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Conservation efficiency and nutritive value of silages made from grass-red clover and multi-species swards compared with grass monocultures

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Abstract

Binary grass-clover and multi-species swards can increase herbage yields or facilitate reduced inputs of inorganic fertiliser nitrogen (N) compared with perennial ryegrass monocultures. However, the efficiency of the ensilage process and the nutritive value of silage produced from multi-species swards has not been documented. Replicate samples from grass-red clover binary mixture and multi-species mixture swards were ensiled in laboratory silos to assess the ensilability, fermentation characteristics, conservation losses and silage nutritive value compared with grass monocultures produced using inorganic N fertiliser. The results suggest that assessment of the ensilability and subsequent ensilage characteristics of binary and multi-species mixtures should be based on direct sampling from such mixtures rather than being predicted from values obtained from monocultures of constituent species. Under favourable ensiling conditions, unwilted binary mixtures and multi-species mixtures are satisfactorily preserved as silage, comparable to a perennial ryegrass monoculture receiving inorganic N fertiliser. However, when ensiled under more challenging crop conditions the mixtures exhibited a greater requirement for their preservation to be aided, compared with the perennial ryegrass monoculture. Despite the application of inorganic N reducing the legume content of multi-species mixture swards, it had relatively little effect on herbage ensilability or silage preservation. For all species treatments, silage nutritive values were primarily dependent on the pre-ensiling values, although herbage digestibility values declined during ensilage where the ensilage process was inefficient. The current study suggests that in order to be satisfactorily preserved as silage, binary grass-clover and multi-species swards have a greater requirement for an adequate rapid field wilt and/or effective preservative application compared with perennial ryegrass produced using inorganic fertiliser N.

Keywords

Conservation losses • ensilability • fermentation • nutritive value • silage

Introduction

In temperate grass-based ruminant production systems such as those in Ireland, grass silage is the primary forage available for livestock during the winter period when weather conditions can make grazing unfeasible. Ideally, for satisfactory preservation as silage, a crop should have an adequate supply of fermentable substrate in the form of water-soluble carbohydrates (WSC) and a relatively low buffering capacity. When WSC is expressed on an aqueous phase basis (WSC_{aq}), then increasing herbage DM content, by means of field wilting, for example, also improves ensilability (Buxton & O'Kiely, 2003).

Perennial ryegrass (*Lolium perenne* L.) is the most commonly sown grass species in temperate regions due to its versatility in both conservation and/or grazing management regimes, exhibiting good yield and silage preservation potential as well as high digestibility and persistence (Frame & Laidlaw, 2011). However, other grass species such as Italian ryegrass (*Lolium multiflorum* L.) are better suited to silage production due to a greater WSC content (Burns *et al*., 2015), while timothy (*Phleum pratense* L.) is preferred in colder regions due to its cold hardy nature (Bélanger *et al*., 2001).

The satisfactory preservation of legume species such as red (*Trifolium pratense* L.) and white (*Trifolium repens* L.) clover can be difficult due to their characteristically low WSC and high buffering capacity (Buxton & O'Kiely, 2003). However, despite their apparently poor ensilability characteristics, legume monocultures and grass–legume binary swards can undergo satisfactory lactic acid-dominant fermentation (Dewhurst *et al*., 2003; King *et al*., 2012a; Copani *et al*., 2014). Red clover is used mainly in swards managed to produce conserved fodder

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and is typically grown with a companion grass species such as perennial ryegrass. The primary function of the companion grass is to increase herbage DM yield, but it can also improve the overall ensilability (Clavin *et al*., 2017). White clover has an important role in grassland swards for grazing (Phelan *et al*., 2015), and its shallow rooting nature has made it a particularly successful partner for perennial ryegrass (Black *et al*., 2009). Alternative forage herbs such as chicory (*Cichorium intybus* L.) and ribwort plantain (*Plantago lanceolata* L.) can contribute to improved herbage yields and nutritive value (Sanderson *et al*., 2003; Deak *et al*., 2007; Pirhofer-Walzl *et al*., 2011), but little research has been reported on the preservation of these species as silage either in monoculture or in mixtures with grasses and/or legumes. However, the relatively high WSC content reported for chicory by Barry (1998), for example, is encouraging.

Sown multi-species grassland swards can transgressively overyield due to temporal and spatial complementarity among the sown species and also to interspecific facilitation such as the transfer of symbiotically fixed N from legumes to neighbouring species (Nyfeler *et al*., 2009; Finn *et al*., 2013; Lüscher *et al*., 2014). Although sward botanical composition can influence herbage chemical composition (Sanderson, 2010) the nutritive value of multi-species swards can differ from what would be expected based on the chemical composition of the component species grown in monoculture (Moloney *et al*. 2020a; Ergon *et al*., 2017). This, in turn, raises the possibility that indices of ensilability and ultimately conservation efficiency may also differ from what might be predicted from values obtained for the constituent species grown in monoculture.

The current paper follows two earlier studies which examined the yield and botanical composition (Moloney *et al*., 2020b) and the nutritive value (Moloney *et al*. 2020a) of binary and multi-species mixtures compared with a number of temperate grassland species monocultures managed under a four-cut annual silage production regime where the response of multi-species mixtures to inorganic nitrogen (N) was also determined.

The objectives of the current study were to quantify the effects on herbage ensilability and silage nutritive value, fermentation characteristics and conservation losses of (1) three common temperate grass monocultures receiving inorganic N or grown in binary mixture with red clover, and a red clover monoculture, (2) a perennial ryegrass monoculture receiving 360 kg N/ha per year compared with a perennial ryegrass/red clover binary mixture and two multi-species mixtures, each receiving no inorganic N fertiliser, (3) a perennial ryegrass monoculture and two multi-species mixtures each receiving 360 kg N/ha per year, and (4) two multi-species mixtures receiving either 0 or 360 kg N/ha per year. Herbage was ensiled without the assistance of either wilting or the application of additives so as to best assess the inherent ensilability of the various sward treatments.

Materials and methods

Field plots

Field plots (each 9 m × 2 m) were established at Teagasc Grange (53.52°N, 6.66°W), and details of soil characteristics, treatment establishment and general plot management have been described by Moloney *et al*. (2020b). The herbages used in this study were obtained from Year 1 of the (Moloney *et al*., 2020b) study which corresponds to the second year after sowing. Eleven treatments (Table 1) from each of four replicate blocks

Table 1: Sward types and the associated species included, rates of seed used, and rates of inorganic N applied

Sward	Species included[1]	Seed rate[2]	N[3]
TIM/360N	Timothy	15	360
IRG/360N	Italian ryegrass	42	360
PRG/0-360N	Perennial ryegrass	32	0, 360
RC	Red clover	15	0
TIM/RC	Timothy, red clover	6, 9	0
IRG/RC	Italian ryegrass, red clover	16.8, 9	0
PRG /RC	Perennial ryegrass, red clover	12.8, 9	0
Mix 1/0-360	Timothy, perennial ryegrass, red clover, white clover	3, 6.4, 5.25, 3	0, 360
Mix 2/0-360	Timothy, perennial ryegrass, red clover, ribwort plantain, chicory	3, 6.4, 5.25, 1.5, 0.63	0, 360

[1]Perennial ryegrass in binary- or multi-species mixtures was always intermediate heading-date, diploid cultivars (as per PRG).
[2]kg seed/ha (values correspond in order with species in the preceding column).
[3]Inorganic fertiliser N input (kg N/ha per year).

were used in this experiment and constituted a randomised complete block design. A single representative sample of harvested herbage was collected from each treatment plot and thus both the experimental unit and replication were at the field plot level. Five monoculture or multi-species mixtures were grown using 360 kg of inorganic fertiliser N/ha per year, and these were Italian ryegrass (IRG/360N), perennial ryegrass (PRG/360N), timothy (TIM/360N), Mix 1 (perennial ryegrass, timothy, red and white clovers; Mix 1/360N) and Mix 2 (perennial ryegrass, timothy, red clover, ribwort plantain and chicory; Mix 2/360N). A further five treatments comprised binary or multi-species mixtures grown without inorganic fertiliser N input. These were binary mixtures of Italian ryegrass (IRG/RC), perennial ryegrass (PRG/RC) and timothy (TIM/RC) with red clover, as well as Mix 1/0N and Mix 2/0N. The final treatment was a red clover monoculture grown without inorganic N input (RC). The species included in each herbage treatment were represented by an equal proportion of three cultivars (two for chicory and one for ribwort plantain). Details of the cultivars used and their seeding rates are presented in Tables 1 and 2. The annual production from each plot was harvested in four cuts (Cuts 1–4) taken on 27 May, 15 July, 2 September and 10 November, with the herbage harvested at Cuts 1–3 being used in this experiment. The inorganic N (calcium ammonium nitrate; 275 g N/kg) was applied at 120, 100, 80 and 60 kg N/ha in mid-March and immediately after Cuts 1–3, respectively. Individual samples of each species growing in Mix 1 and Mix 2 and receiving 120 kg N/ha per year were also taken and their chemical composition determined.

At each cut, the herbage was harvested to a 6-cm stubble height using a Haldrup forage plot harvester (J. Haldrup, Løgstør, Denmark) before being passed through a precision-chop forage harvester (MEX V1, Pottinger, Grieskirchen, Austria) set to a theoretical chop length of 19 mm. A representative 6 kg sample from each plot was immediately ensiled in a laboratory silo (O'Kiely & Wilson, 1991) for 100 days at approximately 15°C. The sealed silos accommodated steel weights to apply practical levels of pressure to the compacted herbage and permitted the drainage of effluent throughout the ensilage and its separate storage beneath the silage. At the silo opening, the silage and effluent were weighed and sampled. Silage DM recovery was calculated as the weight of silage DM removed from the silo expressed as a proportion of the weight of herbage DM ensiled. Samples of herbage taken pre- and post-ensilage were stored at −18°C prior to chemical analysis. Sward botanical composition was determined on pre-ensilage samples and was reported by (Moloney *et al*., 2020b).

Chemical analysis

Representative samples of pre-ensilage herbage were dried at 98°C for 16 h in an oven with forced air circulation, while post-ensilage samples were dried at 40°C for 48 h to estimate silage DM content. Sub-samples of the pre- and post-ensilage herbage were dried at 60°C and 40°C for 48 h, respectively, before being milled through a 1 mm aperture sieve (Wiley mill, 1 mm pore screen). *In vitro* DM digestibility (DMD) was then determined using the method of Tilley and Terry (1963) with the modification that the final residue was isolated by filtration (Whatman GF/A 55 mm, pore size 1.6 μm; Whatman International, Maidstone, UK) rather than by centrifugation. WSC content was measured using the anthrone method on an Autoanalyser 3 (Bran and Leubbe GmbH, Norderstedt, Germany) and, for pre-ensilage herbage only, expressed on an aqueous phase (WSC_{aq}; g/kg aqueous extract) basis, while ash was determined by complete combustion in a muffle furnace at 550°C for 5 h. The crude protein (CP) content (N × 6.25) was determined using a LECO FP 428 N analyser (Leco Instruments, St. Joseph, MI, USA) based on the method 990-03 of the Association of Analytical Chemists (AOAC) (1990). Herbage buffering capacity was determined for pre-ensilage herbage only according to the method of Playne and

Table 2: Species and cultivars used

Species	Cultivar[1]
Timothy (*Phleum pratense* L.) (TIM)	Comer (9/6; H), Erecta (10/6; H), Promesse (10/6; H)
Italian ryegrass (*Lolium multiflorum* Lam.) (IRG)	Fabio (19/5; T), Nabucco (21/5; T), Davinci (23/5; D)
Perennial ryegrass (*Lolium perenne* L.) (PRG)	Premium (23/5; D), Shandon (21/5; D), Solomon (22/5; D)
Red clover (*Trifolium pratense* L.) (RC)	Aberruby (D), Amos (T), Merviot (D) (all early flowering)
White clover (*Trifolium repens* L.)	Aran (very large leaf), Barblanca (large leaf), Chieftain (medium leaf)
Ribwort plantain (*Plantago lanceolata* L.)	Ceres Tonic (D)
Chicory (*Cichorium intybus* L.)	Grasslands Choice (D), Puna (D)

[1]Heading date (date/month), ploidy (D – diploid, T – tetraploid, H – hexaploid) and other cultivar classification characteristics. PRG cultivars were all of intermediate heading date.

McDonald (1966) using an 809 Titrando Universal Titrator and Titrosampler (Metrohm, Herisau, Switzerland). Pre-ensilage DMD, CP, WSC and ash have been reported by Moloney *et al*. (2020a).

Aqueous extracts were obtained from each silage sample, and pH was determined using a pH electrode (HI98127; Hanna Instruments Ltd., Leighton Buzzard, Bedfordshire, UK). L-lactic acid concentration was determined using the SP-Ace Clinical Chemical Analyzer, (Alfa Wassermann Inc., West Caldwell, NJ, USA) and the L-lactic acid ultraviolet (UV)-method test kit (catalogue no. 101309084035; Roche/R-Biopharm, Darmstadt, Germany), whereas D-lactate concentration was determined using the enzyme D-lactate dehydrogenase (catalogue no. 1016941001; Roche/R-Biopharm). The concentration of ammonia (NH_3) was determined using the SP-Ace Clinical Chemical Analyzer and the Thermo Electron Infinity ammonia liquid stable reagent kinetic method (Thermo Fisher Scientific Inc., Waltham, MA, USA).

A fermentation coefficient for pre-ensiled herbage was calculated according to Weissbach and Honig (1996) as follows:

$$\mathrm{DM} + \frac{8 \times \mathrm{WSC_{dm}}}{\mathrm{BC}}$$

where DM = DM g/100 g; WSC_{dm} = WSC of pre-ensiled herbage on a DM basis; BC = buffering capacity expressed as g lactic acid/kg DM, calculated as 0.1545 buffering capacity (mEq/kg DM) – 2.1153.

The index of Flieg's point (Moselhy *et al*., 2015) was used as a gauge of the general standard of silage preservation and was calculated as follows:

$$220 + ((2 \times \mathrm{DM}) - 15) - (40 \times \mathrm{pH})$$

where DM was expressed as g/100 g. According to this index, silage preservation was considered "very bad" at values < 20, "bad" between 21 and 40, "medium" between 41 and 60, "good" between 61 and 80, and "very good" between 81 and 100.

Statistical analysis

The analysis was fitted as a one-way classification of 11 treatments which accounted for the four replicate blocks, using the Mixed procedure in SAS 9.4 (SAS, 2013). Within the 11 treatments, there were two subsets with a factorial structure and two subsets with simple contrasts. These constituted four groupings of treatment that addressed the four objectives of the study described previously. Group 1 included seven treatments (PRG/360N, IRG/360N, TIM/360N, PRG/RC, IRG/RC, TIM/RC and RC) to give a (3 × 2) + 1 arrangement with the +1 (i.e. RC) being a control. Group 2 and Group 3 were simple four- (PRG/360N, PRG/RC, Mix 1/0N and Mix 2/0N) and three- (PRG/360N, Mix 1/360N and Mix 2/360N) level one-way classifications, while Group 4 included four treatments (Mix 1/0N, Mix 1/360N, Mix 2/0N and Mix 2/360N) organised in a 2 × 2 factorial arrangement. In order to accommodate these structures, a series of contrasts were used to evaluate interactions and associated main effects. Main effect means were estimated and compared where appropriate for each of the three cuts separately. Residual checks were made to ensure that the assumptions of the analysis were met. Depending on the results of the interaction tests, multiple comparison adjustments were made (step-down Bonferroni adjustment), for each variable analysed, for each of the relevant sets of comparisons.

Results

Perennial ryegrass, Italian ryegrass and timothy receiving inorganic N or grown with red clover (Group 1; PRG/360N, IRG/360N, TIM/360N, PRG/RC, IRG/RC, TIM/RC and RC)

Herbage ensilability

At Cut 1 (Tables 3 and 4), herbage WSC_{aq} content was greater (P < 0.05) for Italian ryegrass-based treatments (IRG/360N plus IRG/RC) than for perennial ryegrass-based treatments (PRG/360N plus PRG/RC) which were in turn greater (P < 0.05) than timothy-based treatments (TIM/360N plus TIM/RC) and RC. Furthermore, at both Cuts 1 and 2, IRG/RC and IRG/360N had greater (P < 0.01) WSC_{aq} than RC while at Cut 2 (Tables 5 and 6), Italian ryegrass-based treatments had greater (P < 0.01) WSC_{aq} content than both perennial ryegrass- and timothy-based treatments, with the magnitude of difference being greater when each grass was grown with red clover than inorganic N. At Cut 3 (Tables 7 and 8), IRG/360N and PRG/360N had a greater (P < 0.01) WSC_{aq} content than TIM/360N, while IRG/RC had a greater WSC_{aq} than both PRG/RC and TIM/RC with RC being intermediate.

Herbage buffering capacity at Cut 1 was greater (P < 0.05) for timothy-based treatments than for Italian ryegrass-based treatments, with perennial ryegrass-based treatments being intermediate, while RC had a greater (P < 0.01) buffering capacity than all other treatments. At Cut 2, the buffering capacity was greater (P < 0.01) for RC, PRG/RC and TIM/RC than for IRG/RC, while there was no difference (P > 0.05) in the buffering capacity of IRG/360N, PRG/360N or TIM/360N. At Cut 3, the buffering capacity was greatest (P < 0.01) for RC, while perennial ryegrass-based treatments had greater (P < 0.01) buffering capacity than timothy-based treatments which were greater (P < 0.01) than Italian ryegrass-based treatments. Furthermore, values were greater (P < 0.001)

Table 3: Mean herbage ensilability, and silage nutritive value, fermentation and conservation losses, and the associated SEM and *P* values, for all 11 treatments at Cut 1

	IRG/360N	PRG/360N	TIM/360N	RC	IRG/RC	PRG/RC	TIM/RC	Mix 1/0N	Mix 1/360N	Mix 2/0N	Mix 2/360N	SEM	*P*
Herbage ensilability													
WSC_{aq}[1]	32	26	10	9	51	26	15	17	17	24	14	6.0	< 0.01
Buffering capacity[2]	288	284	312	540	237	286	321	295	290	278	311	18.3	< 0.001
Fermentation coefficient	48	43	24	19	70	42	30	33	34	42	29	6.5	< 0.001
Silage fermentation													
pH	4.1	4.1	4.4	5.1	3.7	3.8	4.6	4.3	4.1	3.8	4.1	0.20	< 0.01
LA[3]	59	69	51	12	92	88	38	81	56	83	65	16.4	< 0.05
D-LA[4]	537	560	578	512	531	539	615	610	558	534	574	37.5	0.660
WSC[3]	18	11	10	10	42	18	11	12	12	16	11	3.1	< 0.001
NH_3-N[5]	109	130	154	188	63	86	202	165	109	76	103	46.6	0.333
Flieg's point	75	73	64	30	95	85	52	65	72	86	73	8.6	0.001
Conservation losses													
Effluent output[6]	186	184	202	317	149	122	185	174	215	112	194	20.1	< 0.001
Recovery rate[7]	870	832	952	741	893	899	855	941	893	925	931	31.6	0.002
Silage nutritive value													
DM[8]	174	157	165	149	188	172	156	167	161	170	163	7.2	0.053
DMD[8]	655	656	685	614	677	703	685	695	682	701	674	14.2	< 0.01
Crude protein[3]	123	131	137	176	94	107	117	111	126	110	130	5.6	< 0.001

The above SEM was used in the contrasts of Groups 3 and 4; DMD = DM digestibility; LA = lactic acid; WSC = water-soluble carbohydrates; WSC_{aq} = water-soluble carbohydrates (aqueous phase basis).
[1]g/L.
[2]mEq/kg DM.
[3]g/kg DM.
[4]g D-lactic acid/kg lactic acid.
[5]g/kg N.
[6]g/kg fresh herbage ensiled.
[7]g silage DM/kg herbage DM ensiled.
[8]g/kg.

Table 4: SEM and *P* values for herbage ensilability, and silage nutritive value, fermentation and conservation losses for the main effects and interactions for two groups of treatments at Cut 1

Group[1]	**1**						**4**					
	Species[2]		**N source**[3]		**Species X N source**		**Mix**[4]		**N rate**[5]		**Mix X N rate**	
	SEM	***P***	**SEM**	***P***	**SEM**[6]	***P***	**SEM**	***P***	**SEM**	***P***	**SEM**	***P***
Herbage ensilability												
WSC_{aq}[7]	4.2	< 0.001	3.5	0.105	6	0.289	4.2	0.741	4.2	0.411	6	0.384
Buffering capacity[8]	13	0.021	10.6	0.366	18.3	0.221	13	0.899	13	0.452	18.3	0.307
Fermentation coefficient	4.6	< 0.001	3.7	0.09	6.5	0.181	4.6	0.765	4.6	0.344	0.329	6.5
Silage fermentation												
pH	0.14	0.013	0.11	0.427	0.2	0.225	0.14	0.188	0.14	0.793	0.2	0.227
LA[9]	10.8	0.064	9	0.291	16.4	0.313	10.8	0.691	10.8	0.156	16.4	0.795
D-LA[10]	26.5	0.238	21.7	0.925	37.5	0.729	26.5	0.441	26.5	0.871	37.5	0.228
WSC[9]	2.2	< 0.001	1.8	< 0.001	3.1	0.002	2.2	0.506	2.2	0.419	3.1	0.472
NH3-N[11]	30.6	0.105	25.6	0.692	46.6	0.476	30.6	0.239	30.6	0.712	46.6	0.309
Flieg's point	6.1	0.009	4.9	0.357	8.6	0.169	6.1	0.205	6.1	0.696	8.6	0.254
Conservation losses												
Effluent output[12]	15.5	0.166	12.3	0.03	20.1	0.556	15.5	0.059	15.5	0.006	20.1	0.324
Recovery rate[13]	22.3	0.491	18.2	0.925	31.6	0.038	22.3	0.734	22.3	0.51	31.6	0.389
Silage nutritive value												
DM[14]	5.1	0.016	4.2	0.265	7.2	0.191	5.1	0.756	5.1	0.376	7.2	0.894
DMD[14]	10	0.613	8.2	0.016	14.2	0.536	10	0.937	10	0.171	14.2	0.643
Crude protein[9]	4	0.009	3.3	< 0.001	5.6	0.778	4	0.792	4	0.004	5.6	0.66

DMD = DM digestibility; IRG = Italian ryegrass; LA = lactic acid; PRG = perennial ryegrass; RC = red clover; TIM = timothy; WSC = water-soluble carbohydrates; WSC_{aq} = water-soluble carbohydrates (aqueous phase basis).
[1]Group 1 = IRG/360N, PRG/360N, TIM/360N, IRG/RC, PRG/RC, TIM/RC with RC as a control; Group 4 = Mix 1/0N, Mix 1/360N, Mix 2/0N and Mix 2/360N.
[2]Species = PRG, IRG and TIM.
[3]N source = 360 kg N/ha per year or RC.
[4]Mix = Mix 1 or Mix 2.
[5]N rate = 0 or 360 kg N/ha/year.
[6]This SEM was calculated for the 3 × 2 interaction but is also used when comparing RC to any of the 3 × 2 treatments.
[7]g/L.
[8]mEq/kg DM.
[9]g/kg DM.
[10]g D-lactic acid.
[11]g/kg N.
[12]g/kg fresh herbage ensiled.
[13]g silage DM/kg herbage DM ensiled.
[14]g/kg.

when grasses were grown with red clover (grass + RC) than inorganic N (grass + N).

Fermentation characteristics

Silage pH was greatest ($P < 0.05$) for RC compared with Italian ryegrass-, perennial ryegrass- or timothy-based silages at Cuts 1 (Tables 3 and 4) and 2 (Tables 5 and 6), while at Cut 3 (Tables 7 and 8) TIM/360N had a greater value than PRG/360N and both TIM/RC and PRG/RC had greater values than IRG/RC ($P < 0.05$).

Silage lactic acid concentration at Cut 2 was greater ($P < 0.001$) for perennial ryegrass-based silages than Italian ryegrass- or timothy-based silages as well as RC, while at Cut 3 lactic acid concentration was greater ($P < 0.01$) for

Table 5: Mean herbage ensilability, and silage nutritive value, fermentation and conservation losses, and the associated SEM and *P* values, for all 11 treatments at Cut 2

	IRG/360N	PRG/360N	TIM/360N	RC	IRG/RC	PRG/RC	TIM/RC	Mix 1/0N	Mix 1/360N	Mix 2/0N	Mix 2/360N	SEM	*P*
Herbage ensilability													
WSC_{aq} [1]	44	23	25	15	76	24	22	26	18	23	19	5.1	< 0.001
Buffering capacity	329	409	313	605	219	518	475	447	397	488	425	34.4	< 0.001
Fermentation coefficient	50	32	38	22	81	29	32	32	29	30	29	4.7	< 0.001
Silage fermentation													
pH	3.8	3.9	3.9	4.6	3.8	4.0	4.4	4.0	3.9	4.0	4.1	0.13	< 0.01
LA	104	134	95	71	74	131	76	117	109	106	103	11.0	< 0.01
D-LA	462	487	475	480	479	463	491	479	475	474	524	16.8	0.480
WSC	16	13	11	8	32	17	10	15	10	15	12	3.7	< 0.01
NH_3-N	67	63	46	80	53	69	89	66	60	64	73	6.6	< 0.01
Flieg's point	96	87	98	59	101	85	68	85	87	84	77	6.0	< 0.001
Conservation losses													
Effluent output	39	88	50	73	60	40	35	3	77	62	37	35.9	0.911
Recovery rate	964	994	994	976	915	985	974	985	993	995	969	18.6	0.182
Silage nutritive value													
DM	223	183	238	183	245	190	192	193	197	195	185	5.9	< 0.001
DMD	654	770	690	670	688	747	690	754	728	724	726	15.4	< 0.001
Crude protein	142	175	149	213	90	157	165	158	169	151	162	7.7	< 0.001

DMD = DM digestibility; IRG = Italian ryegrass; LA = lactic acid; PRG = perennial ryegrass; RC = red clover; TIM = timothy; WSC = water-soluble carbohydrates; WSC_{aq} = water-soluble carbohydrates (aqueous phase basis).
[1]See footnotes beneath Table 3.

Table 6: SEM and *P* values for herbage ensilability, and silage nutritive value, fermentation and conservation losses for the main effects and interactions for two groups of treatments at Cut 2

Group[1]	1						4					
	Species		N source		Species X N source		Mix		N rate		Mix X N rate	
	SEM	*P*	SEM	*P*	SEM	*P*	SEM	*P*	SEM	*P*	SEM	*P*
Herbage ensilability												
WSC_{aq}	3.6	< 0.001	2.9	0.022	5.1	0.003	3.6	0.844	3.6	0.24	5.1	0.763
Buffering capacity	24.3	< 0.001	19.8	0.063	34.4	0.001	24.3	0.322	24.3	0.111	34.4	0.838
Fermentation coefficient	3.3	< 0.001	2.7	0.068	4.7	< 0.001	3.3	0.756	3.3	0.717	4.7	0.835
Silage fermentation												
pH	0.09	0.093	0.08	0.065	0.13	0.111	0.09	0.392	0.09	0.731	0.13	0.62
LA	7.8	0.001	6.3	0.063	11	0.46	7.8	0.454	7.8	0.652	11	0.836
D-LA	11.9	0.758	9.7	0.827	16.8	0.391	11.9	0.206	11.9	0.178	16.8	0.125
WSC	2.6	0.003	2.1	0.046	3.7	0.07	2.6	0.822	2.6	0.293	3.7	0.851
NH3-N	4.7	0.508	3.8	0.037	6.6	0.001	4.7	0.428	4.7	0.844	6.6	0.226
Flieg's point	4.2	0.034	3.5	0.067	6	0.016	4.2	0.372	4.2	0.688	6	0.519
Conservation losses												
Effluent output	25.4	0.827	20.7	0.643	35.9	0.636	25.4	0.791	25.4	0.5	35.9	0.178
Recovery rate	13.1	0.023	10.7	0.105	18.6	0.561	13.1	0.714	13.1	0.636	18.6	0.384
Silage nutritive value												
DM	4.2	< 0.001	3.4	0.239	5.9	< 0.001	4.2	0.44	4.2	0.601	5.9	0.277
DMD	10.9	< 0.001	8.9	0.618	15.4	0.194	10.9	0.31	10.9	0.436	15.4	0.383
Crude protein	5.4	< 0.001	4.4	0.008	7.7	0.001	5.4	0.343	5.4	0.175	7.7	0.987

DMD = DM digestibility; LA = lactic acid; WSC = water-soluble carbohydrates; WSC_{aq} = water-soluble carbohydrates (aqueous phase basis).
[1]See footnotes beneath Table 4.

PRG/360N than for RC, PRG/RC and TIM/RC. Furthermore, the proportion of D-lactate was greater ($P < 0.05$) for TIM/RC than for PRG/360N.

Italian ryegrass-based silages had a greater ($P < 0.01$) WSC content than perennial ryegrass- and timothy-based silages and RC at Cuts 1 and 2, with the magnitude of this difference being greater ($P < 0.01$) when each grass was grown with red clover than with inorganic N at Cut 1 only.

Concentrations of NH_3-N at Cut 2 were greater ($P < 0.05$) for TIM/RC than for IRG/360N, PRG/360N, TIM/360N, IRG/RC and PRG/RC, while RC was greater ($P < 0.05$) than TIM/360N and IRG/RC. At Cut 3, TIM/360N, PRG/RC and TIM/RC had greater ($P < 0.05$) NH_3-N concentrations than PRG/360N, while TIM/360N was greater than IRG/360N, PRG/360N and IRG/RC.

Conservation losses

Effluent production ($P < 0.05$) was greater for grass + N than for grass + RC at Cut 1 (Tables 3 and 4), while RC produced more effluent than any other treatment ($P < 0.01$). At Cut 3 (Tables 7 and 8), however, effluent production was greater ($P < 0.01$) for grass + RC than for grass + N. Silage DM recovery rates at Cut 1 were greater ($P < 0.05$) for TIM/360N than for PRG/360N, TIM/RC and RC, while at Cut 2 (Tables 5 and 6) DM recovery was greater ($P < 0.05$) for perennial ryegrass- and timothy-based silages than for Italian ryegrass-based silages.

Silage nutritive value

Silage DM content at Cut 1 (Tables 3 and 4) was greater ($P < 0.01$) for Italian ryegrass-based silages than for RC and at Cut 2 (Tables 5 and 6) it was greater ($P < 0.001$) for IRG/RC and TIM/360N than for TIM/RC, PRG/RC, RC and PRG/360N. Silage DMD at Cut 1 was lower ($P < 0.01$) for RC than for Italian ryegrass-, perennial ryegrass- and timothy-based silages; however, DMD values were greater ($P < 0.05$) for grass + RC than for grass + N. At Cut 2, perennial ryegrass-based silages (PRG/360N and PRG/RC) had greater ($P < 0.001$) values than Italian ryegrass or timothy-based silages (IRG/360,

Table 7: Mean herbage ensilability, and silage nutritive value, fermentation and conservation losses, and the associated SEM and *P* values, for all 11 treatments at Cut 3

	IRG/360N	PRG/360N	TIM/360N	RC	IRG/RC	PRG/RC	TIM/RC	Mix 1/0N	Mix 1/360N	Mix 2/0N	Mix 2/360N	SEM	*P*
Herbage ensilability													
WSC_{aq}[1]	17	16	7	11	14	9	8	8	11	8	10	1.0	< 0.001
Buffering capacity	435	560	476	757	533	775	666	671	544	651	569	26.2	< 0.001
Fermentation coefficient	27	23	19	17	23	17	17	17	21	17	19	0.9	< 0.001
Silage fermentation													
pH	4.9	4.3	5.3	5.4	4.6	5.8	5.9	5.7	5.6	5.6	5.4	0.24	< 0.001
LA	14	42	10	4	25	3	1	7	2	4	2	7.8	< 0.01
D-LA	550	519	594	589	544	603	606	579	590	603	580	17.1	< 0.05
WSC	8	10	7	7	8	7	7	8	8	7	8	0.5	< 0.05
NH_3-N	164	85	362	230	126	326	282	219	231	280	377	64.6	< 0.05
Flieg's point	43	65	26	21	52	5	−3	9	10	9	19	10.6	0.001
Conservation losses													
Effluent output	75	100	97	133	159	189	176	137	66	177	142	34.4	0.177
Recovery rate	857	987	933	970	915	909	937	948	934	923	895	42.0	0.713
Silage nutritive value													
DM	161	168	157	155	166	155	147	155	149	147	145	7.4	0.417
DMD	644	782	661	666	732	683	661	666	706	689	708	16.9	< 0.001
Crude protein	172	207	156	186	192	160	179	195	173	174	168	7.8	< 0.01

DMD = DM digestibility; IRG = Italian ryegrass; LA = lactic acid; PRG = perennial ryegrass; RC = red clover; TIM = timothy; WSC = water-soluble carbohydrates; WSC_{aq} = water-soluble carbohydrates (aqueous phase basis).
[1]See footnotes beneath Table 3.

Table 8: SEM and *P* values for herbage ensilability, and silage nutritive value, fermentation and conservation losses for the main effects and interactions for two groups of treatments at Cut 3

Group[1]	1				4							
	Species		N source		Species X N source		Mix		N rate		Mix X N rate	
	SEM	*P*	SEM	*P*	SEM	*P*	SEM	*P*	SEM	*P*	SEM	*P*
Herbage ensilability												
WSC_{aq}[1]	0.7	< 0.001	0.6	0.001	1.0	0.003	0.7	0.806	0.7	0.011	1.0	0.463
Buffering capacity	18.5	< 0.001	15.1	< 0.001	26.2	0.08	18.5	0.935	18.5	< 0.001	26.2	0.399
Fermentation coefficient	0.6	< 0.001	0.5	< 0.001	0.9	0.159	0.6	0.55	0.6	< 0.001	0.9	0.422
Silage fermentation												
pH	0.17	0.005	0.14	0.003	0.24	0.008	0.17	0.562	0.17	0.562	0.24	0.684
LA	5.1	0.058	4.1	0.035	7.8	0.003	5.1	0.859	5.1	0.655	7.8	0.861
D-LA	12.1	0.012	9.9	0.04	17.1	0.035	12.1	0.7	12.1	0.734	17.1	0.325
WSC	0.4	0.029	0.3	0.046	0.5	0.015	0.4	0.957	0.4	0.122	0.5	0.551
NH3-N	42.5	0.016	33.8	0.385	64.6	0.016	42.5	0.085	42.5	0.351	64.6	0.464
Flieg's point	7.5	0.006	6.1	0.004	10.6	0.01	7.5	0.679	7.5	0.646	10.6	0.678
Conservation losses												
Effluent output	24.3	0.707	19.8	0.005	34.4	0.99	24.3	0.101	24.3	0.131	34.4	0.611
Recovery rate	29.7	0.309	24.3	0.874	42	0.283	29.7	0.456	29.7	0.617	42	0.865
Silage nutritive value												
DM	5.2	0.297	4.3	0.328	7.4	0.463	5.2	0.392	5.2	0.591	7.4	0.767
DMD	11.9	0.008	9.8	0.272	16.9	< 0.001	11.9	0.469	11.9	0.087	16.9	0.553
Crude protein	5.5	0.084	4.5	0.825	7.8	< 0.001	5.5	0.108	5.5	0.084	7.8	0.289

DMD = DM digestibility; LA = lactic acid; WSC = water-soluble carbohydrates; WSC_{aq} = water-soluble carbohydrates (aqueous phase basis).
[1]See footnotes beneath Table 4.

IRG/RC or TIM/360N, TIM/RC, respectively) and RC. At Cut 3 (Tables 7 and 8), PRG/360N had a greater (P < 0.05) DMD than TIM/360N which, in turn, was greater than IRG/360N. Furthermore, at Cut 3, PRG/360N had a greater (P < 0.05) DMD value than PRG/RC, IRG/360N had a lower (P < 0.05) value than IRG/RC, while RC had a lower (P < 0.05) DMD value than both PRG/360N and IRG/RC.

The silage CP content was greater (P < 0.01) for RC than for all other treatments at Cuts 1 and 2. Furthermore, at Cut 1, the silage CP content was greater (P < 0.01) for timothy than for Italian ryegrass-based silages, while grass + N had greater (P < 0.001) values than grass + RC. The silage CP content at Cut 2 was greater for PRG/360N than for TIM/360N and IRG/360N; however, both PRG/RC and TIM/RC had greater values than IRG/RC (P < 0.001). At Cut 3, PRG/360N had a greater (P < 0.01) CP content than PRG/RC and TIM/360N, while IRG/RC was greater than PRG/RC, PRG/360N was greater than PRG/RC, and TIM/RC was greater than TIM/360N (P < 0.05).

Perennial ryegrass at 360N versus binary- and multi-species mixtures at 0N (Group 2; PRG/360N, PRG/RC, Mix 1/0N and Mix 2/0N)

Herbage ensilability

Herbage WSC_{aq} at Cut 3 (Tables 7 and 8) was greatest (P < 0.01) for PRG/360N. Buffering capacity at Cut 2 (Tables 5 and 6) was greater (P < 0.01) for PRG/RC than for PRG/360N, while at Cut 3 PRG/RC had the greatest (P < 0.01) buffering capacity. Furthermore, at Cut 3, Mix 1/0N and Mix 2/0N were greater (P < 0.01) than PRG/360N.

Fermentation characteristics

There was no difference (P > 0.05) in pH or WSC content between species except at Cut 3 (Tables 7 and 8) where PRG/360N had the lowest (P < 0.001) pH value and the highest (P < 0.05) WSC content.

At Cut 3, lactic acid concentration was greatest (P < 0.01) for PRG/360N, while at the same cut D-lactate as a proportion of lactic acid was least (P < 0.01) in PRG/360N silage. Furthermore, at Cut 3, NH_3-N concentrations were greater for PRG/RC and Mix 2/0N (P < 0.05) than for PRG/360N.

Conservation losses

Effluent production was greater (P < 0.05) for PRG/360N than for PRG/RC and Mix 2/0N at Cut 1 (Tables 3 and 4), while DM recovery rates were greater (P < 0.05) for Mix 1/0N and Mix 2/0N than PRG/360N at the same cut.

Silage nutritive value

Silage DMD values at Cut 1 (Tables 3 and 4) were greater (P < 0.01) for PRG/RC and Mix 2/0N than for PRG/360N. At Cut 2 (Tables 5 and 6), PRG/360N had greater (P < 0.01) values than Mix 2/0N, while PRG/360N had the greatest (P < 0.01) DMD at Cut 3 (Tables 7 and 8).

At Cut 1, the silage CP content was greatest (P < 0.01) for PRG/360N, while at Cuts 2 and 3 the values for PRG/360N were greater (P < 0.01) than for Mix 2/0N. Furthermore, at Cut 3 PRG/360N, Mix 1/0N and Mix 2/0N had greater (P < 0.01) CP contents than PRG/RC.

Perennial ryegrass versus two multi-species mixtures at 360N (Group 3; PRG/360N, Mix 1/360N and Mix 2/360N)

Herbage ensilability

There was no difference in the ensilability indices of each species treatment except at Cut 3 (Tables 7 and 8) where the WSC_{aq} content of PRG/360N was greater (P < 0.01) than that of Mix 1/360N and Mix 2/360N.

Fermentation characteristics

There was no treatment effect on silage pH or WSC content except at Cut 3 (Tables 7 and 8) where PRG/360N had the lowest (P < 0.01) pH and a greater (P < 0.01) WSC content than Mix 1/360N. Furthermore, there was no treatment effect on the concentration of LA and NH_3-N at Cuts 1 (Tables 3 and 4) and 2 (Tables 5 and 6). At Cut 3, however, lactic acid concentration was greatest (P < 0.01) for PRG/360N while the proportion of D-lactate was greater for Mix 1/360N and Mix 2/360N than for PRG/360N.

Conservation losses

Silage effluent production was not different between treatments at any cut; however, the DM recovery rate was greater (P < 0.05) for Mix 2/360N than for PRG/360N at Cut 1 (Tables 3 and 4).

Silage nutritive value

Silage DMD was greater (P < 0.05) for PRG/360N than for Mix 2/360N at Cut 2 (Tables 5 and 6), and greater than for both Mix 1/360N and Mix 2/360N at Cut 3, while the silage CP content was greatest (P < 0.01) for PRG/360N at Cut 3 (Tables 7 and 8).

Multi-species mixtures at 0N or 360N (Group 4; Mix 1/0N, Mix 2/0N, Mix 1/360N and Mix 2/360N)

Herbage ensilability

There was no effect of species mixture or rate of inorganic N on the ensilability of herbage except at Cut 3 (Tables 7 and 8) where WSC_{aq} was lower (P < 0.05) and buffering capacity was greater (P < 0.001) for 0N than for 360N.

Fermentation characteristics

There were no effects (P > 0.05) of treatment on fermentation characteristics.

Conservation losses

Effluent production was greater for herbage-receiving 360N than 0N at Cut 1 (Tables 3 and 4).

Silage nutritive value

There was no main or interaction effect (P > 0.05) on the DM, DMD or CP content of silages produced from the four treatments at any cut, except for the CP content at Cut 1 (Tables 3 and 4) where the main effect value was greater (P < 0.01) for 360N than for 0N.

Discussion

In Ireland, herbage harvested from grassland swards sometimes receives relatively little field wilting prior to ensiling due to unsuitable weather conditions. The range of mean DM values of 116–257 g/kg for the 33 unwilted herbages ensiled (three cuts of 11 treatments) reflects the prevailing moist crop-growing conditions, and agrees with previous values reported by Keating & O'Kiely (2000a), Conaghan *et al*. (2012) and Clavin *et al*. (2017), while the corresponding values for WSC_{aq} (7–76 g/L), buffering capacity (219–775 mEq/kg DM) and fermentation coefficient (17–81) represent a broad range in these crop ensilability indicators. Weissbach & Honig (1996) indicated that herbages with fermentation coefficients above 35 were likely to preserve satisfactorily as silage, provided either their nitrate content exceeded 0.5 g/kg DM or their culturable lactic acid bacteria numbers exceeded 10^5 cfu/g. Under Irish climatic conditions, grassland swards managed for commercial silage production commonly reach these nitrate (Muck *et al*., 1991; Lorenzo & O'Kiely, 2008; Navarro-Villa *et al*., 2011) and lactic acid bacteria (Moran *et al*., 1990) thresholds.

The relationship between the fermentation coefficient and Flieg index values for the 132 herbage-silage experimental units was as follows (Figure 1):

$$\text{Flieg index} = ((5.65 \times \text{fermentation coefficient}) - (0.046 \times (\text{fermentation coefficient})^2)) - 61.1, \; (R^2 = 0.66)$$

This indicates that factors other than DM, WSC and buffering capacity influenced the fermentation outcomes. These could include, in individual cases, sub-optimal nitrate content or lactic acid bacteria numbers (Weissbach & Honig, 1996) as well as, among a range of other factors, ambient temperature, speed of availability of WSC to epiphytic microorganisms, content of other fermentable substrate, numbers of other indigenous microorganisms and the speed and extent of effluent outflow. Overall, however, the mean Flieg index point values for Cuts 1–3 of 70, 84 and 23, respectively, highlight the markedly poorer preservation characteristics at Cut 3 and this, in turn, was signposted by the particularly low fermentation coefficients of Cut 3 herbages. In contrast, however, the generally better preservation characteristics of Cut 2 compared with Cut 1 silages were not indicated by their corresponding fermentation coefficient values.

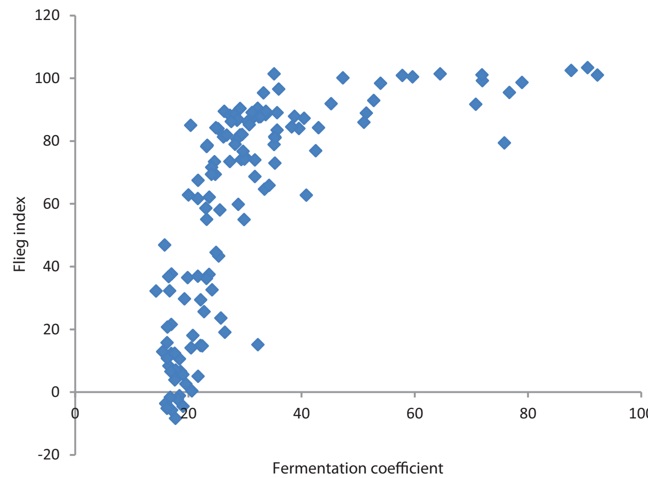

Figure 1. The relationship between the fermentation coefficient and the Flieg index values for the 132 herbage-silage experimental units.

Perennial ryegrass, Italian ryegrass and timothy receiving inorganic N or grown with red clover (Group 1; PRG/360N, IRG/360N, TIM/360N, PRG/RC, IRG/RC, TIM/RC and RC)

The mean annual WSC_{aq} and buffering capacity values of 31, 22 and 14 g/L and 235, 272 and 257 mEq/kg DM for IRG/360N, PRG/360N and TIM/360N, respectively, are of similar rankings of these species for both variables to those reported by Wilson & Collins (1980). The combined effects of these two ensilability indices, in turn, are encapsulated by the single fermentation coefficient index of Weissbach & Honig (1996) where the corresponding values of 42, 33 and 27 suggest IRG/360N was most likely and TIM/360N was least likely to preserve satisfactorily during ensilage. However, these mean annual relativities among grass species were not consistent across cuts. The differences in mean WSC_{aq} across cuts within each grass species likely reflect the direct and indirect effects of the prevailing weather on both DM and WSC values (Deinum, 1984), whereas the general increase in buffering capacity from Cut 1 through to Cut 3 repeats a seasonal pattern previously reported by Muck *et al*. (1991) and can be attributed, at least in part, to the concurrent increase in CP content.

The lower WSC_{aq} content recorded for RC than for IRG/360N and PRG/360N and its consistently greater buffering capacity than any of the grass monocultures is as expected and agrees with the findings of King *et al*. (2012b). Consequently, RC had a lower overall fermentation coefficient (20) than the grass monocultures. The low WSC_{aq} contents for RC are partly due to its lower DM content (Dewhurst *et al*., 2009) and also to some of their non-structural carbohydrates being stored as starch rather than as WSC (Buxton & O'Kiely, 2003).

The expectation that both the WSC_{aq} and buffering capacity of the binary mixtures might be intermediate between the values for the grass and red clover monocultures did not occur, suggesting these traits differed in either or both constituent species compared with when in monoculture. This phenomenon was previously reported by Moloney *et al*. (2020a). It may be, for example, that changes in the amount and/or timing of N provision by red clover to the grass species compared with N provision from inorganic fertiliser when in monoculture could have resulted in considerably greater WSC_{aq} contents and lower buffering capacities for the grasses when in binary mixtures. Both Conaghan *et al*. (2012) and Clavin *et al*. (2017) have demonstrated that lower rates of provision of inorganic N increased grass WSC_{aq} and reduced its buffering capacity. Consequently, the findings by Moloney *et al*. (2020b) of mean annual red clover contents in IRG/RC, PRG/RC and TIM/RC of 25, 55 and 54%, respectively, may explain why the effects of changing from grass plus inorganic N to the corresponding grass plus red clover binary mixture were more dramatic with IRG/RC than with PRG/RC or TIM/RC at both Cuts 1 and 2.

The absence of a difference in the effluent outflow between the three grass species monocultures is in agreement with the study by King *et al*. (2013), while the trend for the red clover monoculture to produce a greater output of effluent was previously identified by King *et al*. (2012a) and can be attributed to the characteristically low DM content of red clover. Thus, the absence of a difference in the effluent outflow between grass plus inorganic N and grass plus red clover treatments is likely due to changed characteristics of grass when in binary mixture with red clover compared with when fertilised with inorganic N. For example, if grass in the binary mixture had a greater DM content than when fertilised with inorganic N, the expected reduced effluent outflow from grass could compensate for the greater effluent outflow of red clover.

King *et al*. (2013) reported mean DM recovery rates for Italian ryegrass, perennial ryegrass and timothy silages of 888, 918 and 936 g/kg, respectively, with King *et al*. (2012a) reporting a corresponding recovery for red clover silages of 874 g/kg. Although the recovery rates for comparable treatments in the current experiment were not statistically different, the numerical trend observed (mean values of 897, 938, 960 and 896 g/kg, respectively) was similar to that in the study by King *et al*. (2013) The absence of a difference in the recovery rate between the binary mixtures and the corresponding grass monoculture treatments was probably strongly influenced by the simultaneous absence of an effect on the effluent outflow discussed earlier.

Silage nutritive value is primarily a product of the nutritive value of the herbage ensiled and the efficiency of the ensilage process (Dulphy & Demarquilly, 1991). Comparison of the three grass monoculture silages to their respective pre-ensiled herbages (Moloney *et al*., 2020a) shows a decline in DMD values at Cuts 1 and 3 where conservation losses were greatest but no such decline at Cut 2 where preservation and recovery rates were more efficient. The absence of a consistent difference in the change in DMD among the three grass species treatments or between these grass and red clover treatments is in agreement with the study by King *et al*. (2012a) and King *et al*. (2013). Thus, for silages at Cuts 2 and 3, the greater DMD observed for PRG/360N than for IRG/360N, TIM/360N and RC, and the absence of a difference among the latter three monoculture treatments, derive mainly from their relative values at the time of ensiling. Similarly, as there was no clear-cut difference between grass plus inorganic N and grass plus red clover treatments in pre-ensilage DMD or a difference in changes in DMD during ensilage, it was therefore to be expected that there would be no clear-cut difference in silage DMD.

Perennial ryegrass at 360N versus binary- and multi-species mixtures at 0N (Group 2; PRG/360N, PRG/RC, Mix 1/0N and Mix 2/0N)

The relatively similar fermentation coefficients for PRG/RC, Mix 1/0N and Mix 2/0N suggest that introducing timothy and white clover into a mixture with perennial ryegrass and red clover (i.e. PRG/RC vs. Mix 1/0N) and then subsequently replacing white clover by ribwort plantain and chicory (i.e. Mix 1/0N vs. Mix 2/0N) did not measurably alter the combined calculated impacts on ensilability of herbage DM, WSC and buffering capacity. This is surprising as the evidence from the monoculture treatments is that timothy, for example, would have an inferior fermentation coefficient to perennial ryegrass because of its lower WSC content (King *et al*., 2012b) and that white clover frequently has a particularly low DM content (Frame & Newbould, 1986). However, the fermentation coefficients for the individual species sampled from Mix 1 and Mix 2 (Table 9), albeit when grown with 120 kg N/ha per year, indicate that timothy growing in multi-species mixtures had a slightly greater fermentation coefficient than a perennial ryegrass monoculture, while red and white clovers had similar fermentation coefficients to each other albeit both were lower than the grasses. The similarly low fermentation coefficients for both herbs and white clover together with their usually relatively small proportions of the harvested biomass (Moloney *et al*., 2020b) explain the muted impact of replacing white clover by herbs.

Perennial ryegrass monocultures managed with the input of inorganic N fertiliser have been the predominant grassland

Table 9: Mean chemical composition of individual species within Mix 1 and Mix 2 at Cuts 1–3 pre-ensiling

Species		Perennial ryegrass[1]	Timothy[2]	Red clover[2]	White clover[2]	Ribwort plantain[2]	Chicory[2]
	Cut						
WSC_{aq}[3]	1	24	61	22	21	16	14
	2	55	28	19	12	24	25
	3	28	50	23	18	31	33
Buffering capacity[4]	1	265	264	544	526	381	469
	2	317	261	589	557	467	473
	3	456	338	649	582	473	513
WSC[5]	1	131	177	120	120	110	117
	2	216	102	89	73	91	128
	3	140	136	100	105	109	166
DM[5]	1	152	257	152	150	125	107
	2	203	213	180	139	206	161
	3	168	267	184	145	220	165
Fermentation coefficient[6]	1	42	62	27	27	28	24
	2	58	43	26	21	31	31
	3	33	48	27	24	34	34

WSC = water-soluble carbohydrates; WSC_{aq} = water-soluble carbohydrates (aqueous phase basis).
[1]Samples were taken from monoculture of diploid perennial ryegrass with an intermediate heading date, grown with 120 kg N/ha per year (Moloney *et al*., 2020a).
[2]Samples were taken from Mix 1 and Mix 2 receiving 120 kg N/ha per year (Moloney *et al*., 2020a).
[3]g/L.
[4]mEq/kg DM.
[5]g/kg.
[6]Weissbach & Honig (1996).

swards sown in Ireland for several decades. It is interesting that the fermentation coefficients of PRG/RC, Mix 1/0N and Mix 2/0N were only slightly lower than for this industry standard (PRG/360N). However, this scale of difference was small compared, for example, to the differences for all treatments between high values for coefficients at Cut 1 and low values at Cut 3.

When compared with pre-ensilage DMD values at Cuts 1 and 3, the corresponding lower post-ensilage values for the four treatments reflect the greater output of effluent and poorer fermentation outcome at those cuts relative to Cut 2 where there was no such decline in DMD. As there was no consistent difference in DMD between these treatments pre-ensilage (Moloney *et al*. 2020a) and no consistent difference in the efficiency of ensilage, it is not surprising that no clearcut differences in silage DMD emerged.

The general trend that occurred at each cut for PRG/360N to have greater CP content than the other three treatments may seem surprising as the direct and indirect effects of the legumes in the binary and multi-species mixture treatments might have been expected to elevate their CP content. However, as previously shown by Keating & O'Kiely (2000b), Conaghan *et al*. (2012) and Clavin *et al*. (2017), high inputs of inorganic N can produce grasses with relatively high CP values.

Perennial ryegrass versus two multi-species mixtures at 360N (Group 3; PRG/360N, Mix 1/360N and Mix 2/360N)

The trend that was evident at each cut of both Mix 1/360N and Mix 2/360N having a numerically lower fermentation coefficient than PRG/360N was caused mainly by their lower WSC_{aq} values. As grass was the dominant functional group in Mix 1/360N and Mix 2/360N (grass contributed 84% and 72% of biomass across all cuts, respectively) and as timothy in these mixtures expressed relatively high fermentation coefficients as did a perennial ryegrass monoculture (Table 9), then the lower coefficients of both mixtures were due to the negative effects of both the legume and herb functional groups.

The trend for the Mix 1 and Mix 2 silages to have lower overall DMD values than the perennial ryegrass treatment (across cut mean values of 705, 703 and 736 g/kg, respectively), although not consistent across cuts, reflected their relative values pre-ensilage (717, 721 and 746 g/kg, respectively; Moloney *et al*., 2020a), with the reductions in the absolute values during ensilage reflecting the effects of conservation losses.

Multi-species mixtures at 0N or 360N (Group 4; Mix 1/0N, Mix 2/0N, Mix 1/360N and Mix 2/360N)

Applying inorganic N to Mix 1 changed the mean annual content of grass from 62 to 84% of herbage biomass and of legume from 38 to 16%. Changes to the grass, legume and herb contents in Mix 2 were from 54 to 72%, 22 to 8% and 24 to 20%, respectively (Moloney *et al*., 2020b). Despite these effects of inorganic N on botanical composition and possibly also on other traits within individual herbage species present (Table 9 and Moloney *et al*., 2020a), the consistent absence of a significant interaction between herbage species mixture and rate of inorganic N applied indicates that species mixture effects on herbage ensilability, silage fermentation, conservation loss and silage nutritive value traits behaved similarly at both rates of inorganic N.

With the exception of Mix 2/0N at Cut 1, both sward species mixtures exhibited lower fermentation coefficients than the threshold value of 35 identified by Weissbach & Honig (1996) as being necessary to exceed in order to ensure successful preservation of ensiled unwilted herbage. The values at Cut 3 therefore appeared particularly challenging. Overall, the low values reflect the effects of the lower coefficients for legume and herb functional groups compared with grasses, as shown in Table 9. In addition, where inorganic N was applied it is likely that this had an effect of reducing WSC_{aq} and increasing buffering capacity values for the grass species present (Keating & O'Kiely, 2000a; Conaghan *et al*., 2012; Clavin *et al*., 2017), and this would have largely cancelled the simultaneous reduction in the proportion of the non-grass functional groups present. The similar fermentation coefficients for the two sward species mixture treatments was a product of there being no significant differences between them in WSC_{aq} or buffering capacity values at any cut. This, in turn, indicates that the replacement of white clover (8–30% of biomass; mean fermentation coefficient of 24) by herbs (12–30% of biomass; mean fermentation coefficient of 30) did not measurably impact the fermentation coefficient. It is noteworthy that within each functional group the fermentation coefficients for the two constituent species were generally comparably high (both grasses) or low (both legumes and both herbs).

Both Pahlow *et al*. (2003) and Clavin *et al*. (2017) suggested that the ensilability index thresholds that would indicate a likelihood of satisfactory silage preservation appear to differ for grasses and legumes. In the case of red clover, for example, Clavin *et al*. (2017) proposed explanations for its better than expected preservation such as a greater quantity of fermentable substrate being available than measured in WSC, the beneficial effects it derives from polyphenol oxidase and a lower water activity compared with grass of the same DM content.

As the herbage from both sward species mixture treatments had similar pre-ensilage DMD and CP values and both treatments underwent similar DM recovery rates and standards of preservation during ensilage, the generally similar DMD and CP of their silages were to be expected. This outcome for DMD also agrees with the finding that the pre-ensilage DMD of white clover was intermediate between that of ribwort plantain and chicory (799 vs. 694 and 832 g/kg, respectively; Moloney *et al*., 2020a) such that replacing this legume by the two herbs caused no net change in value. However, this rationale does not explain the similar CP for Mix 1 and Mix 2 silages as the pre-ensilage values for white clover, ribwort plantain and chicory were 233, 134 and 153 g/kg DM, respectively (Moloney *et al*. 2020a).

Practical implications

Numerous factors such as annual and seasonal herbage yield and nutritive value, and sward persistence, impact on the suitability of different herbage species and cultivars in grassland swards managed for silage production. Other studies from the project with which this experiment is associated have examined these factors (Moloney *et al*., 2020a; 2020b). The current study demonstrates that under favourable ensiling conditions (e.g. Cut 2) unwilted grass-red clover binary mixtures and multi-species mixtures can preserve satisfactorily as silage, comparable to a perennial ryegrass monoculture receiving inorganic N fertiliser. However, when ensiled under more challenging crop conditions (e.g. Cut 3), it appears the binary mixtures and multi-species mixture swards have a greater requirement for an adequate rapid wilt and/or sufficient effective preservative to be evenly applied. Future research should quantify the ease of successfully wilting binary mixture and multi-species mixture swards under practical field conditions, as well as quantifying conservation losses in the field and during ensilage/feedout, and conservation efficiency responses to contrasting additives. Furthermore, the energy and protein values of efficiently conserved silages and the corresponding effects on performance and meat or milk quality should be assessed with appropriate ruminants.

The results of this study suggest that the thresholds of herbage ensilability indices developed to predict the relative ease of successfully preserving grass as silage will need to be adjusted for mixtures containing species from outside of the grass functional group.

Conclusions

Estimation of the ensilability of binary mixtures and the preservation of their resultant silages need to be based on direct analysis of representative binary mixture samples rather than being predicted from measured values for the component species when in monoculture. A major factor impacting negatively on the silage preservation challenge for any of the binary mixtures was the proportion of red clover present.

The broadly similar herbage ensilability and silage fermentation characteristics of PRG/RC, Mix 1/0N and Mix 2/0N suggest that the inclusion of timothy and white clover with perennial ryegrass and red clover (PRG/RC vs. Mix 1/0N) or the replacement of white clover by ribwort plantain and chicory (Mix 1/0N vs. Mix 2/0N) did not negatively affect these traits. There was a trend under difficult ensiling conditions, however, for the reference treatment PRG/360N to be somewhat less difficult to preserve than PRG/RC, Mix 1/0N and Mix 2/0N. This same trend was evident when comparing PRG/360N to Mix 1/360N and Mix 2/360N.

Despite the application of inorganic N to Mix 1 and Mix 2 markedly increasing their grass contents and correspondingly reducing their legume contents, this had relatively little impact on herbage ensilability or silage preservation characteristics. This appeared to be due to expected positive impacts of a reduction in the content of legumes being cancelled by corresponding negative impacts of inorganic N on grass ensilability and on its resultant fermentation during ensilage.

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