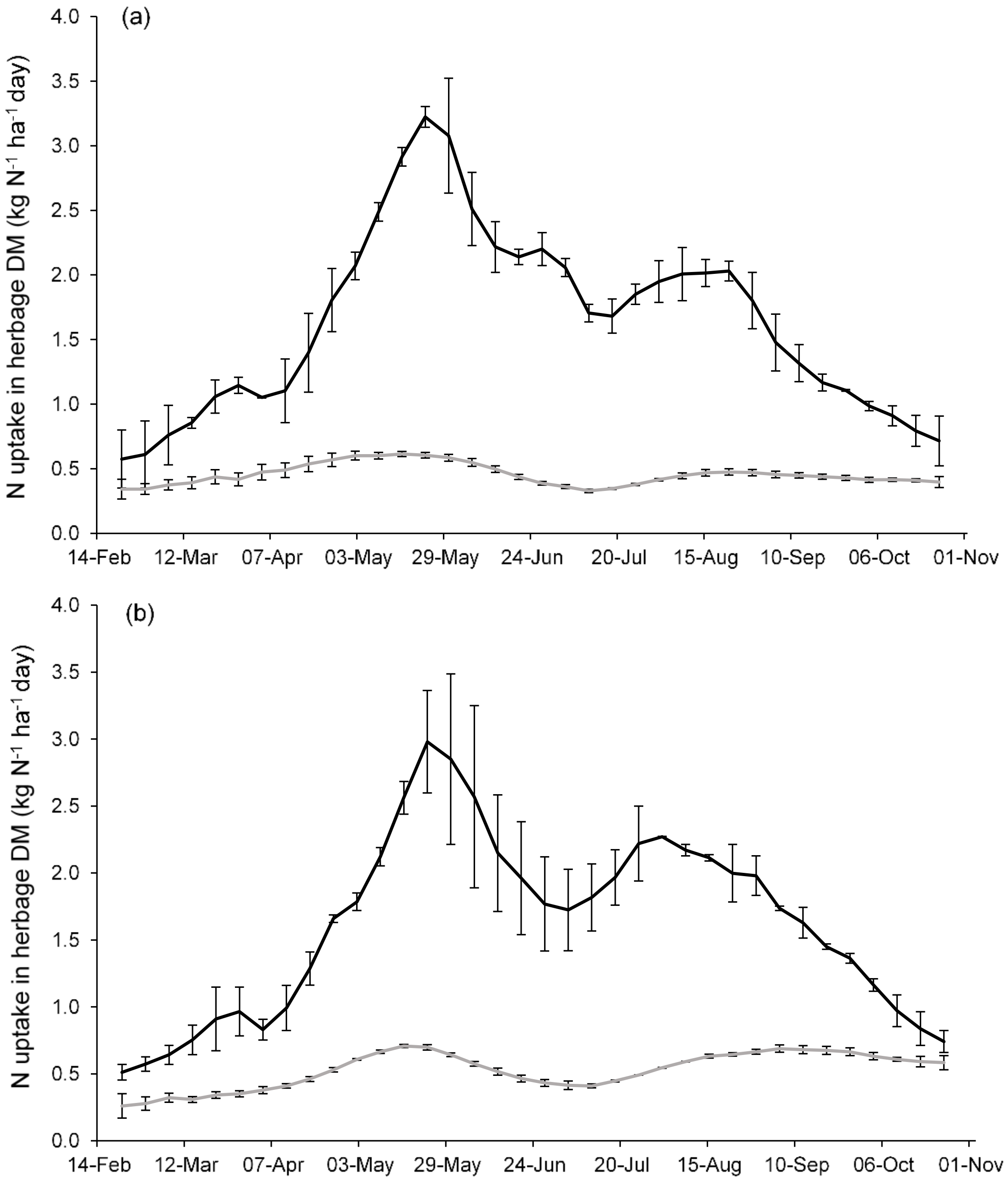

Figure 3.3. Comparison of mean daily N uptake for plots receiving fertiliser input of 350 kg N ha^{-1} (black line) and background N (grey solid line) averaged over two years for (a) Moorepark and (b) Solohead. Error bars represent standard error of the means for each week.

3.4 Discussion

3.4.1 Spatial distribution of background N

Mean annual background N ranged from 67 kg ha^{-1} at Kilmaley (poorly drained clay) to 218 kg ha^{-1} at Pallaskenry (imperfectly drained loam) with a mean of 141 kg ha^{-1} across the 14 sites. Measured using the same plant-N-uptake methodology in the United Kingdom background N ranged between 50 and 250 kg ha^{-1} with an average of 110 kg ha^{-1} (Gill et al., 1995; Hopkins et al., 1990; Richards, 1977). Similarly, in the Netherlands, Hassink (1995a) reported annual background N ranging between 45 and 233 kg ha^{-1} on 10 loam and clay soils with a mean of 137 kg ha^{-1}.

The three factors that best explained annual background N in the present study were soil pH, mean annual air temperature and depth of the A1 horizon. In other studies higher background N tended to be associated with finer textured soils (Herlihy, 1979; Griffin, 2008; Li et al., 2020). However, both Ballinamore and Kilmaley with clay loam soil texture were found to have the lowest annual background N. This can be explained by low soil pH, relatively shallow depth of the A1 horizon and poor drainage status at these sites. Annual background N was generally higher for the finer textured imperfectly drained soils but tended to decline with poorer drainage status. Depth of the A1 horizon also influenced the extent of background N with annual background N increasing by 1.4 kg ha^{-1} for each additional 1 cm depth. Laboratory incubations of soils from different depths have indicated that the majority of background N originated from the top 20 cm (Jarvis et al., 1996). Hence, a shallow depth of the A1 horizon can limit background N in temperate grasslands. This is in accord with the low levels of annual background N at Ballinamore and Kilmaley and serves to explain the absence of a relationship between soil texture and background N in the present study.

The maintenance of optimum pH is essential for optimal soil biological activity, especially the mineralisation of SOM into plant available nutrients (Jansson and Persson, 1982). Similar to Curtin et al. (1998), the present study showed that the variability of background N was mostly explained by soil pH. In contrast, Haynes (1986) found that soil pH was not a significant factor influencing background N across a range of soils. Soil pH was below the optimal level (≥ 6.3) for grassland at Ballinamore (pH 5.3) and Kilmaley (pH 5.4) where effectiveness of background N to contribute to cation exchange capacity below soil pH 5.5 is often minimal (Murphy, 2015). Curtin et al. (1998) found that liming two slightly acid (pH 5.7 and 5.8) soils stimulated C and N mineralisation for background N supply. Culleton et al. (1999) found that the fertiliser N requirement could be lowered by 50 to 70 kg ha^{-1} yr^{-1} when soil pH was raised from 5.5 to 6.3 following the application of lime. Moreover, the results of this study show that a unit increase in soil pH can increase the background N by 42.6 kg ha^{-1}.

3.4.2 Estimating annual background N

The number and variety of laboratory techniques involving chemical extraction or laboratory incubations along with generic simulation models reflect efforts to quantify and describe the complex nature N cycling in soils (Jarvis et al., 1996; McDonald et al., 2014; Ros et al., 2011bb; Bilotto et al., 2021). Despite 80 years of research, these approaches have not been able to adequately predict background N for use in fertiliser N recommendations at farm-scale (Nannipieri and Eldor, 2009; Ros et al., 2011a; Wall and Plunkett, 2020). The prediction of background N would be very helpful to advise farmers on the optimum fertiliser application rates. It has been demonstrated that the prediction of background N by more complicated models has not given better results than simpler ones (Willigen, 1991). The multiple stepwise regression analysis approach in the current study accounted for multicollinearity among soil properties similar to Ros et al. (2011b).

The current study found the moderately strong correlation r^2 = 51 explained greater variability of background N compared with Colman and Schimel (2013) whom found 33% of annual background N variance was explained with mean annual precipitation, clay content, soil C and N concentration. Similarly, Risch et al. (2019) found 33% of annual background N variance was explained by temperature of the wettest quarter of the year, microbial biomass, clay content and bulk density. In contrast to the latter studies, the current study found pH to be main indicator for background N considering a large gradient across sites. Colman and Schimel (2013) and Risch et al. (2019) excluded soil pH from models considering a large gradient across sites similar to the current study. In the present study, some portion of the unexplained variance in annual background N may be explained by a set of factors under the field conditions that were not accounted (Ros et al., 2011a) such as soil wetting and drying cycles and soil microbial dynamics as reported by Xiang et al. (2008).

3.4.3 Temporal pattern of background N

The present study found that there was a significant interaction between site and year in the annual release of background N. It is possible that background N at Pallaskenry in 2003 was influenced by a carry-over effect from the previous year given the annual background N of 283 kg N in 2003 compared with 153 kg N in 2004. Similarly, background N at Kilmaley was 87, 56 and 57 kg ha^{-1} in 2002, 2003 and 2004.

N uptake in herbage followed a similar pattern to the typical growth curve of perennial ryegrass over the course of a growing season in a temperate region (Heide, 1994; Parsons and Chapman, 2000). In temperate regions, the vernalisation process in winter triggers reproductive development of perennial ryegrass in the following spring resulting in a peak of pasture production during late May. The defoliation of all reproductive tillers causes the recommencement of vegetative growth later in the summer and autumn. (Parsons and Chapman, 2000).

In the current study, a peak in background N was observed in May followed by a trough in June and July followed by a secondary peak in August. A similar seasonal pattern of background N release was observed in an incubated pot study of background N in soils of varying texture, moisture and organic matter contents (Herlihy, 1979). The trough in background N in June could be explained by low microbial activity as a consequence of increased temperature and moisture deficit (Bot and Benites, 2005; Herlihy, 1979; Jarvis et al., 1996). While the secondary peak of background N in August could be partly attributed to the drying and rewetting cycles, which stimulate microbial activity and increase mineralisation of SOM (van Gestel et al., 1993; Haynes, 1986; Herlihy, 1979).

Nevertheless it is apparent that the pattern of background N supply over the course of the growing season was influenced by the typical growth pattern of perennial ryegrass during the growing season. There was higher demand for plant-available soil N coinciding with peak growth rates during May, lower demand for N coinciding with the changeover of reproductive to vegetative tillers during June and subsequent higher N uptake coinciding with the growth of vegetative perennial ryegrass tillers during July and August. Hence, the method of measurement used in the present study influenced the within-year variation of background N at each site. These background N results should be interpreted with caution in the context of crops other than perennial ryegrass. This methodology was appropriate for the purpose of the present study, which was to study background N in the context of improving the efficiency of N-fertilisation of permanent grasslands.

3.4.4 Pasture N requirements during the growing season

Using the difference method, the present study showed that the background N had the potential to provide approximately 0.31 of the N uptake in herbage DM by the 350N treatment. The release of weekly background N from Moorepark and Solohead ranged between 0.18 and 0.79 of the requirements of the 350N treatment during the growing season. This result highlights the importance of background N supply to pasture growth at the shoulders (spring and autumn) of the growing season. This contribution to sward N requirement on a week by week basis has clear implications for other sources such as fertiliser N, biological N fixation (BNF) from clover and N recycled in excreta either directly under grazing or in slurry and dirty water (from washing the milking machine, collecting yards etc. Minogue et al., 2015). The demand of the sward for available N from the soil commenced to exceed the background supply in May. Therefore, application of slurry in early spring (late January to March) has clear advantages in terms of the utilisation efficiency of nutrients and minimising ammonia emissions (Maris et al., 2021). Slurry can be efficiently held in the soil in spring due to its composition of organic matter and ammonium, which is not readily leached and can be taken up under cold conditions. Another source of N to increase the proportion of soil N and cut back on fertiliser N is the incorporation of white clover in grasslands (Humphreys et al., 2017). In Ireland, Scully et al. (2021) found BNF ranged 90 to 191 kg N ha^{-1} on grass-clover swards with fertiliser N ranging from 0 to 100 kg N ha^{-1}. The lowest proportion of background N supply to the 350N treatment was found in May, June, July and August (Figure 3.3). During May onwards, BNF by white clover supplies enough N for high levels of pasture production (Humphreys et al., 2009).

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Chapter 4. The productivity of white clover-based grassland for milk production on a poorly drained clay loam soil over nineteen years

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Abstract

The objective of this study was to evaluate the productivity of white clover-based grassland for pasture-based dairy production on poorly drained clay loam soil. Data was collated from a series of system-scale studies conducted between 2000 and 2020 at Solohead Research Farm. N-fertilised grass-only (GO; n = 11) systems stocked at between 2.00 and 2.75 cows ha^{-1} with mean fertiliser N input of 244 kg ha^{-1} averaged over each of eleven years were compared with grass-clover (GC; n = 11) systems stocked at between 1.75 and 2.50 cows ha^{-1} with mean fertiliser N input of 97 kg ha^{-1} within each corresponding year. Across the eleven years, mean annual pasture DM production (12.1 ± 0.25 t DM ha^{-1}) was similar for both systems ($P > 0.05$). Mean milk production per cow did not ($P > 0.05$) differ between systems across the year or during July and August within each year (the peak clover content). Increasing fertiliser N input lowered biological N fixation (BNF; $R^2 = 0.82$, $P < 0.001$). Moreover, sward white clover content of pasture DM was negatively correlated with fertiliser N input ($R^2 = 0.66$, $P < 0.001$). Stolon and root DM mass in February was 55% of that in the previous November ($R^2 = 0.51$, $P < 0.001$). Sward white clover DM content in April was 24% of that in the previous August ($R^2 = 0.25$; $P < 0.001$). Low N fertilisation and maximising overwintering of stolon and root DM mass increased white clover productivity and persistence in grassland.

4.1 Introduction

The environmental impacts of synthetic N are of global concern and there is growing consensus about the need to mitigate its use for agricultural purposes (ECE, 2020). The European Commission (EC) has recently launched the Farm to Fork Strategy as part of the European Green Deal to tackle climate and environmental-related challenges and targets a 20% reduction in chemical fertiliser N use by 2030 (EC, 2019). Biological N fixation (BNF) in association with white clover (*Trifolium repens* L.) can supply 50 to

250 kg ha^{-1} yr^{-1} of plant available N to farming systems (Unkovich et al., 2008). The replacement of fertiliser N with BNF can reduce the carbon footprint and other environmental impacts of milk production (Li et al., 2011; Yan et al., 2013) while remaining economically competitive (Humphreys et al., 2017). These benefits are largely dependent on maintaining the clover component of the sward at agronomically desirable levels of approximately 300 g kg^{-1} of pasture DM (Frame and Newbould, 1986).

The optimal temperatures for growth of white clover are higher than perennial ryegrass (*Lolium perenne*) and this makes clover vulnerable to competition by perennial ryegrass particularly during the winter and early spring (Davies, 1992). Furthermore, poor growth rate of white clover over winter and early spring can compromise the overall annual pasture DM production of mixed swards (Frame and Newbould, 1986). Consequently, strategic application of fertiliser N may be necessary. Fertiliser N can be applied in spring at rates of < 90 kg N ha^{-1} to give improved pasture DM production without affecting annual production of grass-clover swards (Nesheim et al., 1990; Laidlaw, 1980; Enriquez-Hidalgo et al., 2016), although it can cause a lower sward clover DM content later in the growing season (Frame and Boyd, 1987).

The cyclic pattern of change in clover morphology due to seasonal growth, grazing management and fertiliser N can alter the light environment and persistency of clover (Brock and Hay, 1995; Wachendorf et al., 2001; Black et al., 2009). The persistence and spread of white clover in grazed pastures depends largely on the growth of stolons in temperate climates (Collins et al., 1991; Marriott et al., 1997; Wachendorf et al., 2001). A higher rate of stolon survival during the winter favours clover growth during the following growing season and a substantial increase in BNF (Scully et al., 2021). However, low proportions of white clover in the sward in spring can lead to low contents throughout the growing season, resulting in a small contribution to DM pasture production (Collins et al., 1991). As a result, low and inconsistent production within and between years have restricted our ability to capitalise on the benefits of white clover in grazing systems (Chapman et al., 2017; Humphreys et al., 2017).

Previous research has reported conflicting evidence of the effect of sward white clover DM content on milk production per cow. Several studies report no effect of grass-clover systems on milk production per cow when compared to N-fertilised grass systems (Schils et al., 2000b; Leach et al., 2000; Enriquez-Hidalgo et al., 2014). A recent meta-analysis found that a mean annual sward white clover content of 316 g DM kg^{-1} is required to increase daily milk and milk solid yields per cow (Dineen et al., 2018). In Ireland, Egan et al. (2017) reported increased milk yield and milk solid yield from mid-June onwards with approximately 264 g DM·kg^{-1} clover content during the summer and autumn. Furthermore, McClearn et al. (2019) reported cows grazing the grass-clover systems had significantly greater annual milk yields (+ 596 kg cow^{-1}) and milk solid yields (+ 48 kg cow^{-1}) compared with cows grazing N-fertilised grass swards. However, in the latter study, white clover content declined significantly over the four year period; sward white clover content was approximately 360 g DM·kg^{-1} in 2014, 240 g DM·kg^{-1} in 2015, 180 g DM·kg^{-1} in 2016 and 140 g DM·kg^{-1} in 2017 (McClearn, 2019).

Plot-scale experiments are commonly used to examine the productivity and persistency of white clover in mixed swards (Nyfeler et al., 2009, Elgersma and Søegaard, 2016, Enriquez-Hidalgo et al., 2018). Similarly, whole farm studies are limited to two to four years and limit the examination of long-term effects. Therefore, previous research has been limited by being at plot scale, which does not always reflect what happens at system scale, or farm scale studies which tend to be short term in duration. There is a need for system scale studies that examines productivity and persistency in the long term. Hence, the objectives of this study were to (i) compare the performance of N-fertilised grass-only (GO) system to grass-clover (GC) system in terms of pasture and milk production and (ii) examine associations between white clover persistency and sward clover DM content, stolon and root DM mass and BNF using data from several contiguous systems-scale studies over nineteen years.

4.2 Materials and Methods

4.2.1 Dataset

A dataset was compiled for the purpose of this study from system-scale experiments concerned with pasture-based dairy production conducted at Solohead Research Farm (52˚30'N, 08˚12'W, 95 m above sea level) in south-west Ireland between 2000 and 2020 (Table 4.1). Data from 2015 and 2016 were not included in the present study because there were no clover-based systems at Solohead Research Farm in those years (Table 4.1). They were all system-scale studies with between 18 and 27 cows per system examining grassland management practices with spring-calving pasture-based dairy herds over an entire grazing season. There were between two and five experimental systems each year, resulting in a total of n = 59 systems within the 19 years (Table 4.1). The primary database was compiled with both GO and GC systems with n = 22 systems compared within each of 11 years. These systems were representative of fertiliser N input onto low fertiliser N input GC systems and intensively N-fertilised GO systems. Fertiliser N was applied predominantly in early spring to GC systems to increase pasture DM production due to the higher soil temperature threshold for white clover (Laidlaw, 1980). These included N205 and N300 from 2002, GO and GC from 2003 to 2006, S1 and S3 from 2010, HF-L and HF-H from 2011 to 2012, LC and GP25 from 2017 to 2019 (Table 4.1). The GO systems were stocked at between 2.0 and 2.65 cows ha^{-1} with mean fertiliser N input of 244 kg N ha^{-1} (standard deviation [SD] 51.8 kg N ha^{-1}). The GC systems stocked at between 1.75 and 2.65 cows ha^{-1} with mean fertiliser N input of 97 kg N ha^{-1} (SD 36.5 kg N ha^{-1}) within the corresponding year.

A separate dataset included individual paddocks for stolon and root DM mass was compiled for clover-based system-scale studies between 2007 and 2012. Sward clover content compiled for all years of clover-based system-scale studies with application of fertiliser N input of $\leq$ 110 kg N ha^{-1}.

Table 4.1. Details of the 59 grazing systems included in the dataset. Shaded areas represent comparison of system-scale studies for N-fertilised grass-only (GO) systems and grass-clover (GC) systems.

Study	Year of study	System name	Target PGH[1] (cm)	Cows per system	Stocking rate (cows ha^{-1})	Mean fertiliser N (kg ha^{-1})	Mean BNF[2] (kg ha^{-1})	Reference
1	2000	S1	6	18	1.75	81	67	Unpublished
1	2000	S2	6	18	2.10	155	17	Unpublished
1	2000	S3	6	18	2.50	192	9	Unpublished
1	2000	S4	6	18	2.50	309	2	Unpublished
2	2001-2002	N205	6	18	1.75	80	87	Humphreys et al. (2008)
2	2001-2002	N230	6	18	2.10	180	9	Humphreys et al. (2008)
2	2001-2002	N300	6	18	2.50	248	3	Humphreys et al. (2008)
2	2001-2002	N400	6	18	2.50	353	0	Humphreys et al. (2008)
3	2003-2006	WC	6	22-24	2.0-2.2	90	112	Humphreys et al. (2009)
3	2003-2006	FN	6	22-24	2.0-2.2	226	12	Humphreys et al. (2009)
4	2007-2009	6 cm	6	18-27	1.99-2.12	90	99	Phelan et al. (2013a)
4/5	2007-2009	5 cm	5	18-27	1.99-2.12	90	105	Phelan et al. (2013a)
4	2007-2009	4 cm	4	18-27	1.99-2.12	90	135	Phelan et al. (2013a)
5	2008-2009	ES0N	5	18	1.6	0	140	Scully et al. (2021)
5	2008-2010	LS0N	5	18	1.53	0	151	Scully et al. (2021)
6	2010	S1	4	24	2.0	96	111	Unpublished
6	2010	S2	4	24	2.2	101	133	Unpublished
6	2010	S3	4	24	2.2	101	142	Unpublished
6	2010	S4	4	24	2.4	106	134	Unpublished
7	2011-2012	HF-L	4	24	2.35-2.45	110	86	Tuohy et al. (2014)
7	2011-2012	HF-H	4	24	2.56-2.67	280	25	Tuohy et al. (2014)
7	2011-2012	JX-L	4	24	2.39-2.49	110	87	Tuohy et al. (2014)
7	2011-2012	JX-H	4	24	2.64-2.75	280	27	Tuohy et al. (2014)
8	2013-2014	S<7	4	24-25	2.57	280	14	Fenger et al. (2022)
8	2013-2014	S7–6	4	24-25	2.57	280	14	Fenger et al. (2022)
8	2013-2014	S7–5	4	24-25	2.57	280	13	Fenger et al. (2022)
9	2017-2019	LC	4	24	2.5	93	90	Unpublished
9	2017-2019	GP25	4	24	2.5	258	13.2	Fenger et al. (2021)
10	2020	S1	4	27	2.5	0	195	Unpublished
10	2020	S2	4	27	2.5	237	25	Unpublished

[1] PGH= Post-grazing sward height.

[2] BNF = biological nitrogen fixation.

4.2.2 Site description

Soils on the farm are poorly drained Gleys (90%) and Grey Brown Podzolics (10%) with clay loam texture overlying Devonian sandstone. Topographic relief causes variation in shallow ground water table depth ranging from 0 to 2.2 m below ground level. The local climate is humid temperate oceanic with a long potential growing season. The land has been under permanent grassland for over 50 years with predominantly perennial ryegrass (*Lolium perenne*). The white clover-based swards were established between 1999 and 2002 as described by Humphreys et al. (2008). Approximately 0.20 of the grassland area was oversown with white clover (*Trifolium repens* L.) each year to maintain the white clover concentrations as described by Humphreys et al. (2009).

4.2.3 Experimental design

The design and the scale of the experiments were similar in all years. Assignment of cows to herds and paddocks to systems, grazing management and recording of days at pasture was as described by Humphreys et al. (2008; 2009) and Tuohy et al. (2014). Each spring all cows were divided into 4 main groups on the basis of lactation number (1, 2, 3, and $\geq$ 4) and then sub-divided into sub-groups on the basis of calving date (mean 23 February, SD 21.7 days); the number of sub-groups being the same as the number of experimental systems in each year. From within each subgroup, one cow was randomly assigned to each herd. Herds were then randomly assigned to each experimental system. This procedure was repeated each spring. The experimental area was permanent grassland used for grazing and production of silage (ensiled pasture). The mean area per herd was 10.02 ha (range: 7.5 to 14.16 ha). At the beginning of each study, the experimental area was divided into 6 blocks according to soil type and drainage status and one paddock from each block was randomly assigned to a system and remained in that system until the end of the study. Mean paddock size was 1.7 ha (SD 0.42 ha).

4.2.3.1 Management of the grazing systems

The management of the grazing systems generally followed a standard set of rules. Each herd was under rotational strip-grazing management. Cows were turned out to graze approximately three days after calving and remained at pasture until they were dried off between mid-November and mid-December. Pasture was allocated for a 48 h period to dairy cows in a rotational strip-grazing management and each herd was moved to the next pasture area when a target post-grazing height (PGH) was reached (Table 4.1). Back-fencing was used to stop cows returning to previously grazed areas. During the main grazing season (from April to August) in all of the systems included in this study average pasture cover (APC) was measured on a weekly basis and managed to consistently maintain a pre-grazing pasture mass (PGPM; > 4 cm PGH) of 1400 kg ha^{-1}; i.e. an APC of 700 kg ha^{-1}. Excess pasture production was identified throughout the study and removed for silage production. These areas were selected when pasture growth rates exceeded demand, resulting in PGPM > 2000 kg DM ha^{-1} (above PGH). Such areas were generally closed from grazing between early-April and late-May (first-cut silage) or between early-June and mid-July (second-cut silage) of each year. Under exceptional circumstances during the main grazing season when pasture growth rates fell below herd demand, cows were supplemented with concentrates once PGPM was less than 1000 kg ha^{-1}. The rate of supplementation depended on the extent of the pasture deficit. Silage was also fed if the cows had to be housed due to severe pasture deficit due to drought (e.g. 2006 and 2018) or due to excessively wet soil conditions i.e. a volumetric soil moisture content (VSMC m^3 m^{-3}) of between 0.6 and 0.7. The latter tended to be relatively short term (2 or 3 days) during the main grazing season. Supplementation with concentrates to each herd during the main grazing season was allocated at exactly the same rate per cow within each year. Supplementation with silage was allowed to vary in line with the feed budget for each herd. Supplementation with concentrates and silage varied from year to year depending on weather conditions and their impact on pasture growth and ground conditions.

Closing date marked the end of the grazing season and was defined as the final day in winter when all cows of each system were housed for the closed period and did not go out to pasture again until the following spring. The target in all studies was to maintain the cows at pasture until at least 1 December while hitting a target APC of 500 kg ha^{-1} on 1 December. The latter condition was the reason that cows were rarely maintained at pasture until or after 1 December. When the APC fell below the target of 500 kg ha^{-1} during November, cows were housed in order to allow the pasture to grow up to the target. Hence, cows were housed prior to 1 December depending on the current APC and the expected daily pasture growth rate (based on ten-year averages) between the closing date and 1 December. The other criteria governing the decision to close pastures for the winter were ground conditions as described above and drying off the cows at the end of lactation. In all studies drying off of the younger and earlier-calving cows generally commenced in late November and drying off of all cows was completed in the week before Christmas day (25 December).

The annual fertiliser N input to each system is presented in Table 4.1. Fertiliser N was applied for early grazing during late February or early March. The entire area was grazed between turnout and early April. Fertiliser N for the area providing first-cut silage was applied in early April. Fertiliser N for the area providing second-cut silage was applied in early June to the GO system scale studies but not the GC systems. After first-cut silage in late May or early June and after second-cut silage in mid- to late-July the silage areas were subsequently used for grazing and received similar inputs of fertiliser N to that applied to the grazing areas of each GO system. Fertiliser N was applied in the form of urea between February and April and as calcium ammonium nitrate between May and

September. Inorganic phosphorus (P) and potassium (K) were applied across all swards based on yearly soil test results. Cows were housed during the winter and fed together as one group. Slurry produced during housing was collected in a common store and reapplied to grassland on a proportional basis to stocking rate on each system in accordance with regulatory protocols under Statutory Instruments (SI) No. 65/2018. Slurry was applied in late January each year to approximately two-thirds of the area of each system. Slurry was also applied on the area for first-cut silage in late March and after harvests of silage. Slurry was applied using a downward-facing splash-plate up to 2014 and a dribble bar from 2017 onwards.

4.2.4 Measurements

4.2.4.1 Meteorological data

Meteorological data was recorded at the climatological station located on the research farm (Campbell Scientific Ltd, Loughborough, U.K.). Soil temperature was measured daily to a depth of 10 cm. Daily soil moisture deficit (SMD) was calculated for the experimental period of each year using the model developed by Schulte et al. (2005) assuming a poorly drained soil.

4.2.4.2 Pasture DM production, average pasture cover and fertiliser N input

Pre-grazing pasture mass was measured prior to each grazing or silage event by harvesting a strip of pasture using (i) a lawnmower (HRH-536 rotary blade, Honda®, Alpharetta, GA, USA between 2001 and 2009 and (ii) an Etesia Hydro 124DS, (Etesia UK Ltd., Shenington, Oxon, UK) between 2010 and 2020. All mown pasture from each strip was bulked, weighed and a 100 g sub-sample dried for 16 h in a forced-draft oven at 90 °C to determine DM content. Annual pasture yield (kg DM ha^{-1}) was calculated as the sum of pasture removed as pre-grazing and pre-silage cuts. Post-grazing sward height was determined immediately after each grazing using a Filips rising plate meter (Grasstec, Mallow, Cork, Ireland). Pasture growth rate was determined as the mass of pasture grown between two harvests/grazings divided by the number of days in each interval. The compressed sward height of each paddock per system was measured using a Filips rising plate meter once per week during each grazing season and on average once per month during the closed period during which all cows were housed, generally encompassing December and January. Compressed sward height was converted into pasture cover, which was an estimate of mass of pasture > 4 cm PGH per paddock using the following formula:

*Pasture Cover (kg DM ha^{-1}) = (Compressed Sward Height (cm) – Target post-grazing height (cm)) * Sward Density (kg DM cm^{-1} ha^{-1}); assuming a sward density of 240 kg DM cm^{-1} ha^{-1} across all years.*

Average pasture cover of each GO and GC grazing systems on each measurement date was the sum of the pasture covers of all paddocks within each system divided by the total grazing area. The closing pasture cover was measured during the week of the last grazing before the closed period in the preceding year (mean 26 November, SD 14 days). Opening pasture cover was measured at the end of the closed period, i.e. when calved cows were turned back out to pasture; the mean date was 12 February (SD 7 days). Pasture DM production was compiled into four pasture production (PP) timeframes throughout the year; (1) between 11 Jan and 1 May; (2) between 2 May and 3 Jul; (3) between 4 Jul and 11 Sept and (4) 12 Sept and 26 Dec. The fertiliser N input for each GO and GC grazing systems was determined for each of these PP timeframes. Fertiliser N was not applied during the last growing period (12 Sept – 26 Dec) because it was prohibited under Statutory Instruments (SI) No. 605 of 2017 and related earlier instruments.

4.2.4.3 White clover content of pasture DM, biological nitrogen fixation, stolon and root DM mass

The white clover content of the pasture DM (g kg^{-1}) in each paddock was measured using the methodology described by Humphreys et al. (2008) and Phelan et al. (2013ab). These measurements were carried out in April and August each year between 2000 and 2006 and in April, June and August between 2007 and 2020. White clover stolon and root mass was measured in February, May, August, and November in each year between 2007 and 2014 using the methodology as described by Phelan et al. (2013ab). Biological N fixation in each paddock within each year of this study was estimated using a mechanistic model as described by Humphreys et al. (2008).

4.2.4.4 Milk production, days at pasture, concentrates fed, live-weight and body condition score

Cows were milked at 0730 and 1530 h daily throughout lactation in all years of the study. Individual cow milk yield was recorded at each milking. Milk composition (protein, fat and lactose concentrations) from each cow was determined weekly from one successive morning and evening milking sample using a Milkoscan 203 analyser (Foss Electric, Hillerød, Denmark). Milk solids yield was calculated as the sum of milk fat and protein per cow. Additionally, mean daily milk yield per cow was the mean yield of all cows per system each day during a ten-week timeframe (mean date; 13 July (SD 2.0 days) to 21 September (SD 2.0 days)) defined as peak clover content (PCC) timeframe and likewise for other milk production variables. Days at pasture were recorded for each cow with a value of 1.0 ascribed to each 24 h period and a value of 0.5 if the cow was at pasture by day only. The amount of concentrate fed per cow was recorded at each milking (Dairymaster, Causeway, Co. Kerry, Ireland). The liveweight (LW) of each cow were recorded weekly using a weighing scales and the Winweigh software package (Tru-test

Limited, Auckland, New Zealand). Body condition score (BCS) of each cow was recorded fortnightly using the methodology of Edmonson et al. (1989).

4.2.5 Statistical analysis

Days at pasture, concentrates fed, BCS during lactation, LW during lactation, annual and PCC sward white clover content and milk production variables were subjected to an analysis of variance (ANOVA) using the PROC MIXED procedure in SAS (SAS 9.4). For the ANOVA, the dataset was grouped into GO and GC systems within the corresponding years, resulting in n = 22 systems being compared. Grazing system was a fixed effect and year and block were random effects in the ANOVA. Year was considered a random effect as the environmental conditions were assumed to vary year to year and differences in rainfall and SMD between years were small. Pasture DM production and fertiliser N input for GO and GC systems in the PP timeframes were subjected to ANOVA using PROC MIXED and analysed as a two factor (grazing system x pasture DM production) examining the main effects of each factor and interactions between each factor. Individual paddocks were replications for field-based variables and individual cows were replications for animal-related variables. Regression models were fitted with the GLM procedure to determine the relationship between fertiliser N input on sward white clover DM content and BNF across all systems-scale studies (n = 59 systems) independent of the effect of year. Individual white clover paddocks were analysed for associations using the GLM procedure between sward white clover DM content and white clover stolon and root DM mass measurements. Results are presented as mean ± standard error of the mean SEM.

4.3 Results

4.3.1 Meteorological conditions

Mean monthly soil temperature ranged between 3.1 and 8.0 °C during winter (December to February). Likewise it ranged between 12.0 and 17.2°C in the summer (June to August). Mean soil temperature ranged from 7.7 to 10.5°C during the spring (March to May; Figure 4.1a). Mean annual rainfall was 1077 mm (Figure 4.1b). Rainfall amount was 595 mm during the winter 2013/2014 compared to 330 mm recorded across the 21-year mean. Moreover, rainfall amount was 456 mm in summer 2012 relative to the 21-year mean of 236 mm. The mean monthly SMD ranged between -10 and 89 mm (Figure 4.1c). During the summer, cumulative SMD was 169 mm in 2006 and 221 mm in 2018, whereas the 21-year mean was 76 mm.

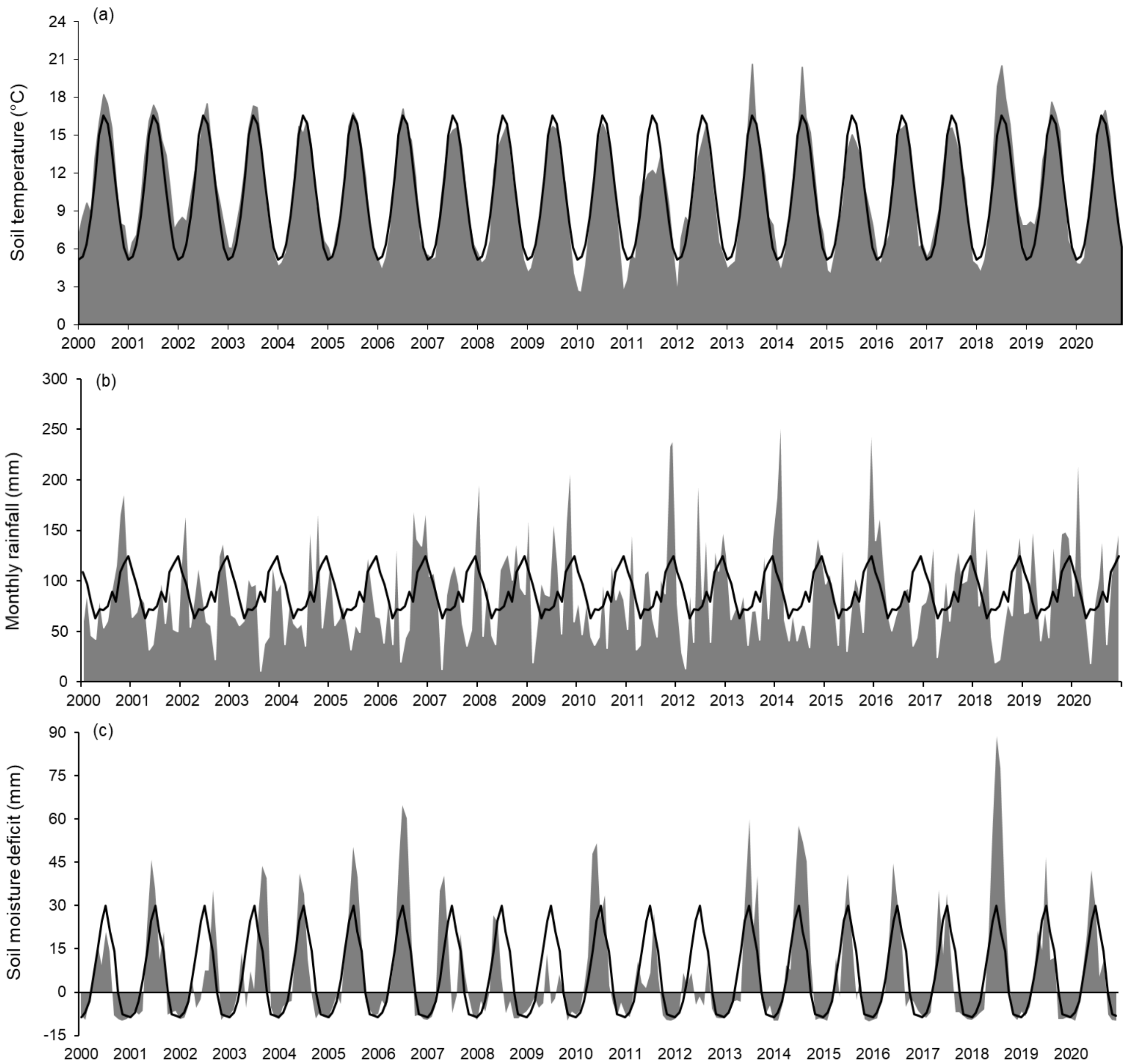

Figure 4.1. Mean monthly (a) soil temperature (b) cumulative rainfall and (c) cumulative soil moisture deficit recorded at Solohead Research Farm. The shaded areas show the recorded values during each individual year and the solid lines show the mean values of 21 years (2000 to 2020).

4.3.2 Pasture DM production, fertiliser N input, days at pasture and average pasture cover

There was no difference in annual pasture DM production between GO and GC (mean = 12.1 ± 0.25 t DM ha^{-1}, $P > 0.05$). There was no ($P > 0.05$) difference in pasture DM production within each of the PP timeframes between the two grassland systems (Figure 4.2). Fertiliser N input was higher ($P < 0.001$) to GO than GC (244 vs 97 kg N ha^{-1}; SEM 12.8). Likewise there was higher ($P < 0.001$) fertiliser N input to GO during each of the

PP timeframes except PP timeframe 4 (Figure 4.2). The application of fertiliser N was 107, 68, 69 and 0 kg ha^{-1} on GO for each of the PP timeframes, respectively. Likewise there were 80, 8, 9 and 0 kg ha^{-1} applied to GC within each PP timeframe, respectively. There was no difference in the number of days at pasture per cow between the two grassland systems (244 ± 3.3 days, P > 0.05, Table 4.2). Mean closing APC for GC paddocks was 466 ± 27.2 kg DM ha^{-1}. Mean opening APC for GC paddocks was 627 ± 27.4 kg DM ha^{-1}. Mean closing APC for GO paddocks was 513 ± 27.8 kg DM ha^{-1} and mean opening APC for GC was 699 ± 27.7 kg DM ha^{-1}.

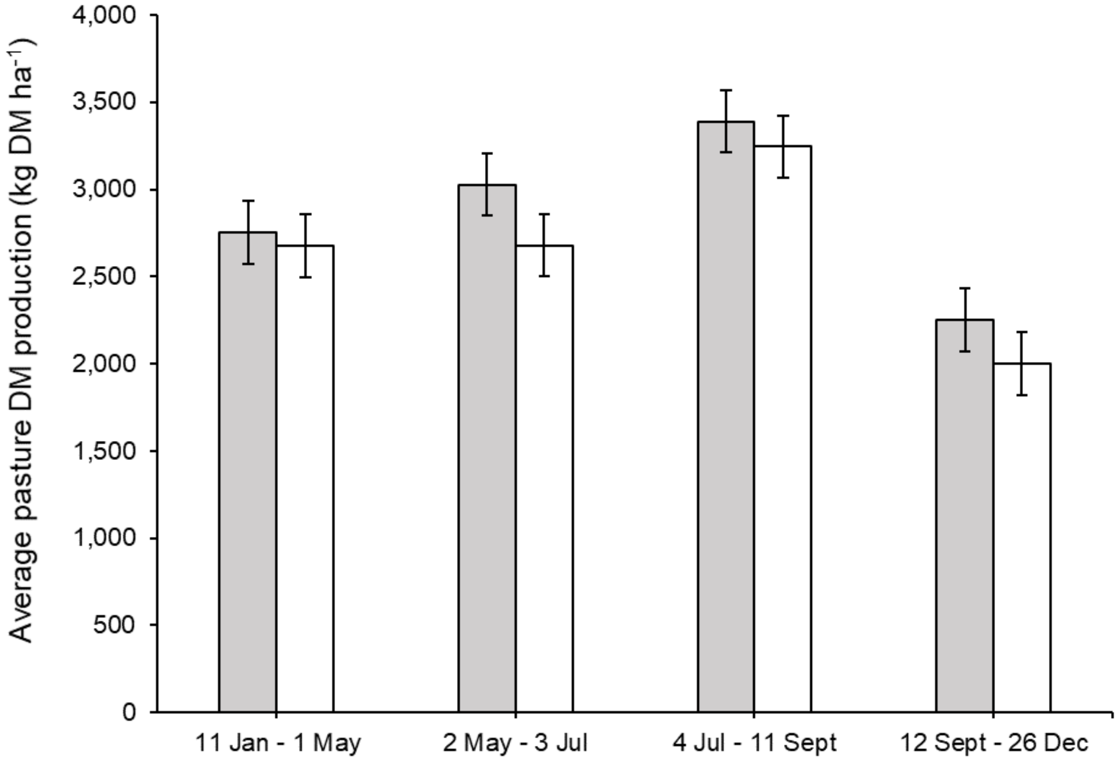

Figure 4.2. Comparison of N-fertilised grass-only (GO; ▢) and grass-clover (GC; □) systems for average accumulated pasture DM production across the four pasture production (PP) timeframes at Solohead Research Farm. Error bars show the SEM for the interaction between grazing system x pasture DM production (P > 0.05).

4.3.3 White clover content of pasture DM, biological nitrogen fixation, stolon and root DM mass

Mean annual sward white clover DM content was significantly ($P < 0.001$) higher on GC compared with GO (190 vs 49 g kg^{-1} DM; SEM 3.7; Table 4.2). Likewise mean PCC sward white clover DM content was ($P < 0.001$) higher on GC systems relative to GO systems (269 vs 66 g DM kg^{-1} SEM 5.0; Table 4.2). Across all individual white clover paddocks, sward white clover DM content in April was associated ($R^2 = 0.25$; $P < 0.001$; Figure 4.3a) with sward white clover DM content in the previous August. Sward white clover DM content in August was not ($P > 0.05$) associated with sward white clover DM content in April of the same year ($R^2 = 0.045$; Figure 4.3b). There was a positive association between stolon and root DM mass in November and stolon and root DM mass in the following February (Figure 4.4a). Stolon and root DM mass in February was 55% of that in the previous November ($R^2 = 0.51$, $P < 0.001$). Stolon and root DM mass in February was not significantly associated with stolon and root DM mass in November of the same year ($R^2 = 0.026$, $P > 0.05$; Figure 4.4b). Across all system-scale studies, there was a significant reduction in sward white clover DM content ($R^2 = 0.66$; $P < 0.001$; Figure 4.5a) and in BNF ($R^2 = 0.82$; $P < 0.001$; Figure 4.5b) with greater annual fertiliser N input.

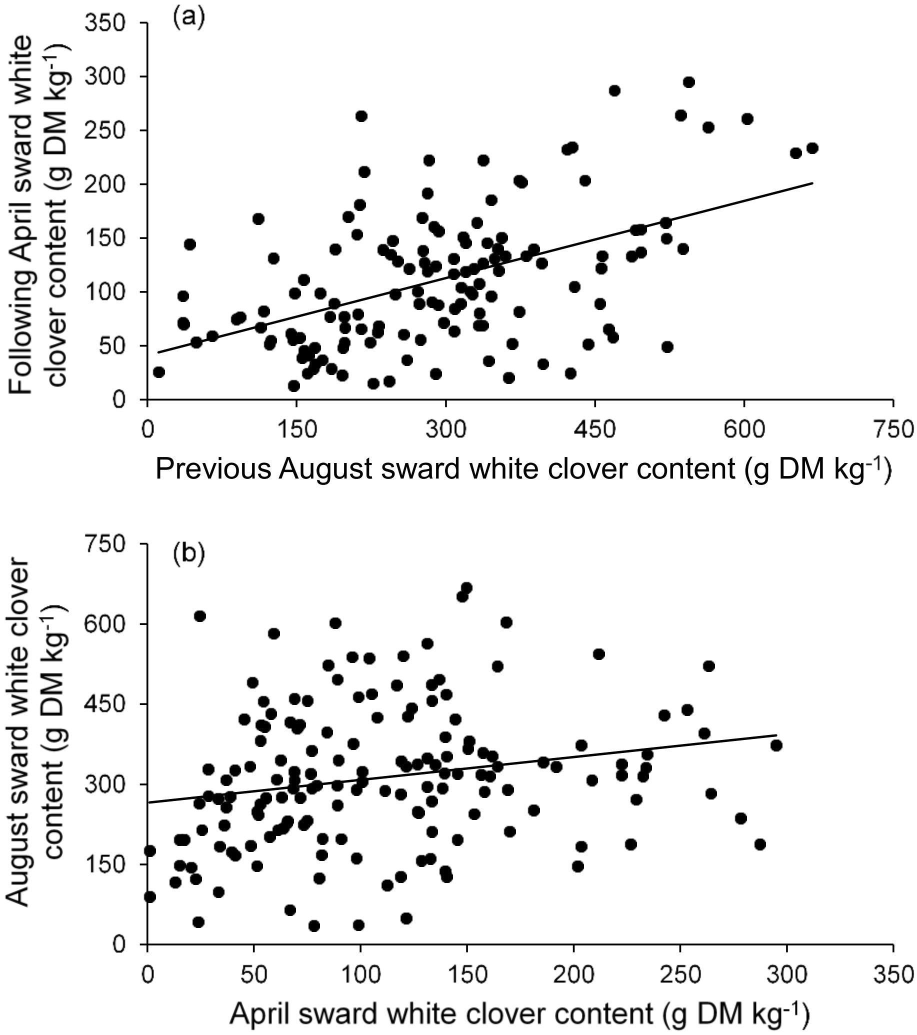

Figure 4.3. Relationship between (a) sward clover DM content in August and the following April (y = 0.2387x + 41.294, $R^2 = 0.25$, $P < 0.001$) and (b) sward clover DM content in April and August of the same year (y = 0.4271x + 265.38, $R^2 = 0.045$, $P > 0.05$) for individual paddocks on Solohead Research Farm; n = 160.

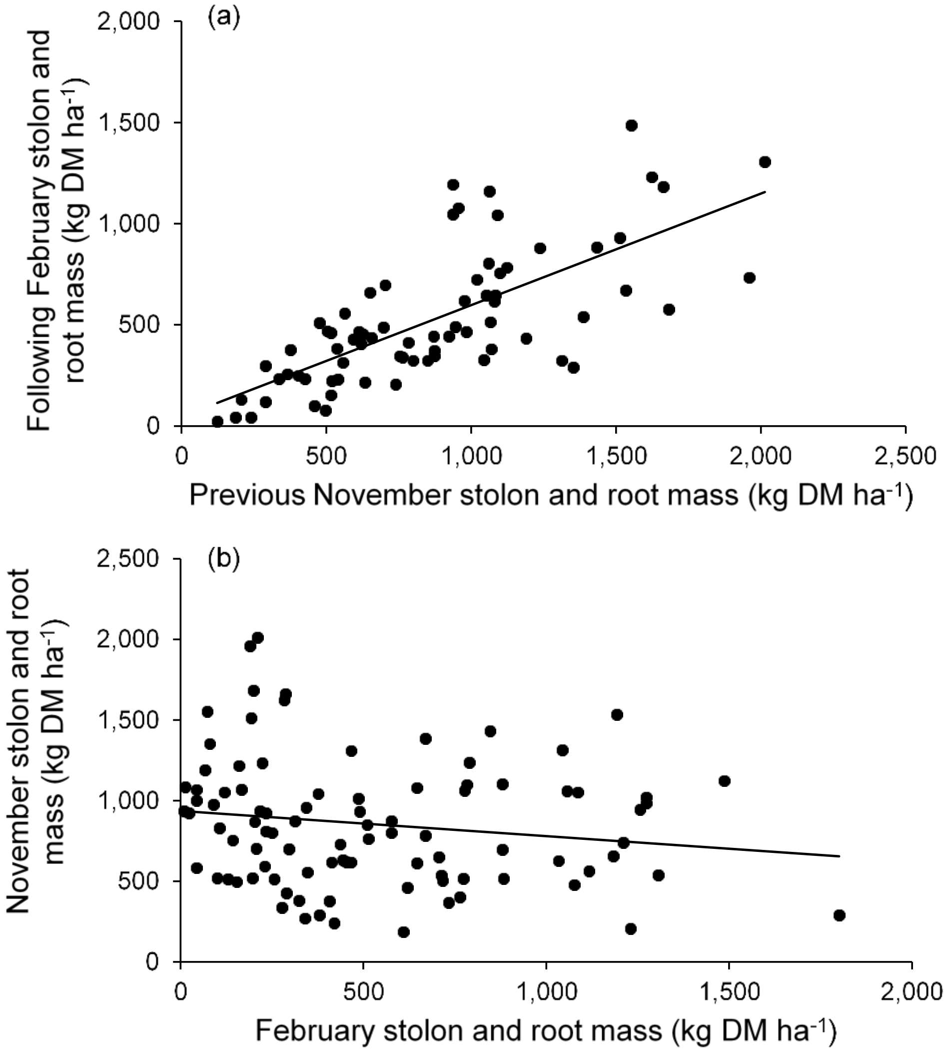

Figure 4.4. Relationship between (a) clover stolon and root DM mass in November and the following February (y = 0.5513x + 45.538, R^2 = 0.51, P < 0.001) and (b) clover stolon and root DM mass in February and November of the same year (y = -0.1571x + 936.97, R^2 = 0.026, P > 0.05) for individual paddocks on Solohead Research Farm; n = 93.

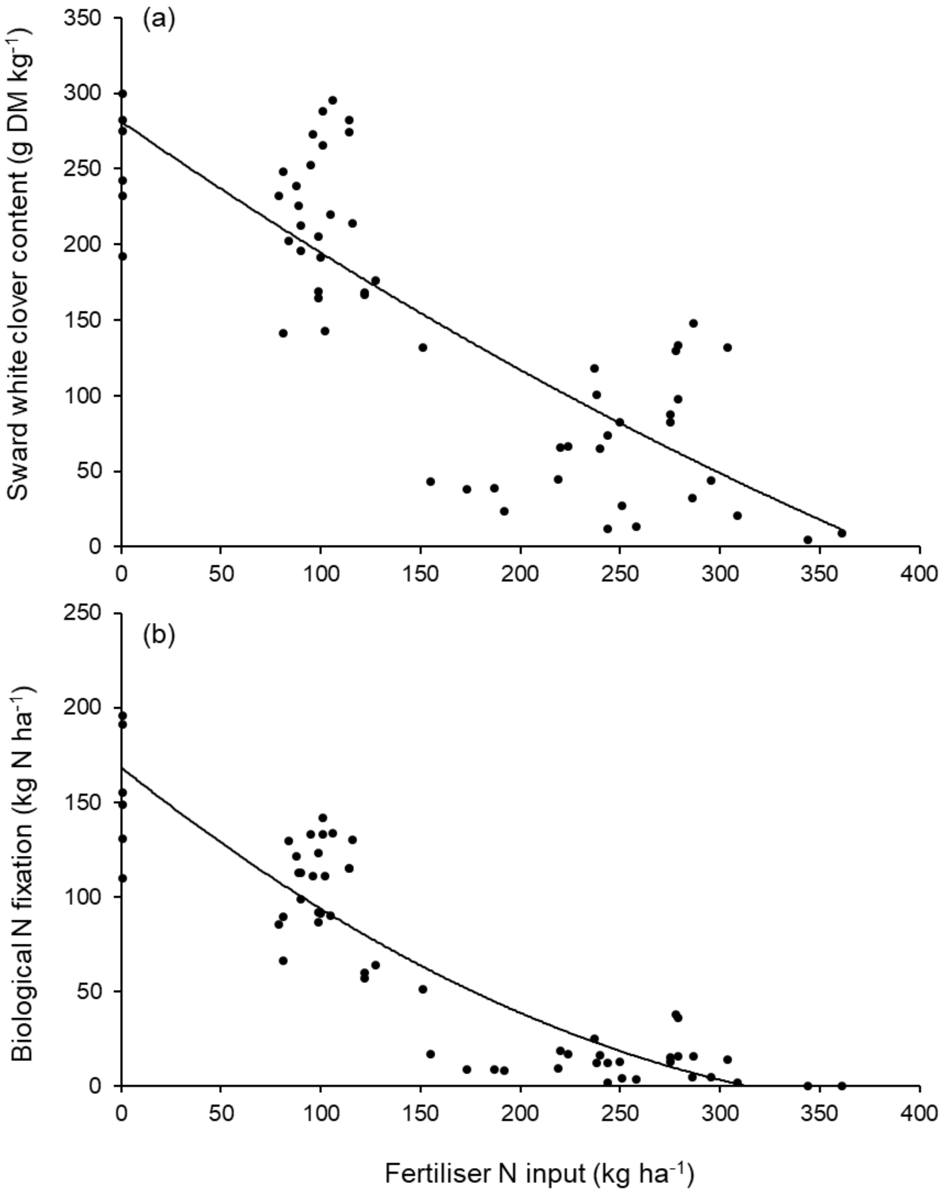

Figure 4.5. The impact of fertiliser N input on annual (a) sward white clover DM content ($y = 0.0005x^2 - 0.9133x + 281.37$, $R^2 = 0.66$, $P < 0.001$) and (b) biological N fixation ($y = 0.001x^2 - 0.8453x + 168.47$, $R^2 = 0.82$, $P < 0.001$) across 59 system-scale studies at Solohead Research Farm.

4.3.4 Milk production, concentrates fed to cows, body weight and body condition score

There was no ($P > 0.05$) difference in annual milk yield (6,320 ± 51.2 kg cow^{-1}), fat (272 ± 1.9 kg cow^{-1}), protein (227 ± 1.7 kg cow^{-1}) and lactose (293 ± 2.7 kg cow^{-1}) yields per cow between GO and GC (Table 4.2). Concentrates fed did not ($P > 0.05$) differ between systems (573 ± 34.4 kg cow^{-1}; Table 4.2). There was no (P > 0.05) difference in dairy cow LW (586 ± 6.1 kg cow^{-1}) or mean BCS (2.95 ± 0.020) during lactation. There was no (P > 0.05) difference for PCC cumulative milk yield (1,566 ± 15.3 kg cow^{-1}) or milk solid yield (123 ± 1.0 kg cow^{-1}) within the PCC timeframe. Likewise, milk composition per cow did not (P > 0.05) differ between grazing systems within the PCC timeframe (Table 4.2).

Table 4.2. Comparison of grass-clover (GC) and N-fertilised grass-only (GO) on the mean annual production of milk, fat, protein and lactose, milk composition, the mean number of days that dairy cows were at pasture, concentrates fed, mean body condition score (BCS; scale 1 to 5 (Edmonson et al., 1989)), dairy cow liveweight (LW) during lactation. Mean cumulative production of milk, fat, protein, lactose and average milk composition for a ten-week period of peak clover content of swards in summer (mean date; 13 July to 21 September), mean sward white clover DM content annually and during August.

Variable[1]	GC	GO	SEM[2]	P-value
<u>Annual</u>				
Milk yield (kg cow^{-1})	6,373	6,266	51.2	NS
Fat (kg cow^{-1})	274	270	1.9	NS
Protein (kg cow^{-1})	227	224	1.7	NS
Lactose (kg cow^{-1})	295	290	4.4	NS
Milk solids yield (kg cow^{-1})	501	494	2.7	NS
Fat (g kg^{-1})	42.8	43.0	0.46	NS
Protein (g kg^{-1})	36.1	36.3	0.44	NS
Lactose (g kg^{-1})	44.3	44.8	0.82	NS
Days at pasture (days cow^{-1})	244	244	3.3	NS
Concentrates (kg cow^{-1})	572	574	34.4	NS
Mean BCS during lactation	3.0	2.9	0.02	NS
Mean LW during lactation (kg cow^{-1})	592	579	6.1	NS
Annual sward white clover (g DM kg^{-1})	190	49	3.7	***
<u>Peak clover content of swards in summer</u>				
Milk yield (kg cow^{-1})	1,573	1,559	15.3	NS
Fat (kg cow^{-1})	65.9	66.0	0.55	NS
Protein (kg cow^{-1})	57.4	57.4	0.77	NS
Lactose (kg cow^{-1})	71.9	71.4	0.50	NS
Milk solids yield (kg cow^{-1})	123.3	123.3	1.0	NS
Fat (g kg^{-1})	42.6	43.1	0.27	NS
Protein (g kg^{-1})	36.9	37.0	0.11	NS
Lactose (g kg^{-1})	45.5	45.7	0.11	NS
August sward white clover (g DM kg^{-1})	269	66	5.0	***

[1] Milk solids yield = kg of milk fat + protein.
[2] Pooled standard error of the mean.
*** P < 0.001; NS, not significant.

4.4 Discussion

4.4.1 Effect of grazing system on pasture DM production and fertiliser N input

The minimum and optimal temperatures for white clover growth are higher than that for perennial ryegrass (Davies, 1992), which can account for the lower growth rates and little to no BNF in winter and early spring (Harris and Clark, 1996; Frame and Newbould, 1986; Davies, 2001). However, the current study found no significant difference in accumulated pasture DM production during the first PP timeframe between the two grassland systems over 11 years. Similarly, Enriquez-Hidalgo et al. (2018) recorded similar pasture production from N-fertiliser grass and grass-clover swards receiving the same N rate in February and April. In contrast, Schils et al. (2000a) found pasture DM production deficit of 0.7 t DM ha^{-1} from January to June timeframe. In the latter study, pasture DM production difference could be attributed to the difference in fertiliser N input during this timeframe with 17 kg N ha^{-1} applied to grass-clover system and 151 kg N ha^{-1} to grass-only system.

In the current study, GC systems received 80 kg ha^{-1} and GO systems received 107 kg ha^{-1} fertiliser N input for first PP timeframe. Laidlaw (1980) and Enriquez-Hidalgo et al. (2016) reported that early spring fertiliser application of < 90 kg N ha^{-1} can increase spring pasture production in grass-clover swards. This strategic N fertilisation in early spring improved pasture growth with reduced reliance on N fertilisation from May onwards as BNF increases with higher soil temperatures and sward white clover content (Laidlaw, 1980; Harris and Clark, 1996). The present study showed substantial differences in fertiliser N inputs between the two systems, particularly between May and mid-September. It was evident that GC paddocks were augmented by BNF during the second and third PP timeframes. Schils et al. (2000a) reported similar findings from July onwards, grass fertiliser-N and grass-clover systems showed similar pasture DM production.

The present study found similar pasture DM production between GC and GO. Similarly, Enriquez-Hidalgo et al. (2016) and Enriquez-Hidalgo et al. (2018) reported that annual pasture production from grass-clover swards receiving fertiliser N input of 60 or 120 kg ha^{-1} was similar to that of grass swards receiving 240 kg ha^{-1}. In contrast, Egan et al. (2018) reported similar annual pasture production between grass-only swards receiving 250 kg N per annum and grass clover swards receiving 250 kg N per annum and also grass clover swards receiving 150 kg N per annum. The 250 kg N ha^{-1} input of fertiliser N on grass-clover swards did not increase pasture DM production and lowered BNF.

4.4.2 The effect of sward composition and dynamics on white clover productivity

Many previous studies have shown that higher annual fertiliser N inputs lowers sward clover DM content and BNF (Harris and Clark, 1996; Ledgard et al., 2001; Burchill et al., 2014; Enriquez-Hidalgo et al., 2016) similar to the results of the present study. Likewise, in a five-year study in Kiel, Germany, Trott et al. (2004) found reductions in sward clover DM content with increasing fertiliser N input under a range of grazing and cutting managements systems. The increasing application of fertiliser N allows perennial ryegrass to take up more N, grow more leaf and tillers and compete with the clover for light (Davies, 2001; Kristensen et al., 2022). As a result, radiation is restricted from reaching the base of the sward, which inhibits branching and leaf emergence of white clover (Frame and Newbould 1986; Black et al., 2009; Phelan et al., 2014).

The current study found that sward clover DM content in April was 24% of that in the previous August. This was within the range previously reported by Woledge et al. (1990). During the winter and spring stolon death and decay exceeds formation of new stolons as larger plants become fragmented into a number of small units (Brock et al., 1988).

Photosynthate is redirected from stolon branches into development of the root system and of leaves. This process explains the loss of stolon and root DM between November and the following February in the present study. Furthermore, the proportion of stolon and root DM mass loss over the winter in the present study was lower than that reported by Hay (1983) and Hay et al. (1987). Guy et al. (2021) reported similar losses of approximately 48% of stolon and root DM mass over the winter.

The over winter period should be taken into careful consideration when planning closing pasture management. Teagasc (2019) recommend a closing APC of 550 to 600 kg DM ha^{-1} for farms stocked at 2.5 cows ha^{-1} in late November on grass-only swards. While it is not possible to clearly identify an optimum closing APC, an APC above 466 kg DM ha^{-1} on approximately 30 November may contribute to greater losses of white clover. High herbage masses carried over 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; Black et al., 2009). Scully et al. (2021) found that ES0N system (with a closing date of 12 November) had lower sward clover contents, clover herbage production and BNF estimates when compared to the LS0N system (with a closing date of 14 January). In contrast, Phelan et al. (2014) found that 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. Further analysis is required to identify optimum autumn management, in terms of autumn closing strategy to support white clover persistency whilst also ensuring sufficient pasture is available for the start of the grazing period in spring.

4.4.3 Effect of grazing system on milk production per cow

The present study found no difference in annual or PCC milk and milk solid yields or milk composition per cow between grazing systems combined over eleven years.

Similarly, Enriquez et al. (2014) did not find an effect on milk productivity per cow for a period in the second part or across the main grazing season. In two short-term indoor feeding trials in New Zealand, Harris et al. (1998) found that the proportion of white clover in the grazed swards needed to be 600 to 700 g DM kg^{-1} for any potential benefit of clover on dry matter intake and milk production during mid and late lactation. Ribeiro Filho et al. (2003) observed a 12% increase in milk yield when average clover content was approximately 420 g kg^{-1}, compared with fertilised grass-only swards. A recent meta-analysis (Dineen et al., 2018) found that a mean annual sward white clover content of 316 g DM kg^{-1} is required to increase daily milk and milk solid yields per cow. In the present study, sward white clover DM content recorded annually (mean 190 g DM kg^{-1}; Table 4.2) or during PCC (mean 269 g DM kg^{-1}; Table 4.2) across GC systems was below the level reported as being necessary for higher milk production per cow. In contrast, Egan et al. (2018) reported increased daily milk and milk solids yields from June to the end of lactation on the grass-clover systems compared to N-fertilised grass with sward clover content of 270 and 230 g DM kg^{-1}, respectively, in July. Moreover, Ribeiro Filho et al. (2005) found that a clover content of 270 g kg^{-1} of herbage DM was not sufficient for increased milk yield. There was no difference in milk protein concentration between grazing systems in the current study, similar to Leach et al. (2000), Enriquez-Hidalgo et al. (2014), Egan et al. (2017) and Egan et al. (2018). Milk fat concentrations were not different between grazing systems similar to Ribeiro Filho et al. (2005) and Enriquez-Hidalgo et al. (2014).

4.5 Conclusions

Annual pasture DM production and milk production per cow was similar for GC systems receiving fertiliser N input of approximately 97 kg N ha^{-1} and GO systems receiving 244 kg N ha^{-1}. Across four PP timeframes, the pasture DM production was similar between the two grassland systems. Sward white clover DM content was greater than that for GO system but had no effect on annual or PCC milk production per cow, cow live weight and body condition score. Increasing annual fertiliser N input lowered BNF and sward white clover DM content. Ultimately, lowering the fertiliser N is required to enhance BNF and sward clover DM content. Stolon and root DM mass in November was positively correlated with clover stolon mass in the following February (R^2 = 0.51). Further investigations into the dynamics of white clover persistency and productivity in pasture-based production systems should focus on identifying the optimum autumn management, in terms of autumn closing strategy.

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Chapter 5. The effect of fertiliser nitrogen input to grass-clover swards and calving date on the productivity of pasture-based dairy production

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Abstract

The objective of this systems-scale study was to investigate grazing season timeframes on pasture and milk production and on milk processability of dairy systems with compact spring calving dairy cows grazing white clover (*Trifolium repens* L.) based grassland. Fifty four primi- and multi-parous Holstein-Friesian dairy cows were used in a one factor study with three systems (n = 18) and repeated over two years (2008/09 and 2009/10). The three systems were: early spring calving with annual fertiliser N input of 100 kg ha^{-1} applied in spring (ES100N; 2.1 cows ha^{-1}; grazing February to November), early spring calving without fertiliser N (ES0N; 1.6 cows ha^{-1}; grazing February to November) and late spring calving without fertiliser N (LS0N; 1.53 cows ha^{-1}; grazing April to January). Annual pasture production was affected by an interaction between grazing system and year: Mean annual pasture yields for 2008 and 2009 were ES100N; 10.35 and 9.88, ES0N; 8.88 and 8.63, LS0N; 9.18 and 10.31 t dry matter (DM) ha^{-1} (SEM 0.39). LS0N had higher pasture DM yield in 2009 due to higher clover DM production and biological N fixation (BNF) compared with the other systems. Clover stolon and root mass in the following February was correlated with stolon and root mass in the previous November with 64% of stolon mass present on LS0N in February (R^2 = 0.84). There were no detectable differences in per-lactation milk yield (6,335 kg cow^{-1}), fat, protein and lactose yields (271, 226, 297 kg cow^{-1}, respectively), cow liveweight (585 kg) or body condition score (BCS; 3.02). Although winter grazing favored subsequent clover DM production, BNF and pasture DM production, delaying calving date in spring and extending lactation into the following winter led to inefficient use of this pasture by the grazing herd and lowered the quality of late lactation milk for processing purposes. Hence, a mean calving date in mid- to late-February is recommended for zero-fertiliser N input clover-based grassland.

5.1 Introduction

The environmental impacts of mineral nitrogen (N) are of global concern and there is growing determination to mitigate its use for agricultural purposes (ECE, 2020). Within the European Union the European Commission (EC) has recently launched the Farm to Fork Strategy as part of the European Green Deal to tackle climate and environmental-related challenges and targets a 20% reduction in fertiliser use by 2030 (EC, 2019). The replacement of synthetic fertiliser N with N fixed by bacteria (biological N fixation; BNF) in association with white clover (*Trifolium repens* L.) offers the potential to lower fertiliser N use, nitrous oxide and ammonia emissions and energy use. This in turn helps reduce the carbon footprint and other environmental impacts of milk production (Li et al., 2011; Yan et al., 2013) while remaining economically competitive (Humphreys et al., 2017). These benefits are largely dependent on maintaining the clover component of the sward at agronomically desirable levels of approximately 300 g kg^{-1} of pasture DM from year to year (Frame and Newbould, 1986).

The optimal temperatures for growth of white clover are higher than perennial ryegrass (*Lolium perenne*) and this makes clover vulnerable to competition by perennial ryegrass particularly during the winter and early spring (Davies, 1992). Previous plot-scale investigations by Laidlaw and Stewart (1987), Laidlaw et al. (1992) and Phelan et al. (2014) showed that carrying high covers of pasture for long periods over the winter and early spring was detrimental to white clover survival and, hence, the subsequent productivity of swards. Furthermore, the application of fertiliser N to grass-clover swards in spring to increase grass production can be detrimental to the white clover component of the sward (Laidlaw et al., 1992; Chapman et al., 2017). Conversely, white clover tends to be prominent in the sward from mid-summer onwards (Frame and Newbould, 1986; Laidlaw et al., 1992; Enriquez-Hidalgo et al., 2018), with high levels of BNF, which provides the potential for higher pasture production to extend the grazing season into the late autumn and winter.

Rates of pasture growth are highly seasonal in temperate latitudes (Hurtado-Uria et al., 2013). The period when pasture growth meets the demand by grazing dairy cows is limited. In Western Europe, pasture deficits due to low growth rates typically occur in late autumn, winter and early spring. Traditional pasture-based dairy systems are designed to match the herd demand for feed to the production of home-grown feed (grazed and ensiled pasture) with minimal purchased concentrate feed. A long grazing season and a short period of winter housing are key elements of low cost pasture-based dairy production (Finneran et al., 2012; Läpple et al., 2012; Hanrahan et al., 2018). Hence, in Ireland, dairy cows are compactly calved with a mean calving date in February and are typically dried off and housed during the winter.

A key component of seasonal pasture-based milk production is matching calving date to the commencement of pasture growth in spring in order to produce as much milk as possible off low-cost grazed pasture during the grazing season. Hence, in the absence of applications of fertiliser N in spring, and consequently lower pasture production in spring, a later calving date might better align pasture supply with the requirement for feed by the spring-calving herd. Under such a management strategy producing milk off pasture can be maintained by extending the grazing season into the following winter. In some instances there is a premium price paid for milk produced during the winter months. Nevertheless, the composition of milk changes during lactation (Lucey, 1996) and milk produced by later calving cows with a later turnout date in spring is likely to reflect lactational and nutritional influences in late lactation (O’Brien et al., 1999). This is likely to have consequences for the yield, composition and quality of milk products (Lucey, 1996; O’Brien et al., 2006; Fox et al., 2017).

The objective of this study was to examine a system of milk production with later than typical mean calving and turnout to pasture dates (mid-April) with the end of lactation in the following January compared with systems with conventional calving and turnout dates (mid-February) with the end of lactation in mid-November. Aspects examined include the productivity of white clover in grassland, BNF, and pasture and milk production as well as indicators of the processability of late lactation milk.

5.2 Materials and Methods

5.2.1 Site characteristics and meteorological data

The study was conducted at Solohead Research Farm in Ireland (52°51´N, 08°21´W; 95 m a.s.l) between February 2008 and February 2010. The soils of the farm are 90% poorly drained gleys and 10% grey-brown podzolics overlaying Devonian sandstone at a depth of 5 to 10 m below ground level. The soil has a clay-loam texture, comprising 36% sand and 28% clay in the A1 horizon. Soil organic matter content was 13%, and soil pH was 6.6. The land has been under permanent grassland for over 50 years and was reseeded with perennial ryegrass (*Lolium perenne*) between 1985 and 1995. Between 2001 and 2006, the grassland sward was oversown with white clover as described by Humphreys et al. (2009b). The botanical composition of the swards in September 2008 (in g kg^{-1} of pasture DM) was predominantly perennial ryegrass (approximately 750 g kg^{-1}) and white clover (approximately 200 g kg^{-1}). Unsown species were primarily dandelion (*Taraxacum officinale*), creeping buttercup (*Ranunculus repens*), daisy (*Bellis perennis*) and ribwort plantain (*Plantago lanceolata*) which in total, accounted for less than 50 g kg^{-1}.Soil temperature (°C at 10 cm depth) and rainfall (mm) were measured every 30 min at an automatic meteorological station on the farm (Campbell Scientific Ltd, Loughborough, U.K.).

5.2.2 Experimental design and grazing systems

Fifty-four (12 primiparous and 42 multiparous) Holstein-Friesian spring-calving dairy cows were used in a one-factor study with 3 grazing systems and 18 cows per system per year (from February to February) and repeated over 2 years. At the end of lactation all cows were housed together and fed together as one group. Each spring all cows were divided into 4 main groups on the basis of lactation number (1, 2, 3, and ≥ 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 herd. Herds were then randomly assigned to the three following systems:

- **ES100N:** Early spring turnout with an annual N fertiliser input of 100 kg ha^{-1}. This grazing system was typical of that recommended for conventional dairy production from cows grazing grass-clover swards in Ireland (Humphreys et al., 2009). The mean calving date was 17 February in each year, and lactating cows were primarily at pasture from 21 February 2008 to 12 November 2008 and from 20 February 2009 to 18 November 2009 (Table 5.1). Overall annual stocking rate was 2.1 cows ha^{-1} (Table 5.1).
- **ES0N:** Early spring turnout without fertiliser-N input. Mean calving date, turnout dates and housing dates were similar to ES100N (Table 5.1) but the overall annual stocking rate was 1.6 cows ha^{-1} as recommended by Culleton and Fox (2001).
- **LS0N:** Late spring turnout without fertiliser-N input. This system had a later mean calving date (12 April) to account for the lower supply of pasture in early spring that occurs in the absence of fertiliser-N applications. Cows were primarily at pasture from 16 April 2008 to 14 January 2009 and from 15 April 2009 to 26 January (Table 5.1). 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 September to January by including

additional land (3.7 ha) in this system. This simulated the housing of calves and heifers off the main grazing block for the winter.

A total of 34 ha in both 2008 and 2009 of permanent grassland was used for the study. The study area was divided into six blocks according to soil type and drainage status in January 2008 with each block containing 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 5.1). Mean paddock sizes were 1.42, 1.88 and 1.76 ha^{-1} (SD = 0.30) in the ES100N, ES0N and LS0N, respectively. However, immediate stocking rates within each system changed over time as surplus pasture was removed for silage production (Table 5.1).

5.2.3 Animal and grazing management

Dairy cows were turned out to pasture three days after calving in spring and remained outside until drying off at the end of lactation in the following winter. Exceptions were made when ground conditions were too wet (soil moisture content > 60%) or when pasture supply was insufficient at pre-grazing pasture masses < 500 kg DM ha^{-1}. Pasture was allocated for a 48 h period to dairy cows under rotational strip-grazing management and each herd was moved to the next pasture area when a post-grazing height (PGH) of 5 cm was reached. The PGH was measured twice per day with 50 measurements of a Filips rising plate meter purchased from www.grasstec.ie. Back-fencing was used to stop cows returning to previously grazed areas. Excess pasture production was identified throughout the study and removed as baled silage. These areas were selected when pre-grazing masses exceeded 1600 kg DM ha^{-1} between April and July and > 2000 kg DM ha^{-1} in August (above PGH of 5 cm). Silage areas were closed off between early-April and late-May (first-cut silage) and between early-June and mid-July (second-cut silage;

Table 5.1). The land area available for grazing in each system at various times of the year and associated stocking rates are outlined in (Table 5.1).

The ES100N system received annual mineral N fertiliser input of 100 kg ha^{-1}. Nitrogen fertiliser was applied as urea between February and April for early grazing and as calcium ammonium nitrate (CAN) during May of each year. Slurry produced during housing was collected in a common store and reapplied to grassland on a proportional basis to stocking rate on each system (see Table 5.1) as described by Humphreys et al. (2008).

5.2.3.1 Concentrates and silage fed

Cows received concentrate feed supplementation (26% barley, 26% maize gluten, 35% beet pulp, and 12% soybean meal) at rates of between 3 and 5 kg cow^{-1} between February and April and 0 and 4 kg cow^{-1} between April to November for cows on ES100N and ES0N. Cows on LS0N received between 4 and 5 kg cow^{-1} from September through to drying off in late January. Rates of supplementation were dependent on pasture availability and nutritive value (see results). When housed, cows were fed grass-clover silage *ad libitum*.

5.2.4 Sward measurements

5.2.4.1 Pasture production and nutritive value

Pasture DM production was measured prior to each grazing event by cutting four random strips (each 5 m x 0.55 m) using a HRH-536 lawn-mower (Honda, Alpharetta, GA, USA) at a cutting height of 5 cm above ground level. Prior to harvesting grass-clover for silage, an Agria auto-scythe (Etesia UK Ltd., Warwick, UK) was used to cut three strips (5.0 m × 1.1 m). All the harvested pasture samples were bulked, weighed and a 100 g sub-sample dried for 16 h in a forced-draft oven at 95°C to determine DM content. Annual pasture yield (kg DM ha^{-1}) was calculated as the sum of pasture removed as pre-grazing and pre-

Table 5.1. Details of the 3 clover-based grazing systems[1] in 2008 and 2009.

Year	2008			2009		
Grazing system	ES100N	ES0N	LS0N	ES100N	ES0N	LS0N
Start of grazing year	Feb. 21	Feb. 19	Apr. 16	Feb. 20	Feb. 22	Apr. 15
End of grazing year	Nov. 12	Nov. 12	Jan. 14	Nov. 18	Nov. 18	Jan. 26
Mean grazing days[2] cow^{-1}	220	234	231	218	234	235
Mean grazing days[3] ha^{-1}	462	374	353	458	374	360
Total land area (ha)	8.5	11.3	10.5(14.2)	8.5	11.3	10.5(14.2)
Overall stocking rate[4] (cows ha^{-1})	2.1	1.6	1.7(1.3)	2.1	1.6	1.7(1.3)
Bi-monthly stocking rates when silage areas were accounted for (cows ha^{-1})						
February and March	2.1	1.6		2.1	1.6	
April and May	4.2	2.3	3.4	3.4	2.0	3.2
June and July	2.1	2.8	2.3	2.4	2.4	3.3
August and September	2.1	1.6	1.7	2.1	1.6	1.7
October and November	2.1	1.6	1.3	2.1	1.6	1.3
December and January			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 (April and May)	0.5	0.3	0.5	0.4	0.2	0.5
2nd cut (June and July)	0.0	0.4	0.2	0.1	0.3	0.5
N-inputs (kg ha^{-1})						
Fertiliser	105	-	-	96	-	-
Slurry	90	65	65	93	69	65
BNF[5]	113	131	110	90	149	191
Total	308	196	175	279	218	256

[1]ES100N = early spring calving with annual fertiliser N input of 100 kg·ha^{-1} applied in spring; ES0N = early spring calving without fertiliser N; LS0N = late spring calving without fertiliser N.
[2]24 hr periods, a value of 0.5 was used when cows were kept indoors at night.
[3]The number of dairy cow grazing days per ha averaged over the entire area of each system.
[4]From September to January each year, additional land was included in the LS0N system (adjusted land area and stocking rate are in parentheses). During the rest of the year this area was used for grazing heifers and calves.
[5]BNF = biological nitrogen fixation.

silage cuts. Growth rates for each pre-grazing and pre-silage cut were calculated by dividing the pasture mass by the regrowth interval. A second 100 g sub-sample of composite pasture was freeze-dried and milled through a 0.2 mm sieve before chemical analyses for ash content (550ºC muffle furnace for 12 h), crude protein (CP; N content × 6.25; Leco N analyser FP-528, Leco Corporation, St. Joseph, MI, USA), and *in vitro* organic matter digestibility (IVOMD) as described by Morgan et al. (1989). Silage grab samples of approximately 100 g were randomly collected (n = 88) prior to feeding to housed animals throughout the study. This was analysed for ash, IVOMD and CP using near infra-red spectroscopy (Model 6500, Foss-NIR System, Hillerød, Denmark). The NIR machine was calibrated on a monthly basis using twelve samples of varying nutritive value determined using laboratory techniques similar to that described above.

5.2.4.2 White clover content in pasture DM, biological nitrogen fixation, stolon and root DM mass

The white clover content of the pasture DM (g kg^{-1}) in each paddock was measured from 30 randomly distributed strips (each 10 cm × 30 cm) of pasture sampled at a cutting height of 5 cm with electric hand shears (Accu-shears Gardena, Ulm, Germany). This measurement was carried out in April, June, August and November of each year. All samples were manually separated into clover or other grassland species, and DM was determined as described for pasture production. Annual grass and clover pasture production in each paddock was calculated from the mean annual grass and clover content of each paddock and its respective annual pasture yield.

Clover stolon and root DM mass was measured in February, March, August and November of each year by cutting 30 random sods (each measuring 10 cm × 10 cm) to a depth of approximately 8 cm in each paddock. Stolons with attached roots were manually separated from the sods, washed and analysed for DM as described above. The annual

BNF was estimated from annual clover pasture yield, sward age and soil type using the model by Høgh-Jensen et al. (2004).

5.2.5 Animal production measurements

5.2.5.1 Milk production, liveweight and body condition score

Cows were milked at 0730 and 1530 h daily throughout lactation in both years of the study. Individual cow milk yield was recorded at each milking. Milk composition from each cow was determined weekly from one successive morning and evening milking sample from each cow using a Milkoscan 203 analyser (Foss Electric, Hillerød, Denmark). The liveweight (LW) of cows were recorded weekly using a weighing scales and the Winweigh software package (Tru-test Limited, Auckland, New Zealand). Body condition score of each cow was recorded fortnightly using the methodology described by Edmonson et al. (1989).

5.2.5.2 Days at pasture and intake estimates

Days at pasture were recorded for each cow with a value of 1.0 ascribed to each 24 hour 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 measured as silage fed to cows when housed. Intake of grazed pasture DM by each cow was estimated as the difference between net energy (NE) provided from silage and concentrates and that needed to meet the NE requirements for milk production, maintenance and pregnancy (Jarrige et al., 1986; Jarrige 1989; O'Mara 1996). Feed costs were based on prices at that time (Teagasc, 2013).

5.2.5.3 Milk processability

During the second year of the study, milk samples were collected fortnightly from each system and analysed for milk processability characteristics. Milk processability was assessed from 1 August 2009 (approximately week 23 of lactation) until 2 December

2009 (end of lactation) for ES100N and ES0N, and from 1 September 2009 (approximately week 23 of lactation) until 23 January 2010 (end of lactation) for LS0N. Pooled morning and evening bulk milk samples from each system were collected, stored overnight at 4ºC and analysed in triplicate on the following day. An aliquot of each pooled milk sample was analysed for fat, protein and lactose concentrations using a Milkoscan 203 analyser (Foss Electric, Hillerød, Denmark). Somatic cell count was measured by laser-based flow cytometry (Somacount 300, Bentley Instruments Inc., Chaska, MN). Milk samples were also analysed for casein concentration, nonprotein nitrogen (NPN) and total protein content. These were measured using the Kjeldahl method [methods 29 (IDF, 1964); 20-4 (ISO/ IDF, 2001) and 20-3 (ISO/ IDF, 2004), respectively] using a Tecator Digestor Auto and Kjeltec 8400 distiller (Foss Electric, Hillerød, Denmark). Casein number was expressed as casein ÷ total protein x 100.

Rennet gelation characteristics were determined using low-amplitude strain oscillation (Advanced Rheometer AR550, T.A Instruments Ltd, Crawley, RH10 9NB, UK). The pH of 100 mL of milk was standardised to 6.55 at room temperature. The temperature of the milk was then brought to 31ºC by immersing the milk sample in a water bath, and if required, the pH was readjusted to 6.55. Rennet (Chymax Plus, Pfizer Inc., Milwaukee, WI, USA), diluted to 1:20 with deionised water, was then added to milk at a level 0.18 mL of undiluted rennet per litre of milk. The sample was subjected to a low-amplitude shear strain of 0.025 at a frequency of 1 Hz and the elastic shear modulus (G′) was measured continuously as a function of time (Guinee et al., 1997). The following variables were calculated from the resultant G′-time profiles: gelation time (GT), defined as the time in seconds for G′ to reach a value ≥ 0.2 Pa; maximum curd firming rate (CFR_{max}), defined as the maximum slope of the G′-time curve; and gel strength (GS) defined as the G′ value at 50 min from rennet addition.

5.2.6 Statistical analyses

ANOVA was conducted with MIXED procedure in SAS 9.3 (SAS, 2011) 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} = mean dependent variable, μ = 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 sampling date and e_{jkl} = residual error term. Year and sampling date were entered as 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 and animal measurements that were calculated on a herd basis for each system. Grass and clover measurements were analysed separately using the model. The relationships between November clover stolon and root DM mass and February clover stolon and root DM mass were analysed for effect of the system with simple linear regression using the GLM procedure in SAS.

Data was analysed with a repeated measures linear model fitted using the MIXED procedure in SAS 9.3. Responses analysed were GT, CFR_{max}, GS, total protein, casein, NPN, casein number, fat, protein, lactose and SCC. Mean values for main effects or interaction, as appropriate, were compared with a Tukey adjustment for multiplicity effects. Residual checks were made to ensure that the assumptions of the analyses were met.

5.3 Results

5.3.1 Meteorological data

Mean daily soil temperature and monthly rainfall amounts for the study period and the previous ten-year means are shown in Figure 5.1. Mean soil temperature was 9.5ºC in 2008 and 9.6ºC in 2009, whereas the previous ten-year mean was 10.9ºC (range: 10.0 to 11.8ºC; Figure 5.1a). Total rainfall was 1,228 mm in 2008 and 1,296 mm in 2009, both of which substantially exceeded the previous ten-year annual average of 1,004 mm (range 797 to 1,150 mm; Figure 5.1b). Monthly rainfall was higher particularly in the summer months of both years. Nevertheless, some months, such as February and December in both years had lower rainfall than the previous ten-year means (Figure 5.1b).

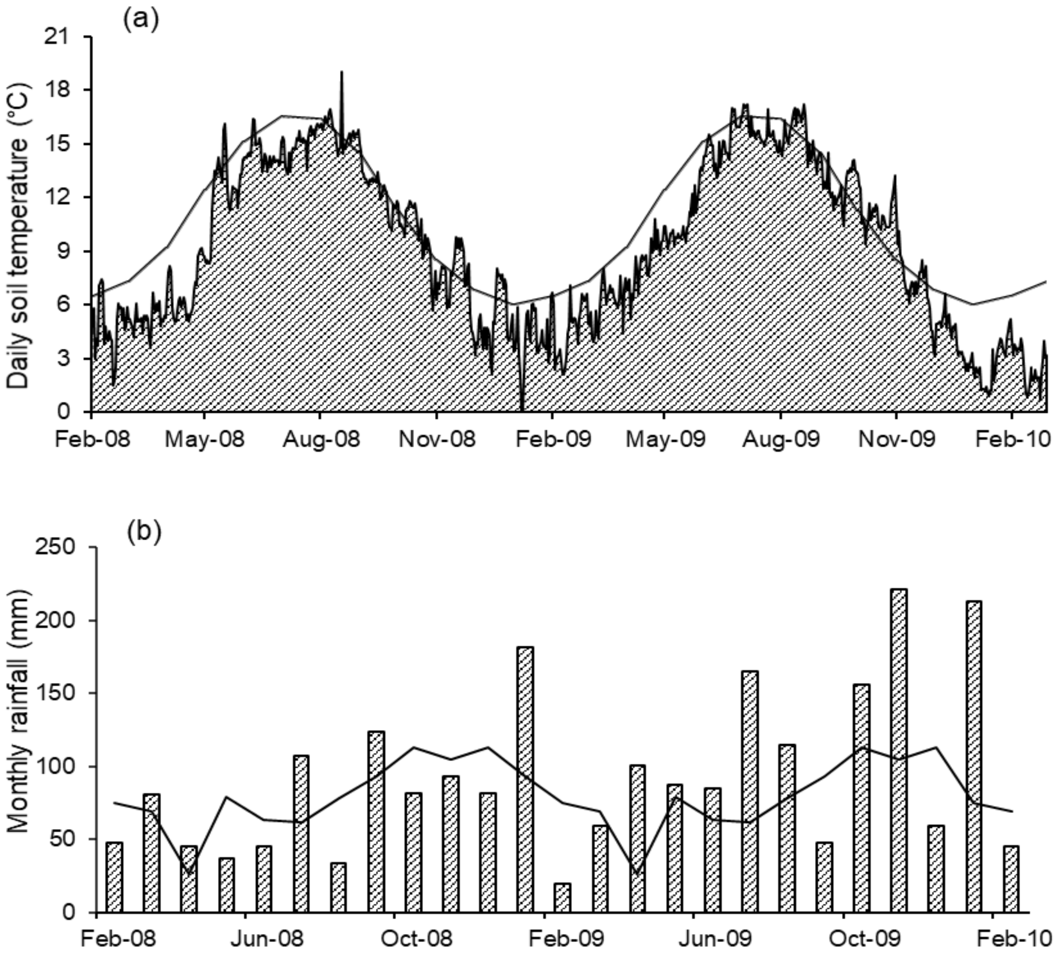

Figure 5.1. Mean daily soil temperature (a) and monthly rainfall (b) recorded at the meteorological station at Solohead Research Farm between February 2008 and February 2010. The shaded areas show the recorded values during the study period and the solid lines show the previous ten-year mean values (1998 to 2007).

5.3.2 Pasture production and nutritive value

Averaged over both years annual pasture DM production on ES100N was similar to LS0N and was higher ($P < 0.05$) on both of these systems than ES0N (Table 5.2). Grass DM production was higher ($P < 0.001$) on ES100N than on the other systems in both years. Clover DM production was affected by an interaction between grazing system and year.

There was no difference in clover DM production between systems in 2008. Clover DM production was higher in 2009 than 2008 on the ES0N and LS0N and significantly so ($P < 0.05$) on LS0N. In 2009 LS0N had higher ($P < 0.05$) clover DM production than ES100N and was not different from ES0N. Mean post-grazing sward height was not affected by grazing system (mean = 4.97 cm, SEM = 0.042, $P > 0.05$). There were no ($P > 0.05$) differences between systems in mean IVOMD (Figure 5.2a), CP (Figure 5.2b) and ash content, which averaged 102 g kg^{-1} (SEM ±8.23) for each system averaged over both years. There were no significant differences in composition of silage fed to cows during this study. The mean composition (±SD) of silage DM was 78 g kg^{-1} ash (±13.3), 784 g kg^{-1} OMD (±6.3), and 122 g kg^{-1} CP (±18.9).

Table 5.2. The effect of grazing system[1] on pasture DM production and annual estimated biological nitrogen fixation (BNF, kg ha^{-1}).

Grazing system	ES100N	ES0N	LS0N
2008		Pasture DM production (t ha^{-1})	
Grass	8.22	6.41	7.11
Clover	2.13	2.47	2.07
Total	10.35	8.88	9.18
2009			
Grass	8.18	5.83	6.73
Clover	1.69	2.80	3.58
Total	9.88	8.63	10.31
		BNF (kg ha^{-1})	
2008	113	131	110
2009	90	149	191

	S.E. of the means		
	System	Year	System x Year
Grass	0.293***	0.239	0.414
Clover	0.262*	0.137	0.373*
Total pasture	0.389*	0.319	0.553
BNF	16.6*	10.8*	18.8**

[1]ES100N = early spring calving with annual fertiliser N input of 100 kg·ha^{-1} applied in spring; ES0N = early spring calving without fertiliser N; LS0N = late spring calving without fertiliser N.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

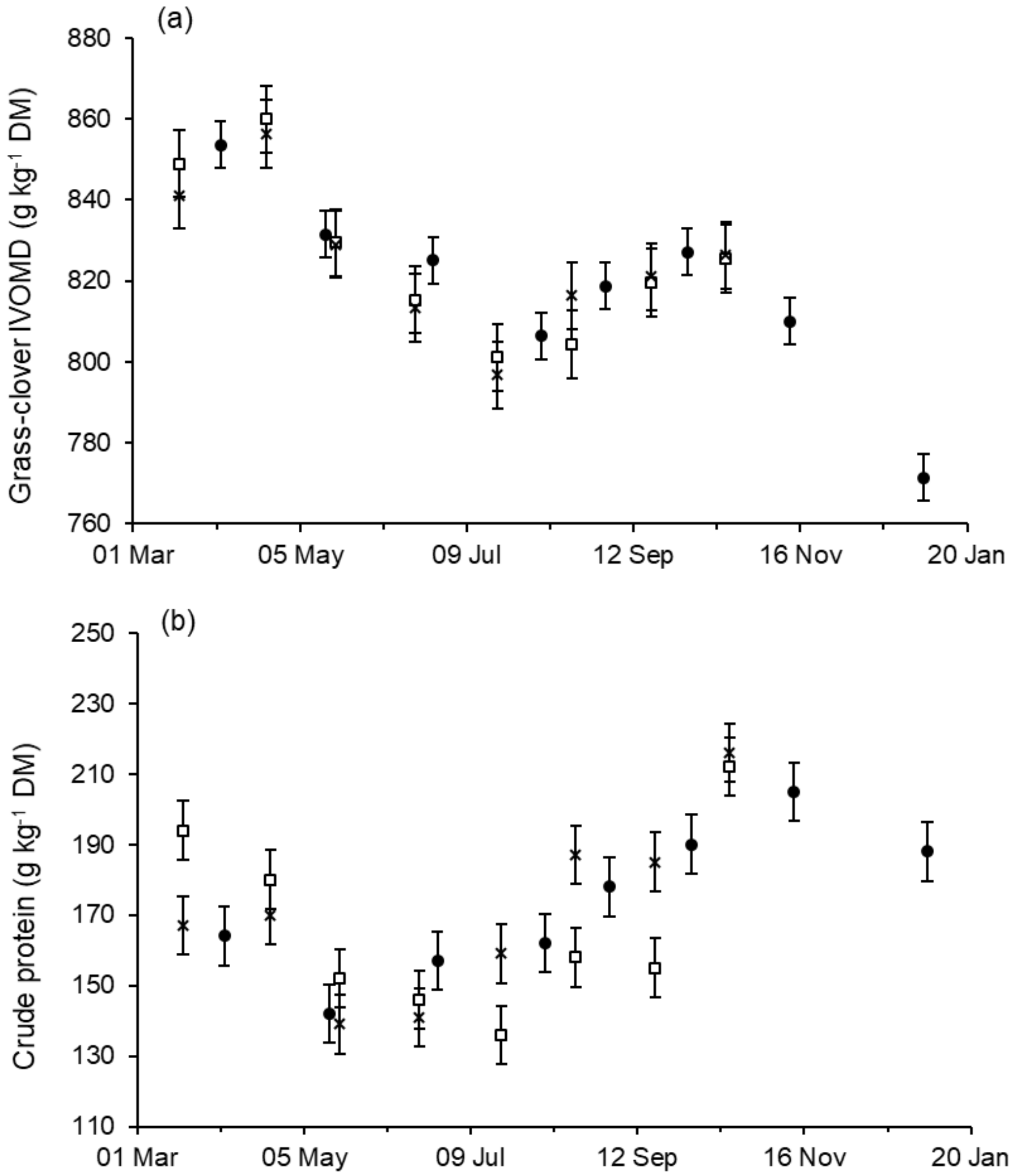

Figure 5.2. Effect of pasture nutritive value of grass-clover in vitro organic matter digestibility (IVOMD) (a) and crude protein (b) for each system, ES100N (□), ES0N (x) and LS0N (●). Error bars show the SEM for the interaction between grazing rotation and system, $P > 0.05$. ES100N = early spring calving with annual fertiliser N input of 100 kg·ha^{-1} applied in spring; ES0N = early spring calving without fertiliser N; LS0N = late spring calving without fertiliser N.

5.3.3 Clover content of pasture DM, biological nitrogen fixation, stolon and root DM mass

Clover content of pasture DM was affected by an interaction between grazing system, month and year ($P < 0.001$; Table 5.3). ES0N and LS0N had similar clover contents throughout 2008 whereas LS0N had higher clover content in August 2009 ($P < 0.01$;

Table 5.3). In 2008, ES100N had lower clover content than the other two systems in June, while in 2009 ES100N had lower clover contents in both June and August (P < 0.01; Table 5.3). Biological N fixation was affected by an interaction between grazing system and year (P < 0.01; Table 5.2). The mean BNF was significantly lower in ES100N than the other systems in 2009 (P < 0.05; Table 5.2). Clover stolon and root DM mass were affected by an interaction between grazing system and year, being similar across systems in 2008 but significantly lower in ES100N in 2009 (P < 0.01; Table 5.3). There were positive correlations between stolon and root DM mass in November and stolon and root DM mass in the following February in both years (Figure 5.3). Stolon and root DM mass in February was 64% of that in the previous November on LS0N (R^2 = 0.84, P < 0.01) and approximately 50% on ES0N (R^2 = 0.34, P < 0.05) and ES100N (R^2 = 0.59, P < 0.01).

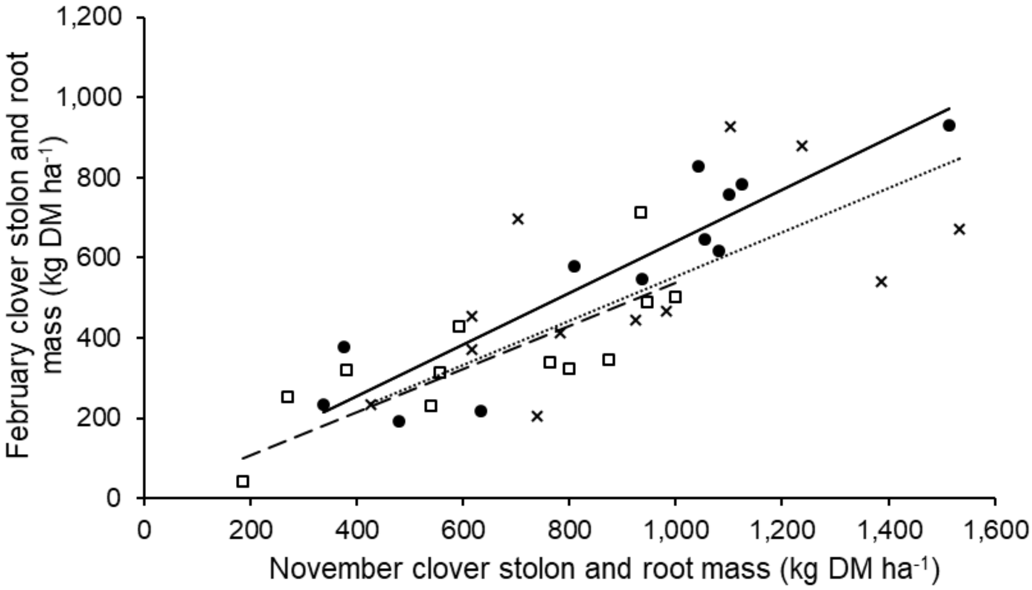

Figure 5.3. Relationship between clover stolon and root DM mass in November and the following February for three grazing systems, ES100N (□, long-dash fitted line: y = 0.538x, R^2 = 0.59, P = 0.002), ES0N (x, dotted fitted line: y = 0.553x, R^2 = 0.34, P = 0.03) and LS0N (●, solid fitted line: y = 0.641x, R^2 = 0.84, P = 0.004). Data is from both years of the study (2008/09 and 2009/10). ES100N = early spring calving with annual fertiliser N input of 100 kg·ha^{-1} applied in spring; ES0N = early spring calving without fertiliser N; LS0N = late spring calving without fertiliser N.

Table 5.3. The effect of grazing system[1] on clover content of pasture DM production and mass of clover stolon and root.

Grazing system		ES100N	ES0N	LS0N
		Clover content of pasture 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	58	62	119
	June	165	322	294
	August	321	421	557
	November	112	161	156
	Mean	164	242	282
		Clover stolon and root DM (kg 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	339	527	608
	March	344	710	797
	August	581	807	1,066
	November	610	885	1,052
	Mean	469	732	881
2010	February	400	572	709

S.E. of the means

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

[1]ES100N = early spring calving with annual fertiliser N input of 100 kg·ha^{-1} applied in spring; ES0N = early spring calving without fertiliser N; LS0N = late spring calving without fertiliser N.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

5.3.4 Days at pasture, feed intake per cow, silage surpluses and deficits

The number of days at pasture per cow for ES100N was ($P < 0.001$) less than ES0N and LS0N (219 versus 234 and 233 days, respectively; Table 5.1). On the other hand the number of grazing days per ha was higher with higher stocking rate (Table 5.1). Cows on LS0N system consumed a mean additional 136 kg cow^{-1} of concentrates compared with ES100N and ES0N ($P < 0.001$). Intake of grazed pasture per cow was higher on ES0N and silage DM intake was correspondingly lower (Table 5.4). Combined over two years ES100N had a silage DM deficit of 0.44 kg cow^{-1}, whereas ES0N and LS0N had silage surpluses of 0.09 and 1.03 kg DM cow^{-1}, respectively.

Table 5.4. Effect of grazing system[1] on silage dry matter (DM) ensiled, annual budgets of feed intake per cow with the end-of year surpluses or deficits (negative values) per cow, purchases (negative values) and sales (positive values) of concentrate DM and silage DM per cow and per ha over 2 years.

Year		2008			2009	
Grazing system	ES100N	ES0N	LS0N	ES100N	ES0N	LS0N
Silage DM ensiled[2]	0.83	0.96	1.41	0.71	1.15	2.12
Intake per cow[3]:			(t cow^{-1})			
Grazed pasture DM intake	3.23	3.41	3.12	3.13	3.33	3.15
Concentrate DM intake	0.50	0.50	0.72	0.65	0.58	0.62
Silage DM intake	1.46	1.32	1.35	1.47	1.31	1.30
Silage DM surplus or deficit[4]	-0.36	-0.04	0.54	-0.53	0.23	1.52
Purchases/sales of feed per cow			(€ cow^{-1})			
Concentrate DM	-165	-165	-237	-213	-190	-206
Silage DM	-46	-5	70	-69	29	198
Purchases/sales of feed per ha			(€ ha^{-1})			
Concentrate DM	-347	-264	-363	-448	-304	-315
Silage DM	-97	-8	107	-144	47	302

[1]ES100N = early spring calving with annual fertiliser N input of 100 kg·ha^{-1} applied in spring; ES0N = early spring calving without fertiliser N; LS0N = late spring calving without fertiliser N.
[2]Proportional loss during ensilage assumed to be 0.25 (McGechan, 1989, 1990).
[3]Daily herbage intake at pasture, estimated from energy balances.
[4]Silage DM ensiled minus silage intake.

5.3.5 Milk production, liveweight, body condition score and milk processability

There was no (P > 0.05) difference in per-lactation milk yield (6,335 ± 119.8 kg cow^{-1}), fat (271 ± 6.0 kg cow^{-1}), protein (226 ± 4.0 kg cow^{-1}) and lactose (297 ± 5.9 kg cow^{-1}) yields per cow between the three grazing systems. There was no (P > 0.05) difference in dairy cow LW during (585 ± 6.7 kg) or at the end of lactation (585 ± 6.7 kg). Likewise for BCS; during (3.02 ± 0.029) or at the end of lactation (2.98 ± 0.044). Somatic cell counts in late lactation were not influenced by the grazing system (P > 0.05; Table 5.5). LS0N system had lower (P < 0.001) concentrations of fat, protein, casein and total protein between week 23 and the end of lactation compared with ES100N and ES0N (Table 5.5). There were no significant differences in lactose, casein number and NPN of late lactation milk between the systems. Rheological measurements indicated that CFR_{max} and GT were (P < 0.001) shorter for milk produced on LS0N compared with ES100N and ES0N (Table 5.5). Gel strength at 50 min was (P < 0.001) greater for ES100N and ES0N than LS0N (Table 5.5).

Table 5.5. Milk composition and processing characteristics of milk from spring calved cows in late lactation 2009/10 (week 23 to end of lactation)[1].

Item[2]	System				*P*-value
	ES100N	ES0N	LS0N	SEM[3]	System
Fat (g kg^{-1})	49.3	47.8	46.2	0.23	***
Protein (g kg^{-1})	38.2	38.6	36.7	0.31	***
Lactose (g kg^{-1})	45.1	45.0	44.7	0.12	NS
Casein (g kg^{-1})	29.1	29.4	28.6	0.09	***
Total Protein (g kg^{-1})	37.8	38.2	36.8	1.20	***
Casein number[3] (g kg^{-1})	77.9	77.2	76.7	0.66	NS
NPN (g kg^{-1})	0.26	0.27	0.28	0.30	NS
SCC	203	195	198	7.49	NS
GT (s)	1,243	1,141	1,106	3.91	***
CFR_{max} (Pa min^{-1})	3.41	3.50	3.22	0.005	***
GS (Pa)	79.5	78.6	71.0	0.18	***

[1]ES100N = early spring calving with annual fertiliser N input of 100 kg·ha^{-1} applied in spring; ES0N = early spring calving without fertiliser N; LS0N = late spring calving without fertiliser N. SCC = Somatic cell count values reported are cells/mL ÷ 1,000; NPN = nonprotein nitrogen; GT = gelation time; CFR_{max} = maximum curd firming rate; GS = gel strength.
[2]Casein number = (casein ÷ total protein) x 100.
[3]Pooled standard error of the mean.
*** P < 0.001; NS, not significant.

5.4 Discussion

5.4.1 Pasture and milk production

In previous studies at this site, the average number of grazing days per year was 255 per cow (Humphreys et al., 2008, 2009). Likewise, with similar input of fertiliser N to grass-clover swards and using the same methods of measuring pasture DM production, Humphreys et al. (2009) recorded a mean annual pasture DM production of 11.5 t ha^{-1} averaged over four years between 2003 and 2006. Hence the lengths of the grazing season and annual yields of pasture on ES100N (10.35 and 9.88 t ha^{-1}) in the present study were lower than in earlier similar studies at this site. Lower pasture production can be attributed to annual average soil temperatures in both years that were > 1.0°C below average and annual volumes of rainfall that were > 20% above average on this site with impeded drainage that is prone to waterlogging; excess rainfall lowered pasture production and caused inferior grazing conditions compared with the earlier studies. Furthermore, the stocking rate imposed on ES100N was based on previous research at this site (Humphreys et al., 2009) and during the present study required the purchase of an additional 0.45 t silage DM per cow (Table 5.4). Nevertheless, taking into account the adverse weather conditions during the present study, the stocking rate and feed demand on ES100N was reasonably well aligned with the pasture production potential at this site.

The two zero-fertiliser N input systems had lower milk output per ha than ES100N. However, milk output per ha was constrained by the stocking rates of dairy cows imposed on systems in this study. In contrast to ES100N there was a surplus of silage generated on both zero-fertiliser N systems averaged over both years, which indicates that stocking rates on both of these systems were sub-optimal to fully utilise available pasture, particularly LS0N. Averaged over both years annual pasture DM production on ES100N was similar to LS0N and certainly in 2009 LS0N produced sufficient pasture to carry a similar stocking rate of dairy cows to ES100N.

5.4.2 Milk production per cow

There were no significant differences in annual milk production and milk composition per cow between the three grazing systems in the current study. Similarly, Glassey et al. (2013) reported no difference in annual milk production per cow by cows grazing grass-clover swards receiving and not receiving input of fertiliser N. The IVOMD and CP of grazed swards did not differ between the systems, which partly accounts for the absence of a difference in dairy cow performance in the present study. A recent meta-analysis (Dineen et al., 2018) found that a mean sward white clover content of approximately 320 g kg^{-1} is required to increase in daily milk and milk solid yields per cow. The annual white clover content (range 164 to 282 g kg^{-1}; Table 5.3) across all systems in the present study was below the level required for higher milk production per cow.

5.4.3 Clover production and biological nitrogen fixation

In the former study (Humphreys et al., 2009) clover content of pasture DM averaged 219 g kg^{-1} (range: 203 to 239 g kg^{-1} DM) and annual clover DM production averaged 2.52 t ha^{-1} (range 2.10 to 2.85 t ha^{-1}) averaged over the four years in a system with similar stocking rates and fertiliser N input to ES100N. The annual clover content of pasture DM on ES100N in the present study (164 and 203 g kg^{-1}; Table 5.3) were lower than the former study. Likewise the annual yields of clover pasture DM in the present study (range: 1.69 to 2.13 t ha^{-1}; Table 5.2) were lower than the previous study, partly due to lower clover content of pasture DM and partly due to lower annual pasture DM production. It seems that the adverse weather conditions experienced during the present study lowered the clover DM production proportionally more than overall pasture DM production on ES100N.

The absence of fertiliser N input increased clover DM production on ES0N from 2.47 t ha^{-1} in 2008 to 2.80 t ha^{-1} in 2009 (Table 5.2). Likewise clover DM production on LS0N increased from 2.07 t ha^{-1} in 2008 to 3.58 t ha^{-1} in 2009. Early spring has previously been identified as the optimal time to apply fertiliser N to grass-clover swards (Laidlaw, 1980; Harris and Clark, 1996; Humphreys et al., 2008). Little or no BNF takes place and clover growth rates are low at this time of year (Davies, 2001). Fertiliser N applied to ES100N and higher recycling of N in excreta increased spring and annual pasture production compared with ES0N. Higher grass production following the application of fertiliser N was associated with lower clover DM production on ES100N in 2009, which can be attributed to greater competition from the grass component of the sward. It is well established that there is a negative relationship between N fertilisation and the clover content of swards and hence, BNF (Laidlaw et al., 1992; Harris and Clark, 1996; Burchill et al., 2014a). On the other hand grazing during the first half of the winter (December 2008 and January 2009) combined with no fertiliser N input substantially increased clover DM production on LS0N in 2009 compared with ES0N. The higher pasture production on LS0N in 2009 can be attributed to higher annual clover DM production along with higher BNF.

It is apparent that the higher clover DM production and BNF on LS0N in 2009 was influenced by a carry-over effect from the previous year. The mass of stolon surviving over the winter is an important indicator of clover persistence (Frame and Newbould, 1986; Collins et al., 1991; Wachendorf et al., 2001). LS0N had higher stolon masses than the other systems in February 2009 and February 2010. There was a net loss of stolon mass of 22% between November 2008 and February 2009 on LS0N compared with 55% on the two early spring grazing systems, which had an earlier closing date (Table 5.1). As a consequence of this the LS0N had higher stolon mass throughout 2009. The net loss of stolon over the winter 2009/10 was similar in the three systems (approximately 34%). Nevertheless the LS0N had higher stolon mass than the other systems at the end of the study in February 2010 as a consequence of having higher stolon mass during the 2009 growing season.

It seems that grazing during the winter by the cows on LS0N favored clover stolon survival, perhaps by allowing greater light penetration to the base of the sward during the winter (Phelan et al., 2014). A similar result was found in Northern Ireland by Laidlaw and Stewart (1987) and Laidlaw et al. (1992) who found that grazing by sheep over the winter improved clover content of pasture DM in the following spring relative to no grazing between November and March. The beneficial effect on clover was attributed to lower competition from the accompanying grass. On the other hand, in Switzerland Lüscher et al. (2001) found that frequent defoliation (7 times between November and March) caused a net loss in clover stolon DM mass by the spring. The differences in responses in the latter study and those conducted on the island of Ireland could be attributed to climatic differences between Ireland (relatively mild weather during the winter) and Switzerland and perhaps to the much greater severity of the defoliation treatments in the latter study.

5.4.4 Milk constituents and processing characteristics

The measurements of the processability of late lactation milk indicated that there were no detectable differences in lactose, casein number or NPN between the three systems. Somatic cell count was not influenced by grazing systems; mean SCC values were within the range of normality ($< 200 \times 10^3$ cell mL^{-1}) previously reported by O'Brien et al. (2006) and Guinee and O'Brien (2010). The decrease in milk volume with the later stages of lactation coincided with increases in total protein and fat levels, and a reduction in that of lactose similar to that reported by Guinee et al. (2007). However, the late lactation milk of the LS0N system had lower concentrations of fat, protein, casein and total protein relative to ES100N and ES0N. While LS0N had lowest mean concentrations of milk composition and nitrogen fractions, these results were still within the range of values reported previously for spring-calved herds (Mehra et al., 1999; O'Brien et al., 1999).

The current study found that LS0N had shorter coagulation times and weaker gel structures. The low firmness of LS0N gels made from late lactation milk may be due to proteolysis of casein by plasmin (Lucey, 1996). The higher casein content of milk is expected to enhance coagulation properties with higher cheese yields (Guinee et al., 2006). In contrast to previous studies, the current study found that gelation time was longer for ES100N and ES0N milk (Guinee et al., 1997; Auldist et al., 2010). However, the higher protein content in the late lactation milk from the latter systems formed a firmer gel than LS0N, which was influenced by higher concentrations of protein in the milk (Guinee et al., 1997). Guinee et al. (2006) found that during cheesemaking where curds are cut based on time, then an increase in milk protein content is likely to coincide with an increase in the moisture content of the curd, because the stiffer gel at cutting has a lower rate of syneresis. Consequently, both ES100N and ES0N potentially would have higher cheese yield. Cheddar cheese yield increases by approximately 0.25 to 0.30 kg/100 kg of milk for every 0.1 g/100 g increase in milk protein in the range 3.0 to 4.5 g/100 g, while retaining the protein to fat ratio constant at 0.96 (Guinee et al., 2006).

5.4.5 Calving date and grazing season timeframe

A key component of seasonal pasture-based milk production is achieving good synchronicity between calving date and the demand of the lactating herd for pasture and the commencement of pasture growth in spring. Whereas grazing during the winter in LS0N promoted BNF and pasture production in the following year in the present study, the later calving strategy resulted in less pasture consumed per cow and higher supplementation with concentrates compared with ES0N (Table 5.4). These concentrates were mostly fed during the winter. Furthermore, on LS0N there was an excessive supply of pasture available when lactating cows were turned out to pasture in mid-April making it necessary to close some of the area available for grazing for silage in April (Table 5.1). Hence, there was poor synchronicity between the herd demand for pasture and pasture availability resulting in a less efficient and higher cost system. Furthermore, extending lactation into the early winter lowered the quality of late lactation milk for processing purposes.

In terms of producing low-cost milk for processing into long shelf-life products, the ES0N was more efficient than LS0N. Under such circumstances it is advisable that calving date is not changed from mid-February, even under zero-fertiliser N input. It’s possible that the beneficial effects of winter grazing on subsequent clover productivity could be achieved on an ES0N-type system by grazing later and to a lower post-grazing height before housing for the winter rather than delaying calving date. Such an approach warrants further investigation. The economic feasibility of the LS0N approach depends on the purpose for which the milk is used; such as liquid milk and short shelf-life products, and any additional price paid to promote the production of this milk during the winter months.

5.5 Conclusions

The zero-fertiliser N input systems had lower milk production per ha than ES100N because milk output was constrained by stocking rate. Pasture production on LS0N in 2009 had the potential to carry a similar stocking rate of dairy cows as ES100N. Delaying calving date until mid-April on LS0N resulted in inefficient use of pasture for milk production. Extending lactation into early winter lowered the quality of late lactation milk for processing purposes. It is recommended that a mean calving date in mid-February under zero-fertiliser N input is a better option than a later calving date. It's possible that the beneficial effects of winter grazing on clover productivity could be achieved by grazing later and to a lower post-grazing height before housing for the winter. Such an approach could carry a higher stocking rate of dairy cows under zero-fertiliser N input; perhaps in the region of 2.0 to 2.5 cows ha^{-1}.

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Chapter 6. Discussion, Implications and Future Work

6.1 Overall Discussion

6.1.1 Soil nitrogen supply

Chapter 3 quantified the background N release of SOM under 14 Irish permanent grassland sites. Annual background N ranged from 67 kg ha^{-1} to 218 kg ha^{-1} with a mean of 141 kg ha^{-1}. Similar observations have been reported elsewhere (Hopkins et al., 1990; Gill et al., 1995; Hassink, 1995a). The variation in annual background N across sites was accounted for by a stepwise regression model; Annual background N (kg N ha^{-1}) = -735.6 + 42.6 (soil pH) + 55.9 (air temperature) + 1.4 (A1 depth) ($R^2 = 0.51$; $P < 0.001$). When data from Chapter 3 and 4 from Solohead Research Farm are combined, the supply of non-fertiliser sources and fertiliser N input can be compared for an inefficient and efficient grazing system scenario (Table 6.1).

Table 6.1. Comparison of total soil N supply and cost of fertiliser N for an inefficient and efficient grazing system scenario.

	Inefficient	Efficient
Soil pH	5.5	6.5
Slurry management	After silage and last grazing	Jan/Feb and March silage
White clover DM content (g DM kg^{-1})	0	220
Background N (kg ha^{-1})	97	140
Available N slurry (kg ha^{-1})	5	35
N fixed by clover (kg ha^{-1})	0	120
Supply of non-fertiliser N (kg ha^{-1})	102	295
Fertiliser N requirement (kg ha^{-1})	293	100
Total soil N supply (kg ha^{-1})	395	395
Cost of fertiliser N (€ ha^{-1})	293	100
Cost on 50 ha farm (€)[1]	14,650	5,000

Chapter 3 found that a unit difference in soil pH can increase the background N by 42.6 kg ha^{-1}. The maintenance of optimum soil pH (≥ 6.3) for grassland is essential for optimal soil biological activity, especially the mineralisation of SOM into plant available nutrients (Jansson and Persson, 1982). Moreover, the BNF process of rhizobia bacteria can produce excess H^+ in the soil solution (Crush, 1987). Hence, lime application to ameliorate soil acidity is required to increase soil populations of rhizobia and enhance root nodulation, stimulate nodule nitrogenase activity and manage acidification of soil in grasslands (Brock et al., 1989; Hayes et al., 2019).

The management and timing of slurry application has an impact for utilisation efficiency of nutrients and minimising ammonia emissions (Maris et al., 2021). The most efficient use of nutrients in slurry is early in the growing season in cool, damp condition. Warm, dry conditions increase NH_3 volatilisation. Therefore, application of slurry in early spring (late January to March) has clear advantages according to weekly background N supply in Chapter 3. Biological N fixation from white clover supplies another soil N source. At Solohead Research Farm mean sward white clover DM content was 220 g kg^{-1} with a mean BNF rate of 120 kg N ha^{-1} (Chapter 4). This soil N source is prominent from May onwards as observed in GC systems described in Chapter 4. According to Chapter 3, the lowest proportion of background N supply to the N requirement of the 350N treatment was found in May, June, July and August. Biological N fixation has the potential to meet this N deficit.

Using the difference method the relative contributions of different sources to total N supply can be calculated. The supply of non-fertiliser N accounted for 0.75 of the total N supply for an efficient system compared to 0.26 of an inefficient system. This is a substantial difference in the supply of non-fertiliser N which has implications for the fertiliser N recommendations with greater cost associated with an inefficient system (Table 6.1).

6.1.2 White clover productivity and persistency

Chapter 4 and Chapter 5 demonstrated that higher annual fertiliser N inputs lowered sward clover DM content and BNF as previously reported by several authors, including Ledgard and Steele (1992), Harris and Clark (1996), Trott et al. (2004), Burchill et al. (2014) and Enriquez-Hidalgo et al. (2016). The increasing application of fertiliser N allows the taller companion grasses to restrict light penetration through the canopy for white clover growth. Light penetrating to the base of the sward is particularly important for white clover stolon between November and February. The mass of white clover stolon surviving over the winter impacts on the persistency of white clover in the sward. It was presented in Chapter 4 that stolon and root DM mass in February was 55% of that in the previous November. Moreover, it was presented in Chapter 5 that stolon and root DM mass in February was 64% of that in the previous November on LS0N system. This high survival rate of stolon and root DM mass in Chapter 5 was attributed to winter grazing and resulted in increased sward clover DM content, clover pasture DM production and total annual pasture DM production during the following growing season. The results of Chapter 5 are in line with previous experiments where winter grazing was beneficial to subsequent pasture DM production and sward white clover DM content (Laidlaw and Stewart, 1987; Laidlaw et al., 1992). Moreover, adjusting defoliation frequency and post-grazing sward height reduces competition from companion grasses during the autumn and winter period prior housing of livestock (Phelan et al., 2013a, 2014).

In a series of plot-scale experiments Enriquez-Hidalgo et al. (2016) and Enriquez-Hidalgo et al. (2018) reported that annual pasture production from grass-clover swards receiving fertiliser N input of 60 or 120 kg ha^{-1} was similar to that of grass swards receiving 240 kg ha^{-1}. Chapter 4 found that over a long term dataset that annual and seasonal pasture DM production was similar for GC systems receiving an average fertiliser N input of 97 kg N ha^{-1} and GO systems receiving an average of 244 kg N ha^{-1}. Moreover, across four PP timeframes, the pasture DM production was similar between the two grassland systems. The GC paddocks were augmented by BNF during the second and third PP timeframes. The total fertiliser N input was 17 kg N ha^{-1} from May to December for the GC systems.

6.1.3 Dairy production systems

The perception that there is lower pasture availability with grass-clover systems in spring remains a key deterrent for many farmers to incorporate clover into their farming system. Traditional seasonal pasture-based dairy systems are designed to maximise the synchrony between calving date and the herd demand for home-grown feed (grazed and ensiled pasture) with minimal purchased concentrate feed. The results of Chapter 5 demonstrated that a later than typical spring calving date of mid-April for zero-fertiliser N white clover-based systems resulted in inefficient use of pasture for milk production. This later calving strategy also resulted in less pasture consumed per cow and higher supplementation with concentrates resulting in a higher cost system compared to the earlier calved systems. An excessive supply of pasture when lactating cows were turned out in April resulted in a greater proportion ensiled and higher silage surpluses. Ensilage is associated with an increase in production costs (Finneran et al., 2012).

Collectively across eleven years, Chapter 4 found no difference in annual milk production per cow between a GO compared with GC system scale studies. A recent meta-analysis reported that a mean annual sward white clover DM content of 316 g DM kg^{-1} is required to increase daily milk and milk solid yields per cow (Dineen et al., 2018). In Chapter 4 the mean annual sward white clover DM content was 190 g DM kg^{-1}. Moreover, annual sward white clover DM content (range 164 to 282 g kg^{-1}) reported in Chapter 5 was below the level required for higher milk production per cow. Previous system scale studies reporting similar annual sward white clover DM content found no difference in annual milk production per cow by cows grazing grass-clover swards receiving and not receiving input of fertiliser N or N-fertilised grass-only (Ribeiro Filho et al., 2005; Glassey et al., 2013; Enriquez-Hidalgo et al., 2014).

Chapter 5 found that extending lactation into early winter on LS0N system lowered the concentrations of milk constituents and processing characteristics of late lactation milk.

The rennet gelation properties showed that the higher concentrations of protein in ES100N and ES0N milk formed a firmer gel than LS0N. Consequently, these firmer gels would result in higher cheese yield as reported by Guinee et al. (2006).

There is positive export prospects for organic premium products from Ireland based on sustained demand from China, Indonesia, Vietnam, Africa and the Middle East (OECD-FAO, 2019). However, current supply of organic milk is failing to meet market requirement for added value products as the small milk pool is processed for liquid milk and short shelf-life products. The establishment of an organic supply chain for premium products such as cheese and long shelf-life milk powders in Ireland has immense potential for exports due to greater attention in recent decades due to consumer preference related to environmental, biodiversity and animal welfare issues.

6.2 Overall Implications

The escalating costs of synthetic fertiliser combined with regulations have created pressure to increase NUE and concomitant reductions in fertiliser N use could be gained from optimising soil nutrient status and adding N-fixing legumes onto swards (Humphreys et al., 2017; Hoekstra et al., 2020). The EU Green Deal Farm to Fork Strategy requires a 20% reduction in fertiliser use and an increase in organic farming. The wider adoption of white clover in Irish grassland has the potential to supply up to 190 kg N ha^{-1} through BNF. Moreover, maximising the supply of background N from soil organic matter and increasing slurry management can further increase the proportion of N available in the soil for plant growth. These aspects can improve both the environmental and economic sustainability of dairy production through improved resource use efficiencies.

This thesis was timely as both increased environmental legislation and tighter economic margins place an increasing worldwide pressure on dairy farmers. While clover has long been promoted as a sustainable alternative to fertiliser N applications, this thesis develops a further understanding of the impact of white clover on the whole farm system from its effect on milk and pasture DM production. The experiments in this thesis assessed aspect of white clover management strategies and nitrogen inputs on seasonal pasture-based milk production. Mean background N was 141 kg ha^{-1} with variation explained by soil pH, air temperature and depth of A1 horizon. Dairy production based on low-input clover-based grassland was similar to N-fertilised grass-only in long term studies. Low N fertilisation and maximising overwintering of stolon and root DM mass by tight winter grazing increased white clover productivity and persistence in grassland. A conventional compact spring calving in mid-February is recommended for zero-fertiliser N white clover-based systems. This system maximises dairy herd alignment for pasture and pasture availability resulting in higher concentrations of milk constituents for processing purposes of late lactation milk.

6.3 Recommendations for future work

- The dataset for background N could be used for future modelling validation purposes.

- Evaluate N use efficiency and mitigation measures linked to the Marginal Abatement Cost Curve (MACC) such as dairy economic breeding index (EBI), low emissions slurry spreading (LESS), protected urea, reducing crude protein content, increasing soil fertility and white clover.

- Rates of adoption of white clover will require further grassland management practices to improve persistency and productivity. Irish pasture management strategies are exclusively developed for grass-only swards. However, grass-clover swards may benefit from alternative grazing strategies, particularly optimum winter closing covers.

- Evaluate the adoption of clover-based grassland by commercial organic and conventional dairy farmers and assess farm productivity, economic competitiveness and the carbon and ammonia footprints of milk.

- Further developments of a blueprint for low or zero nitrogen fertiliser use for low-emissions pasture-based dairy farming.

- It is targeted by policy makers that the proportion of Irish utilised agricultural area under organic production should increase fivefold by 2030. While the organic farming scheme supports farmers financially during the 2 year conversion period, there is an opportunity for the Irish dairy industry to establish a premium market for organic milk internationally. The investigation into potential supply chains warrants further research. This organic value chain will require interaction of diverse sectoral stakeholders.

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