

Crops and Soils Research  
Paper

**Cite this article:** Guy C, Hennessy D, Gilliland T J, Coughlan F, McCarthy B (2018). Growth, morphology and biological nitrogen fixation potential of perennial ryegrass-white clover swards throughout the grazing season. *The Journal of Agricultural Science* **156**, 188–199. <https://doi.org/10.1017/S0021859618000199>

Received: 11 October 2017

Revised: 8 February 2018

Accepted: 21 February 2018

First published online: 5 April 2018

**Key words:**

Herbage DM production; herbage nutritive value; *Lolium perenne* L.; perennial ryegrass ploidy; stolon morphology; *Trifolium repens* L.

**Author for correspondence:**

C. Guy, E-mail: [clare.guy@teagasc.ie](mailto:clare.guy@teagasc.ie)

## Growth, morphology and biological nitrogen fixation potential of perennial ryegrass-white clover swards throughout the grazing season

C. Guy<sup>1,2</sup>, D. Hennessy<sup>1</sup>, T. J. Gilliland<sup>2,3</sup>, F. Coughlan<sup>1</sup> and B. McCarthy<sup>1</sup>

<sup>1</sup>Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland; <sup>2</sup>The Institute for Global Food Security, Queen's University Belfast, Belfast, Ireland and <sup>3</sup>Agri-food and Biosciences Institute, Large Park, Hillsborough, BT26 6DR, Ireland

**Abstract**

Sustainable ruminant production systems depend on the ability of livestock to utilize increased quantities of grazed herbage. The current study aimed to compare the effect of white clover (WC) inclusion and perennial ryegrass (PRG) ploidy on herbage dry matter (DM) production, plant morphology, nutritive value and biological nitrogen (N) fixation (BNF) under high N fertilizer use (250 kg N/ha) and high stocking rates (2.75 livestock units/ha). Four sward treatments (diploid-only, tetraploid-only, diploid-WC, tetraploid-WC) were evaluated over a full grazing season at a farmlet scale. White clover inclusion had a significant effect on herbage DM production, herbage growth rate, tiller density, organic matter digestibility, crude protein and BNF. Tetraploid swards had a lower tiller density, lower sward WC content and post-grazing sward height and increased organic matter digestibility and crude protein than diploid swards. White clover inclusion improved herbage DM production and nutritive value across a full grazing season, with tetraploid and diploid swards producing similar herbage DM yields across the year. Perennial ryegrass ploidy had an effect on WC morphology as plants in diploid-WC swards had narrower, longer stolons, fewer branches and more petioles than tetraploid-WC swards. The current study highlights the benefit of including WC in grass-based systems under a high N fertilizer regime and high stocking rate.

**Introduction**

Agriculture in the 21st century faces multiple challenges, with a requirement to feed an ever-increasing population, produce food more efficiently and adapt to climate change (Godfray *et al.* 2010). In temperate regions worldwide, grass-based livestock production systems can improve the efficiency and sustainability of agriculture through lower input costs (Shalloo *et al.* 2004) and reduced environmental pressure (Bleken *et al.* 2005; Arsenault *et al.* 2009), when compared with confinement systems, thereby overcoming some of these challenges.

Perennial ryegrass (*Lolium perenne* L.: PRG) dominates temperate grass-based systems and provides high nutritive value feed for livestock while maintaining a high regrowth capacity and good grazing persistence (Wilkins & Humphreys 2003). In grass-based systems, achieving high herbage dry matter (DM) production and high herbage nutritive value throughout an extended grazing season is important. Perennial ryegrass can maintain high herbage DM production in nitrogen (N)-fertilized grass-based systems: however, the nutritive value of grass-only (GO) swards declines as the grazing season progresses (Enriquez-Hidalgo *et al.* 2016). The inclusion of white clover (*Trifolium repens* L.: WC) in PRG swards can increase herbage growth rates (Frame & Newbould 1986), maintain a high nutritive value (Beever *et al.* 1986) and improve animal intake (Ribeiro Filho *et al.* 2005) throughout the grazing season (Frame & Newbould 1986; Ledgard & Steele 1992). However, many studies suggest that WC in grass-clover (GC) swards does not persist under high N fertilizer levels and high stocking rates (Frame & Newbould 1986). At the fertilizer levels required to maintain high herbage DM production under intensive grazing, there is little information on the interaction between high N fertilizer use (>200 kg N/ha) and high stocking rates (>2.5 livestock units (LU)/ha) on WC persistence and plant morphology.

The rise of inorganic N fertilizer prices (Prince *et al.* 2009) and increased environmental legislation may lead to an increase in the use of GC swards on-farm to provide N from biological N fixation (BNF), which can introduce an additional 100–300 kg N/ha/year, depending on soil type, inorganic N fertilizer and sward WC content (Carlsson & Huss-Danell 2003; Lüscher *et al.* 2014). Studies examining the environmental impacts of combining high levels of N fertilizer and WC show that, at similar N inputs, GC swards can be more energy efficient than N-fertilized GO swards and that nutrient losses to ground water are similar for both sward types (Ledgard *et al.* 2009).

Previous studies on WC inclusion in grass-based systems have concentrated on agronomic aspects (Frame & Newbould 1986; Baker & Williams 1987), animal nutritional aspects (Thomson 1984; Beever & Thorp 1996) and environmental aspects (Jarvis 1992). Whole-farm grazing system studies have been undertaken (Humphreys *et al.* 2009; Enriquez-Hidalgo 2014; Egan *et al.* 2018); however, few have looked at the detailed sward level interaction between PRG and WC. This has left a knowledge gap on how to manage WC plants to withstand grazing system stresses (grazing and poaching damage) and identify the morphological characteristics that contribute to herbage growth and plant survival under high N fertilizer use (>200 kg N/ha) and high stocking rates (>2.5 LU/ha). The objective of the current study was to investigate herbage DM production, plant morphology, herbage nutritive value and N fixation rates of GO and GC swards, and tetraploid and diploid swards, over a full grazing season under these conditions, at a farmlet scale.

## Materials and methods

### Site

The study was conducted during the 2015 grazing season at Teagasc, Clonakilty Agricultural College, Co. Cork, Ireland (Latitude: 51°63'N; Longitude: -08°85'E; 25–70 m asl), on a free-draining acid brown earth soil of light loam to gley loam in texture.

### Experimental design

The swards used were a component of a larger farm-systems study (43.6 ha) that commenced in 2014; 0.75 of the farm area was reseeded in 2012 and the remaining 0.25 was reseeded in 2013. The larger study consisted of four sward treatments: diploid-only (DO); tetraploid-only (TO); diploid-WC (DC); tetraploid-WC (TC). Four diploid cultivars (sown at 30 kg/ha) and four tetraploid cultivars (sown at 37.5 kg/ha) were sown as monocultures in five paddocks each around the farm. This was repeated using the same diploid and tetraploid cultivars and also included medium-leaved WC cultivars (0.50 Chieftain, 0.50 Crusader; sown at 5 kg/ha). This resulted in a total of 80 paddocks. Thus, 20 blocks (balanced for location, soil type and soil fertility) of four paddocks each were created and each treatment was assigned randomly to one of the four paddocks within each block. For the purpose of the current study, a sub-set of three of the 20 blocks were used, giving three experimental paddocks per grazing treatment (DO, TO, DC, TC). The blocks consisted of three tetraploid cultivars (Tymax, AstonEnergy, Kintyre) and two diploid cultivars (Glenveagh, Aberchoice).

Treatments were grazed rotationally ten and 11 times for GO and GC swards, respectively, in 2015 with 30 dairy cows per treatment (2.75 LU/ha). Daily herbage allowance was 17 kg DM/cow/day (>4 cm) and concentrate supplementation was 300 kg/cow/year. All sward treatments were managed similarly in terms of grazing management and average farm cover targets (O'Donovan 2000) using a decision support tool, PastureBase Ireland (Hanrahan *et al.* 2017), which produced a weekly grass wedge, a visual aid to assess herbage DM supply to facilitate management decisions on-farm. Grazing commenced between 4 February 2015 and 27 March 2015 for each of the study paddocks and ceased between 29 September and 16 November 2015. Target pre-grazing herbage mass was 1300–1600 kg DM/ha (>4 cm) and the target post-grazing sward height was ≤4 cm for rotations 1–3 (February to end April) and 4 cm for the remainder of the study period.

Rotation length targets in the May–July period were 18–21 days. All paddocks were mechanically harvested at least once during the previous year; however, study paddocks were grazed exclusively for the 2015 grazing season with no silage harvesting or mechanical topping of swards occurring. All paddocks received 250, 8, 47 kg/ha/year of N, phosphorus (P) and potassium (K), respectively (Table 1). Nitrogen application was in the form of urea (46% N) early in the grazing season (rotations 1 + 2; mid-January to March) and calcium ammonium nitrate (CAN; 27% N) was applied in subsequent rotations (April to mid-September). All sward treatments received 7.05 m<sup>3</sup>/ha of cattle slurry applied in spring and summer.

### Herbage dry matter production, grazing efficiency and nutritive value

#### Pre-grazing herbage mass and sward density

Pre-grazing herbage mass was calculated prior to each grazing from two strips harvested in each paddock to 4 cm (1.2 m wide; 10 m long), as per Creighton *et al.* (2012), using an Etesia mower (Etesia UK Ltd., Warwick, UK). Harvested herbage was collected and weighed, and a sub-sample collected for DM content determination. Dry matter content was calculated by drying a 100 g sub-sample in an oven at 90 °C for 15 h. To calculate sward density, ten sward height measurements were recorded along the cut area immediately pre-harvest and post-harvest using a Jenquip rising plate meter (Castle 1976; Jenquip 2016). Sward density was calculated using these measurements according to the following equation:

$$\text{Sward density (kg DM/cm)} = \frac{\text{herbage mass}/(\text{pre-cutting height} - \text{post-cutting height})}{\text{pre-cutting height} - \text{post-cutting height}}$$

Herbage mass above 4 cm was calculated using sward density according to the following equation:

$$\text{Herbage mass above 4 cm (kg DM/ha)} = [\text{pre-grazing sward height (cm)} - 4 \text{ cm}] \times \text{sward density}$$

Pre-grazing and post-grazing sward heights (cm) were calculated by measuring compressed sward height at 50 locations within each paddock at each grazing using the plate meter described above.

**Table 1.** Nitrogen (N), Phosphorus (P) and Potassium (K) application rates for the study paddocks in 2015

Date	Fertilizer compound	Units	N	P	K
28 January 2015	Urea	kg/ha	28	0	0
20 March 2015	Urea + 0:10:20	kg/ha	33	8	16
28 April 2015	CAN <sup>1</sup>	kg/ha	33	0	0
29 April 2015	Muriate of Potash	kg/ha	0	0	31
20 May 2015	CAN	kg/ha	33	0	0
10 July 2015	CAN	kg/ha	16	0	0
27 July 2015	CAN	kg/ha	16	0	0
04 August 2015	CAN	kg/ha	33	0	0
18 August 2015	CAN	kg/ha	17	0	0
31 August 2015	CAN	kg/ha	42	0	0

CAN<sup>1</sup> = calcium ammonium nitrate (CAN; 27% N).

### Grazing efficiency

Grazing efficiency was calculated using the method of Delaby *et al.* (1998) and McCarthy *et al.* (2013), whereby grazing efficiency = total herbage removed/pre-grazing herbage mass (>4 cm).

Total herbage removed (kg DM/ha) = (pre-grazing sward height (cm) – post-grazing sward height (cm)) × sward density.

Grazing efficiency is an estimation of how efficiently the sward was grazed relative to herbage removed from the sward and pre-grazing herbage mass available. A higher value indicates more herbage was removed relative to the herbage available (i.e. herbage mass >4 cm) for grazing.

Daily herbage growth rate (kg DM/ha/day) was calculated by dividing the herbage mass on a given measurement date by the number of days between that measurement date and the previous grazing date.

### Herbage nutritive value

Herbage sub-samples, collected when measuring herbage mass at each grazing event, were freeze-dried and milled through a 1 mm sieve. Sub-samples were selected from each treatment and each cultivar within treatment at four time-points (February–March, mid-May to mid-June, mid-June to mid-July, September) across the year. Samples were analysed for DM, ash concentrations, acid detergent fibre (ADF), neutral detergent fibre (NDF) (Van Soest 1963), crude protein (CP) (AOAC 1990) and organic matter digestibility (OMD) (Morgan *et al.* 1989).

### Sward composition and dynamics

#### Sward white clover content

Sward WC content in each GC treatment was determined at each grazing by taking 15 random samples in a 'W' formation across each paddock at 4 cm above ground level using Gardena (Accu 60, Gardena International GmbH, Ulm, Germany) hand shears. Two 70 g samples were separated into PRG (and other grass or weed species) and WC components, dried at 60 °C for 24 h and the proportion of each component of the sward calculated on a DM basis.

#### Tiller density and stolon mass

Twenty turves (10 × 10 cm<sup>2</sup>) were removed from each paddock five times during the year (dates: 2 February, 6 April, 3 June, 3 August, 1 October) to estimate PRG tiller density and WC stolon mass (tillers/m<sup>2</sup> and g/m<sup>2</sup>, respectively) as described by Evans *et al.* (1998). The botanical composition was determined from these turves by counting PRG tillers and other grass species. White clover stolons were separated from individual turves, the roots and leaves removed, and gently washed to remove excess soil. Stolons were dried at 40 °C for 48 h to estimate DM content.

#### Stolon morphology

Fifteen WC stolons were marked along three random 1 m transects using coloured wire in each GC treatment. Five stolons were marked per transect, and markers were placed behind the node bearing the oldest leaf present on the stolon, as per Pinxterhuis (2000). Stolons were measured at each grazing for main stolon length (cm), number of nodes on main and branch stolons, and number of branches on the main stolon at each rotation. The presence or absence of leaf, petiole, root and branch was noted for each

node. The rate of node production on the main stolon was calculated. Stolon width (cm) was measured using digital callipers.

#### Leaf, stem and dead proportions

Four samples (approximately 80 g in total) were cut at random using a scalpel to ground level in each paddock pre-grazing. From each sample, 40 g were weighed and separated into two portions: above and below 4 cm. The portion below 4 cm was discarded and that above 4 cm was separated into leaf, stem and dead material, and the proportion of each component of the sward calculated on a DM basis.

#### Leaf area

Leaf area (cm<sup>2</sup>) was calculated for 50 randomly selected leaves in each paddock pre-grazing using a portable laser leaf area meter (CI-202, CID Bio-Science, Washington, USA).

#### Tiller weight

Individual tiller weights (g) were calculated for 50 tillers in each paddock pre-grazing, as described by Enriquez-Hidalgo (2014).

### Biological nitrogen fixation

Biological N fixation was estimated using the N difference method (Munro & Davies 1974). This method estimates BNF by calculating the difference between N yields of GC and GO swards within the same block:

Quantity of N fixed (kg N/ha) = Total N yield in GC swards – Total N yield in GO swards.

### Statistical analyses

Analyses were undertaken on all variables using the mixed model procedure (PROC MIXED) in the statistical package SAS 9.3 (SAS 2011). Fixed effects included in the model were PRG ploidy, WC inclusion, rotation, PRG ploidy × WC inclusion interaction, PRG ploidy × rotation interaction and WC inclusion × rotation interaction, with block as a random effect. Grazing characteristics were analysed using the fixed effect rotation. Time period and date were used when analysing herbage nutritive values and tiller density and stolon mass measurements, respectively, in place of rotation. Differences were considered significant if  $P < 0.05$ . There was no significant effect of PRG ploidy × WC inclusion × rotation and so this interaction has been omitted from the text.

## Results

### Herbage dry matter production, herbage removed and grazing efficiency

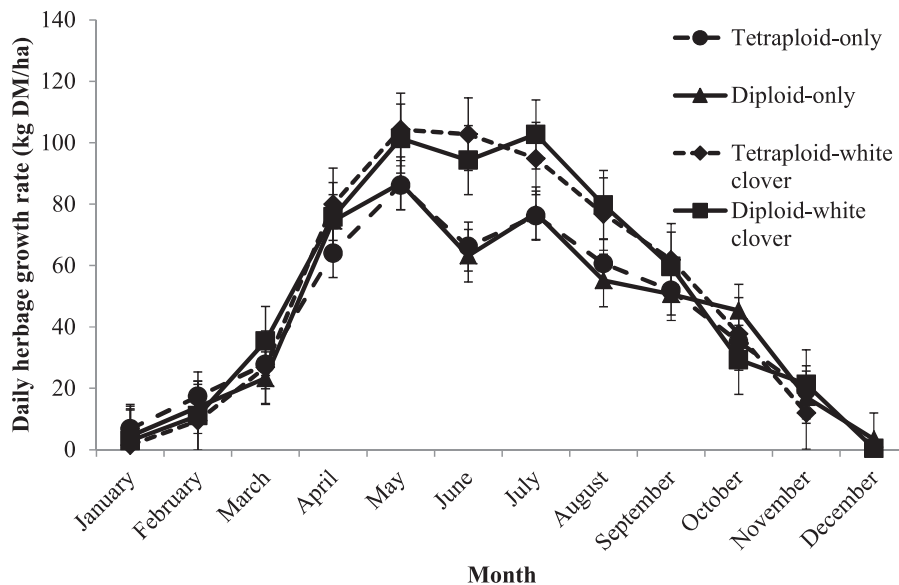
Mean pre-grazing herbage mass did not differ between diploid (DO, DC) and tetraploid (TO, TC) swards (Table 2; diploid: 1600 ± 64.4 kg DM/ha; tetraploid: 1538 ± 63.8 kg DM/ha). The inclusion of WC reduced mean pre-grazing herbage mass on GC (DC, TC) swards by 212 kg DM/ha compared with GO (DO, TO) swards ( $P < 0.05$ ; GO: 1675 ± 67.4 kg DM/ha; GC: 1463 ± 61.2 kg DM/ha). Annual herbage DM production did not differ between diploid and tetraploid swards (Table 2; diploid: 17 377 ± 375.8 kg DM/ha; tetraploid: 17 155 ± 375.8 kg DM/ha) but including WC increased herbage DM production by more than 2500 kg DM/ha (GO: 16 013 ± 375.8 kg DM/ha; GC: 18

**Table 2.** Grazing characteristics and herbage nutritive value of each sward type

Grazing characteristics	DO	TO	DC	TC	S.E.D.	Ploidy (P)	Clover (C)	Time <sup>a</sup>	P × C	P × time	C × time
Cumulative production (kg DM/ha)	16 325	15 694	18 423	18 610	531.5	NS	0.01	n/a	NS	n/a	n/a
Herbage removed (kg DM/ha)	12 892	14 345	15 996	15 673	1393.3	NS	NS	n/a	NS	n/a	n/a
Sward WC content (%)	n/a	n/a	29	21	2.6	0.05	n/a	0.05		NS	n/a
Pre-grazing herbage mass (kg DM/ha)	1681	1669	1519	1407	86.3	NS	0.05	0.001	NS	0.001	0.001
Post-grazing sward height (cm)	4.31	3.91	4.10	3.68	0.129	0.001	0.05	0.001	NS	0.001	0.01
Number of grazings	10	10	11	11	.	.	.	.	.	.	.
Growth rate (kg DM/ha)	49.9	50.4	60.9	60.9	2.05	NS	0.001	0.001	NS	NS	NS
Grazing efficiency	0.97	1.03	1.01	1.14	0.076	NS	NS	0.001	NS	0.05	0.01
Herbage nutritive value											
Crude protein (g/kg DM)	188	191	229	254	5.5	0.05	0.001	0.001	0.05	NS	NS
Organic matter digestibility (g/kg DM)	781	796	785	810	7.0	0.01	NS	0.001	NS	NS	0.001
Neutral detergent fibre (g/kg DM)	432	400	381	354	7.6	0.001	0.001	0.001	NS	NS	NS
Acid detergent fibre (g/kg DM)	253	241	252	208	5.2	0.001	0.01	0.001	0.01	NS	NS
Ash (g/kg DM)	119	114	131	111	5.4	0.05	NS	0.001	NS	NS	NS

Data are presented as least-squares means. DO, diploid-only, TO, tetraploid-only, DC, diploid-white clover, TC, tetraploid-white clover; S.E.D., standard error of the difference between means; NS, not significant:  $P > 0.05$ ; n/a, not applicable.

<sup>a</sup>Time = rotation is used for grazing characteristics, and period is used for herbage nutritive values: (1) February–March, (2) Mid May–mid June, (3) Mid June–mid July or (4) September.



**Fig. 1.** Mean daily herbage growth rates (kg DM/ha) from 1 January to 31 December 2015 for diploid-only (DO), tetraploid-only (TO), diploid-white clover (DC) and tetraploid-white clover (TC) swards.

519 ± 375.8 kg DM/ha). An extra 95 kg DM/ha was produced for every 1% increase in sward WC content ( $R^2 = 0.696$ ;  $P < 0.001$ ). The grazing efficiency of each sward (herbage utilized as a proportion of the pre-grazing herbage mass) is presented in Table 2. Although non-significant, tetraploid and GC swards maintained a higher grazing efficiency value than diploid and GO swards. There were significant effects of PRG ploidy ( $P < 0.001$ ), WC inclusion ( $P < 0.01$ ) and rotation ( $P < 0.001$ ) on post-grazing sward height throughout the grazing season (Table 2). Tetraploid swards had a lower post-grazing sward height than diploid swards ( $P < 0.001$ ; tetraploid: 3.79 cm; diploid: 4.20 cm) and GC swards had a lower post-grazing sward height when compared with GO swards ( $P < 0.01$ ; GO: 4.11 cm; GC: 3.89 cm).

### Growth rates

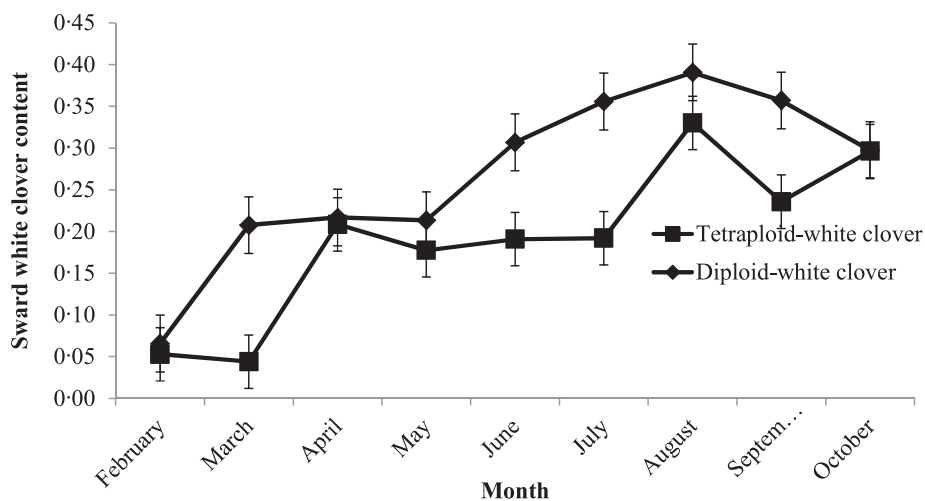
There were no significant differences in mean daily herbage growth rates between diploid and tetraploid swards. Mean daily herbage growth rates for GC swards were significantly greater than GO swards ( $P < 0.001$ ; Figure 1; DO: 50 ± 2.1 kg DM/ha/day; TO: 50 ± 2.1 kg DM/ha/day; DC: 61 ± 2.0 kg DM/ha/day; TC: 61 ± 2.1 kg DM/ha/day).

### Sward composition and dynamics

Mean sward WC content differed between PRG ploidy ( $P < 0.05$ ; DC: 0.29 ± 0.026; TC: 0.21 ± 0.026). White clover content in both swards was low at the beginning of the grazing season (February; 0.06) increasing to a peak of 0.39 (±0.072) and 0.33 (±0.072) in August in DC and TC swards, respectively (Fig. 2).

Stolons in DC swards were 0.58 cm longer than in TC swards (Table 3;  $P < 0.01$ ; TC: 4.33 cm; DC: 4.91 cm) and as the grazing season progressed, stolons elongated significantly more in DC swards (Fig. 3;  $P < 0.001$ ; TC: 3.18 cm; DC: 3.47 cm). Stolons in TC swards were approximately 0.53 mm wider than stolons in DC swards ( $P < 0.05$ ; TC: 2.88 mm; DC: 2.35 mm) and had more branches than in DC swards (Fig. 4;  $P < 0.01$ ; TC: 1.9 ± 0.52; DC: 1.0 ± 0.51). Stolons in DC swards had 0.14 more petioles than in TC swards ( $P < 0.05$ ; TC: 3.4 ± 0.59; DC: 4.0 ± 0.59). White clover petioles in DC swards were 0.94 cm longer than in TC swards ( $P < 0.05$ ; TC: 5 ± 1.1 cm; DC: 6 ± 1.1 cm).

Tiller density and stolon mass varied with PRG ploidy ( $P < 0.001$ ), WC inclusion ( $P < 0.001$ ) and between measurement dates ( $P \leq 0.001$ ; Table 3). Diploid swards maintained a consistently higher tiller density (+1225 tillers/m<sup>2</sup>) compared with



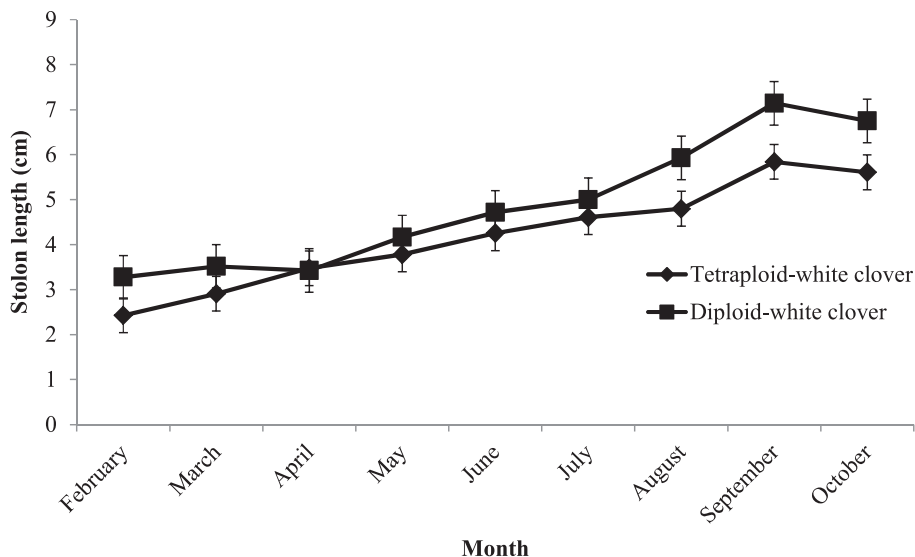
**Fig. 2.** Monthly sward white clover content between 1 February 2015 and 31 November 2015 for grass-clover swards.

**Table 3.** Perennial ryegrass tiller density and morphology, and white clover stolon mass and morphology for each sward type

<i>Tiller density (tillers/m<sup>2</sup>)</i>	DO	TO	DC	TC	S.E.D.	Ploidy (P)	Clover (C)	P × C	Time <sup>a</sup>	P × time	C × time
Average	6952	4631	4206	4079	351.1	0.001	0.001	0.001	0.001	NS	0.05
February	7447	4683	4753	3939	565.4	0.001	0.001	0.001	0.001	NS	0.05
April	8950	5608	4684	3958	565.4	0.001	0.001	0.001	0.001	NS	0.05
June	5346	4093	3517	3908	565.4	0.001	0.001	0.001	0.001	NS	0.05
August	6647	4340	4675	4691	565.4	0.001	0.001	0.001	0.001	NS	0.05
October	6372	4430	3401	3900	565.4	0.001	0.001	0.001	0.001	NS	0.05
<i>Grass morphology</i>											
Leaf proportion	0.66	0.70	0.56	0.65	0.025	0.01	0.01	NS	0.001	0.001	0.001
Pseudo-stem proportion	0.17	0.14	0.22	0.17	0.012	0.01	0.001	NS	0.001	NS	NS
True-stem proportion	0.05	0.06	0.02	0.07	0.021	NS	0.05	NS	0.001	NS	0.05
Dead proportion	0.13	0.10	0.13	0.10	0.020	0.05	NS	NS	NS	NS	NS
Leaf area (cm <sup>2</sup> )	3.24	4.27	3.37	3.79	0.346	0.05	NS	NS	0.001	NS	NS
Tiller weight (g)	0.32	0.41	0.37	0.44	0.031	0.01	NS	NS	0.001	NS	NS
<i>Stolon mass (g/m<sup>2</sup>)</i>											
Average	.	.	85.4	70.3	12.32	NS	n/a	n/a	0.05	NS	n/a
February	.	.	58.2	61.0	19.01	NS	n/a	n/a	0.05	NS	n/a
April	.	.	105.2	120.7	19.01	NS	n/a	n/a	0.05	NS	n/a
June	.	.	89.9	57.6	19.01	NS	n/a	n/a	0.05	NS	n/a
August	.	.	80.8	45.3	19.01	NS	n/a	n/a	0.05	NS	n/a
October	.	.	92.9	66.9	19.01	NS	n/a	n/a	0.05	NS	n/a
<i>Stolon morphology</i>											
Stolon length (cm)	.	.	4.91	4.33	0.317	0.01	n/a	n/a	0.001	NS	n/a
Stolon width (mm)	.	.	2.35	2.88	0.234	0.05	n/a	n/a	NS	NS	n/a
Stolon branching	.	.	1.01	1.86	0.565	0.01	n/a	n/a	NS	NS	n/a
Stolon petiole length (cm)	.	.	5.73	4.79	0.535	0.05	n/a	n/a	NS	NS	n/a

Data are presented as least-squares means. DO, diploid-only, TO, tetraploid-only, DC, diploid-white clover, TC, tetraploid-white clover; S.E.D., standard error of the difference between means; NS, not significant:  $P > 0.05$ ; n/a, not applicable.

<sup>a</sup>Time = date is used for tiller density and stolon mass, and rotation is used for grass morphology and stolon morphology.



**Fig. 3.** Average stolon length (cm) during each rotation of grazing between 1 February 2015 and 31 November 2015 for grass-clover swards (diploid-white clover (DC) and tetraploid-white clover (TC) swards).

tetraploid swards (diploid:  $5579 \pm 314.2$  tillers/m<sup>2</sup>; tetraploid:  $4354 \pm 314.2$  tillers/m<sup>2</sup>) and the inclusion of WC reduced tiller density by 1649 tillers/m<sup>2</sup> (GO:  $5792 \pm 314.2$  tillers/m<sup>2</sup>; GC:  $4143 \pm 314.2$  tillers/m<sup>2</sup>). Tiller density decreased by 58 tillers/m<sup>2</sup> ( $R^2 = 0.408$ ) with every 1% increase in sward WC content. There was no difference in stolon mass between DC and TC swards (Table 3; TC:  $70 \pm 12.3$  g/m<sup>2</sup>; DC:  $85 \pm 12.3$  g/m<sup>2</sup>). Overall, stolon mass decreased by 25% across the grazing season ( $P < 0.05$ ).

Tetraploid swards had a greater leaf proportion (Table 3;  $P < 0.01$ ; tetraploid:  $0.71 \pm 0.025$ ; diploid:  $0.61 \pm 0.025$ ) and less dead ( $P < 0.05$ ; tetraploid:  $0.10 \pm 0.020$ ; diploid:  $0.13 \pm 0.020$ ) and pseudo-stem ( $P < 0.01$ ; tetraploid:  $0.16 \pm 0.012$ ; diploid:  $0.20 \pm 0.012$ ) proportions than diploid swards. Sward leaf content was less ( $P < 0.01$ ; GO:  $0.68 \pm 0.025$ ; GC:  $0.61 \pm 0.025$ ) and pseudo-stem ( $P < 0.001$ ; GO:  $0.16 \pm 0.012$ ; GC:  $0.20 \pm 0.012$ ) and true-stem ( $P < 0.01$ ; GO:  $0.06 \pm 0.021$ ; GC:  $0.05 \pm 0.021$ ) proportions greater in GC swards than GO swards.

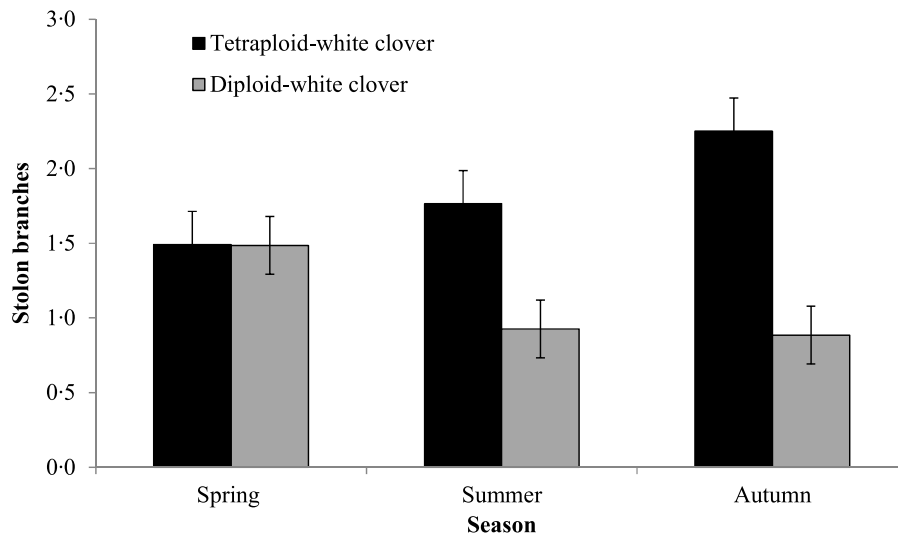
Perennial ryegrass leaves in tetraploid swards were larger than in diploid swards ( $P < 0.05$ ; tetraploid:  $4.0 \pm 0.35$  cm<sup>2</sup>; diploid:  $3.3 \pm 0.35$  cm<sup>2</sup>) and total leaf area increased in all sward treatments as the grazing season progressed ( $P < 0.001$ ; Table 3). White clover

inclusion did not affect leaf area (GO:  $3.8 \pm 0.35$  cm<sup>2</sup>; GC:  $3.6 \pm 0.35$  cm<sup>2</sup>).

Perennial ryegrass tillers in tetraploid swards were heavier than PRG tillers in diploid swards ( $P < 0.01$ ; Table 3; tetraploid: 0.42 g; diploid: 0.35 g) and tiller weight increased in all sward treatments as the grazing season progressed ( $P < 0.001$ ). However, tiller weight in GC swards was similar to GO swards (GO: 0.36 g; GC: 0.41 g).

#### Biological nitrogen fixation and herbage nutritive value

Grass-clover swards fixed 151 kg N/ha/year when compared with GO swards ( $P < 0.05$ ). Significant seasonal changes in BNF rates were observed ( $P < 0.001$ ), as rates in spring were very low and were greatest in August. All the measured herbage nutritive value characteristics differed significantly when compared between PRG ploidies ( $P < 0.05$ ; Table 2). Greater OMD ( $P < 0.01$ ; +21 g/kg DM) and CP ( $P < 0.05$ ; +14 g/kg DM) was recorded in tetraploid swards than in diploid swards throughout the grazing season. Structural fibres (NDF and ADF) were significantly lower in tetraploid swards than in diploid swards



**Fig. 4.** Average number of stolon branches during Spring, Summer and Autumn (1 February 2015 and 31 November 2015) for grass-clover swards (diploid-white clover (DC) and tetraploid-white clover (TC) swards).

( $P = 0.001$ ; NDF:  $-29$  g/kg DM;  $P < 0.001$ ; ADF:  $-27$  g/kg DM). White clover had a significant effect on all herbage nutritive value characteristics with the exceptions of OMD and ash. Structural fibres (NDF and ADF) were lower in GC swards than in GO swards ( $P < 0.001$ ; NDF:  $367$  v.  $416$  g/kg DM;  $P < 0.01$ ; ADF:  $230$  v.  $247$  g/kg DM). Examining nutritive value changes on a seasonal basis, GC swards maintained consistently greater CP values than GO swards throughout the grazing season ( $P < 0.001$ ;  $241$  v.  $189$  g/kg DM). Organic matter digestibility decreased to the same extent in both GO and GC sward types until late summer, after which the GC swards maintained a more uniform OMD content than GO swards (September:  $801$  v.  $787$  g/kg DM). Organic matter digestibility and CP declined significantly from spring to autumn; OMD declined from  $832$  to  $756$  g/kg and CP values declined from  $241$  to  $193$  g/kg DM, respectively. Structural fibres (NDF and ADF) increased throughout the grazing season; NDF and ADF values increased from  $371$  to  $431$  and  $224$  to  $266$  g/kg DM ( $P < 0.001$ ) from spring to autumn, respectively.

## Discussion

Perennial ryegrass swards in Ireland can produce in excess of  $16\ 000$  kg DM/ha (Brereton 1995; O'Donovan *et al.* 2011), with the national average on Irish dairy farms estimated at  $7000$ – $8000$  kg DM/ha (Creighton *et al.* 2011). Swards in the current study produced an average of  $17\ 266$  kg DM/ha, with GO swards producing  $16\ 013$  kg DM/ha and GC swards producing  $18\ 519$  kg DM/ha. These high DM production yields were achieved by a combination of high N fertilizer use and the grazing management used which ensured a pre-grazing herbage mass ( $>4$  cm height) of  $1300$ – $1600$  kg DM/ha/rotation.

Additional herbage DM production on GC swards was a result of the greater herbage growth rates compared with GO swards from May until October, when sward WC content reached  $0.20$ . Above a sward WC content of  $0.20$ , herbage DM production increases (Thomas 1973; Ribeiro Filho *et al.* 2003, 2005) and animal performance improves (Harris *et al.* 1997). Soil temperatures reached  $16.2$  °C in July, leading to sward WC content peaking at  $0.45$  and  $0.35$  in DC and TC swards, respectively. Prior to this, both GO and GC swards maintained similar herbage growth rates under similar management practices. In GC swards, high N fertilizer use can reduce sward WC content and WC contribution to herbage growth rate and DM production (Frame & Newbould 1986; Carlsson & Huss-Danell 2003; Lüscher *et al.* 2014). Previous reductions of  $27$ – $41\%$  sward WC content have been reported when a range of  $180$ – $200$  kg N/ha has been used (Elgersma *et al.* 2000; Ledgard 2001; Enriquez-Hidalgo *et al.* 2016); however, increased herbage growth rates were maintained with high N fertilizer use in the current study, similar to Enriquez-Hidalgo *et al.* (2016). This increased the herbage DM production difference between GC and GO swards receiving the same high N fertilizer levels. Increased stocking rates (and the resulting frequent, tight grazing) at high N levels has been shown to minimize the negative effects of increased grass growth on sward WC content (Harris & Clark 1996) and the high stocking rate which led to frequent, tight grazing in the current study would have led to increased light penetration to the sward base, favouring WC growth. The combination of high N fertilizer use and high stocking rates also maintained herbage DM production in GO swards.

Perennial ryegrass ploidy can limit sward WC content as previous research suggests that tetraploid swards can result in greater

sward WC content compared with diploid swards due to the more open structure of tetraploid swards (Gooding *et al.* 1996; Stewart & Hayes 2011). However, in the current study, DC swards achieved higher sward WC contents than TC swards ( $0.29$  v.  $0.21$ ). Both DC and TC swards were grazed to a similar post-grazing sward height and managed similarly during the grazing season, reducing competition for light and removing any PRG ploidy sward canopy structure effects on sward WC content. However, PRG ploidy did not limit WC stolon mass, which can affect above-ground processes and WC persistence (Caradus & Williams 1981; Curl & Wilkins 1982; Hay *et al.* 1987). Stolon mass in the current study was similar for both DC and TC swards and was greater than that reported by Phelan (2013), under limited N fertilizer use, and Egan (2015) under similar grazing managements, at different sites.

Similar to reducing sward WC content, high N fertilizer use can result in stolon mass reduction (Davies 1992); however, in the current study, high stolon mass persisted at high N fertilizer levels, albeit for a single grazing season in young swards. Pinxterhuis (2000) reported that high N fertilizer use ( $100$  kg N/ha) increased stolon elongation in GC swards, increasing the spreading ability of WC and the ability to escape unfavourable grazing conditions. This is thought to be due to an increased internode length which occurs due to increased shading at the node. In the current study, similar to Pinxterhuis (2000), the high N fertilizer use resulted in greater herbage mass, thereby increasing shading at the WC nodes due to increased PRG growth, than would be expected with zero N fertilizer use. This may have increased the spreading ability of WC. White clover plants in DC swards had longer stolons and fewer branches than TC swards which are a possible response to reduced light availability in swards with greater tiller density, similar to Brock *et al.* (1988). Longer petioles allow leaves to be brought up higher into the canopy and thereby intercept more light: DC swards had narrower stolons, with longer and more numerous petioles, thereby potentially allowing for greater light interception by these swards. White clover plants in TC swards had wider stolons, which could result in the allocation of more resources to stolon tissue and fewer resources for the production of above-ground biomass which may have resulted in the shorter and less numerous petioles in TC swards compared with DC swards. Although growing points were not measured in the current experiment, the implication of lower petiole numbers is that WC plants in TC swards had fewer growing points. This can lead to reduced persistence of WC in tetraploid swards over time, as growing points are fundamental to the horizontal expansion of WC in the sward (Laidlaw *et al.* 1992). Despite these morphological differences, cumulative herbage DM production was similar in both GC swards.

Sward structure can affect herbage utilization and grazing efficiency (Barrett *et al.* 2001; Gilliland *et al.* 2002) and, although all sward treatments in the current study were managed similarly, GC swards had lower tiller density than GO swards, allowing for a greater grazing intensity. This was indicated by the lower post-grazing sward height, which can lead to increased DM intake (Harris *et al.* 1997; Cosgrove *et al.* 2006) and in the current study, estimated herbage removed per cow was  $2.8$  kg DM/day greater for the GC swards compared with the GO swards ( $19.9$  v.  $17.1$  kg DM/cow/day). The lower tiller density in GC swards has been shown previously and is due to interspecific competition between PRG and WC plants (Harris 1987; Caradus *et al.* 1996), whereas less intraspecific competition exists in GO swards.



However, despite the reduction in tiller density in GC swards, higher daily herbage growth rates and herbage DM production were maintained in GC swards. This is directly attributed to the WC contribution to the sward in the latter part of the grazing season, which provided additional herbage DM production when compared with GO swards.

The suppression of BNF by high N fertilizer use is widely accepted (Ledgard *et al.* 2001; Carlsson & Huss-Danell 2003; Unkovich 2012). However, the amount of N fixed ultimately depends on several factors, including sward WC content. In the current study, sward WC contents were higher (0.25 sward WC content average in TC and DC swards) than Egan *et al.* (2018; 0.23) and Enriquez-Hidalgo *et al.* (2016; 0.19) at similar N fertilizer use. These high sward WC contents led to an extra 151 kg N fixed per ha. In spring, low levels of BNF were due to the lower herbage DM yields of WC in GC swards (GC: 19.7 kg N/ha; GO: 63.6 kg N/ha). However, as sward WC content increased from May, and soil N mineralization likely increased as temperatures increased, N content in GC swards began to increase (GC: 69 kg N/ha; GO: 53 kg N/ha). Biological N fixation values presented here accounted for herbage growth above 4 cm, and stolons, stubble or root N contents were not taken into account, where 0.25–0.70 of the plant N content occurs (Jorgensen & Ledgard 1997; Høgh-Jensen *et al.* 2004). It is possible that greater BNF values would have been obtained than those recorded in the current study were below-ground structures included.

Grass-clover swards had higher CP and lower fibre contents than GO swards, favourable characteristics for increased DM intake, digestion and utilization (Ayres *et al.* 1998). Crude protein contents in GC swards were 5.3% higher than GO swards, similar to Ribeiro Filho *et al.* (2003) and Enriquez-Hidalgo *et al.* (2014), and OMD increased as sward WC content increased, similar to Leach *et al.* (2000) and Ribeiro Filho *et al.* (2003). Previous investigations into GC cut plots have shown that NDF contents between PRG and WC sward components can vary as much as 171 g/kg DM (Søegaard 1993) and the lower NDF contents are due to lower WC stem proportions compared with GO swards (Ulyatt *et al.* 1977; Buxton 1996). Seasonal variation in the current study was similar to Ulyatt (1980) and Ulyatt *et al.* (2002); fibre digestion rates decline with increased plant maturity (Chaves *et al.* 2006), resulting in variation throughout the year.

Diploid and tetraploid swards achieved similar herbage growth rates throughout the grazing season (diploid: 55.4 kg DM/ha/day; tetraploid: 55.7 kg DM/ha/day), resulting in similar cumulative herbage DM production (diploid: 17 374 kg DM/ha; tetraploid: 17 152 kg DM/ha). Previous reports of PRG ploidy effects on herbage DM production have provided contrasting results. In GO swards, diploid swards were more productive than tetraploid swards in earlier studies: by 139 kg DM/ha/grazing (O'Donovan & Delaby 2005) or 1141 kg DM/ha/year (Balocchi & López 2009). Connolly (2001) reported that plots of tetraploid monocultures could produce greater cumulative herbage DM yields (18 336 v. 17 388 kg DM/ha/year) than diploid monocultures under simulated grazing protocols, indicating that grazing pressure may affect PRG ploidies. However, these studies were undertaken before 2002 and so used different cultivars to the current study. The development of modern tetraploid cultivars has ensured optimum herbage DM production and so differences between PRG ploidies have become minimal. More recent studies also found similar herbage DM yields from diploid and tetraploid cultivars under animal grazing (Cashman

*et al.* 2016). In an evaluation of cultivars on commercial farms under animal grazing, cultivars were found to perform similarly in terms of herbage DM production regardless of PRG ploidy (Byrne *et al.* 2017). In the current study, excess herbage did not accumulate between grazings, and despite sward structural differences between PRG ploidies (Gilliland *et al.* 2002), neither PRG ploidy provided superior herbage DM production under similar grazing managements.

Previous studies have shown tetraploid cultivars to have greater leaf proportions and lower stem proportions (Gowen *et al.* 2003) than diploid cultivars. The higher post-grazing sward height and greater dead proportions in diploid swards in the current study may have led to the accumulation of dead material in subsequent rotations that can affect nutritive value negatively (Fulkerson & Donaghy 2001), leading to loss of herbage DM available through leaf senescence and reduced rates of tillering (Grant *et al.* 1981). In contrast, the lower post-grazing sward height and reduced dead proportions of tetraploid swards in the current study led to superior herbage nutritive value during the grazing season similar to O'Riordan *et al.* (1998) and Wims *et al.* (2013). In addition, previous reports have shown tetraploid swards remain highly digestible (Cashman 2014) and support a higher DM intake (Lantinga & Groot 1996; Gowen *et al.* 2003; Wims *et al.* 2013) throughout the grazing season. The greater leaf proportions in tetraploid swards led to higher CP (+6.2%) and OMD (+2.6%) contents than diploid swards. Tiller density in tetraploid swards was lower than diploid swards and PRG tillers in tetraploid swards had reduced dead proportions, due to a lower post-grazing sward height, and greater leaf area, due to the nature of tetraploid morphology. The potential for increased grazing intensity due to this lower tiller density and post-grazing sward height led to higher levels of sward digestibility and CP as per McCarthy *et al.* (2013). Additionally, sward DM content was lower in tetraploid swards compared with diploid swards, similar to Wims *et al.* (2013) and tetraploid swards had enhanced nutritive value due to an increased ratio of cell contents to cell walls (Smith *et al.* 2001), leading to easier grazing of tetraploid swards. In the current study, tetraploid tillers were 20% heavier than diploid tillers, due to the decreased competition for space and light in the sward as shown previously by Davies & Thomas (1983).

### Practical implications and conclusions

Including WC in PRG swards resulted in an increase in herbage DM production of 2507 kg DM/ha compared with GO swards at 250 kg/ha N and 2.75 LU/ha. However, the current study was conducted for one full grazing season on young swards (2–3 years old) and so may not represent older sward types (+5 years old), as older swards typically have a lower sward WC content and higher soil N. The frequent and tight grazing management (post-grazing sward height  $\leq$  4 cm) used in the current study maintained a sward WC content of  $>$ 0.20 despite high N fertilizer use. Although the GC swards produced an extra 2507 kg DM/ha, herbage CP content was also greater on these sward types, thereby increasing the potential for N to be lost from the system. A reduction in N fertilizer use as per Enriquez-Hidalgo *et al.* (2016) could result in lower CP content and similar herbage DM production and OMD at levels required for animal production. Although tetraploid swards maintained a lower tiller density, sward WC content was higher in diploid swards. This may have been due to the lower tetraploid tiller density promoting a lower post-grazing sward height that may have

removed more WC from TC swards. However, as tetraploid swards had a greater leaf proportion and increased CP and OMD levels than diploid swards, they provided better quality feed at a similar herbage DM production and herbage growth rate.

**Acknowledgements.** The authors wish to gratefully acknowledge the invaluable assistance provided by the farm and technical staff at Teagasc, Clonakilty and Teagasc, Moorepark, Co. Cork, Ireland. This research was funded by the Irish Dairy Levy administered by Dairy Research Ireland. The first author was in receipt of a Teagasc Walsh Fellowship.

## References

- AOAC (1990) *Official Methods of Analysis*, 15th ed. Method 990-03. Volume 1. Arlington, VA, USA: Association of Official Analytical Chemists.
- 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.
- Ayres JF, Nandra KS and Turner AD (1998) A study of the nutritive value of white clover (*Trifolium repens* L.) in relation to different stages of phenological maturity in the primary growth phase in spring. *Grass and Forage Science* **53**, 250–259.
- Baker M and Williams W (1987) *White Clover*. Wallingford, UK: CAB International.
- Balocchi OA and López IF (2009) Herbage production, nutritive value and grazing preference of diploid and tetraploid perennial ryegrass cultivars (*Lolium perenne* L.). *Chilean Journal of Agricultural Research* **69**, 331–339.
- Barrett PD, Laidlaw AS, Mayne CS and Christie H (2001) Pattern of herbage intake rate and bite dimensions of rotationally grazed dairy cows as sward height declines. *Grass and Forage Science* **56**, 362–373.
- Beever DE and Thorp C (1996) Advances in the understanding of factors influencing the nutritive value of legumes. In Younie D (ed.). *Legumes in Sustainable Farming Systems*, British Grassland Society Occasional Symposium no. 30. Reading, UK: British Grassland Society, pp. 194–207.
- Beever DE, Dhanoa MS, Losada HR, Evans RT, Cammell SB and France J (1986) The effect of forage species and stage of harvest on the processes of digestion occurring in the rumen of cattle. *British Journal of Nutrition* **56**, 439–454.
- Bleken MA, Steinshamm 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. J. (1995) Regional and year-to-year variation in yield. In Jeffrey DW, Jones MB and McAdam JH (eds). *Irish Grasslands: Their Biology and Management*, Dublin, Ireland: Dublin Royal Irish Academy, pp. 12–22.
- Brock JL, Hay MJM, Thomas VJ and Sedcole JR (1988) Morphology of white clover (*Trifolium repens* L.) plants in pastures under intensive sheep grazing. *Journal of Agricultural Science, Cambridge* **111**, 273–283.
- Buxton DR (1996) Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Animal Feed Science and Technology* **59**, 37–49.
- Byrne N, Gilliland TJ, McHugh N, Delaby L, Geoghegan A and O'Donovan M (2017) Establishing phenotypic performance of grass varieties on Irish grassland farms. *Journal of Agricultural Science, Cambridge* **155**, 1633–1645.
- Caradus JR and Williams WM (1981) Breeding for improved white clover production in New Zealand hill country. In Wright CE (ed.). *Plant Physiology and Herbage Production*. British Grassland Society Occasional Symposium, Nottingham, UK: Nottingham University, pp. 163–168.
- Caradus JR, Hay RJM and Woodfield DR (1996) The positioning of white clover cultivars in New Zealand. In Woodfield DR (ed.). *White Clover: New Zealand's Competitive Edge*, Agronomy Society of New Zealand Special Publication 11, Grassland Research and Practice Series no. 6. Palmerston North, New Zealand: New Zealand Grassland Association, pp. 45–50.
- Carlsson G and Huss-Danell K (2003) Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil* **253**, 353–372.
- Cashman PA (2014) *Differential productivity and persistency responses to simulated and animal grazing of perennial ryegrass genotypes*. PhD Thesis, Queen's University Belfast.
- Cashman PA, McEvoy M, Gilliland TJ and O'Donovan M (2016) A comparison between cutting and animal grazing for dry-matter yield, quality and tiller density of perennial ryegrass cultivars. *Grass and Forage Science* **71**, 112–122.
- Castle ME (1976) A simple disc instrument for estimating herbage yield. *Grass and Forage Science* **31**, 37–40.
- Chaves AV, Waghorn GC, Brookes IM and Woodfield DR (2006) Effect of maturation and initial harvest dates on the nutritive characteristics of ryegrass (*Lolium perenne* L.). *Animal Feed Science and Technology* **127**, 293–318.
- Connolly V (2001) *Breeding Improved Varieties of Perennial Ryegrass*. End of Project Report. Oak Park, Carlow, Ireland: Teagasc.
- Cosgrove GP, Burke JL, Death AF, Lane GA, Fraser K and Pacheco D (2006) The effect of clover-rich diets on cows in mid lactation: production, behaviour and nutrient use. *Proceedings of the New Zealand Grassland Association* **68**, 267–273.
- Creighton P, Kennedy E, Shalloo L, Boland TM and O'Donovan M (2011) A survey analysis of grassland dairy farming in Ireland, investigating grassland management, technology adoption and sward renewal. *Grass and Forage Science* **66**, 251–264.
- Creighton P, Gilliland TJ, Delaby L, Kennedy E, Boland TM and O'Donovan M (2012) Effect of *Lolium perenne* sward density on productivity under simulated and actual cattle grazing. *Grass and Forage Science* **67**, 526–534.
- Curl ML and Wilkins RJ (1982) Frequency and severity of defoliation of grass and clover by sheep at different stocking rates. *Grass and Forage Science* **37**, 291–297.
- Davies A (1992) White clover. *Biologist* **39**, 129–133.
- Davies A and Thomas H (1983) Rates of leaf and tiller production in young spaced perennial ryegrass plants in relation to soil temperature and solar radiation. *Annals of Botany* **57**, 591–597.
- Delaby L, Peyraud J-L, Bouittier A and Peccatte JR (1998) Effet d'une réduction simultanée de la fertilisation azotée et du chargement sur les performances des vaches laitières et la valorisation du pâturage. *Annales de Zootechnie* **47**, 17–39.
- Egan M (2015) *Strategies to increase white clover in intensive dairy production systems*. PhD Thesis, University College Dublin.
- Egan M, Galvin N and Hennessy D (2018) Incorporating white clover (*Trifolium repens* L.) into perennial ryegrass (*Lolium perenne* L.) swards receiving varying levels of nitrogen fertilizer: effects on milk and herbage production. *Journal of Dairy Science* (in press). doi: 10.3168/jds.2017-13233.
- Elgersma A, Schlepers H and Nassiri M (2000) Interactions between perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) under contrasting nitrogen availability: productivity, seasonal patterns of species composition, N<sup>2</sup> fixation, N transfer and N recovery. *Plant and Soil* **221**, 281–299.
- Enriquez-Hidalgo D (2014) *Strategies to optimise white clover (Trifolium repens L.) content in grass white clover swards to consistently replace inorganic nitrogen in grazing systems*. PhD Thesis, Queen's University Belfast.
- Enriquez-Hidalgo D, Gilliland T, Deighton MH, O'Donovan M and Hennessy D (2014) Milk production and enteric methane emissions by dairy cows grazing fertilized perennial ryegrass pasture with or without inclusion of white clover. *Journal of Dairy Science* **97**, 1400–1412.
- Enriquez-Hidalgo D, Gilliland TJ 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 DR, Williams TA, Jones S and Evans SA (1998) The effect of cutting and intensive grazing managements on sward components of contrasting ryegrass and white clover types when grown in mixtures. *Journal of Agricultural Science, Cambridge* **130**, 317–322.
- Frame J and Newbould P (1986) Agronomy of white clover. *Advances in Agronomy* **40**, 1–88.
- Fulkerson WJ and Donaghy DJ (2001) Plant-soluble carbohydrate reserves and senescence-key criteria for developing an effective grazing management

- system for ryegrass-based pastures: a review. *Australian Journal of Experimental Agriculture* **41**, 261–275.
- Gilliland TJ, Barrett PD, Mann RL, Agnew RL and Fearon AM** (2002) Canopy morphology and nutritional quality traits as potential grazing value indicators for *Lolium perenne* varieties. *Journal of Agricultural Science, Cambridge* **139**, 257–273.
- Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM and Toulmin C** (2010) Food security: the challenge of feeding 9 billion people. *Science* **327**, 812–818.
- Gooding RF, Frame J and Thomas C** (1996) Effects of sward type and rest periods from sheep grazing on white clover presence in perennial ryegrass/white clover associations. *Grass and Forage Science* **51**, 180–189.
- Gowen N, O'Donovan M, Casey I, Rath M, Delaby L and Stakelum G** (2003) The effect of grass cultivars differing in heading date and ploidy on the performance and dry matter intake of spring calving dairy cows at pasture. *Animal Research* **52**, 321–336.
- Grant SA, Barthram GT and Torvell L** (1981) Components of regrowth in grazed and cut *Lolium perenne* swards. *Grass and Forage Science* **36**, 155–168.
- Hanrahan L, Geoghegan A, O'Donovan M, Griffith V, Ruelle E, Wallace M and Shaloo L** (2017) Pasturebase Ireland: a grassland decision support system and national database. *Computers and Electronics in Agriculture* **136**, 193–201.
- Harris SL and Clark DA** (1996) Effect of high rates of nitrogen fertiliser on white clover growth, morphology, and nitrogen fixation activity in grazed dairy pasture in northern New Zealand. *New Zealand Journal of Agricultural Research* **39**, 149–158.
- Harris SL, Clark DA, Auldish MJ, Waugh CD and Laboyrie PG** (1997) Optimum white clover content for dairy pastures. *Proceedings of the New Zealand Grassland Association* **59**, 29–34.
- Harris W** (1987) Population dynamics and competition. In Baker MJ and Williams WM (eds). *White Clover*, Wallingford, UK: CAB International, pp. 203–297.
- Hay MJM, Chapman DE, Hay RJM, Pennell CGL, Woods PW and Fletcher RH** (1987) Seasonal variation in the vertical distribution of white clover stolons in grazed swards. *New Zealand Journal of Agricultural Research* **30**, 1–8.
- Høgh-Jensen H, Loges R, Jørgensen FV, Vinther FP and Jensen ES** (2004) An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. *Agricultural Systems* **82**, 181–194.
- Humphreys J, Casey IA and Laidlaw AS** (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.
- Jarvis SC** (1992) Nitrogen cycling and losses in clover based pastures. *Herba* **5**, 56–59.
- Jenquip** (2016) *Rising Plate Pasture Meters*. New Zealand: Jenquip. Available at <http://jenquip.co.nz/categories/rising-plate-pasture-meters> (Accessed 9 February 2018).
- Jørgensen FV and Ledgard SF** (1997) Contribution from stolons and roots to estimates of the total amount of N<sub>2</sub> fixed by white clover (*Trifolium repens* L.). *Annals of Botany* **80**, 641–648.
- Laidlaw AS, Teuber NG and Withers JA** (1992) Out-of-season management of grass/clover swards to manipulate clover content. *Grass and Forage Science* **47**, 220–229.
- Lantinga EA and Groot JC** (1996) Optimization of grassland production and herbage feed quality in an ecological context. In Groen AF and van Bruchem J (eds). *Utilization of Local Feed Resources by Dairy Cattle*, Wageningen, The Netherlands: Wageningen Press, pp. 58–66.
- Leach KA, Bax JA, Roberts DJ and Thomas C** (2000) The establishment and performance of a dairy system based on perennial ryegrass – white clover swards compared with a system based on nitrogen fertilized grass. *Biological Agriculture and Horticulture* **17**, 207–227.
- Ledgard S, Schils R, Eriksen J and Luo J** (2009) Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* **48**, 209–226.
- Ledgard SF** (2001) Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/ grass pastures. *Plant and Soil* **228**, 43–59.
- Ledgard SF and Steele KW** (1992) Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil* **141**, 137–153.
- Ledgard SF, Sprosen MS, Penno JW and Rajendram GS** (2001) Nitrogen fixation by white clover in pastures grazed by dairy cows: temporal variation and effects of nitrogen fertilization. *Plant and Soil* **229**, 177–187.
- Lüscher A, Mueller-Harvey I, Soussana JF, Rees RM and Peyraud JL** (2014) Potential of legume-based grassland-livestock systems in Europe: a review. *Grass and Forage Science* **69**, 206–228.
- McCarthy B, Pierce KM, Delaby L, Brennan A, Fleming C and Horan B** (2013) The effect of stocking rate and calving date on grass production, utilization and nutritive value of the sward during the grazing season. *Grass and Forage Science* **68**, 364–377.
- Morgan DJ, Stakelum G and O'Dwyer J** (1989) Modified neutral detergent cellulase digestibility of herbage by dairy cows. *Irish Journal of Agricultural and Food Research* **26**, 23–24.
- Munro JMM and Davies DA** (1974) Potential pasture production in the uplands of Wales. 5. The nitrogen contribution of white clover. *Grass and Forage Science* **29**, 213–213.
- O'Donovan M** (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, Lewis E and O'Kiely P** (2011) Requirements of future grass-based ruminant production systems in Ireland. *Irish Journal of Agricultural and Food Research* **50**, 1–21.
- O'Riordan EG, O'Kiely P and Keane MG** (1998) *Efficient Beef Production From Grazed Pasture*. End of Project Report. Oak Park, Ireland: Teagasc.
- Phelan P** (2013) *Aspects of grazing management to improve productivity and persistence of white clover in Irish grassland*. PhD Thesis, Waterford Institute of Technology.
- Pinxterhuis JB** (2000) *White clover dynamics in New Zealand pastures*. PhD Thesis, Wageningen University.
- Prince AJ, Apostolopoulou E and Simonova M** (2009) *Nitrogen Fertiliser Supply and Demand: Balances, Outlook and Drivers of Change*. *Proceedings of the International Fertiliser Society*. Colchester, UK: International Fertiliser Society.
- Ribeiro Filho HMN, Delagarde R and Peyraud JL** (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.
- Ribeiro Filho HMN, Delagarde R and Peyraud JL** (2005) Herbage intake and milk yield of dairy cows grazing perennial ryegrass swards or white clover/perennial ryegrass swards at low-and medium-herbage allowances. *Animal Feed Science and Technology* **119**, 13–27.
- SAS** (2011) *SAS version 9.3*. Cary, North Carolina: SAS Institute.
- Shaloo 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.
- Smith KE, Simpson RJ, Culvenor RA, Humphreys MO, Prud'Homme MP and Oram RN** (2001) The effects of ploidy and a phenotype conferring a high water-soluble carbohydrate concentration on carbohydrate accumulation, nutritive value and morphology of perennial ryegrass (*Lolium perenne* L.). *Journal of Agricultural Science, Cambridge* **136**, 65–74.
- Soegaard K** (1993) Nutritive value of white clover. In Frame J (ed.). *White Clover in Europe: State of the Art*, 17A23. REUR Technical Series 29. Rome, Italy: FAO.
- Stewart A and Hayes R** (2011) Ryegrass breeding-balancing trait priorities. *Irish Journal of Agricultural and Food Research* **50**, 31–46.
- Thomas VJ** (1973) Competition among pasture plants. III. Effects of frequency and height of cutting on competition between white clover and two ryegrass cultivars. *New Zealand Journal of Agricultural Research* **16**, 49–58.
- Thomson DJ** (1984) The nutritive value of white clover. In Thomson DJ (ed.). *Forage Legumes*, Occasional Symposium no. 16. Berkshire, UK: British Grassland Society, pp. 78–92.
- Ulyatt MJ** (1980) The feeding value of temperate pastures. In Morley FHW (ed.). *Grazing Animals*, Amsterdam, The Netherlands: Elsevier Scientific Publishing Company, pp. 125–141.

- Ulyatt MJ, Lancashire JA and Jones WT** (1977) The nutritive value of legumes. *Proceedings of the New Zealand Grassland Association* **38**, 107–118.
- Ulyatt MJ, Lassey KR, Shelton ID and Walker CF** (2002) Seasonal variation in methane emission from dairy cows and breeding ewes grazing ryegrass/white clover pasture in New Zealand. *New Zealand Journal of Agricultural Research* **45**, 217–226.
- Unkovich M** (2012) Nitrogen fixation in Australian dairy systems: review and prospect. *Crop and Pasture Science* **63**, 787–804.
- Van Soest PJ** (1963) Use of detergents in the analysis of fibrous feeds. 2. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Agricultural Chemists* **46**, 829–835.
- Wilkins PW and Humphreys MO** (2003) Progress in breeding perennial forage grasses for temperate agriculture. *Journal of Agricultural Science, Cambridge* **140**, 129–150.
- Wims CM, McEvoy M, Delaby L, Boland TM 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.