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Red clover for silage: management impacts on herbage yield, nutritive value, ensilability and persistence, and relativity to perennial ryegrass

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Abstract

This 6-year experiment quantified the impacts of management factors on red clover yield, persistence, nutritive value and ensilability, and compared these with perennial ryegrass receiving inorganic N fertilizer. Within a randomized complete block design, field plots were used to evaluate a 2 (cultivar, Merviot and Ruttinova) × 2 (alone and with perennial ryegrass) × 2 (0 and 50 kg fertilizer N ha^{-1} in mid-March) × 2 (harvest schedule) combination of the factors relating to red clover, and a 2 (harvest schedule) × 4 (0, 50, 100 and 150 kg N ha^{-1} for each cut) combination of the factors relating to perennial ryegrass. The early and late harvest schedules both involved four cuts per year, but commenced a fortnight apart. Red clover treatments averaged 14 906 kg dry matter (DM) ha^{-1} per year, whereas perennial ryegrass receiving 600 kg inorganic N fertilizer per year averaged 14 803 kg DM ha^{-1} per year. There was no yield decline evident across years despite a decline in the proportion of red clover. The early harvest schedule and sowing ryegrass with red clover increased the herbage yield and digestibility. March application of fertilizer N to red clover treatments reduced the annual yield. Early harvest schedule increased and both fertilizer N and sowing with ryegrass decreased the proportion of red clover. Sowing with ryegrass improved the indices of ensilability, but reduced the crude protein content. Both red clover cultivars had similar performance characteristics. A selected red clover-based treatment, considered to exhibit superior overall production characteristics, outyielded N-fertilized perennial ryegrass in mid-season. However, it had poorer digestibility and ensilability indices.

Keywords: harvest schedule, N fertilizer, seed mixture, cultivar

Introduction

Red clover (*Trifolium pratense* L.) offers a number of attractions as a home-produced forage on grassland farms involved in ruminant production (Frame *et al.*, 1998; Phelan *et al.*, 2015), and on such farms it is primarily used for forage conservation rather than grazing. Nevertheless, in many areas of Europe, its use has been in decline (Phelan *et al.*, 2015). The main problem perceived by farmers is that its yield is less reliable and may not match that of grass receiving inorganic fertilizer N and that this yield will progressively decline over a duration of several years (i.e., persistency). There can be additional challenges regarding its successful establishment following reseeding and with its successful conservation by ensilage compared to that of grass reseeds (Laidlaw and Frame, 1988; Phelan *et al.*, 2015).

Given the potential benefits of using red clover for silage production on grassland farms, there exists the need to quantify its yield and persistence and to identify the management opportunities to enhance these traits without compromising nutritive value or ensilability. Modifying harvest schedule provides one such management opportunity. Thus, where the annual production of herbage from a red clover-based sward is harvested in a set number of cuts, altering the timing of the primary growth cut will change the distribution of yield across cuts (Rinne and Nykänen, 2000; King *et al.*, 2012b) and this may influence the persistence of red clover. A second management opportunity is the application of a moderate input of

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inorganic N fertilizer to red clover in spring to elevate herbage growth at this time of year when low soil temperatures limit N fixation for red clover (Liu *et al.*, 2011). However, repercussions for persistence must be considered (Nyfeler *et al.*, 2009). An alternative management strategy to improve herbage production both early and late in the growing season, and potentially improve annual yield, is by sowing red clover in combination with an appropriate grass (Frame and Harkess, 1987). Furthermore, differences in seasonal or annual yield, persistence, nutritive value or ensilability, even among the early-flowering cultivars of red clover which may be the best suited to temperate grassland climates, may offer potential benefits (Drobná and Jančovič, 2006; Gilliland and Meehan, 2014). Finally, if interactions occur among the above factors, this would influence the optimum management strategy selected for red clover.

The objectives of this study, conducted within a simulated silage production regimen, were therefore to (i) quantify the seasonal and annual yield and the persistence of red clover over a series of years and to determine the nutritive value and ensilability indices for this crop; (ii) determine the extent to which the above are altered by changing the harvest schedule, applying inorganic fertilizer N in spring, including perennial ryegrass (*Lolium perenne* L.) in the reseeding mixture with red clover, and the cultivar of red clover chosen. It was also an objective to determine whether there were interactions among these factors; (iii) select a red clover-based treatment that best balances yield, nutritive value and ensilability and identify the rate of inorganic fertilizer N application to perennial ryegrass that provides the comparable results.

Materials and methods

Site and experimental treatments

The experiment was conducted at Teagasc Grange (53°30′N, 6°40′W; elevation 83 m above sea level). The site had been in permanent grassland for at least 30 years prior to the experiment, and existing grasses and broad-leaved species were sprayed with glyphosate (1·8 kg ha^{-1}; Roundup, Monsanto) 10 d prior to ploughing in August 2001. The soil was an imperfectly drained eutric gleysol of the Ashbourne series (Finch *et al.*, 1983), and prior to the experiment it had a pH of 6·6 and phosphorus (P) and potassium (K) values of 7·9 and 88 mgL^{-1} respectively. The ploughed ground then had 35 kg P and 150 kg K applied per hectare (ha).

The twenty-four treatments consisted of sixteen treatments containing red clover and eight treatments with monocultures of perennial ryegrass (cv. Greengold – an intermediate heading date tetraploid). There were two different harvest schedules, each comprising four consecutive growths within a year but differing in the date of the primary growth harvest (early vs. late; Table 1). The red clover treatments assessed the effects of all combinations of (i) two harvest schedules differing in primary growth harvest date, (ii) applying inorganic fertilizer N to the primary growth (0 vs. 50 kg N ha^{-1}), (iii) sowing red clover in monoculture or in a binary mixture with perennial ryegrass (cv. Greengold) and (iv) red clover cultivar (Merviot vs. Ruttinova). Both Merviot and Ruttinova are early-flowering diploid cultivars with Ruttinova (a Swiss 'Mattenklee') considered more persistent than Merviot (Kölliker *et al.*, 2003). The perennial ryegrass monoculture treatments were used to assess the response to applying inorganic fertilizer N (0, 50, 100 and 150 kg N ha^{-1} for each growth). All treatments were maintained on the same plots for six consecutive years.

The twenty-four treatments were randomly allocated among twenty-four plots (each 10 m × 2·5 m) within each of four replicate blocks. Monocultures of perennial ryegrass and red clover were sown at 30 and 15 kg seed ha^{-1}, respectively, while the red clover and perennial ryegrass in the binary mixture were sown at 10 kg and 10 kg seed ha^{-1} respectively. Seed was manually sown in September into a finely tilled seedbed, lightly raked and then rolled. All plots were sprayed with a herbicide containing 2,4-DB sodium salt, MCPA potassium salt, benazolin potassium salt, trisodium nitrilotriacetate and potassium hydroxide (Legumex Extra; Aventis CropScience, Cambridge, UK) in March 2002.

Table 1 Harvest dates (day/month) each year for the four harvests within the early and late primary growth harvest schedules.

Year of harvest	2002		2003		2004		2005		2006		2007	
Primary growth harvest schedule	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late	Early	Late
Primary growth (Cut 1)	2/6	17/6	31/5	13/6	26/5	10/6	25/5	10/6	24/5	8/6	24/5	11/6
Regrowth 1 (Cut 2)	22/7	2/8	16/7	30/7	13/7	3/8	6/7	25/7	11/7	28/7	20/7	30/7
Regrowth 2 (Cut 3)	4/9	13/9	2/9	10/9	30/8	17/9	23/8	5/9	29/8	7/9	30/8	18/9
Regrowth 3 (Cut 4)	10/12	10/12	29/10	29/10	1/12	1/12	11/11	11/11	2/11	2/11	5/11	5/11

The annual yield of each plot was the sum of its four separate cuts within a growing season. Inorganic N fertilizer was applied as calcium ammonium nitrate (275 g N kg^{-1}) and the perennial ryegrass plots assigned to the 0, 50, 100 and 150 kg N ha^{-1} treatments received these inputs of N for each of the four cuts per year. Nitrogen for the primary growth was applied in spring during the second half of March and that for subsequent regrowths was applied within two days of the preceding harvest. Where N was applied to red clover treatments, it was during the second half of March. All plots received 17·5 kg P ha^{-1} and 75 kg K ha^{-1} after each of the first three harvests per year and 35 kg P ha^{-1} and 150 kg K ha^{-1} after the fourth harvest each year.

Plots were harvested to a 5-cm stubble height by a Haldrup plot harvester (J. Haldrup, Løgstør, Denmark). Herbage yield was measured on a 1·5-m-wide swath harvested from the centre of each 2·5-m-wide plot. Herbage yield was measured in all years of the experiment, but due to the loss of some 2003 data, yield data for 2003 are not included. During 2002 and 2003, a representative sample of *c.* 500 g fresh forage was taken from each harvest of each plot and stored at −18°C prior to chemical analysis. For all other harvests, a sample was taken for dry-matter (DM) determination.

The proportion of visible biomass contributed on a fresh weight basis by red clover was assessed for the 16 red clover treatments for years 2003–2007 inclusive. This was undertaken throughout the experiment by the same trained assessor, immediately before harvesting the early harvest schedule primary growth (termed May) and immediately before the final harvest of the year (termed November). Red clover growth stages were classified according to the phenological staging scheme developed by Ohlsson and Wedin (1989), while correspondingly the maturity of perennial ryegrass at each harvest was categorized as described by Moore and Moser (1995). Weather data were recorded on a daily basis at a meteorological station situated within 2 km of the experimental site.

Chemical analysis

Frozen forage samples were thawed and individually chopped in an industrial food processor (Muller food processor, type MTK 204 special, Saarbrucken, Germany). Subsamples were dried in a forced air circulation oven at 98°C for 16 h for the determination of DM. Further chemical analyses were carried out using subsamples dried at 40°C for 48 h and milled through a 1-mm pore screen. *In vitro* dry-matter digestibility (DMD) was determined using the method of Tilley and Terry (1963) with the modification that the final residue was isolated by filtration rather than by centrifugation. Ash was estimated following the complete combustion in a muffle furnace at 550°C for 5 h. Crude protein (CP; N × 6·25) was measured using a LECO FP-428N analyser based on method 990-03 of the Association of Official Analytical Chemists (AOAC, 1990). The cold water-soluble carbohydrate (WSC) concentration was determined using the anthrone method (Thomas, 1977), and the results are expressed on a DM (WSC_{dm})- and aqueous (WSC_{aq})-phase basis. Buffering capacity (BC) was determined according to Playne and McDonald (1966). A fermentation coefficient was calculated according to Weissbach (1996) as DM (g/100 g) + 8 WSC (g/kg DM)/BC (g lactic acid (LA)/kg DM). Buffering capacity, expressed as g LA/kg DM, was calculated as 0·0154 BC (mEq/kg DM) − 0·2115 (R^2 = 0·95; O'Kiely and Pahlow, 2003).

Statistical analysis

The MIXED procedure in SAS 9.4 (2014) was used to perform an analysis of variance for red clover treatments. A factorial model was fitted for each cut separately with the factors harvest schedule, N rate, sward mixture and cultivar of red clover, as well as year and replicate blocks. A similar model without the year factor was fitted to the annual DM yield data. Comparisons of means were made, within interactions as appropriate, using a Tukey adjustment for multiplicity effects.

Analysis of response curves for perennial ryegrass monoculture plots receiving varying inorganic fertilizer N rates was conducted by regression using the GLM procedures in SAS 9.4 (2014). Analysis was conducted separately for each variable, harvest schedule and cut. It was also conducted for annual DM yield. In the linear regressions, quadratic terms were tested and retained if statistically significant.

Residual checks were made to ensure that the assumptions of all analyses were met. Where appropriate, log transformation was used to correct for skew and non-constant variance. Means from the log-scale analysis were back-transformed as medians on the data scale. As the log-scale standard error could not be straightforwardly back-transformed, 95% confidence limits were produced on the log scale and the end-points were back-transformed to produce asymmetric confidence intervals on the data scale.

Results

Meteorological conditions

The accumulated annual precipitation for each of the harvest years 1–6 was 1066, 743, 864, 812, 969 and 799 mm, respectively, with corresponding mean minimum and maximum monthly soil temperatures

(10 cm depth) of 4·8–15·2, 3·0–16·2, 3·5–15·8, 3·8–16·6, 3·4–18·0 and 4·5–14·8°C. The long-term (1971–2014) average values for annual precipitation and mean minimum and maximum monthly soil temperatures were 856 mm and 3·7–15·8°C respectively.

Growth stage at harvest

The herbages were harvested at contrasting phenological growth stages. For perennial ryegrass, the primary growth herbage in the early harvest schedule was at reproductive stage R1 (index 3·1) where there was a visible first spikelet of inflorescence emerging, whereas the herbage in the late harvest schedule was at reproductive stage R2 (index 3·3) where there were spikelets fully emerged but the peduncle was still absent. The mean growth stage at the three subsequent cuts (for both harvest schedules) was E2 (stem elongation and with second node palpable), V4 (vegetative and with fourth leaf collared) and V4 respectively. For red clover, the primary growth herbage in the early harvest schedule was at maturity stage 4 where there were three nodes with buds, but there were neither flowers nor seed pods, while the herbage in the late harvest schedule was at maturity stage 5 where there were open flowers on the main stem, but no seeds in flower heads. The mean growth stages at the three subsequent cuts were mainly at development maturity Stages 3 (1–2 nodes with buds; no flowers or seed buds), 2 (stem length ≥31 cm; no buds, flowers or seed pods) and 1 (stem length >10 and <30 cm; no buds, flowers nor seed pods) respectively. There was no visible difference between the two cultivars of red clover.

Yield and chemical composition for red clover-based treatments

The effects of harvest schedule, N rate, sward mixture and cultivar on yield and chemical composition in Cuts 1, 2, 3 and 4 are shown in Tables 2, 3, 4 and 5 respectively. The effect of harvest schedule, N rate, sward mixture and cultivar on annual yield is shown in Table 6.

Table 2 Main effects of harvest schedule, N rate, sward mixture and cultivar in Cut 1. Interactions are in footnotes.

	Harvest schedule			N rate (kg N ha^{-1} in March)			Sward mixture			Cultivar of red clover			
	Early	Late	Sig.	0 N	50 N	Sig.	Mono	Binary	Sig.	Merviot	Ruttinova	Sig.	s.e.
Yield (kg DM/ha)	5947	6699	<0·001	6221	6425	<0·05	6016	6630	<0·001	6211	6436	<0·05	63·0
DM (g/kg)	153	184	<0·001	169	168	ns	165	172	<0·001	170	167	ns	1·4
DMD (g/kg)	723	660	<0·001	691	692	ns	677	706	<0·001	689	694	ns	3·0
Ash (g/kg DM)	107	101	<0·001	105	102	ns	104	104	ns	104	104	ns	1·0
CP (g/kg DM)	163	164	ns	166	162	ns	177	151	<0·001	159	169	<0·01	2·2
WSC_{dm} (g/kg DM)	84	45	<0·001	63	67	ns	55	75	<0·001	66	64	ns	2·6
WSC_{aq} (g/L)	13·8[2]	8·1[3]	<0·001	10·1[4]	11·1[5]	ns	9·4[6]	11·9[7]	<0·01	11·1[8]	10·1[9]	ns	–[1]
BC (mEq/kg DM)	425	440	<0·05	443	423	<0·01	455	410	<0·001	425	440	<0·05	5·0

Interactions: CP: Harvest schedule × Cultivar, $P < 0{\cdot}05$; 162, 165, 156, 173 g/kg DM for early Merviot, early Ruttinova, late Merviot and late Ruttinova respectively.

WSC_{dm}: Harvest schedule × Sward mixture, $P < 0{\cdot}01$; 68, 101, 41, 49 g/kg DM for early mono, early binary, late mono and late binary respectively.

BC: Harvest schedule × Cultivar, $P < 0{\cdot}05$; 425, 425, 426, 455 mEq/kg DM for early Merviot, early Ruttinova, late Merviot and late Ruttinova respectively.

All other two-, three- and four-way interactions were not significant ($P > 0{\cdot}05$).

DM, dry matter; DMD, Dry-matter digestibility; CP, crude protein; WSC_{dm}, water-soluble carbohydrates (dry-matter basis); WSC_{aq}, water-soluble carbohydrates (aqueous basis); BC, buffering capacity.

[1]No s.e. but lower and upper 95% confidence limits as follows: [2]12·5, 15·2; [3]7·4, 9·0; [4]9·1, 11·2; [5]10·0, 12·3; [6]8·5, 10·4; [7]10·8, 13·2; [8]10·1, 12·3; [9]9·1, 11·1.

Table 3 Main effects of harvest schedule, N rate, sward mixture and cultivar in Cut 2. Interactions are in footnotes.

	Harvest schedule			N rate (kg N ha^{-1} in March)			Sward mixture			Cultivar of red clover			
	Early	Late	Sig.	0 N	50 N	Sig.	Mono	Binary	Sig.	Merviot	Ruttinova	Sig.	s.e.
Yield (kg DM/ha)	4423	4532	ns	4677	4278	<0·001	4673	4282	<0·001	4425	4530	ns	56·7
DM (g/kg)	146	152	<0·01	148	149	ns	148	150	ns	149	148	ns	1·7
DMD (g/kg)	726	708	<0·01	710	723	<0·05	708	725	<0·01	716	717	ns	4·0
Ash (g/kg DM)	116	114	ns	115	116	ns	112	118	<0·001	114	116	ns	1·0
CP (g/kg DM)	187	200	<0·001	195	192	ns	202	185	<0·001	190	196	<0·05	2·1
WSC_{dm} (g/kg DM)	77	56	<0·001	66	67	ns	63	70	<0·05	72	61	<0·01	2·4
WSC_{aq} (g/L)	12·9[2]	8·0[3]	<0·01	10·0[4]	10·3[5]	ns	9·6[6]	10·8[7]	ns	11·2[8]	9·2[9]	<0·01	–[1]
BC (mEq/kg DM)	513	484	<0·001	504	493	ns	517	480	<0·001	493	504	ns	4·8

Interactions: Yield: Harvest schedule × Sward mixture, $P < 0·01$; 4511, 4335, 4836, 4229 kg DM/ha for early mono, early binary, late mono and late binary respectively.
Harvest schedule × Cultivar, $P < 0·05$; 4472, 4374, 4379, 4686 kg DM/ha for early Merviot, early Ruttinova, late Merviot and late Ruttinova respectively.
All other two-, three- and four-way interactions were not significant ($P > 0·05$).
DM, dry matter; DMD, dry-matter digestibility; CP, crude protein; WSC_{dm}, water-soluble carbohydrates (dry-matter basis); WSC_{aq}, water-soluble carbohydrates (aqueous basis); BC, buffering capacity.
[1]No s.e. but lower and upper 95% confidence limits as follows: [2]11·9, 14·0; [3]7·4, 8·7; [4]9·3, 10·9; [5]9·5, 11·2; [6]8·9, 10·4; [7]9·9, 11·7; [8]10·3, 12·2; [9]8·5, 10·0.

There were no four-way interactions ($P > 0·05$), and two- and three-way interactions were not significant unless otherwise specified.

Cut 1 (Table 2)

Harvest schedule

Yield and DM were higher ($P < 0·001$) and DMD, ash, WSC_{dm} and WSC_{aq} were lower ($P < 0·001$) for the late harvest schedule. However, there was a harvest schedule × sward mixture interaction ($P < 0·01$) for WSC_{dm}, with the magnitude of the reducing effect of late harvest schedule being greater for the binary mixture rather than for the monoculture. The BC was higher ($P < 0·05$) for the late rather than for the early harvest schedule, but there was a harvest schedule × cultivar interaction ($P < 0·05$), with the harvest schedule effect only being significant in the case of Ruttinova. There was no main effect ($P > 0·05$) of harvest schedule on CP although there was a harvest schedule × cultivar interaction ($P < 0·05$) with a trend for the late harvest schedule to produce a lower CP value with Merviot, but a higher value with Ruttinova.

N rate

Yield was higher ($P < 0·05$) and BC was lower ($P < 0·01$) where there was spring application of fertilizer N. There was no effect ($P > 0·05$) of N rate on the other variables.

Sward mixture

Yield, DM, DMD, WSC_{dm} ($P < 0·001$) and WSC_{aq} ($P < 0·01$) were higher and CP and BC were lower ($P < 0·001$) for the binary sward mixture. However, for WSC_{dm}, there was a harvest schedule × sward mixture interaction ($P < 0·01$) such that the binary mixture had a higher WSC_{dm} than the monoculture only with the early harvest schedule. There was no effect ($P > 0·05$) of sward mixture on ash.

Cultivar

Yield ($P < 0·05$), CP ($P < 0·01$) and BC ($P < 0·05$) were higher for Ruttinova. However, there was a harvest schedule × cultivar interaction ($P < 0·05$) for CP

Table 4 Main effects of harvest schedule, N rate, sward mixture and cultivar in Cut 3. Interactions are in footnotes.

	Harvest schedule			N rate (kg N ha^{-1} in March)			Sward mixture			Cultivar of red clover			
	Early	Late	Sig.	0 N	50 N	Sig.	Mono	Binary	Sig.	Merviot	Ruttinova	Sig.	s.e.
Yield (kg DM/ha)	3678	2759	<0·001	3313	3124	<0·001	3218	3219	ns	3290	3147	<0·05	39·4
DM (g/kg)	132	126	<0·001	128	129	ns	127	131	<0·05	130	128	ns	1·0
DMD (g/kg)	746	719	<0·001	730	735	ns	726	739	<0·01	732	733	ns	3·4
Ash (g/kg DM)	124	119	<0·05	120	123	ns	117	125	<0·001	121	121	ns	1·2
CP (g/kg DM)	226	227	ns	230	223	ns	237	216	<0·001	223	231	ns	3·2
WSC_{dm} (g/kg DM)	64	36	<0·001	48	52	ns	49	51	ns	52	48	ns	1·7
WSC_{aq} (g/L)	9·3[2]	5·3[3]	<0·001	6·7[4]	7·4[5]	<0·05	6·7[6]	7·4[7]	<0·05	7·5[8]	6·6[9]	<0·01	–[1]
BC (mEq/kg DM)	516	483	<0·05	495	505	ns	520	480	<0·01	497	502	ns	10·8

Interactions: Yield: Harvest schedule × Cultivar × N rate, $P < 0·05$; 3801, 3760, 3700, 3449, 2972, 2717, 2685, 2663 kg DM/ha for early Merviot 0 N, early Ruttinova 0 N, early Merviot 50 N, early Ruttinova 50 N, late Merviot 0 N, late Ruttinova 0 N, late Merviot 50 N and late Ruttinova 50 N respectively.

DM: Harvest schedule × Sward mixture, $P < 0·05$; 132, 132, 122, 129 g/kg for early mono, early binary, late mono and late binary respectively.All other two-, three- and four-way interactions were not significant ($P > 0·05$).

DM, dry matter; DMD, dry-matter digestibility; CP, crude protein; WSC_{dm}, water-soluble carbohydrates (dry-matter basis); WSC_{aq}, water-soluble carbohydrates (aqueous basis); BC, buffering capacity.

[1]No s.e. but lower and upper 95% confidence limits as follows: [2]8·7, 9·9; [3]5·0, 5·7; [4]6·3, 7·2; [5]6·9, 7·8; [6]6·3, 7·1; [7]7·0, 7·9; [8]7·1, 8·0; [9]6·2, 7·0.

and BC such that the effect of cultivar was significant only with the late harvest schedule.

Cut 2 (Table 3)

Harvest schedule

The DM ($P < 0·01$) and CP ($P < 0·001$) were higher and DMD ($P < 0·01$), WSC_{dm} ($P < 0·001$), WSC_{aq} ($P < 0·01$) and BC ($P < 0·001$) were lower for the late harvest schedule. There was no main effect ($P > 0·05$) of harvest schedule on yield or ash. However, there was a harvest schedule × sward mixture interaction ($P < 0·01$) such that the late harvest schedule increased the yield only for the monoculture. There was also a harvest schedule × cultivar interaction ($P < 0·05$) such that the late harvest schedule increased the yield only for Ruttinova.

N rate

Yield was lower ($P < 0·001$) and DMD was higher ($P < 0·05$) where there was spring application of N. There was no effect ($P > 0·05$) of N rate on the other variables.

Sward mixture

DMD ($P < 0·01$), ash ($P < 0·001$) and WSC_{dm} ($P < 0·05$) were higher and yield, CP and BC were lower ($P < 0·001$) for the binary sward mixture. For yield, there was a harvest schedule × sward mixture interaction ($P < 0·01$) where the magnitude of reduction in yield for the binary sward mixture was greater for the late harvest schedule compared to the early harvest schedule. There was no effect ($P > 0·05$) of sward mixture on DM.

Cultivar

The CP ($P < 0·05$) was higher and WSC_{dm} and WSC_{aq} ($P < 0·01$) were lower for Ruttinova. There was no main effect ($P > 0·05$) of cultivar on the other variables. However, there was a harvest schedule × cultivar interaction ($P < 0·05$) whereby the yield was higher for Ruttinova than for Merviot only for the late harvest schedule.

Table 5 Main effects of harvest schedule, N rate, sward mixture and cultivar in Cut 4. Interactions are in footnotes.

	Harvest schedule			N rate (kg N ha^{-1} in March)			Sward mixture			Cultivar of red clover			
	Early	Late	Sig.	0 N	50 N	Sig.	Mono	Binary	Sig.	Merviot	Ruttinova	Sig.	s.e.
Yield (kg DM/ha)	991	782	<0·001	827	946	<0·001	747	1027	<0·001	870	904	ns	22·8
DM (g/kg)	153	151	ns	153	152	ns	149	156	<0·001	153	152	ns	1·3
DMD (g/kg)	744	763	<0·001	752	755	ns	744	764	<0·001	751	756	ns	2·7
Ash (g/kg DM)	110	113	ns	111	112	ns	110	113	<0·01	110	113	ns	0·9
CP (g/kg DM)	272	268	ns	276	264	ns	284	256	<0·001	270	270	ns	4·2
WSC_{dm} (g/kg DM)	55	69	<0·001	59	63	ns	55	67	<0·001	61	61	ns	2·1
WSC_{aq} (g/L)	12·4[2]	15·4[3]	<0·001	13·9[4]	13·8[5]	ns	12·2[6]	15·6[7]	<0·001	13·9[8]	13·7[9]	ns	–[1]
BC (mEq/kg DM)	488	475	ns	486	477	ns	509	454	<0·001	476	487	ns	5·5

Interactions: Yield: Harvest schedule × Cultivar, $P < 0·001$; 916, 1067, 823, 742 kg DM/ha for early Merviot, early Ruttinova, late Merviot, late Ruttinova respectively.

WSC_{dm}: Harvest schedule × Sward mixture, $P < 0·05$; 53, 58, 58, 76 g/kg DM for early mono, early binary, late mono and late binary respectively.

BC: Harvest schedule × Sward mixture, $P < 0·05$; 506, 470, 513, 437 mEq/kg DM for early mono, early binary, late mono and late binary respectively.

All other two-, three- and four-way interactions were not significant ($P > 0·05$).

DM, dry matter; DMD, dry-matter digestibility; CP, crude protein; WSC_{dm}, water-soluble carbohydrates (dry-matter basis); WSC_{aq}, water-soluble carbohydrates (aqueous basis); BC, buffering capacity.

[1]No s.e. but lower and upper 95% confidence limits as follows: [2]11·6, 13·3; [3]14·4, 16·5; [4]13·0, 14·9; [5]12·9, 14·8; [6]11·4, 13·1; [7]14·6, 16·7; [8]13·0, 14·9; [9]12·8, 14·7.

Table 6 Main effects of harvest schedule, N rate, sward mixture and cultivar on annual yield.

	Harvest schedule			N rate (kg N ha^{-1} in March)			Sward mixture			Cultivar of red clover			
	Early	Late	Sig.	0 N	50 N	Sig.	Mono	Binary	Sig.	Merviot	Ruttinova	Sig.	s.e.
Annual yield (kg DM ha^{-1})	15 039	14 773	<0·05	15 039	14 773	<0·05	14 654	15 159	<0·001	14 795	15 017	ns	89·1

All two-, three- and four-way interactions were not significant ($P > 0·05$).

Cut 3 (Table 4)

Harvest schedule

Yield ($P < 0·001$), DM ($P < 0·001$), DMD ($P < 0·001$), ash ($P < 0·05$), WSC_{dm} ($P < 0·001$), WSC_{aq} ($P < 0·001$) and BC ($P < 0·05$) were lower for the late harvest schedule. There was a harvest schedule × cultivar × N rate interaction, but this did not contradict any of the associated main effects. The harvest schedule × sward mixture interaction for DM ($P < 0·05$) resulted in the early harvest schedule having a higher value than the late harvest schedule only for the monoculture. There was no effect ($P > 0·05$) of harvest schedule on CP.

N rate

Yield was lower ($P < 0·001$) and WSC_{aq} higher ($P < 0·05$) where there was spring application of N. There was no effect ($P > 0·05$) of N rate on the other variables.

Sward mixture

The DM ($P < 0{\cdot}05$), DMD ($P < 0{\cdot}01$), ash ($P < 0{\cdot}001$) and WSC_{aq} ($P < 0{\cdot}05$) were higher and CP ($P < 0{\cdot}001$) and BC ($P < 0{\cdot}01$) were lower for the binary sward mixture. However, for DM, there was a harvest schedule × sward mixture interaction ($P < 0{\cdot}05$) such that the higher value for the binary sward mixture was only significant for the late harvest schedule. There was no effect ($P > 0{\cdot}05$) of sward mixture on yield or WSC_{dm}.

Cultivar

Yield ($P < 0{\cdot}05$) and WSC_{aq} ($P < 0{\cdot}01$) were lower for Ruttinova. There was no effect ($P > 0{\cdot}05$) of cultivar on the other variables.

Cut 4 (Table 5)

Harvest schedule

Yield ($P < 0{\cdot}001$) was lower and DMD, WSC_{dm} and WSC_{aq} ($P < 0{\cdot}001$) were higher for the late harvest schedule. However, for yield there was a harvest schedule × cultivar interaction with the reduction in yield with the late harvest schedule being greater for Ruttinova than for Merviot. For WSC_{dm}, there was a harvest schedule × sward mixture interaction ($P < 0{\cdot}05$), which resulted in the increase in response to the late harvest schedule being significant only for the binary sward mixture. There was no effect ($P > 0{\cdot}05$) of harvest schedule on DM, ash or CP. For BC there was a harvest schedule × sward mixture interaction ($P < 0{\cdot}05$) with the reduction associated with the late harvest schedule only being significant for the binary sward mixture.

N rate

Yield ($P < 0{\cdot}001$) was higher where there was spring application of N. There was no effect ($P > 0{\cdot}05$) of N rate on the other variables.

Sward mixture

Yield ($P < 0{\cdot}001$), DM ($P < 0{\cdot}001$), DMD ($P < 0{\cdot}001$), ash ($P < 0{\cdot}01$), WSC_{dm} ($P < 0{\cdot}001$) and WSC_{aq} ($P < 0{\cdot}05$) were higher and CP and BC were lower ($P < 0{\cdot}001$) for the binary sward mixture. For WSC_{dm} and BC, there was a harvest schedule × sward mixture interaction ($P < 0{\cdot}05$) where the magnitude of the response to the binary sward mixture was greater for the late harvest schedule.

Cultivar

There was no main effect ($P > 0{\cdot}05$) in any of the variables measured. However, there was a harvest schedule × cultivar interaction ($P < 0{\cdot}001$) such that Ruttinova had a higher yield only in the case of the early harvest schedule.

Annual yield (Table 6)

Late harvest schedule and spring application of inorganic fertilizer N decreased ($P < 0{\cdot}05$), while the binary sward mixture increased ($P < 0{\cdot}001$) annual yield. There was no significant effect ($P > 0{\cdot}05$) of cultivar. The average annual yield across all red clover treatments was 13 008, 15 495, 14 566, 15 604 and 15 857 kg DM ha^{-1} for 2002, 2004, 2005, 2006 and 2007 respectively.

Proportion of red clover (Table 7)

The proportion of red clover in the sward decreased ($P < 0{\cdot}001$) from 2003 to 2004 and from 2005 to 2006. However, the year × harvest schedule interaction reflected that between 2006 and 2007, the proportion decreased ($P < 0{\cdot}05$) in the early harvest schedule and increased ($P < 0{\cdot}001$) in the late harvest schedule. The year × N rate interaction ($P < 0{\cdot}05$) did not alter the overall effects of year.

Although the main effect indicated that the proportion of red clover present was greater in May than in November ($P < 0{\cdot}01$), the two-way interactions found this to be significant only when 0 kg N ha^{-1} was applied in March ($P < 0{\cdot}001$), only with Ruttinova ($P < 0{\cdot}01$) and only with the red clover monoculture treatments ($P < 0{\cdot}01$). The main effect of harvest schedule indicated that the proportion of red clover present was higher ($P < 0{\cdot}001$) for the early than for the late schedule. The two-way interactions of harvest schedule with N rate ($P < 0{\cdot}05$), sward mixture ($P < 0{\cdot}05$) and red clover cultivar ($P < 0{\cdot}01$), or the three-way interaction with mixture and red clover cultivar ($P < 0{\cdot}05$), did not alter the overall effect of harvest schedule. However, the interaction with year ($P < 0{\cdot}001$) indicated that the effect occurred only in 2005 and 2006. The proportion of red clover was higher for 0 N ($P < 0{\cdot}001$) and this was not contradicted by the interactions of rate of N with harvest schedule ($P < 0{\cdot}05$), sward mixture ($P < 0{\cdot}001$), time of year when the proportion of red clover was assessed ($P < 0{\cdot}001$) or year ($P < 0{\cdot}05$).

The main effect of sward mixture showed that overall, sowing monocultures of red clover rather than binary mixtures with perennial ryegrass resulted in a

Table 7 Main effects of year, month of observation, harvest schedule, N rate, sward mixture and cultivar on the proportion of red clover. Interactions are in footnotes.

Year	2003	2004	2005	2006	2007	s.e.	Sig.
	0·749	0·569	0·574	0·364	0·437	0·015	<0·001

Month of observation	May	November	s.e.	Sig
	0·563	0·523	0·01	<0·01

Harvest schedule	Early	Late	s.e.	Sig.
	0·620	0·464	0·01	<0·001

N rate (kg N ha^{-1} in March)	0 N	50 N	s.e.	Sig.
	0·609	0·476	0·01	<0·001

Sward mixture	Mono	Binary	s.e.	Sig.
	0·595	0·490	0·06	<0·001

Cultivar of red clover	Merviot	Ruttinova	s.e.	Sig.
	0·550	0·530	0·01	NS

Interactions: Year × Harvest schedule, $P < 0·001$; 0·705, 0·554, 0·619, 0·497, 0·427, 0·789, 0·584, 0·529, 0·249, 0·449 for 2003 early; 2004 early, 2005 early, 2006 early, 2007 early, 2003 late, 2004 late, 2005 late, 2006 late, 2007 late respectively.

Year × N rate, $P < 0·05$, 0·790, 0·662, 0·661, 0·413, 0·474, 0·704, 0·471, 0·483, 0·317, 0·402 for 2003 0 N, 2004 0 N, 2005 0 N, 2006 0 N, 2007 0 N, 2003 50 N, 2004 50 N, 2005 50 N, 2006 50 N, 2007 50 N respectively.

Month of observation × N rate, $P < 0·001$; 0·655, 0·560, 0·466, 0·486 for May 0 N, Nov 0 N, May 50 N, Nov 50 N respectively.

Month of observation × Cultivar, $P < 0·001$; 0·550, 0·557, 0·576, 0·489 for May Merviot, Nov Merviot, May Ruttinova, Nov Ruttinova respectively.

Month of observation × Sward mixture, $P < 0·01$; 0·635, 0·554, 0·488, 0·493 for May mono, Nov mono, May binary, Nov binary respectively.

Harvest schedule × N rate, $P < 0·05$; 0·664, 0·574, 0·550, 0·380 for early 0 N, early 50 N, late 0 N, late 50 N respectively.

Harvest schedule × Sward mixture, $P < 0·001$; 0·646, 0·593, 0·542, 0·388 for early mono, early binary, late mono, late binary respectively.

Harvest schedule × Cultivar, $P < 0·01$; 0·651, 0·587, 0·451, 0·477 for early Merviot, early Ruttinova, late Merviot, late Ruttinova respectively.

N rate × Sward mixture, $P < 0·001$; 0·626, 0·591, 0·563, 0·390 for 0 N mono, 0 N binary, 50 N mono, 50 N binary respectively.

Harvest schedule × Cultivar × Sward mixture, $P < 0·05$; 0·654, 0·638, 0·649, 0·536, 0·542, 0·542, 0·364, 0·413 for early Merviot mono, early Ruttinova mono, early Merviot binary, early Ruttinova binary, late Merviot mono, late Ruttinova mono, late Merviot binary and late Ruttinova binary respectively.

All other two-, three- and four-way interactions were not significant ($P > 0·05$).

greater ($P < 0·001$) red clover content in the sward. This was not contradicted by the two-way interaction ($P < 0·001$) with harvest schedule. However, the interaction with rate of N ($P < 0·001$) indicated that the higher proportion with the monoculture was only significant when inorganic fertilizer N was applied in spring. The three-way interaction with both harvest schedule and cultivar ($P < 0·05$) highlighted that the higher red clover value with the monoculture was not significant for Merviot managed within the early harvest schedule.

Overall, the main effect of red clover cultivar was not significant ($P > 0·05$). However, two-way interactions ($P < 0·01$) indicated that the proportion was higher for Merviot than for Ruttinova in the early harvest schedule only and for the November observation only. The three-way interaction with harvest schedule and mixture ($P < 0·05$) found Merviot to have a higher proportion of red clover than Ruttinova only in the early harvest schedule of the binary mixture.

The main non-sown species in the red clover plots were annual meadow grass (*Poa annua* L.) and

Table 8 Best-fit response curves of perennial ryegrass monoculture yield (kg DM/ha), DM (g/kg), DMD (g/kg), CP (g/kg DM), WSC_{dm} (g/kg DM), WSC_{aq} (g/L) and BC (mEq/kg DM) to increasing rates of fertilizer nitrogen (kg N/ha).

Harvest schedule	Cut	Intercept	b	c	RSD	R^2	Sig.	Harvest schedule	Cut	Intercept	b	c	RSD	R^2	Sig.
Yield (kg DM/ha)								WSC_{dm} (g/kg DM)							
Early	1	4325	45·86	−0·2231	1052·6	0·43	<0·001	Early	1	233	−2·2	0·0084	54·9	0·51	<0·001
	2	2276	23·52	−0·1037	942·8	0·24	<0·001		2	271	−2·0	0·0060	29·2	0·84	<0·001
	3	1742	26·59	−0·1194	511·0	0·56	<0·001		3	166	−0·37	–	43·2	0·20	<0·05
	4	1335	19·47	−0·1118	498·9	0·30	<0·001		4	126	−0·35	–	38·5	0·21	<0·01
Late	1	5968	36·45	−0·1975	904·7	0·33	<0·001	Late	1	124	−0·63	–	51·2	0·34	<0·001
	2	1941	26·44	−0·1286	749·7	0·33	<0·001		2	143	−0·57	–	46·2	0·34	<0·001
	3	1670	26·30	−0·1167	592·5	0·49	<0·001		3	96	−0·22	–	28·3	0·17	<0·05
	4	1022	17·74	−0·0885	615·1	0·23	<0·001		4	134	−0·35	–	30·9	0·30	<0·01
DM (g/kg)								WSC_{aq} (g/L)							
Early	1	188	−0·36	–	26·0	0·36	<0·001		1	61	−0·83	0·0035	18·5	0·53	<0·001
	2	183	−0·32	–	27·8	0·30	<0·001		2	78	−0·85	0·0031	11·1	0·83	<0·001
	3	155	−0·18	–	24·3	0·15	<0·001		3	39	−0·14	–	10·6	0·37	<0·001
	4	162	−0·08	–	66·5	0·01	ns		4	35	−0·12	–	10·1	0·31	<0·001
Late	1	205	−0·15	–	25·8	0·38	<0·001		1	31	−0·18	–	14·9	0·33	<0·001
	2	165	−0·16	–	27·7	0·31	<0·001		2	27	−0·13	–	10·3	0·36	<0·001
	3	141	−0·08	–	24·4	0·16	<0·01		3	18	−0·06	–	6·0	0·25	<0·001
	4	155	−0·04	–	66·9	0·01	ns		4	33	−0·10	–	7·2	0·40	<0·001
DMD (g/kg)								BC (mEq/kg DM)							
Early	1	795	−0·28	–	27·2	0·26	<0·01	Early	1	302	0·70	–	62·2	0·30	<0·01
	2	812	−0·17	–	17·8	0·24	<0·01		2	288	0·99	–	43·90	0·63	<0·001
	3	808	−0·11	–	19·0	0·10	ns		3	323	0·57	–	52·9	0·28	<0·01
	4	788	−0·08	–	41·4	0·01	ns		4	340	0·77	–	44·2	0·50	<0·001
Late	1	727	−0·26	–	35·8	0·15	<0·05	Late	1	353	0·27	–	84·2	0·03	ns
	2	796	−0·54	0·0029	19·7	0·20	<0·05		2	343	0·40	–	51·1	0·17	ns
	3	784	0·11	−0·0008	14·4	0·03	ns		3	373	0·54	–	73·6	0·15	<0·05
	4	796	0·24	−0·002	31·2	0·03	ns		4	355	0·70	–	33·6	0·59	<0·001
CP (g/kg DM)															
Early	1	98	0·49	–	22·4	0·62	<0·001								
	2	94	0·72	–	17·5	0·85	<0·001								
	3	137	0·54	–	25·9	0·59	<0·001								
	4	184	0·59	–	14·4	0·83	<0·001								
Late	1	99	0·42	–	28·3	0·42	<0·001								
	2	129	0·69	–	27·0	0·68	<0·001								
	3	160	0·046	0·0041	17·4	0·80	<0·001								
	4	197	0·42	–	20·8	0·58	<0·001								

DM concentration, dry-matter concentration; DMD, dry-matter digestibility; CP, crude protein; WSC_{dm}, water-soluble carbohydrates (dry matter); WSC_{aq}, water-soluble carbohydrates (aqueous phase); BC, buffering capacity; RSD, residual standard deviation.

b & c are linear and quadratic coefficients respectively.

Note: Ash is omitted as it was not significant for 6 of the 8 cuts.

perennial ryegrass and, to a much lesser extent and in declining incidence, creeping buttercup (*Ranunculus repens* L.), broad-leaved dock (*Rumex obtusifolius* L.) and curled dock (*Rumex crispus* L.).

Yield and chemical composition of grass monocultures

The equations that describe the relationship between the rate of N applied and yield (average over 5 years) and chemical composition (average over 2 years) for early and late harvest schedules per cut are summarized in Table 8.

Table 9 shows the relationship between the rate of N applied and annual yield for early and late harvest schedules. The average annual yields for plots receiving 0 kg N ha^{-1} were 9452, 9873, 9278, 9785 and 11 108 kg DM ha^{-1} for 2002, 2004, 2005, 2006 and 2007, respectively, and the average annual yields for plots receiving 600 kg N ha^{-1} were 14 994, 15 313, 14 088, 13 907 and 15 714 kg DM ha^{-1} for 2002, 2004, 2005, 2006 and 2007 respectively.

Discussion

The experimental design strategy for this study was to randomly distribute 24 treatments among 24 plots in each of four replicate blocks: 16 treatments were associated with two levels of each of four factors being applied to red clover and the remaining eight treatments were associated with two factors applied to perennial ryegrass monocultures. The purpose of these latter eight treatments was to provide a benchmark against which the outcomes from the red clover treatments could be related.

Red clover treatments – yield

The annual yield of harvested herbage when averaged across the 16 treatments containing red clover and also across five of the first 6 years after they were sown (14 906 kg DM ha^{-1}) is less than the average yield (16 500 kg DM ha^{-1}) over a three-year duration achieved across all recommended red clover varieties in the Recommended Variety Trials in Northern Ireland 2014/2015 (Gilliland and Meehan, 2014). However, it is greater than the values reported by Laidlaw and Frame (1988) in their review of European studies, with the yield advantage in the present study arising from the absence of a decline through to the end of the sixth year after sowing. The persistence of yield for the red clover treatments over the six years of this study was matched over a 4-year period by Marshall *et al.* (2014). The mean yield is also greater than that achieved in the present study with perennial ryegrass monocultures that received no inorganic fertilizer N (10 140 kg DM ha^{-1}) but within the range achieved when 200–600 kg N ha^{-1} year^{-1} was applied. The reported decline in annual yield of red clover-based swards after 3 years from sowing (McBratney, 1981, 1984, 1987; Laidlaw and Frame, 1988) increases the frequency with which reseeding is required and can be an important impediment to the more widespread use of red clover in permanent grassland swards. Thus, the finding in this study that yields were showing no signs of decline by the end of six years after sowing is important. This improved persistence may reflect the very good initial establishment of the crop post-sowing and/or a low apparent challenge from pests and diseases. Frame *et al.* (1998) stressed the potential negative impact of pests and diseases on red clover persistence, and the undertaking of this study at a site where red clover had not previously been grown may have provided particularly favourable conditions for the treatments being studied.

Although red clover has an asymmetrical growth profile during the year, the actual yield distribution across cuts depends on the harvest schedule operated. Thus, e.g., Frame *et al.* (1998) cited a schedule involving a late first cut, a second cut and a subsequent aftermath as contributing 50–60, 30–40 and 10–20% of annual DM production respectively. In general, the highest individual cut yields in this study were achieved when a first cut was harvested during late May to early June, with progressively lighter yields in subsequent harvests (42, 30, 22 and 6% for Cuts 1–4 respectively). The likely costs associated with harvesting and ensiling the relatively low yield at Cut 4 could be difficult to justify economically (Finneran *et al.*, 2010) and, in

Table 9 Best-fit response curves of annual perennial ryegrass monoculture yield (kg DM ha^{-1}) to increasing increments of fertilizer nitrogen (kg N ha^{-1}).

Harvest schedule	Intercept	b	c	RSD	R^2	Sig.
Early	9679	28·86	−0·034878	1484·6	0·71	<0·001
Late	10 601	26·73	−0·03321	1385·4	0·69	<0·001

b & c are linear and quadratic coefficients respectively.
RSD: residual standard deviation.

commercial farming practice, consideration would need to be given to utilizing it by grazing or mulching instead.

The overall positive effect of an early harvest schedule on annual yield masks quite contrasting harvest schedule effects on individual cuts. As expected, delaying Cut 1 for a mean duration of 15 d resulted in a 13% yield advantage for the late harvest schedule. This trend agrees with the findings of Rinne and Nykänen (2000) and King *et al.* (2012b). However, the mean growth rate of 49 kg DM ha^{-1} d^{-1} for the period between the early and late harvest for Cut 1 suggests that herbage growth was slowing as higher yields were achieved. This is in contrast to King *et al.* (2012b) who showed that growth rate did not decline as the date of first cut was delayed.

The similar growth rates of herbage for the 48 d after Cut 1 for both early and late harvest schedules suggest that growth rates were constant for at least part of this period until Cut 2 was harvested. In contrast, the lower yields associated with the late compared to the early harvest schedule during the periods up to Cuts 3 and 4 can be explained by the approximately 15-d later timing of the late harvest schedule growth during a stage of the year when declining temperatures led to progressively slower rates of herbage growth. In the case of Cut 4, the approximately 13-d shorter growth duration for the late harvest schedule also contributed to its lower yield.

The positive yield response for Cut 1 to spring application of N agrees with Frame *et al.* (1976) and Frankow-Lindberg (1989), while the corresponding negative effect on the annual average proportions of red clover present in these swards agrees with Frame and Newbould (1986). The decline in red clover content as a result of spring application of N likely explains the subsequent lower yields associated with this treatment at Cuts 2 and 3. This would be due to the direct effect of a lower yield of red clover and to the indirect effect of the lesser amount of red clover present, which would probably have resulted in the fixation of a lesser amount of N (Walker *et al.*, 1956; McAuliffe *et al.*, 1958), and no inorganic fertilizer N was applied after Cut 1. The magnitude of this negative effect of spring applied N on Cuts 2 and 3 yields was sufficiently large to result in an overall reduction in annual yield. The positive response at Cut 4 to spring applied N may be due to its suppressive effect on the proportion of red clover and the corresponding higher proportion of perennial ryegrass. As shown with the grass monocultures, perennial ryegrass has a higher growth rate than red clover at this stage late of the year when soil temperatures are relatively low.

The inclusion of grass with red clover resulted in a significant increase in annual yield, although the scale of this increase was modest in practical terms. However, the positive response to inclusion of grass agrees with other research (McBratney, 1981; Camlin *et al.*, 1983; Frame and Harkess, 1987). This was the result of higher yields achieved by the binary mixture both early (Cut 1) and late (Cut 4) in the season when colder temperatures limit N fixation for red clover (Liu *et al.*, 2011). This is supported by the findings in this study that perennial ryegrass monocultures receiving inorganic fertilizer N had a higher yield than red clover monocultures for these two cuts. In contrast, the higher yield for the red clover monoculture treatments at Cut 2 is likely associated with its better response than for perennial ryegrass to warmer temperatures at that time. This was demonstrated by the higher yield obtained by the red clover monocultures at Cut 2 compared to the grass monocultures that received inorganic N.

Even though both cultivars of red clover produced similar total annual yields, their patterns of production across the season differed, with Ruttinova producing more of its yield earlier in the season. This difference in pattern, despite similar overall annual yields, has been observed previously in comparisons of cultivars by Olson *et al.* (2014). The similarity in the total annual yield masked the differences between the cultivars in Year 1 whereby Ruttinova demonstrated significantly higher yields than Merviot (results not presented). This is supported by Depez *et al.* (2004) who also found that Ruttinova produced higher yields than Merviot in the 2 years after sowing.

Red clover treatments – nutritive value

Animal performance within livestock systems based on permanent grassland depends on the supply of sufficient energy and protein from forage. Both DMD and CP are commonly used indices of these quality characteristics. In this study, these values ranged from 644 to 782 g kg^{-1} and 139–301 g kg^{-1} DM, respectively, among the four cuts of the 16 treatments containing red clover. They are comparable to other published values of 616–762 g kg^{-1} (organic matter digestibility) and 152–277 g CP kg^{-1} DM (www.feedipedia.org; accessed 10 September 2015). The seasonal pattern of a marked increase in CP from Cut 1 through to Cut 4 with a corresponding but less marked decline in DMD agrees with the observations of Drobná and Jančovič (2006).

Because the digestibilities of both red clover and perennial ryegrass are largely determined by their phenological growth stage (Fales and Fritz, 2007), the lower digestibility for the late harvest at Cut 1 reflects the 15-d delay in harvest date, with this decline in DMD agreeing with Rinne and Nykänen (2000) and King *et al.* (2012b). This effect was amplified by the simultaneous large reduction in WSC_{dm} concentration. For Cut 4, the lower DMD for the early harvest schedule is explained by its 13-d longer growth period and also the corresponding lower WSC_{dm}.

Spring application of 50 kg N ha^{-1} could be expected to have an effect on digestibility and protein by two methods. First, it could have an effect on Cut 1 by decreasing WSC_{dm}, and therefore DMD, while simultaneously increasing CP (Keating and O'Kiely, 2000a; King *et al.*, 2012b). Second, spring application of inorganic fertilizer N might be expected to increase DMD and reduce CP by increasing the proportion of grass in the sward. The offsetting of these two effects may explain the lack of an effect of inorganic fertilizer N applied in spring on DMD and CP for Cut 1.

The consistent effect of sward mixture on both indices of nutritive value, with the binary mixture having a greater DMD and a lower CP at each cut compared to the red clover monoculture, reflects the ranking of values for these indices for the perennial ryegrass and red clover monocultures in this study. These relativities between the perennial ryegrass and red clover monocultures were previously reported for CP in the primary growth by King *et al.* (2012b), but these latter authors did not find a marked difference in DMD as seen in the present study. Overall, however, caution is required when interpreting the lower DMD values recorded for the red clover compared to the perennial ryegrass monoculture, because the relationship between *in vitro* DMD and either intake or performance by ruminants differs between clovers and ryegrass (Frame *et al.*, 1998; Dewhurst, 2013).

A feature of the findings in this study has been the similarity between the two cultivars of red clover in characteristics such as annual yield and DMD. In this context, it is not evident why Ruttinova exhibited a higher CP than Merviot at Cuts 1 and 2.

Red clover treatments – ensilability

Ensilability is an important determinant of how well forages would likely be preserved if they were ensiled using good management practices. Two commonly used indices of ensilability are WSC_{aq} and BC. In this study, these values ranged from 4·6 to 18·2 g/L and 386–548 mEq/kg DM, respectively, among the four cuts of the 16 treatments containing red clover. The range of WSC_{aq} found in this study is within the range found by King *et al.* (2012b). Likewise, the ranges for BC found in this study are within the ranges (390–570 mmol kg^{-1} DM) reported for lucerne (McDonald *et al.*, 1991). In general, the relatively small impact of applying inorganic fertilizer N in March on WSC_{aq} and BC values throughout the year reflects the relatively modest input of N.

The reduction in WSC_{aq} observed with the late harvest schedule at Cuts 1–3 was simultaneously observed for the perennial ryegrass monocultures. Herbage WSC can be influenced by factors such as species and growth stage, but prevailing weather conditions can have a much larger short-term effect as demonstrated by Keating and O'Kiely (2000a). Consequently, despite King *et al.* (2012b) showing WSC_{aq} decline with a later primary growth harvest, the findings of Keating and O'Kiely (2000a) indicate that this can be quite variable depending on prevailing weather conditions. In the present study, it is suggested that the late harvest schedule effect likely reflected prevailing weather conditions.

Buffering capacity has been shown to be positively related to CP content (Muck *et al.*, 1991), and it generally declines with advancing herbage growth stage (King *et al.*, 2012b). These findings contrast with those recorded in the present study where the later harvest schedule resulted in a higher BC at Cut 1. However, this greater value for the late schedule at Cut 1 was also observed for the grass monoculture receiving the lower rates of inorganic N. The reasons for the lower values in the late harvest schedule for Cuts 2 and 3 are not apparent.

McDonald *et al.* (1991) indicated that temperate grasses generally have higher WSC_{dm} and lower BC values than temperate legumes. Consequently, the higher WSC_{dm} and lower BC of the binary mixture relative to the red clover monoculture in the present study reflect its corresponding higher content of grass and a lower content of red clover. Overall, however, the similar low WSC_{aq} and high BC values recorded for the treatments associated with both cultivars of red clover are indicative of conditions that are challenging for a successful fermentation. Combining these two indices into a single fermentation coefficient (Weissbach, 1996) produces values between 17 and 27 and these are below the threshold value of 45 considered necessary for good preservation of silage. Thus, all the red clover treatments would be adjudged to require an aid to preservation such as adequate effective wilting or the adequate application of effective preservative. However, the results of King *et al.* (2012a) suggest that red clover with fermentation coefficients beneath the thresholds of Weissbach (1996) can sometimes undergo a satisfactory lactic acid-dominant fermentation with limited protein breakdown to ammonia. This indicates that this fermentation coefficient may not be appropriate for legumes such as red clover, a finding previously identified by Pahlow *et al.* (2002). The contrasting outcome relative to the fermentation coefficient developed for grasses may reflect the effects on silage fermentation of substrates not measured in WSC but fermented during ensilage, of compounds in red clover such as polyphenol oxidase (Lee, 2014) or of the lower water activity of legumes compared to grasses at a similar DM content (Muck, 2010).

Perennial ryegrass monocultures

The yield of 10 140 kg DM ha^{-1} for perennial ryegrass monocultures grown without the addition of inorganic fertilizer N was high in comparison with the results reported by Keating and O'Kiely (2000b), but similar to yields reported under comparable conditions by Conaghan *et al.* (2012). They were considerably higher than observed by Hopkins *et al.* (1990) in a study on a series of sites throughout England and Wales. However, the persistence of annual DM yields across the range of inorganic fertilizer N treatments and across the six years of this study was as expected for well-managed perennial ryegrass. Furthermore, the quadratic response of annual yield to inorganic fertilizer N agrees with Keating and O'Kiely (2000b) even though the rates of N that gave the maximum yield for individual cuts differed somewhat from those reported by Keating and O'Kiely (2000b) [values of 102, 113, 111 and 86 kg N ha^{-1} for Cuts 1, 2, 3 and 4, respectively, in the early harvest schedule, compared to corresponding values of 250, 122, 112 and 104 kg N ha^{-1} in Keating and O'Kiely (2000b)]. The average seasonal profile of grass production in the present study showed a greater proportion being produced earlier in the season (43, 23, 20 and 14% of annual DM yield at Cuts 1–4, respectively) compared to the values reported by Conaghan *et al.* (2012; 33, 25, 25 and 17% of annual DM yield at Cuts 1–4 respectively) under comparable climatic and management conditions.

The perennial ryegrass cultivar used in this study was typical of its species, and its ranges in indices of nutritive value and ensilability were comparable across four cuts to those reported for other cultivars by Keating and O'Kiely (2000b, c) and Conaghan *et al.* (2012). Similarly, the general negative response of DMD and WSC_{aq} and positive response of CP to incremental rates of application of inorganic fertilizer N agree with Keating and O'Kiely (2000b).

Equivalence of red clover to grass plus N

In order to relate the performance of the perennial ryegrass monoculture to which inorganic fertilizer N was applied with an alternative strategy based on red clover, a red clover treatment considered to best balance superior characteristics for yield, nutritive value and ensilability was selected. This treatment was the early harvest schedule that received no inorganic fertilizer N in March from the binary mixture including Ruttinova. Its performance was compared to that of perennial ryegrass treatments (Table 10).

Although the yield for the selected red clover treatment outperformed the perennial ryegrass treatments within the range of the data for Cuts 2, 3 and 4, the annual yield was matched by the perennial ryegrass monoculture receiving an annual input of 412 kg N ha^{-1}. However, the distribution of yield across the four harvests differed between the grass and clover. An important consideration when interpreting the findings in this study is the local site characteristics, particularly with respect to the apparent availability of soil N. This site supported 6-year average annual yields of 10 140 kg DM ha^{-1} for the grass monoculture receiving no inorganic fertilizer N input, and many other sites would likely require higher application rates of inorganic fertilizer N to achieve the comparable yields.

The consistently higher DMD of the perennial ryegrass monoculture across all rates of inorganic N application when compared to the selected red clover treatment must be interpreted within the context of the different animal responses to *in vitro* DMD for grasses and legumes (Frame *et al.*, 1998; Dewhurst, 2013). In contrast, for CP, rates of 116–150 kg fertilizer N ha^{-1} were required to produce equivalent values for individual cuts of red clover, thus reflecting the ability of red clover to produce a high-protein forage.

Despite the application of inorganic N to the perennial ryegrass monocultures reducing its ensilability indices, even at the maximum rate of fertilizer N input the ensilability indices of grass were consistently more favourable than those of the selected red clover treatment. However, as discussed earlier, caution must be exercised in imposing the thresholds appropriate for grass on red clover.

Conclusions

A mean annual yield of 14 906 kg DM ha^{-1} across all 16 red clover treatments and 15 785 kg DM ha^{-1} for the selected red clover treatment (early harvest schedule, 0 kg N ha^{-1} in March, binary mixture, Ruttinova), together with no trend towards a decline in annual yield over the six years of this study, shows the potential of red clover to contribute productively to the provision of forage on grassland farms. However, the general decline in the proportion of red clover in swards by the end of the sixth full season after sowing suggests that persistence was unlikely to have continued indefinitely beyond this study.

Altering harvest schedule, applying inorganic fertilizer N in spring, including perennial ryegrass in the seed mixture with red clover and the cultivar of red

Table 10 Mean values for the selected red clover treatment*, the rate of fertilizer N applied to perennial ryegrass (PRG) to match the selected red clover treatment and the maximum value achieved with PRG (with the corresponding fertilizer N rate in parentheses).

	Cut 1			Cut 2			Cut 3			Cut 4		
	Selected red clover treatment value	N (kg/ha)†	Maximum PRG‡ value (N rate; kg N/ha)	Selected red clover treatment value	N (kg/ha)†	Maximum PRG‡ value (N rate; kg N/ha)	Selected red clover treatment value	N (kg/ha)†	Maximum PRG‡ value (N rate; kg N/ha)	Selected red clover treatment value	N (kg/ha)†	Maximum PRG‡ value (N rate; kg N/ha)
Yield (kg DM/ha)	6364	65	6682 (102)	4459	No eq.§	3610 (113)	3847	No eq.	3222 (111)	1115	No eq.	2183 (86)
DMD (g/kg)	739	No eq.	795 (0)	736	No eq.	812 (0)	752	No eq.	808 (0)	749	No eq.	788 (0)
CP (g/kg DM)	155	116	172 (150)	187	129	202 (150)	218	150	218 (150)	263	134	273 (150)
WSC_{dm} (g/kg DM)	99	96	233 (0)	73	No eq.	271 (0)	59	No eq.	166 (0)	59	No eq.	126 (0)
WSC_{aq} (g/L)	15·6	No eq.	61 (0)	12·5	No eq.	78 (0)	8·5	No eq.	39 (0)	15·5	No eq.	31 (0)
BC (mEq/kg DM)	419	No eq.	407 (150)	516	No eq.	437 (150)	508	No eq.	409 (150)	475	No eq.	456 (150)

*Early harvest schedule, no inorganic N applied in March and the binary mixture containing Ruttinova.
†N required (kg/ha) on the perennial ryegrass monoculture in the early harvest schedule to equal the selected red clover treatment.
‡PRG = perennial ryegrass monoculture in the early harvest schedule.
§no eq. = no equivalent within the range of the data.

clover chosen all had contrasting and interacting effects on herbage yield, nutritive value and ensilability and on red clover persistence. Thus, a harvest schedule in which the primary growth of red clover-based swards was harvested in late May rather than in early June provided an overall advantage in terms of annual yield, DMD and the proportion of red clover in the sward. It had little overall impact on either crude protein content or ensilability. In contrast, although the application of inorganic fertilizer N to these swards in mid-March increased the primary growth yield, it reduced both annual yield and the proportion of red clover in the sward. Furthermore, it has no consistent effect on indices of nutritive value or ensilability. The inclusion of perennial ryegrass with red clover in the seed mixture (equal weight of seeds from both species) increased the annual yield by increasing the yield harvested at the primary and final cuts. It also improved the indices of digestibility and ensilability, but consistently reduced the crude protein content of the herbage. The inclusion of perennial ryegrass in the seed mixture also resulted in a lower proportion of red clover in the sward throughout the six years of this study, and this could potentially result in a shorter productive lifespan for a red clover-based sward. Finally, the two cultivars of red clover had relatively similar yield, nutritive value, ensilability and persistence characteristics.

Under the conditions of this study, the selected red clover-based treatment provided an annual yield and crude protein content that equated with a perennial ryegrass monoculture receiving 412 and 529 kg fertilizer N ha^{-1} $year^{-1}$, respectively, but with a different seasonal distribution of yield. Its indices of digestibility and ensilability were not as good as those of the perennial ryegrass monoculture. However, these indices should not be interpreted similarly for these two sward types.

A significant finding in this 6-year field plot study has been the persistence in annual yield of red clover-based swards. There is a need for farm-scale research to confirm whether such persistence can be repeated where red clover is subjected to the effects of machinery during fertilizer or manure spreading, and silage harvesting, on a number of occasions during the year and under a range of soil and weather conditions. It will also be important to quantify herbage yield and red clover persistence if some of the lighter yielding cuts (e.g., the final cut) were harvested by grazing livestock as a lower cost option. Finally, the potential agronomic benefits of red clover must be manifest in more profitable livestock systems, and thus, an appropriate whole-farm economic assessment is required.

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References

AOAC. (1990) *Official methods of analysis: 1st supplement*, 15th edn, pp. 3–4. Washington, DC: Association of Official Analytical Chemists.

Camlin M.S., Gilliland T.J. and Stewart R.H. (1983) Productivity of mixtures of Italian ryegrass and red clover. *Grass and Forage Science*, **38**, 73–78.

Conaghan P., O'Kiely P., Halling M.A., O'Mara F.P. and Nesheim L. (2012) Yield and quality response of perennial ryegrass selected for high water-soluble carbohydrate to nitrogen application rate. *Crop Science*, **52**, 2839–2851.

Depez B., Lambert R., Decamps C. and Peeters A. (2004) Production and quality of red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) in pure stand or in grass mixture in Belgium. *Grassland Science in Europe*, **9**, 498–500.

Dewhurst R.J., Delaby L., Moloney A., Boland T. and Lewis E. (2009) Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research*, **48**, 167–187.

Drobná J. and Jančovič J. (2006) Estimation of red clover (*Trifolium pratense* L.) forage quality parameters depending on the variety, cut and growing year. *Plant Soil and Environment*, **52**, 468–475.

Fales S.L. and Fritz J.O. (2007) Factors affecting forage quality. In: Barnes R.F., Nelson C.J., Collins M. and Moore K.J. (eds) *Forages. The science of grassland agriculture*, 6th edn, pp. 569–571. Wiley-Blackwell: Oxford, UK.

Finch T.F., Gardiner M.J., Comey A. and Radford T. (1983) *Soils of Co. Meath*. Dublin, Ireland: An Foras Talúntais.

Finneran E., Crosson P., O'Kiely P., Shalloo L., Forristal D. and Wallace M. (2010) Simulation modelling of the cost of producing and utilising feeds for ruminants on Irish farms. *Journal of Farm Management*, **14**, 95–116.

Frame J. and Harkess R. (1987) The productivity of four forage legumes sown alone and with each of five companion grasses. *Grass and Forage Science*, **42**, 213–223.

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

Frame J., Harkess R.D. and Hunt I.V. (1976) The effect of variety and fertilizer nitrogen level on red clover production. *Grass and Forage Science*, **31**, 111–115.

Frame J., Charlton J.F.L. and Laidlaw A.J. (1998) Red clover. In: Frame J., Charlton J.F.L. and Laidlaw

A.J. (eds) *Temperate forage legumes*, pp. 181–224. Wallingford, UK: CAB International.
Frankow-Lindberg B.E. (1989) The effect of nitrogen and clover proportion on yield of red clover-grass mixtures. *Proceedings of the XVI International Grassland Congress*, Nice, France, pp. 173–174
Gilliland T.J. and Meehan E.J. (2014) *Grass and clover varieties for 2014/2015 Grass and Clover Recommended Varieties for Northern Ireland 2014/2015*. Belfast: AFBI. Department of Agriculture and Rural Development.
Hopkins A., Gilbey J., Dibb C., Bowling P.J. and Murray P.J. (1990) Response of permanent and reseeded grassland to fertilizer nitrogen. 1. Herbage production and herbage quality. *Grass and Forage Science*, **45**, 43–55.
Keating T. and O'Kiely P. (2000a) Comparison of old permanent grassland *Lolium perenne* and *Lolium multiflorum* swards grown for silage. 2. Effects on conservation characteristics in laboratory silos. *Irish Journal of Agricultural and Food Research*, **39**, 25–33.
Keating T. and O'Kiely P. (2000b) Comparison of old permanent grassland *Lolium perenne* and *Lolium multiflorum* swards grown for silage. 3. Effects of varying fertiliser nitrogen application rate. *Irish Journal of Agricultural and Food Research*, **39**, 35–53.
Keating T. and O'Kiely P. (2000c) Comparison of old permanent grassland *Lolium perenne* and *Lolium multiflorum* swards grown for silage. 4. Effects of varying harvesting date. *Irish Journal of Agricultural and Food Research*, **39**, 55–71.
King C., McEniry J. and O'Kiely P. (2012a) A note on the fermentation characteristics of red clover silage in response to advancing stage of maturity in the primary growth. *Irish Journal of Agricultural Research*, **51**, 79–84.
King C., McEniry J., Richardson M. and O'Kiely P. (2012b) Yield and chemical composition of five common grassland species in response to nitrogen fertilizer application and phenological growth stage. *Acta Agriculturae Scandanavia, Section B - Soil & Plant Science*, **2**, 644–658.
Kölliker R., Herrmann D., Boller B. and Widmer F. (2003) Swiss Mattenklee landraces, a distinct and diverse genetic resource of red clover (*Trifolium pratense* L.). *Theoretical Applied Genetics*, **107**, 306–315.
Laidlaw A.S. and Frame J. (1988) Maximising the use of the legume in grassland systems. *Proceedings of the 12th General Meeting of the European Grassland Federation*. Dublin, Ireland, pp. 199–203.
Lee M.R.F. (2014) Forage polyphenol oxidase and ruminant livestock nutrition. *Frontiers in Plant Science*, **5**, 1–9.
Liu Y., Wu L., Baddeley J.A. and Watson C.A. (2011) Models of biological nitrogen fixation of legumes. A review. *Agronomy for Sustainable Development*, **31**, 155–172.
Marshall A.H., Lowe M. and Vale J.E. (2014) Persistence of red clover (*Trifolium pratense* L.) varieties in mixed swards over four harvest years. *Grassland Science in Europe*, **19**, 867–869.
McAuliffe C., Chamblee D.S., Uribe-Arango H. and Woodhouse W.W. (1958) Influence of inorganic nitrogen on nitrogen fixation by legumes as revealed by N^{15}. *Agronomy Journal*, **50**, 334–337.
McBratney J.M. (1981) Productivity of red clover grown alone and with companion grasses over a four-year period. *Grass and Forage Science*, **36**, 267–279.
McBratney J.M. (1984) Productivity of red clover grown alone and with companion grasses; further studies. *Grass and Forage Science*, **39**, 167–175.
McBratney J.M. (1987) Effect of fertilizer nitrogen on six-year-old red clover/perennial grass swards. *Grass and Forage Science*, **42**, 147–152.
McDonald P., Henderson A.R. and Heron S.J.E. (1991) *The biochemistry of silage*, 2nd edn. Marlow, UK: Chalcombe Publications.
Moore K.J. and Moser L.E. (1995) Quantifying developmental morphology of perennial grasses. *Crop Science*, **35**, 37–43.
Muck R.E. (2010) Silage microbiology and its control through additives. *Revista Brasileira de Zootecnia*, **39**, 183–191.
Muck R.E., O'Kiely P. and Wilson R.K. (1991) Buffering capacities in permanent pasture grasses. *Irish Journal of Agricultural Research*, **30**, 129–141.
Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A. (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology*, **46**, 683–691.
Ohlsson C. and Wedin W.F. (1989) Phenological staging schemes for predicting red clover quality. *Crop Science*, **29**, 416–420.
O'Kiely P. and Pahlow G. (2003) Comparison of buffering capacity of grasses determined using two different techniques. 2003 Research Report Beef Production. Teagasc Grange Research Centre.
Olson G.L., Smith S.R. and Lacefield G.D. (2014) *2014 Red and White Clover Report*. University of Kentucky, College of Agriculture, Food and Environment. Available at: https://www.uky.edu/Ag/Forage/PR677.pdf (accessed 10 December 2015).
Pahlow G., Rammer C., Slottner D. and Tuori M. (2002) Ensiling of legumes. *Landbauforschung Volkenrode*, **234**, 27–31.
Phelan P., Moloney A.P., McGeough E.J., Humphreys J., Bertilsson J., O'Riordan E.G. and O'Kiely P. (2015) Forage legumes for grazing and conserving in ruminant production systems. *Critical Reviews in Plant Sciences*, **34**, 281–326.
Playne M.J. and McDonald P. (1966) The buffering constituents of herbage and of silage. *Journal of the Science of Food and Agriculture*, **17**, 264–268.
Rinne M. and Nykänen A. (2000) Timing of primary growth harvest affects the yield and nutritive value of timothy-red clover mixtures. *Agricultural and Food Science*, **9**, 121–134.

SAS 9.4. (2014) *SAS Institute Inc. 2014. SAS/STAT® 13.2 User's Guide*. Cary, NC: SAS Institute Inc.

Thomas T.A. (1977) An automated procedure for the determination of soluble carbohydrates in herbage. *Journal of the Science of Food and Agriculture*, **28**, 639–642.

Tilley J.M.A. and Terry R.A. (1963) A two-stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society*, **18**, 104–111.

Walker T.W., Adams A.F.R. and Orchiston H.D. (1956) Fate of labelled nitrate and ammonium nitrogen when applied to grass and clover grown separately. *Soil Science*, **81**, 339–351.

Weissbach F. (1996) New developments in crop conservation. *Proceedings of the XIth International Silage Conference. Aberystwyth, UK*, 1996, pp. 11–25.