Original Study • DOI: 10.15212/ijafr-2020-0132 IJAFR • 60(1) • 2021 • 114-128

Irish Journal of Agricultural and Food Research

Changes in sward structure, plant morphology and growth of perennial ryegrass–white clover swards over winter

C. Guy[1,2], T.J. Gilliland[2,3], D. Hennessy[1], F. Coughlan[1], B. McCarthy[1†]

[1]Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, P61 C996, Ireland
[2]The Institute for Global Food Security, Queen's University Belfast, Belfast, Northern Ireland
[3]Agri-food and Biosciences Institute, Large Park, Hillsborough, BT26 6DR, Northern Ireland

Abstract

White clover (Trifolium repens L.) is at a disadvantage to perennial ryegrass (Lolium perenne L.; PRG) due to its limited cold tolerance and low growth rates at colder temperatures, which can affect subsequent spring herbage dry matter (DM) availability. The effect of PRG ploidy on white clover morphology and growth over winter, and its subsequent recovery in spring and the following growing season, is poorly understood. The objective of this study was to compare the effect of white clover inclusion and PRG ploidy on sward structure, plant morphology and growth of PRG–white clover swards over winter. Four swards (diploid PRG only, tetraploid PRG only, diploid PRG–white clover and tetraploid PRG–white clover) were evaluated over a full winter period (November–February) at a farmlet scale. The PRG ploidy had no effect on herbage DM production, white clover content or tissue turnover (P > 0.05) over winter. However, white clover inclusion caused a significant decrease in herbage DM production (P < 0.001; −254 kg DM/ha) and tiller density (P < 0.001; −1,953 tillers/m²) over winter. Stolon mass was not affected by PRG ploidy (P > 0.05); however, stolon length and number of leaves per stolon were affected by PRG ploidy (P < 0.05). Including white clover in PRG swards can alter winter sward dynamics, potentially causing difficulties in subsequent spring management and performance due to the reduced over-winter growth rate when compared with PRG.

Keywords

Perennial ryegrass ploidy • stolon morphology • tissue turnover • Trifolium repens *L.*

Introduction

The efficiency of grass-based dairy production systems lies in the correct and appropriate use of forage species to lower input costs (Shalloo *et al.*, 2004) and reduce environmental pressure (Bleken *et al.*, 2005; Arsenault *et al.*, 2009; O'Brien *et al.*, 2014) whilst maintaining high levels of animal performance. As grazed herbage is of high nutritive value (O'Neill *et al.*, 2011) and the cheapest source of feed for animal production in Ireland (Shalloo, 2009; Finneran *et al.*, 2012) and other temperate regions (Dillon *et al.*, 2005), its optimisation at farm level can promote sustainable livestock systems (Lorenz *et al.*, 2019). Within spring-calving dairy production systems, ensuring adequate quantities of grazed herbage in the diet of the lactating cow in early spring can improve animal performance (Sayers & Mayne, 2001; Dillon *et al.*, 2002; Kennedy *et al.*, 2005) and increase herbage dry matter (DM) utilisation and herbage nutritive value (O'Donovan *et al.*, 2004). However, spring herbage availability is dictated by herbage growth in autumn and over winter (O'Donovan *et al.*, 2002; Hennessy *et al.*, 2006; Ryan *et al.*, 2010), and the provision of spring herbage can be altered depending on the composition of forage species within the sward (Lüscher *et al.*, 2014). Previous research has examined factors affecting perennial ryegrass (*Lolium perenne* L.; PRG) over-winter growth, including heading date (Brereton & McGilloway, 1999; Tozer *et al.*, 2014), nitrogen (N) fertiliser (O'Donovan *et al.*, 2004), temperature responses (Bullock *et al.*, 1994; Hurtado-Uria *et al.*, 2013) and autumn closing dates (Hennessy *et al.*, 2008; Ryan *et al.*, 2010). However, few studies have examined the effect of PRG ploidy (i.e. diploid [2N] or tetraploid [4N]) on sward morphology and over-winter growth despite the well-known differences in morphology, growth habit and sward structure between diploid and tetraploid PRG swards in the main grazing season (Frame & Boyd, 1986).

†Corresponding author: Brian McCarthy
E-mail: brian.mccarthy@teagasc.ie

Furthermore, few studies have investigated the interaction between PRG and white clover (*Trifolium repens* L.) on over-winter herbage growth, and of those that have, most have been plot-based rather than on grazed swards (Collins & Rhodes, 1995; Frankow-Lindberg & Von Fircks, 1998; Lüscher *et al.*, 2001). These plot experiments expose plant communities to minimal levels of stress resulting in differing levels of persistence being achieved in plot-based and farm system experiments over the same period (Kerr *et al.*, 2012).

White clover is typically included in PRG swards to increase herbage nutritive value (Ribeiro Filho *et al.*, 2003) and to substitute inorganic N fertiliser with biological N fixation (Enriquez-Hidalgo *et al.*, 2016). White clover has a lower growth rate over winter than PRG (Hoglind & Frankow-Lindberg, 1998), resulting in reduced winter and early spring growth (Collins *et al.*, 1991; Davies, 1992; Frankow-Lindberg & Von Fircks, 1998; Lüscher *et al.*, 2001). The lower growth rate of white clover at cooler soil temperatures may lead to reduced spring herbage availability in PRG–white clover swards, and subsequently reduced annual herbage DM yields (Frame & Newbould, 1986; Woledge *et al.*, 1990; Hoglind & Frankow-Lindberg, 1998).

Investigating the comparative performance of diploid and tetraploid PRG cultivars as companion grasses with white clover to maximise over-winter DM production and white clover contribution to the sward is important in spring-calving livestock production systems to ensure adequate spring herbage availability for grass-based production systems. The morphological characteristics that contribute to over-winter growth and survival of white clover remain an important knowledge gap in intensive grass-based production systems. The hypotheses to be tested in this study were (1) PRG-only swards would have greater over-winter growth than PRG–white clover swards and (2) tetraploid PRG swards would have greater over-winter growth than diploid PRG swards. Consequently, the objective of this study was to investigate the growth patterns, morphology and structural characteristics of PRG-only and PRG–white clover swards varying in ploidy in grazing swards over winter.

Materials and methods

Location

The experiment was conducted at Teagasc Clonakilty Agricultural College, Cork, Ireland (latitude: 51°63′N; longitude: −08°85′E; 25–70 m above sea level) on a free-draining acid brown earth of light to gley loam texture. From 1981–2010 (Cork Airport, Met Éireann), the mean annual precipitation was 1,228 mm, monthly air temperature was 9.9°C and soil temperature was 9.6°C.

Experimental design

The swards used were a component of a larger farm system experiment (43.6 ha) that commenced in 2014 (McClearn *et al.*, 2019). Paddock sizes ranged from 0.43 ha to 0.71 ha. Briefly, the larger experiment consisted of four sward treatments: diploid PRG only (DGO), tetraploid PRG only (TGO), diploid PRG-white clover and (DWC) and tetraploid PRG-white clover (TWC). Four diploid (sown at 30 kg/ha) and four tetraploid (T; sown at 37.5 kg/ha) cultivars were sown as monocultures with and without white clover (50% Chieftain, 50% Crusader, sown at 5 kg/ha) in five different blocks around the farm giving a total of 20 blocks each containing four paddocks (80 paddocks in total). Each sward treatment received 250 kg N/ha per year between mid-January and mid-September 2014. The swards were grazed by 120 dairy cows (30 cows per sward treatment) stocked at 2.75 dairy cows/ha in a rotational grazing system. All sward treatments were managed similarly in terms of grazing management and average farm cover targets (O'Donovan, 2000) during the grazing season. A subset of five blocks, each consisting of four paddocks, was used for this experiment. The four diploid cultivars included in the blocks were Tyrella, Aberchoice, Glenveagh and Drumbo, and the four tetraploid cultivars were Aston Energy, Kintyre, Twymax and Dunluce (Table 1). Data for each cultivar within each ploidy level were grouped for sampling purposes. All paddocks were closed from grazing between 13 and 22 October (Table 2). Sward measurements were undertaken on five occasions during a 13-wk period on 3 November 2014 (measurement date 1 [MD1]), 24 November 2014 (MD2), 17 December 2014 (MD3), 16 January 2015 (MD4) and 26 January 2015 (MD5). Blocks were chosen based on location, soil topography and closing date. Measurement period (MP) denoted the time between each MD, 3–24 November 2014 (MP1), 24 November–17 December 2014 (MP2), 17 December 2014–16 January 2015 (MP3), 16–26 January 2015 (MP4).

Table 1: Perennial ryegrass cultivar heading dates (DAFM[1], 2014)

Diploid cultivars	Heading date	Tetraploid cultivars	Heading date
Glenveagh	3 June	Dunluce	29 May
Tyrella	4 June	AstonEnergy	2 June
Drumbo	7 June	Twymax	7 June
Aberchoice	10 June	Kintyre	8 June

Data from the Grass and Clover Recommended List Varieties for Ireland.
[1]DAFM = Department of Agriculture, Food and the Marine.

Table 2: Sward closing dates and growing season grazing records, and sward herbage mass and sward white clover proportion at the beginning of the experiment (3 November 2014; MD1) for each sward treatment

Growing season grazing records and sward condition				
Sward treatment	**Grazing turnout (2014)**	**Grazing closure (2014)**	**Herbage mass (kg DM/ha)**	**Sward white clover proportion**
DGO	6 March	22 October	423	N/A
TGO	5 March	22 October	369	N/A
DWC	5 March	13 October	400	0.36
TWC	5 March	13 October	362	0.24

DGO = diploid perennial ryegrass (PRG) only, TGO = tetraploid PRG only, DWC = diploid PRG–white clover, TWC = tetraploid PRG–white clover, MD = measurement date, DM = dry matter, N/A = not applicable.

Sward measurements

Herbage mass and daily herbage growth rate

Herbage mass was calculated to approximately 4 cm above ground level at each MD from two strips harvested in each paddock (1.2 m wide; 10 m long), as per Kennedy *et al.* (2009), using an Etesia mower (Etesia UK Ltd., Warwick, UK). Harvested herbage was collected and weighed, and a sub-sample was collected for DM content determination. DM content was calculated by drying a 100-g sub-sample in an oven at 90°C for 15 h. To calculate sward density, 10 sward height measurements were recorded within the cut area immediately pre- and post-harvest using a Jenquip rising plate meter (Jenquip, Feilding, New Zealand; Castle, 1976). Sward density was calculated using these measurements according to the following formula: Sward density (kg DM/cm) = pre-grazing herbage mass (kg DM/ha)/(pre-cutting height [cm] − post-cutting height [cm]). Daily herbage growth rate (kg DM/ha) was calculated by dividing the herbage accumulated between MDs by the number of days between each MD.

Sward white clover content

Sward white clover content in each PRG–white clover treatment was determined at each MD. Sward white clover content was measured by taking 15 random samples in a "W" formation across each paddock at 4 cm above ground level using Gardena hand shears (Accu 60, Gardena International GmbH, Ulm, Germany). Two 70-g samples were separated into PRG (and other grass or weed species) and white clover components and dried at 90°C for 15 h and the proportion of each component of the sward was calculated on a DM basis. Tiller density and stolon mass samples were taken from each paddock at MD1, MD3 and MD5 to estimate PRG tiller density and white clover stolon mass (tiller/m^2 and g/m^2, respectively) as described by Evans *et al.* (1998). Twelve turves (10 cm × 10 cm) were removed from 20 paddocks (five paddocks per sward treatment). The botanical composition was determined from these turves by counting PRG tillers and other grass species. White clover stolons were separated from each turve, and roots and leaves were removed and gently washed to remove excess soil. Stolons were dried at 90°C for 15 h to estimate DM content. Grass morphology was determined using four grab samples (totalling 80 g), cut to ground level at MD1, MD3 and MD5 using a scalpel. The vertical structure of the sample was preserved and extended tiller height (from ground level to the highest point of the tiller) and extended sheath height from ground level to the point of the highest ligulae) were measured for each individual tiller in each sample (Gilliland *et al.*, 2002). Free leaf lamina was calculated as: extended tiller height − extended sheath height (Gilliland *et al.*, 2002). From each sample, 40 g was cut at 4 cm and the above 4-cm portion was separated into leaf, stem and dead material and the DM proportion was determined by drying at 90°C for 15 h.

Leaf extension rate and leaf appearance rate

Thirty tillers were marked at random using coloured wire in each paddock, at 10-cm intervals along three 1-m transects (Hennessy *et al.*, 2008) and measured at each MD for length of each green leaf (mm) and number of leaves per tiller to calculate mean leaf extension rate (LER; mm/tiller per day) and leaf appearance rate (LAR), respectively. The number of secondary and tertiary tillers was counted at each MD and marked with a different colour and followed in the same manner. Fifteen white clover stolons along each of the three 1-m transects were marked using coloured wire behind the node bearing the oldest leaf present on the stolon, as per Pinxterhuis (2000) to measure stolon morphology. Each stolon was measured for length of main stolon, number of nodes on main and branch stolons and number of branches on the main stolon at each MD.

Meteorological data

Meteorological data were recorded using a BSW-200 weather station (Campbell Scientific, Loughborough, UK) in Timoleague, Cork, 4.5 km from the experimental site.

Statistical analyses

Analyses were undertaken on all variables (herbage mass, sward white clover content, tiller density, stolon mass, grass morphology, LER, LAR and stolon morphology) using the mixed-model procedure (PROC MIXED) in the statistical package SAS 9.4 (SAS Institute, 2014). Fixed effects included in the model were PRG ploidy, white clover inclusion, MD, white clover inclusion × ploidy interaction, white clover × MD interaction, and ploidy × MD interaction, with block included as a random effect. Tukey's test was used to determine differences between treatment means. Statistical significance was considered at $P \leq 0.05$ and trends were considered at $0.05 < P \leq 0.10$.

Results

Meteorological data

The meteorological data recorded between November 2014 and February 2015 followed similar trends to the long-term figures (1981–2010) with only a few notable exceptions (Table 3). Rainfall in November 2014 was greater than during the other months of the experiment and also compared with the long-term average for November. The 2014/2015 November–January period was also slightly warmer (6.8°C) than previous figures (6.5°C). Overall, no extreme weather conditions were experienced during the experimental period.

Herbage mass

Herbage mass was reduced by white clover inclusion (Tables 4 and 5; $P < 0.001$); on average, over the experimental period, PRG-only swards (DGO and TGO) had 254 kg DM/ha greater herbage mass than PRG–white clover swards (DWC and TWC; PRG only: 789 kg DM/ha; PRG–white clover: 535 kg DM/ha). On MD1, the mean herbage mass was approximately 400 kg DM/ha across all sward treatments. On the TWC swards, this declined to 240 kg DM/ha by MD2, but increased on the DGO swards to approximately 577 kg DM/ha, with the other two swards (TGO and DWC) remaining largely unchanged (Figure 1). There was little change in herbage mass between MD2 and MD3, but this was followed by a period of rapid growth to 1,100–1,300 kg DM/ha on the PRG-only swards and 900–1,000 kg DM/ha on the PRG–white clover swards. Thereafter, the only significant change was

Table 4: Herbage mass (kg DM/ha), sward white clover content (%), tiller density (tillers/m^2) and stolon mass (g/m^2) for each measurement date for each treatment

	Treatment			
	DGO	TGO	DWC	TWC
Herbage mass (kg DM/ha)				
Mean	806	771	549	521
3 November 2014	423	369	400	362
24 November 2014	577	438	415	240
17 December 2014	534	454	345	236
16 January 2015	1,172	1,318	981	929
26 January 2015	1,326	1,277	605	836
Sward white clover content (%)				
Mean	–	–	23	24
3 November 2014	–	–	36	24
24 November 2014	–	–	31	33
17 December 2014	–	–	18	21
16 January 2015	–	–	20	23
26 January 2015	–	–	8	17
Tiller density (tillers/m^2)				
Mean	5,460	4,119	2,939	2,734
3 November 2014	5,552	4,792	2,342	2,112
17 December 2014	4,323	3,207	1,985	1,443
26 January 2015	6,505	4,358	4,490	4,648
Stolon mass (g/m^2)				
Mean	–	–	160.0	152.1
3 November 2014	–	–	196.1	174.1
17 December 2014	–	–	184.2	203.6
26 January 2015	–	–	99.8	78.6

DGO = diploid perennial ryegrass (PRG) only, TGO = tetraploid PRG only, DWC = diploid PRG–white clover, TWC = tetraploid PRG–white clover, DM = dry matter.

Table 3: Monthly mean air temperature (°C), precipitation (mm), humidity (%), soil temperature (°C) and wind speed (m/s) collected at Timoleague, Co. Cork for winter 2014/2015 (mean of 1981–2010 values collected at Cork Airport in parentheses)

Date	Air temperature (°C)	Precipitation (mm)	Humidity (%)	Soil temperature (°C)	Wind speed (m/s)
October 2014	11.5 (10.5)	148.0 (138.2)	93.4 (85.3)	12.7 (10.0)	3.53 (n/a)
November 2014	8.6 (7.8)	176.2 (120.0)	93.3 (87.1)	9.8 (7.2)	2.77 (n/a)
December 2014	6.5 (6.1)	84.6 (133.1)	93.1 (88.0)	7.6 (5.6)	3.35 (n/a)
January 2015	5.6 (5.6)	120.0 (131.4)	92.4 (86.8)	6.7 (4.8)	3.75 (n/a)
February 2015	4.9 (5.7)	58.2 (97.8)	91.9 (84.2)	5.6 (4.8)	2.92 (n/a)

n/a = not available.

Table 5: Herbage mass (kg DM/ha), sward white clover content, tiller density (tillers/m^2) and stolon mass (g/m^2) and associated significance

		Significance				
	s.e.	Ploidy	Clover	Ploidy × clover	MD	Ploidy × MD
Herbage mass (kg DM/ha)	128.7	0.590	<0.001	0.949	<0.001	–
Sward white clover content	0.038	0.624	–	–	<0.001	0.068
Tiller density (tillers/m^2)	514	0.007	<0.001	0.044	<0.001	–
Stolon mass (g/m^2)	16.80	0.534	–	–	<0.001	0.305

DM = dry matter, s.e. = pooled standard error, MD = measurement date.

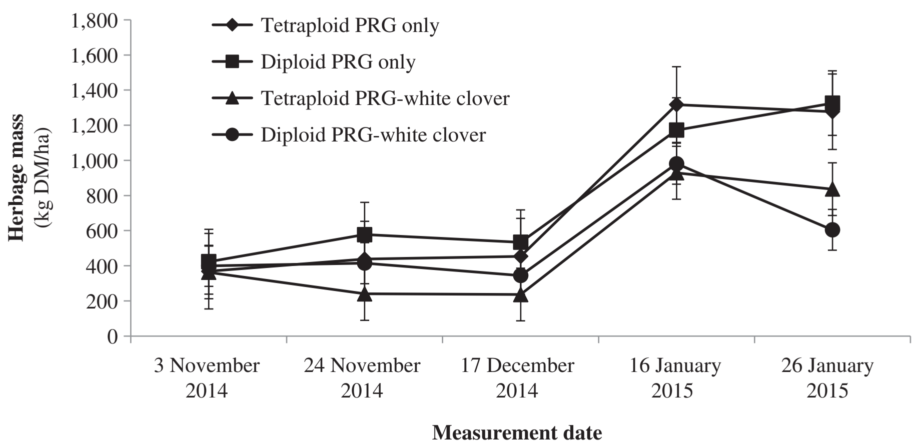

Figure 1. Herbage mass for each grazing treatment at each measurement date over the winter period in 2014. DM = dry matter, PRG = perennial ryegrass.

that the DWC and TWC swards declined to 605 kg DM/ha and 836 kg DM/ha, respectively. Mean herbage mass was similar on tetraploid (TGO + TWC) and diploid (DGO + DWC) swards. The overall increase in herbage mass between MD1 and MD5 (i.e., herbage production; calculated by subtracting herbage mass at MD1 from herbage mass at MD5) was greatest on the PRG-only swards compared with the PRG–white clover swards. Cumulative herbage DM production over the experimental period was 902 kg DM/ha and 908 kg DM/ha on the DGO and TGO swards, respectively, whereas cumulative herbage DM production on the DWC and TWC swards was 205 kg DM/ha and 474 kg DM/ha, respectively. For every 100 kg DM/ha present in the swards on MD1, there was 329 kg DM/ha at MD5 on the PRG-only swards, and 189 kg DM/ha on the PRG–white clover swards.

Sward white clover content

Average mean sward white clover content during the experiment did not differ between DWC and TWC swards ($P > 0.05$; 23% and 24%, respectively); however, differences did occur during the winter period. On MD1, sward white clover content was significantly greater on the DWC swards compared with the TWC swards ($P < 0.001$; Figure 2). However, between MD1 and MD2, white clover content of the DWC swards declined by 5%, down to 31%, whereas it increased in the TWC swards by 9%, up to 33%. Thereafter, sward white clover content declined throughout the winter period, as sward white clover content on the DWC and TWC swards at MD5 was 8% and 17%, respectively.

Tiller density, stolon mass and grass morphology

PRG tiller density was significantly different between PRG-only and PRG–white clover swards ($P < 0.001$) and diploid and tetraploid swards ($P < 0.01$). Tiller density was significantly affected by MD with significant differences occurring between each MD (Tables 4 and 5). Diploid swards had a greater tiller density than tetraploid swards (TGO + TWC; $P < 0.01$; diploid: 4,200 tillers/m^2; tetraploid: 3,426 tillers/m^2) throughout the winter period. Including white clover in the sward significantly reduced tiller density; PRG-only swards had approximately

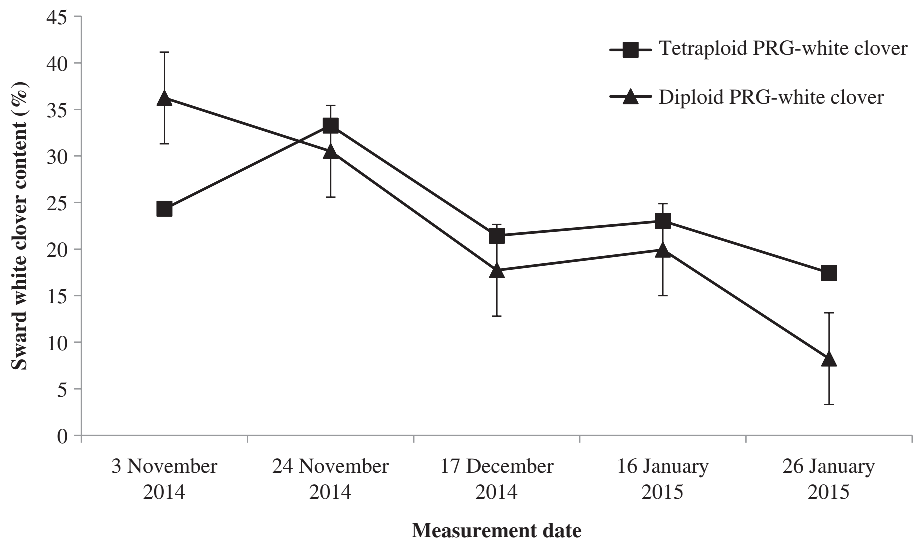

Figure 2. Sward white clover content for diploid PRG-white clover and tetraploid PRG-white clover at each measurement date over the winter period in 2014.

1,953 more tillers/m² than PRG–white clover swards (P < 0.001; PRG-only 4,789 tillers/m²; PRG–white clover; 2,837 tillers/m²). On average, tiller density decreased by 26% in each sward between MD1 and MD3 and increased by 46% between MD3 and MD5. There was a moderate relationship between herbage mass and tiller density (R^2 = 0.473; P < 0.001; Figure 3).

Greater accumulation of stolon mass was associated with lower tiller density, as there was a significant negative relationship between tiller density and stolon mass across ploidies (R^2 = 0.941; P < 0.001; Figure 4).

White clover stolon mass did not differ significantly between DWC and TWC swards at any MD (160 g/m² vs. 152 g/m², respectively; Tables 4 and 5). There was a significant decline

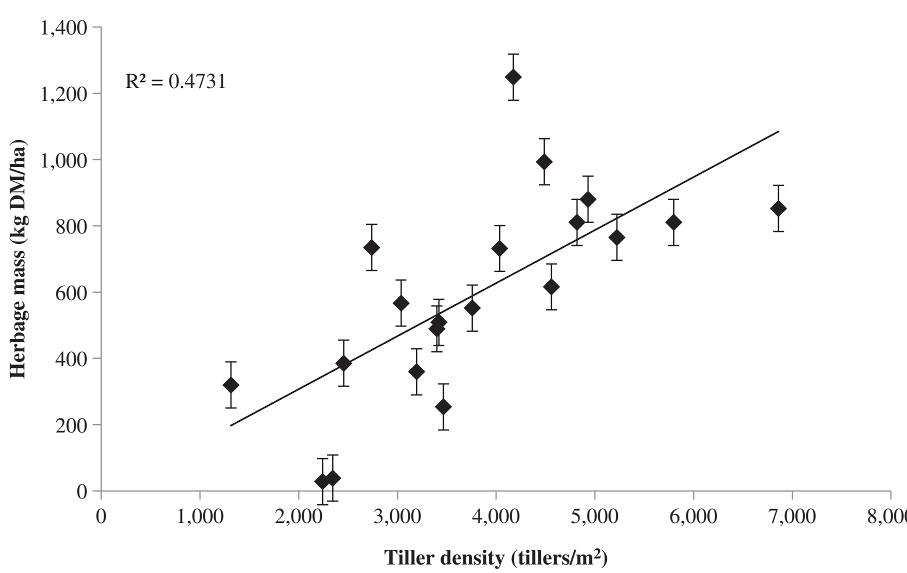

Figure 3. Relationship between tiller density (tillers/m²) and herbage mass (kg DM/ha). Points are the four treatments (DGO, TGO, DWC and TWC) × five recordings (MD1–MD5). DGO = diploid perennial ryegrass (PRG) only, TGO = tetraploid PRG only, DWC = diploid PRG–white clover, TWC = tetraploid PRG–white clover, MD = measurement date, DM = dry matter.

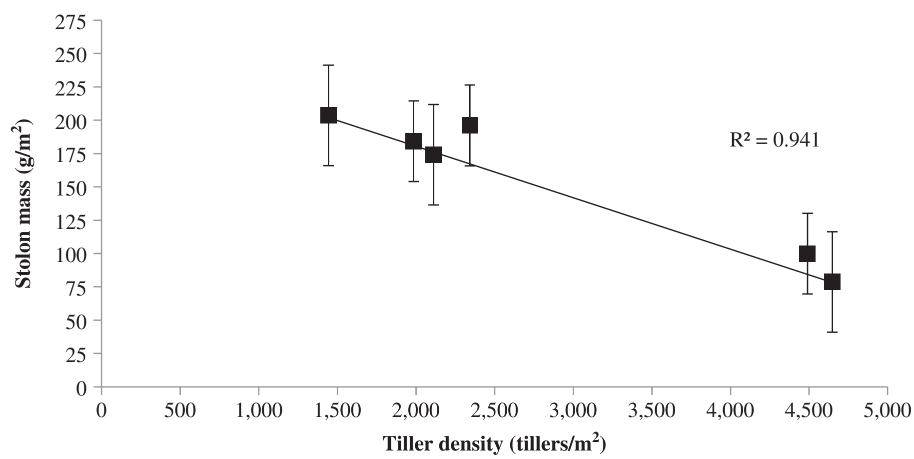

Figure 4. Relationship between tiller density (tillers/m^2) and stolon mass (g/m^2) in perennial ryegrass–white clover swards.

in stolon mass over the winter (P < 0.001, 52% decline), with the greatest decline in total stolon mass occurring between MD3 and MD5.

Grass morphological components did not differ with white clover inclusion or between diploid and tetraploid swards, with the exception of free leaf lamina, which was greater for tetraploid compared to diploid swards (17.8 cm vs. 16.2 cm; Table 6). Stem proportion and extended sheath height decreased significantly (P < 0.05) from MD1 (19%, 6.10 cm, respectively) to MD5 (13%, 5.01 cm, respectively). The PRG leaf length (mm) was significantly different between PRG tillers in PRG-only and PRG–white clover swards (P < 0.001) and diploid and tetraploid swards (P < 0.01; Table 7). Primary PRG tillers in PRG–clover swards were longer (113.1 mm) than primary PRG tillers in PRG-only swards (85.8 mm). Primary PRG tillers were longer than both secondary and tertiary PRG tillers. Secondary PRG tiller appearance rate was greater in PRG–white clover swards than PRG-only swards (P < 0.05; 0.21 vs. 0.14) and greater in tetraploid than diploid swards (P < 0.05; 0.21 vs. 0.14). MD affected secondary PRG tiller appearance rate (P < 0.01) as appearance rate was greatest at MD4 (0.30). Tertiary PRG tiller appearance rate did not differ between PRG-only and PRG–clover swards, PRG ploidies or MD (P > 0.05).

Leaf extension rate and leaf appearance rate

The LER decreased significantly from MP1 to MP2 across all sward types, from 7.8 mm/d to 4.4 mm/d (P < 0.05; Table 8). LAR followed a similar trend, decreasing from 0.083 leaves/tiller per day to 0.046 leaves/tiller per day between MP1 and MP2 (P < 0.05). LER and LAR did not differ between PRG-only and PRG–white clover swards or between PRG ploidies over the winter period (Table 8; P > 0.05). Although non-significant, LER and LAR decreased at a faster rate in PRG–white clover

Table 6: Perennial ryegrass morphological components as measured during winter 2014/2015 (each parameter is given as a mean of the five measurement dates)

	Treatment					Significance		
	DGO	TGO	DWC	TWC	s.e.	Ploidy	Clover	MD
Leaf proportion	0.64	0.65	0.63	0.69	0.026	0.230	0.629	0.094
Stem proportion	0.16	0.14	0.17	0.16	0.016	0.353	0.353	0.029
Dead proportion	0.22	0.21	0.21	0.17	0.019	0.230	0.125	0.064
Extended tiller height (cm)	21.0	22.8	22.3	23.3	1.19	0.131	0.329	0.172
Extended sheath height (cm)	5.48	5.31	5.32	5.47	0.200	0.601	0.630	0.013
Free leaf lamina (cm)	15.7	17.5	16.7	18.0	1.01	0.042	0.317	0.006

DGO = diploid perennial ryegrass (PRG) only, TGO = tetraploid PRG only, DWC = diploid PRG–white clover, TWC = tetraploid PRG–white clover, s.e. = pooled standard error, MD = measurement date.

Table 7: Perennial ryegrass leaf length (mm) for each treatment and tiller stage (primary, secondary or tertiary)

	Treatment					Significance		
Tiller	**DGO**	**TGO**	**DWC**	**TWC**	**s.e.**	**Ploidy**	**Clover**	**Tiller stage**
Mean	41.1	43.8	47.9	67.6	3.45	0.001	<0.001	<0.001
Primary	87.3	84.3	96.9	129.3	5.96			
Secondary	30.4	36.0	44.6	66.7	6.00			
Tertiary	5.6	11.1	2.2	6.74	5.96			

DGO = diploid perennial ryegrass (PRG) only, TGO = tetraploid PRG only, DWC = diploid PRG–white clover, TWC = tetraploid PRG–white clover, s.e. = pooled standard error.

Table 8: Perennial ryegrass leaf extension rate (mm/tiller per day) and leaf appearance rate (leaves/tiller per day) during winter

	Treatment					Significance		
	DGO	**TGO**	**DWC**	**TWC**	**s.e.**	**Ploidy**	**Clover**	**MP**
Leaf extension rate								
Mean	5.7	5.5	4.5	5.5	0.99	0.689	0.537	0.060
MP1	6.5	7.1	8.6	9.1	1.87			
MP2	4.7	5.2	3.5	4.8	1.87			
MP3	7.6	3.8	3.9	2.8	1.87			
MP4	4.1	6.1	2.2	5.3	1.87			
Leaf appearance rate								
Mean	0.065	0.059	0.043	0.051	0.0097	0.893	0.180	0.027
MP1	0.084	0.077	0.082	0.090	0.0188			
MP2	0.052	0.055	0.034	0.042	0.0188			
MP3	0.080	0.043	0.039	0.026	0.0188			
MP4	0.043	0.063	0.016	0.044	0.0188			

DGO = diploid perennial ryegrass (PRG) only, TGO = tetraploid PRG only, DWC = diploid PRG–white clover, TWC = tetraploid PRG–white clover, s.e. = pooled standard error, MP = measurement period. MP1 = 3 November 2014–24 November 2014, MP2 = 24 November 2014–17 December 2014, MP3 = 17 December 2014–16 January 2015 and MP4 = 16 January 2015–26 January 2015.

swards when compared with PRG-only swards (Table 8). The relationship between LAR and soil temperature indicated that LAR decreased from 0.083 leaves/tiller per day when soil temperature was between 8°C and 10°C, to 0.045 leaves/tiller per day when soil temperature was between 4°C and 8°C (Figure 5). Similarly, LER decreased from 7.5 mm/d when temperatures were between 8°C and 10°C to approximately 4.5 mm/d when soil temperature was between 4°C and 8°C (Figure 6).

White clover stolon morphological components

The greatest above-ground stolon loss occurred between MD4 and MD5, when senescence of plant material occurred and stolons were buried or a number of stolon markers were lost. Stolon length ($P < 0.001$) and number of leaves per stolon ($P < 0.05$) were significantly different between DWC and TWC swards (Table 9). The number of green leaves per marked stolon remained constant for the experimental period. Stolons present in DWC swards were approximately 1.7 cm longer than those in TWC swards across the winter period (11.3 cm and 9.6 cm, respectively; Table 9), with stolons in DWC and TWC swards growing 0.5 cm over the winter period. White clover plants present in DWC swards had 0.1 more leaves on the main stolon than those in TWC swards ($P < 0.05$). Between MD1 and MD5, plants in DWC and TWC swards produced 0.3 and 0.8 new leaves, respectively. Branch appearance rate, LAR, stolon elongation rate or node appearance did not differ significantly between sward treatments ($P > 0.05$).

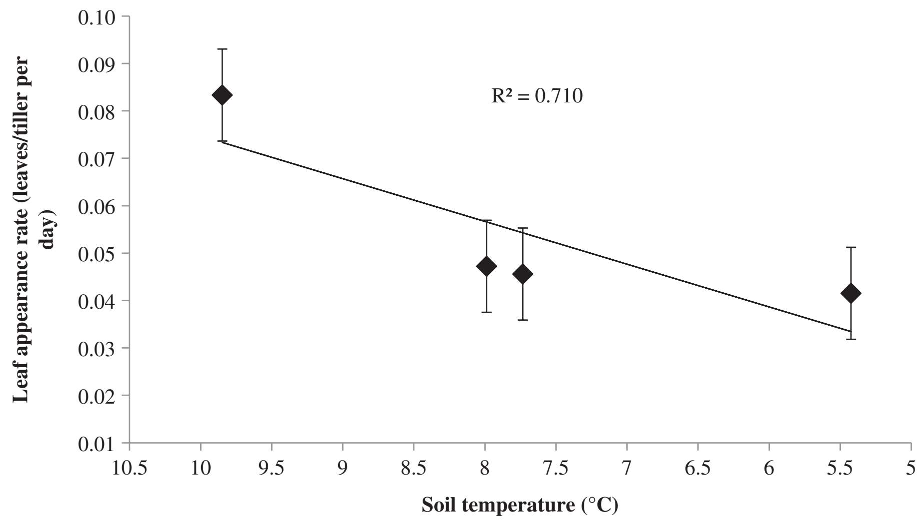

Figure 5. Relationship between perennial ryegrass leaf appearance rate (leaves/tiller per day) and soil temperature.

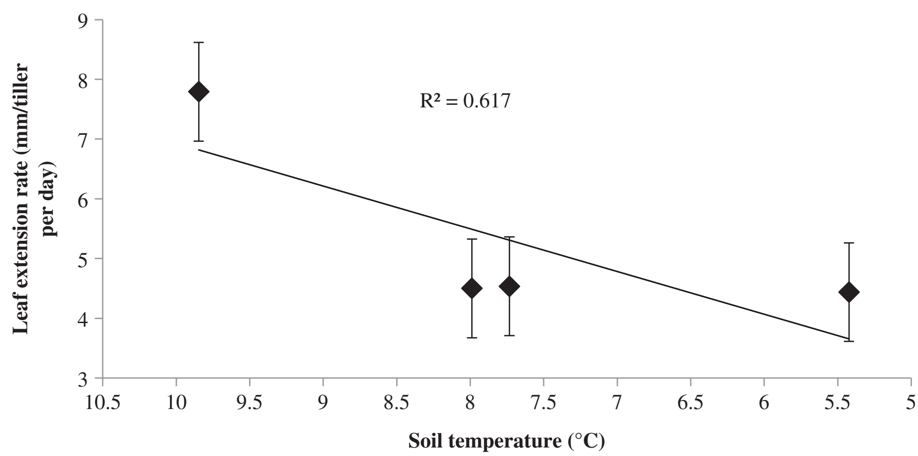

Figure 6. Relationship between perennial ryegrass leaf extension rate per day (mm/tiller per day) and soil temperature.

Discussion

Over-winter herbage growth influences spring herbage availability (O'Donovan *et al.*, 2002; Hennessy *et al.*, 2006; Ryan *et al.*, 2010), and as a consequence, can greatly alter spring grazing management in PRG-based production systems. However, reduced herbage production on PRG–white clover swards over winter (Collins *et al.*, 1991) due to low white clover growth at temperatures less than 9°C (Frame & Newbould, 1986; Hart, 1987) means that spring herbage availability of PRG–white clover swards can be reduced.

The effect of white clover on herbage mass, over-winter growth and sward structure

In this experiment, soil temperatures were below 5°C (minimum temperature for PRG growth) and 9°C (minimum temperature for white clover growth) for 16 and 118 d, respectively, resulting in herbage growth rates of 2.4, 5.6, 10.7 and 10.8 kg DM/ha per day on DWC, TWC, DGO and TGO swards, respectively. Previously O'Donovan *et al.* (2002) and Lawrence (2015) showed over-winter herbage growth rates of 13–15 kg DM/ha per day in the South of Ireland, albeit at a different site and for a slightly different period. As a result of this reduced over-winter herbage growth rate, over-winter herbage production and spring herbage availability on PRG–white clover swards

Table 9: Mean white clover morphological components on DWC and TWC swards at each measurement date

		Treatment			Significance	
Morphological measurement	**Measurement date**	**DWC**	**TWC**	**s.e.**	**Ploidy**	**MD**
Stolon length (cm)	Mean	11.33	9.65	0.99	0.010	0.871
	3 November 2014	10.94	9.25	1.114		
	24 November 2014	11.41	9.65	1.114		
	17 December 2014	11.47	9.74	1.114		
	16 January 2015	11.43	9.83	1.114		
	26 January 2015	11.40	9.74	1.154		
Petioles (number/stolon)	Mean	3.75	3.30	0.181	0.049	0.393
	3 November 2014	3.62	3.00	0.288		
	24 November 2014	3.95	3.30	0.288		
	17 December 2014	3.92	3.33	0.288		
	16 January 2015	3.45	3.20	0.288		
	26 January 2015	3.80	3.64	0.317		
Leaves (number/stolon)	Mean	3.54	3.15	0.184	0.112	0.760
	3 November 2014	3.24	2.80	0.321		
	24 November 2014	3.80	3.13	0.321		
	17 December 2014	3.78	3.17	0.321		
	16 January 2015	3.32	3.09	0.321		
	26 January 2015	3.53	3.56	0.355		
Nodes (number/stolon)	Mean	3.22	3.05	0.148	0.300	0.944
	3 November 2014	3.32	3.02	0.245		
	24 November 2014	3.43	3.17	0.245		
	17 December 2014	3.43	3.24	0.245		
	16 January 2015	2.93	2.96	0.245		
	26 January 2015	2.99	2.86	0.270		

DWC = diploid perennial ryegrass (PRG)-white clover, TWC = tetraploid PRG-white clover, s.e. = pooled standard error, MD = measurement date.

was 566 kg DM/ha less than PRG-only swards. Reduced spring herbage availability results in reduced proportions of grazed herbage in the diet of the lactating animal in spring, leading to reduced animal performance (Sayers & Mayne, 2001; Dillon *et al.*, 2002; Kennedy *et al.*, 2005) and negative impacts on herbage nutritive value and sward utilisation (O'Donovan *et al.*, 2004) in subsequent grazing rotations.

There was no difference in sward white clover content between diploid and tetraploid swards over winter. Other studies have reported various white clover responses to differences in PRG sward structure, such as Swift *et al.* (1993) who found tetraploids more suited to white clover growth and Tozer *et al.* (2014) and Elgersma & Schlepers (1997) who found no difference between tetraploid and diploid for white clover growth. However, the majority of previous studies focused on growth during the main growing season (Ribeiro Filho *et al.*, 2003; Humphreys *et al.*, 2009; Tozer *et al.*, 2014), and do not provide clear comparisons for over-winter sward white clover content. Although herbage DM production was reduced in PRG–white clover swards, there was no difference in spring herbage availability between DWC and TWC swards (Guy *et al.*, 2018). The steady decline in white clover content over winter was expected given similar declines reported by Hoglind & Frankow-Lindberg (1998), Pinxterhuis (2000) and Wachendorf *et al.* (2001), who attributed the decline to colder temperatures over winter, and possibly the lower light levels over winter.

Tiller density was lower in the PRG–white clover swards compared with PRG-only swards, similar to Garay *et al.* (1997). The presence of white clover stolons in the PRG–white clover swards provided competition for space with the PRG tillers (Brereton *et al.*, 1985; Swift *et al.*, 1993; Garay *et al.*, 1997). Mean tiller density in the PRG–white clover swards was 2,163 tillers/m^2 below the 5,000 tillers/m^2 at which white clover growth is compromised as suggested by Brereton *et al.* (1985). The reduction in tiller density was part of the reason for the reduced spring herbage availability, as a lack of tillering can lead to reduced herbage growth (Hennessy *et al.*, 2008). Tiller density was not static over winter and increased towards the end of the winter period and early spring, most likely due to moderate levels of tillering and low levels of tiller death, as also reported by Hunt & Field (1979) and Korte (1986). A build-up of tillers in the later stage of the study was important in providing a dense sward for the main spring growth period (Korte, 1986; Hennessy *et al.*, 2006; Ryan *et al.*, 2010). Tetraploid PRG-only and TWC swards followed a similar pattern, providing fewer tillers/m^2 than DGO and DWC swards. This is due to the difference in sward structure between diploid and tetraploid PRG (Frame & Boyd, 1986). Overall, tiller density initially decreased but began to rapidly increase in the later stage of the study resulting in increased tiller production, a similar tiller production curve as observed by Thomas & Norris (1981). Tiller density increased on all swards over winter as expected (Davies, 1977) but the final number of tillers was lower on PRG–white clover compared with PRG-only swards. Although PRG–white clover swards had a lower tiller density than PRG-only swards, tiller density on PRG–white clover swards had a greater overall increase (an increase of 43%) whereas tiller density on PRG-only swards increased in total by 29%. The appearance of secondary PRG tillers was greater on PRG–white clover swards despite lower initial tiller density at MD1, indicating no reduction in the overall tillering capacity on PRG–white clover swards.

Over-winter stolon mass is a good indicator of future white clover DM yield potential, and is often considered an important indicator of persistence (Beinhart *et al.*, 1963; Frame & Newbould, 1986; Collins *et al.*, 1991). In this study, over-winter stolon mass was greater than reported by Hay (1983), Hay *et al.* (1987) and Phelan *et al.* (2014) and was an indication of the high mean white clover content throughout the previous grazing season (0.38–0.41; McCarthy, personal communication). Stolon mass remained relatively steady up to the middle of December but declined thereafter as shown by Hay *et al.* (1987) and Phelan *et al.* (2014). The number of buried stolons increased over winter. This was similar to Hay (1983) and Hay *et al.* (1987), though their stolon burial levels of 87%–99% were considerably higher than in the current study, which ranged from 7% in mid-January to 14% buried at the end of January. This large difference was likely due to the variation in sampling method (tiller cores vs. marked stolons) and may also be due to the previous grazing management (sheep vs. dairy cows). Longer stolons with more leaves and petioles were present in DWC compared with TWC swards, a response likely linked to reduced light availability as described by Brock & Hay (1996). This induces white clover plants to produce longer petioles and an abundance of leaves to capture all available light energy (Frame & Newbould, 1986).

The effect of perennial ryegrass ploidy on herbage mass, over-winter growth and sward structure

Although variations in growth habit and morphology of PRG diploid and tetraploid swards impact sward performance (Frame & Boyd, 1986), little evidence exists from cattle grazing studies that clearly show advantages in using one PRG ploidy over another for improved herbage growth rates or herbage DM production. In this study, cumulative herbage production over winter was similar on the diploid and tetraploid PRG swards, regardless of MD or meteorological conditions, similar to Brereton & McGilloway (1999) who did not find significant differences between diploid and tetraploid cultivars from the November to March winter period. This indicates that no difference in cold weather performance exists between diploid and tetraploid cultivars and therefore significant deficits in spring herbage availability are unlikely to occur if either PRG ploidy is used in a grazing system.

Although no herbage DM production differences were found between diploid and tetraploid PRG swards over winter, structural differences were observed similar to those previously reported during the main growing season (Gilliland *et al.*, 2002; O'Donovan & Delaby, 2005). Tiller density was greater in diploid swards compared with tetraploid swards, similar to Vipond *et al.* (1997) and Orr *et al.* (2005). In contrast, free leaf lamina was greater for tetraploid swards compared to diploid swards over winter, which is similar to Wims *et al.* (2013) during the main grazing season. There was a poor relationship between herbage mass and tiller density, hence the similar herbage mass on tetraploid and diploid swards despite the greater tiller density on diploid swards. This may be due to low over-winter herbage growth rates but could also be due to lower light quality between November and January, and a greater sward density may not be an advantage in terms of herbage DM production. Previous research has found that both positive (King *et al.*, 1984; Davies, 1988) and negative (Langer, 1963, 1979) yield:density and yield:morphology relationships (Griffiths *et al.*, 2016) can exist during the main growing season, leading to difficulties in interpreting the relationship between tiller density and herbage DM production. Ryan *et al.* (2010) found that herbage mass increased directly after the period in which PRG tiller density increased, as the newly established tillers' leaves begin to expand, increasing leaf area index and maximising leaf production (Davies, 1988) and sward growth. However, high leaf area index can also result in the restriction of light reaching the base of the sward, resulting in a reduction of tiller density and increased levels of senescence (Hunt & Field, 1979). Tiller density can decline as ceiling herbage mass is achieved (Laidlaw & Mayne, 2000; Hennessy *et al.*, 2006), although the reduction in tiller density may not have a negative effect on herbage mass (Ryan *et al.*, 2010), as was found in this study. New leaves appeared on the main tillers during this study at a rate of one new leaf every seven weeks similar to Davies (1977). This differed between MP, reflecting winter temperatures and their effects on PRG plant growth. In this study, sward leaf proportion increased by 0.10 from the start of November to the end of January. This led to a decrease in dead proportion of 0.06 across the winter period. Although leaf senescence was not examined during this study, the implication of the increase in leaf proportion is that LER was consistently greater than senescence during the study period. This net positive leaf growth contributed to the increase in herbage DM production over winter. LER declined over winter, slowing as the temperatures decreased. From MD4 to MD5, a significant increase in LER was observed, similar to Hennessy *et al.* (2008) and Ryan *et al.* (2010). A similar pattern was noted for LAR, with leaves appearing at a slower rate between MD1 and MD4 but increasing as temperatures started to increase and growth was initiated between MD4 and MD5. As with cumulative over-winter herbage production, no differences between diploid and tetraploid swards existed for tissue turnover. The sample size may have been a contributing factor to this lack of difference. An increase in total number of samples may have provided more information on structural variations between diploid and tetraploid swards.

Practical implications

The results from this experiment imply that white clover can have a negative effect on herbage DM production, morphology and structural characteristics in the November to January period and this can have a negative effect on spring herbage availability if it carries through to the onset of grazing (early February). Few differences were found in PRG morphology with the exception of reduced tiller density in TGO compared to DGO swards. The presence of white clover in the sward reduced PRG tiller density; this is not an issue during the main growing season due to white clover inhabiting the spaces between PRG plants but in the winter period this can present itself as vacant spaces in the sward. This may have been a factor contributing to the spring herbage availability deficit, which can only be replaced with conserved forage and/or concentrate supplementation, reducing feed quality and adding cost per unit output. The swards in this experiment experienced high sward white clover contents in the previous grazing season which may have led to a greater decrease in over-winter growth due to white clover contributing a greater proportion of the sward than PRG. Swards with lower white clover contents in the previous grazing season may not have displayed such large differences in over-winter growth and spring herbage availability. Further research into the dynamics of PRG–white clover mixtures in grass-based production systems should focus on identifying the optimum autumn management, in terms of autumn sward closure and herbage production over winter to support white clover production. Additional research is required to underpin the methods of achieving high levels of over-winter herbage growth without compromising the balance of companion PRG and white clover content in the sward.

Conclusion

Overall, a superior agronomic performance from PRG–white clover swards over PRG-only swards was not experienced in the November to January period, as PRG–white clover swards were associated with reduced PRG tiller density, reduced herbage growth and herbage DM production. The presence of white clover in PRG swards altered winter dynamics and reduced herbage DM availability in early spring. Growth in the November to January period did not differ between the two PRG ploidies. Tetraploid and diploid swards displayed similar herbage DM production and daily herbage growth rates in the November to January period and consequently did not differ in spring herbage availability. Over-winter growing conditions imposed a herbage DM production ceiling, and both PRG ploidies and white clover were similarly inhibited and unable to express any differences until conditions improved in spring. Although not measured in this particular study, it is possible that the PRG–white clover swards were higher in herbage nutritive value, which may offset the lower herbage DM production in these swards. Further studies should investigate whether this has an impact on early spring grazing in PRG–white clover swards.

Acknowledgements

This research was funded by the Irish Dairy Levy administered by Dairy Research Ireland. The first author was in receipt of a Teagasc Walsh Scholarship. The authors would like to gratefully acknowledge the invaluable assistance of the farm and technical staff based at Teagasc Clonakilty and Teagasc Moorepark.

Conflicts of interest

The authors declare no conflicts of interest.

References

Arsenault, N., Tyedmers, P. and Fredeen, A. 2009. Comparing the environmental impacts of pasture-based and confinement-based dairy systems in Nova Scotia (Canada) using life cycle assessment. *International Journal of Agricultural Sustainability* **7**: 19–41.

Beinhart, G., Gibson, P.B., Halpin, J. and Hollowell, E. 1963. Selection and evaluation of white clover clones. III. Clonal differences in branching in relation to leaf area production and persistence. *Crop Science* **3**: 89–92.

Bleken, M.A., Steinshamn, H. and Hansen, S. 2005. High nitrogen costs of dairy production in Europe: worsened by intensification. *AMBIO: A Journal of the Human Environment* **34**: 598–606.

Brereton, A., Carton, O. and Conway, A. 1985. The effect of grass tiller density on the performance of white clover. *Proceedings of the XV International Grassland Congress, The National Grassland Research Institute: Tochigi-Ken, Japan*, pages 756–757.

Brereton, A.J. and McGilloway, D.A. 1999. Winter growth of varieties of perennial ryegrass (*Lolium perenne* L.). *Irish Journal of Agricultural and Food Research* **38**: 1–12.

Brock, J.L. and Hay, M.J. 1996. A review of the role of grazing management on the growth and performance of white clover cultivars in lowland New Zealand pastures. White clover: New Zealand's Competitive Edge. *Agronomy Society of New Zealand Special Publication No.11/New Zealand Grassland Association, Grasslands Research and Practice Series, 6*, pages 65–70.

Bullock, J., Hill, B.C. and Silvertown, J. 1994. Tiller dynamics of two grasses – responses to grazing, density and weather. *Journal of Ecology* **82**: 331–340.

Castle, M. 1976. A simple disc instrument for estimating herbage yield. *Grass and Forage Science* **31**: 37–40.

Collins, R.P. and Rhodes, I. 1995. Stolon characteristics related to winter survival in white clover. *Journal of Agricultural Science* **124**: 11–16.

Collins, R.P., Glendining, M.J. and Rhodes, I. 1991. The relationships between stolon characteristics, winter survival and annual yields in white clover (*Trifolium repens* L.). *Grass and Forage Science* **46**: 51–61.

DAFM. 2014. Grass and Clover Recommended List Varieties for Ireland 2014. Department of Agriculture, Food and the Marine. Available online: https://www.teagasc.ie/media/website/crops/grassland/GrassCloverRecommendedListVarietiesIreland06022014.pdf.

Davies, A. 1977. Structure of the grass sward. *Proceedings of the International Meeting on Animal Production from Temperate Grassland*, *An Foras Taluntais*, *Dublin, Ireland*, pages 36–44.

Davies, A. 1988. The regrowth of grass swards. In: "The Grass Crop". Springer, Dordrecht, pages 85–127.

Davies, A. 1992. White clover. *Biologist* **39**: 129–133.

Dillon, P., Crosse, S., O'Brien, B. and Mayes, R.W. 2002. The effect of forage type and level of concentrate supplementation on the performance of spring-calving dairy cows in early lactation. *Grass and Forage Science* **57**: 212–223.

Dillon, P., Roche, J., Shalloo, L. and Horan, B. 2005. Optimising financial return from grazing in temperate pastures. In: "Proceedings of a Satellite Workshop of the XXth International Grassland Congress" (ed. J.J. Murphy.), Wageningen Academic Publishers, Wageningen, the Netherlands, pages 131–147.

Elgersma, A. and Schlepers, H. 1997. Performance of white clover/perennial ryegrass mixtures under cutting. *Grass and Forage Science* **52**: 134–146.

Enriquez-Hidalgo, D., Gilliland, T.J. and Hennessy, D. 2016. Herbage and nitrogen yields, fixation and transfer by white clover to companion grasses in grazed swards under different rates of nitrogen fertilization. *Grass and Forage Science* **71**: 559–574.

Evans, D., Williams, T., Jones, S. and Evans, S. 1998. The effect of cutting and intensive grazing managements on sward components of contrasting ryegrass and white clover types when grown in mixtures. *The Journal of Agricultural Science* **130**: 317–322.

Finneran, E., Crosson, P., O'Kiely, P., Shalloo, L., Forristal, D. and Wallace, M. 2012. Stochastic simulation of the cost of home-produced feeds for ruminant livestock systems. *The Journal of Agricultural Science* **150**: 123–139.

Frame, J. and Boyd, A. 1986. Effect of cultivar and seed rate of perennial ryegrass and strategic fertilizer nitrogen on the productivity of grass/white clover swards. *Grass and Forage Science* **41**: 359–366.

Frame, J. and Newbould, P. 1986. Agronomy of white clover. *Advances in Agronomy* **40**: 1–88.

Frankow-Lindberg, B. and Von Fircks, H. 1998. Population fluctuations in three contrasting white clover cultivars under cutting, with particular reference to overwintering properties. *The Journal of Agricultural Science* **131**: 143–153.

Garay, A.H., Matthew, C. and Hodgson, J. 1997. Effect of spring grazing management on perennial ryegrass and ryegrass-white clover pastures: 2. Tiller and growing point densities and population dynamics. *New Zealand Journal of Agricultural Research* **40**: 37–50.

Gilliland, T., Barrett, P., Mann, R., Agnew, R. and Fearon, A. 2002 Canopy morphology and nutritional quality traits as potential grazing value indicators for *Lolium perenne* varieties. *The Journal of Agricultural Science* **139**: 257–273.

Griffiths, W., Matthew, C., Lee, J. and Chapman, D. 2016. Is there a tiller morphology ideotype for yield differences in perennial ryegrass (*Lolium perenne* L.)? *Grass and Forage Science* **72**: 700–713.

Guy, C., Hennessy, D., Gilliland, T.J., Coughlan, F., McClearn, B., Dineen, M. and McCarthy, B. 2018. Comparison of perennial ryegrass, *Lolium perenne* L., ploidy and white clover, *Trifolium repens* L., inclusion for herbage production, utilization and nutritive value. *Grass and Forage Science* **73**: 865–877.

Hart, A.L. 1987. Physiology. In: "White Clover" (eds. M.J. Baker and W.M. Williams), CAB International, Wallingford, pages 125–155.

Hay, M. 1983. Seasonal variation in the distribution of white clover (*Trifolium repens* L.) stolons among 3 horizontal strata in 2 grazed swards. *New Zealand Journal of Agricultural Research* **26**: 29–34.

Hay, M., Chapman, D., Hay, R., Pennell, C., Woods, P. and Fletcher, R. 1987 Seasonal variation in the vertical distribution of white clover stolons in grazed swards. *New Zealand Journal of Agricultural Research* **30**: 1–8.

Hennessy, D., O'Donovan, M., French, P. and Laidlaw, A. 2006. Effects of date of autumn closing and timing of winter grazing on herbage production in winter and spring. *Grass and Forage Science* **61**: 363–374.

Hennessy, D., O'Donovan, M., French, P. and Laidlaw, A. 2008. Factors influencing tissue turnover during winter in perennial ryegrass-dominated swards. *Grass and Forage Science* **63**: 202–211.

Hoglind, M. and Frankow-Lindberg, B. 1998. Growing point dynamics and spring growth of white clover in a mixed sward and the effects of nitrogen application. *Grass and Forage Science* **53**: 338–345.

Humphreys, J., Casey, I. and Laidlaw, A. 2009. Comparison of milk production from clover based and fertilizer-N-based grassland on a clay-loam soil under moist temperate climatic conditions. *Irish Journal of Agricultural and Food Research* **48**: 189–207.

Hunt, W.F. and Field, T.R.O. 1979. Growth characteristics of perennial ryegrass. *Proceedings of the New Zealand Grassland Association, Invercargill, New Zealand* **40**: 104–113.

Hurtado-Uria, C., Hennessy, D., Shalloo, L., O'Connor, D. and Delaby, L. 2013. Relationships between meteorological data and grass growth over time in the south of Ireland. *Irish Geography* **46**: 175–201.

Kennedy, E., O'Donovan, M., Murphy, J.P., Delaby, L. and O'Mara, F.P. 2005. Effects of grass pasture and concentrate-based feeding systems for spring-calving dairy cows in early spring on performance during lactation. *Grass and Forage Science* **60**: 310–318.

Kennedy, E., McEvoy, M., Murphy, J.P. and O'Donovan, M. 2009. Effect of restricted access time to pasture on dairy cow milk production, grazing behavior, and dry matter intake. *Journal of Dairy Science* **92**: 168–176. [CrossRef].

Kerr, G., Chapman, D., Thom, E., Matthew, C., Van Der Linden, A., Baird, D., Johnston, E. and Corkran, J. 2012. Evaluating perennial ryegrass cultivars: improving testing. *Proceedings of the New Zealand Grassland Association, Gore, New Zealand, 5–8 November 2012*, pages 127–136.

King, J., Grant, S., Torvell, L. and Sim, E. 1984. Growth rate, senescence and photosynthesis of ryegrass swards cut to maintain a range of values for leaf area index. *Grass and Forage Science* **39**: 371–380.

Korte, C. 1986. Tillering in 'Grasslands Nui'perennial ryegrass swards 2. Seasonal pattern of tillering and age of flowering tillers with two mowing frequencies. *New Zealand Journal of Agricultural Research* **29**: 629–638.

Laidlaw, A. and Mayne, C. 2000. Setting management limits for the production and utilization of herbage for out-of-season grazing. *Grass and Forage Science* **55**: 14–25.

Langer, R.H.M. 1963. Tillering in herbage grasses. A review article. *Herbage Abstracts* **33**: 141–148.

Langer, R.H.M. 1979. "How Grasses Grow". Edward Arnold, London, UK.

Lawrence, D.C. 2015. Development of production guidelines to improve efficiency in autumn calving herds. PhD thesis, University College Dublin.

Lorenz, H., Reinsch, T., Hess, S. and Taube, F. 2019. Is low-input dairy farming more climate friendly? A meta-analysis of the carbon footprints of different production systems. *Journal of Cleaner Production* **211**: 161–170.

Lüscher, A., Stäheli, B., Braun, R. and Nösberger, J. 2001. Leaf area, competition with grass, and clover cultivar: key factors to successful overwintering and fast regrowth of white clover (*Trifolium repens* L.) in spring. *Annals of Botany* **88**: 725–735.

Lüscher, A., Mueller-Harvey, I., Soussana, J.F., Rees, R.M. and Peyraud, J.L. 2014. Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science* **69**: 206–228.

McClearn, B., Gilliland, T.J., Delaby, L., Guy, C., Dineen, M., Coughlan, F. and McCarthy, B. 2019. Milk production per cow and per hectare of spring-calving dairy cows grazing swards differing in *Lolium perenne* L. ploidy and *Trifolium repens* L. composition. *Journal of Dairy Science* **102**: 8571–8585.

O'Brien, D., Capper, J., Garnsworthy, P., Grainger, C. and Shalloo, L. 2014. A case study of the carbon footprint of milk from high-performing confinement and grass-based dairy farms. *Journal of Dairy Science* **97**: 1835–1851.

O'Donovan, M.A. 2000. The relationship between the performance of dairy cows and grassland management on intensive dairy farms in Ireland. PhD thesis, University College Dublin.

O'Donovan, M. and Delaby, L. 2005. A comparison of perennial ryegrass cultivars differing in heading date and grass ploidy with spring calving dairy cows grazed at two different stocking rates. *Animal Research* **54**: 337–350.

O'Donovan, M., Dillon, P., Reid, P., Rath, M. and Stakelum, G. 2002. A note on the effects of herbage mass at closing and autumn closing date on spring grass supply on commercial dairy farms. *Irish Journal of Agricultural and Food Research* **41**: 265–269.

O'Donovan, M., Delaby, L., Stakelum, G. and Dillon, P. 2004. Effect of autumn/spring nitrogen application date and level on dry matter production and nitrogen efficiency in perennial ryegrass swards. *Irish Journal of Agricultural and Food Research* **43**: 31–41.

O'Neill, B.F., Deighton, M.H., O'Loughlin, B.M., Mulligan, F.J., Boland, T.M., O'Donovan, M. and Lewis, E. 2011. Effects of a perennial ryegrass diet or total mixed ration diet offered to spring-calving Holstein-Friesian dairy cows on methane emissions, dry matter intake, and milk production. *Journal of Dairy Science* **94**: 1941–1951.

Orr, R., Cook, J., Young, K., Champion, R. and Rutter, S. 2005. Intake characteristics of perennial ryegrass varieties when grazed by yearling beef cattle under rotational grazing management. *Grass and Forage Science* **60**: 157–167.

Phelan, P., Casey, I. and Humphreys, J. 2014. The effects of simulated summer-to-winter grazing management on herbage production in a grass–clover sward. *Grass and Forage Science* **69**: 251–265.

Pinxterhuis, J.B. 2000. White clover dynamics in New Zealand pastures. PhD thesis, Wageningen Agricultural University, The Netherlands.

Ribeiro Filho, H., Delagarde, R. and Peyraud, J. 2003. Inclusion of white clover in strip-grazed perennial ryegrass swards: herbage intake and milk yield of dairy cows at different ages of sward regrowth. *Animal Science* **77**: 499–510.

Ryan, W., Hennessy, D., Murphy, J.J. and Boland, T.M. 2010. The effects of autumn closing date on sward leaf area index and herbage mass during the winter period. *Grass and Forage Science* **65**: 200–211.

SAS Institute. 2014. SAS User's Guide: Statistics, SAS Institute Inc., Cary, NC, USA.

Sayers, H. and Mayne, C. 2001. Effect of early turnout to grass in spring on dairy cow performance. *Grass and Forage Science* **56**: 259–267.

Shalloo, L. 2009. Pushing the barriers on milk costs/output. *Proceedings of the National Dairy Conference, Westmeath, Ireland*, page 19.

Shalloo, L., Dillon, P., O'Loughlin, J., Rath, M. and Wallace, M. 2004. Comparison of a pasture-based system of milk production on a high rainfall, heavy-clay soil with that on a lower rainfall, free-draining soil. *Grass and Forage Science* **59**: 157–168.

Swift, G., Vipond, J., McClelland, T., Cleland, A., Milne, J. and Hunter, E. 1993. A comparison of diploid and tetraploid perennial ryegrass and tetraploid ryegrass/white clover swards under continuous sheep stocking at controlled sward heights. 1. Sward characteristics. *Grass and Forage Science* **48:** 279–289.

Thomas, H. and Norris, I. 1981. The influence of light and temperature during winter on growth and death in simulated swards of *Lolium perenne*. *Grass and Forage Science* **36**: 107–116.

Tozer, K.N., Chapman, D.F., Bell, N.L., Crush, J.R., King, W.M., Rennie, G.M., Wilson, D.J., Mapp, N.R., Rossi, L., Aalders, L.T. and Cameron, C.A. 2014. Botanical survey of perennial ryegrass-based dairy pastures in three regions of New Zealand: implications for ryegrass persistence. *New Zealand Journal of Agricultural Research* **57**: 14–29.

Vipond, J., Swift, G., Cleland, A., Fitzsimons, J. and Hunter, E. 1997. A comparison of diploid and tetraploid perennial ryegrass and tetraploid ryegrass/white clover swards under continuous sheep stocking at controlled sward heights. 3. Sward characteristics and animal output, years 4–8. *Grass and Forage Science* **52**: 99–109.

Wachendorf, M., Collins, R., Connolly, J., Elgersma, A., Fothergill, M., Frankow-Lindberg, B., Ghesquiere, A., Guckert, A., Guinchard, M. and Helgadottir, A. 2001. Overwintering of *Trifolium repens* L. and succeeding growth: results from a common protocol carried out at twelve European sites. *Annals of Botany* **88**: 669–682.

Wims, C.M., McEvoy, M., Delaby, L., Boland, T.M., and O'Donovan, M. 2013. Effect of perennial ryegrass (*Lolium perenne* L.) cultivars on the milk yield of grazing dairy cows. *Animal* **7**: 410–421.

Woledge, J., Ubbi, A. and Tewson, V. 1990. Photosynthesis of white clover petioles. *Photosynthetica* **24**: 56–62.