Plant Soil (2010) 326:355–367
DOI 10.1007/s11104-009-0016-z

REGULAR ARTICLE

Botanical composition, production and nutrient status of an originally *Lolium perenne*-dominant cut grass sward receiving long-term manure applications

Wenju Liu · Yong-Guan Zhu · Peter Christie · Adam Scott Laidlaw

Received: 21 November 2008 / Accepted: 27 April 2009 / Published online: 19 May 2009

Abstract Effects of long-term applications (50, 100 and 200 m^3 $ha^{-1}y^{-1}$) of pig and cow slurries on yield, botanical composition and nutrient content of herbage of an original perennial ryegrass sward were assessed in a three-cut silage system and compared with unamended and fertilized controls in the 36th year of the experiment. Cow slurry at 50 m^3 ha^{-1} produced similar annual herbage DM yield to 200 kg ha^{-1} fertilizer N in 2006, whereas about 100 m^3 ha^{-1} pig slurry were required to produce a similar amount of DM. The highest slurry application rate significantly influenced sward botanical composition without depressing DM yield. The principal invading species were creeping bent and meadow grasses (similar to findings at a previous assessment in 1981) except in the unamended control (which were common bent and Yorkshire fog). Perennial ryegrass remained a main species in plots receiving fertilizer (31 % annual DM yield) and low slurry rates (38 %) but declined to 3 % annual DM yield at the highest slurry rate where the ability of ryegrass to utilize slurry N and P may have been affected by chemically or physically induced deficiencies of other nutrients (e.g. Ca) or direct physical effects such as smothering.

Keywords Slurry · *Lolium perenne* · *Agrostis stolonifera* · *Poa* spp. · Nitrogen · Mineral nutrients

Responsible Editor: Elizabeth Stockdale.

W.J. Liu
College of Natural Resources and Environmental Sciences,
Hebei Agricultural University,
Baoding 071000, China

W.J. Liu · Y.-G. Zhu
Research Centre for Eco-Environmental Sciences,
Chinese Academy of Sciences,
Beijing 100085, China

P. Christie (✉)
Agri-Environment Branch, Agriculture,
Food and Environmental Science Division,
Agri-Food and Biosciences Institute (AFBI),
Newforge Lane,
Belfast BT9 5PX, UK
e-mail: peter.christie@afbini.gov.uk

A. S. Laidlaw
AFBI Plant Testing Station,
50 Houston Road,
Belfast BT6 9SH, UK

Introduction

Many modern intensively-managed livestock production systems produce large amounts of liquid animal manures or slurries. In general, while recycling of animal manures is a source of plant nutrients, repeated slurry applications may alter chemical and physical properties of soils and the botanical composition of herbage and pollute the soil in various ways (Christie 1987; Haynes and Naidu 1998; Hao and Chang 2002; Murphy et al. 2005). A field experiment was established in 1970 to assess the effects of frequent regular applications of slurry to grassland on the yield and mineral composition of herbage and on physical and chemical properties of the soil. The results from the first 16 years of this long-term slurry experiment have shown that herbage dry-matter yields increased with increasing slurry rates applied and the sward botanical composition changed (Christie 1987). Furthermore, at the highest rate of pig slurry Cu and Zn accumulated in the soil and soil microbial biomass decreased significantly (Christie and Beattie 1989) and with increasing application rates of both types of slurry arbuscular mycorrhizal infection decreased (Christie and Beattie 1992). The build-up of soil P due to slurry application may increase the risk of P loss to surface water bodies from soil receiving high rates of cow slurry (Anderson and Wu 2001; Murphy et al. 2005).

The botanical composition of grassland has an impact on herbage production potential and nutritive value for animals, including mineral and crude protein content (Frame 1989, 1991). Botanical composition of herbage in treatments in the long-term slurry experiment was first determined in 1981–1982 (after 16 years) by Christie (1987). Whether the botanical composition had changed markedly or not after a further 24 years of slurry treatments was investigated by comparing the results in 2006 with those reported by Christie (1987).

Perennial ryegrass (*Lolium perenne* L.) is the most important forage grass in European agriculture where it provides the major supply of nutrients for grazing sheep and cattle. It has been reported that increasing rates of N fertilizer to perennial ryegrass alters the ratios of N: S and of K :(Ca and Mg) (Whitehead et al. 1978). Hopkins et al. (1994) concluded that applied N reduced concentrations of Ca, Mn and S and increased those of Mg, Na and Zn, with no consistent effects on concentrations of K and Cu in herbage of perennial ryegrass. These results were derived mainly from short-term experiments and so may not have been sufficient to detect long-term processes.

The present study was carried out on the long term slurry experiment to determine the extent of changes in herbage production and botanical composition caused by slurry treatments since the previous study in 1985. As an indication of the competitive ability of the main species in response to the various treatments and to help explain differences between treatments in botanical composition, the mineral composition of these species was determined.

Materials and methods

The long-term slurry experiment

The long-term field experiment was established on a sown sward of perennial ryegrass at Hillsborough, Northern Ireland (54°27′ N, 6°4′ W) in 1970. The site is considered typical of a large proportion of the grassland area in Northern Ireland and similar to that of much of the northwest of the UK. The soil is a clay loam (42% sand, 24% silt and 34% clay). There were eight treatments: unfertilized control (CU), fertilized control (CF: 200 kg N, 32 kg P, and 160 kg K $ha^{-1}y^{-1}$), pig slurry at 50, 100 and 200 m^3 $ha^{-1}y^{-1}$ (Pig50, Pig100 and Pig200) and cow slurry at the same three rates of slurry (Cow50, Cow100 and Cow200). Average total concentrations of N, NH_4^+-N, P and K (mg g^{-1} fresh weight) over the three applications were, respectively, 3.7, 2.1, 0.8 and 5.4 for cow and 1.1, 0.8, 0.2 and 0.9 for pig slurry. The experimental design comprised six replicates of each treatment in three randomized blocks with two replicates of each treatment fully randomized within each block, giving a total of 48 plots. Because of the large amount of work required to determine the species composition of the herbage samples the two replicates of each treatment in each block were combined to give three replicates. The plots were cut three times each year at the silage stage (mid-May, mid-July and mid-September). In 2006 they were cut on 17 May, 18 July and 19 September. The fertilizers and slurries were applied in three equal dressings, first in the early spring and then immediately after the first two cuts. The batches of pig and cow slurries used throughout the study were supplied by the farm of the Agricultural Research Institute of Northern Ireland (renamed AFBI-Hillsborough since April 2006). Each 29.75 m^2 rectangular plot with harvestable area of 18.75 m^2 was harvested at a cutting height of 5 cm with a plot harvester. Total fresh herbage biomass was weighed in the field at each harvest. Two subsamples were taken. One of about 500 g fresh weight was oven dried at 80°C for dry matter (DM) determination and ground to pass a 0.5-mm sieve prior to chemical analysis. In addition in 2006 a subsample of fresh herbage of approximately 1 kg was taken from each plot. The samples were paired, bulked and stored at −20°C for subsequent determination of the botanical composition of the herbage and mineral composition of the main plant species fractions.

Sward botanical composition

A subsample of about 500 g fresh weight drawn from each bulked sample was separated as far as possible into individual plant species which were then oven dried at 60°C for 24 h and their contribution to herbage dry matter calculated. The proportion of each species for each treatment was calculated and the main species for all treatments were determined. The three principal species fractions identified for each treatment were perennial ryegrass, creeping bent (*Agrostis stolonifera*) and meadow grasses (*Poa* spp.; mainly *Poa annua* and *Poa trivialis*), except for the control treatment without fertilizer (CU) in which the main species were perennial ryegrass, common bent *(Agrostis tenuis*) and Yorkshire fog (*Holcus lanatus*).

Chemical analysis of main species

A further subsample of about 1 kg fresh herbage was taken from the bulked sample and divided into the 3 principal species fractions and remainder. These were oven dried at the same temperature as the botanical samples for 48 h prior to chemical analysis and then ground to pass a 0.2-mm sieve in an Ultra centrifugal mill ZM200 (Retsch Company, Germany). Total nitrogen and carbon in plants were measured with a LECO 2000 dry combustion analyzer (LECO Equipment Corp., St. Joseph, MI). The other elements were detected using an Axios x-ray fluorescence spectrometer (XRF) (PANalytical BV, the Netherlands). The concentration of nutrient elements was the average of cuts 1 and 2.

Data analysis

Analysis of variance was carried out using the Genstat (11th Edition) package by considering the experimental design to be a 2 x 3 factorial for slurry type and rate with two additional control treatments. In addition to means within the factorial and responses (linear contrasts) to be tested for significance, the analysis allowed comparison between control means and between each control and overall mean of slurry treatments. The least significant difference applicable to this comparison was used to make a judgment about the closeness of CF to either the mean for slurry type or slurry rate. Means of each slurry type at a given rate are only considered if the type×rate interaction is significant. Regressions between mineral offtake of individual species and total offtake in herbage were determined by fitting trend lines using MS Excel.

Results

Slurries

Mean annual composition of slurries applied in 2006 was relatively close to the average for those applied over the previous 20 years, considering the wide range in annual composition over these years, although the slurries applied at the beginning of the three regrowth periods in 2006 varied widely (Table 1). The lower dry matter, N and K content in pig than in cow slurry applied over the 20 years was reflected in the mean composition of slurries applied in 2006.

Harvested DM

Considering the yields at the first two harvests and for the total annual, expressed as a mean over the three rates of application, cow slurry produced significantly higher yields than pig slurry, and at all harvests yields increased significantly with rate of slurry application (Table 2), with the response being significantly linear. A significant interaction between slurry type and rate of application for yield at the first two harvests and for the year was due to cow slurry producing a higher response of DM yield than pig slurry up to 100 m^3 ha^{-1}. Responses to the first 50 m^3 slurry of total annual yield were 139 and 87 kg DM m^{-3} for cow and pig slurry, respectively, with corresponding responses to an increment of 50 $m^3 ha^{-1}$ and a further 100 m^3 ha^{-1} of 114 and 73 and 24 and 51 kg m^{-3}, respectively. The greatest differences in response between the slurry types at individual cuts were at the two lower rates of slurry application at the first cut and at the intermediate rate at the second cut. The

Table 1 Mean annual composition of slurry applied from 1986 to 2006 and of slurry applied prior to re-growth contributing to each cut in 2006

Slurry type		%DM	$\%NH_4^+$ N	% Total N	% P	% K
Pig	**Annual mean 1986–2006**	1.23	0.17	0.22	0.05	0.15
	SE	0.135	0.011	0.013	0.007	0.022
	Range: from	0.33	0.05	0.06	0.01	0.01
	to	3.31	0.25	0.33	0.14	0.36
	2006					
	Cut					
	1	0.49	0.06	0.08	0.017	0.113
	2	0.74	0.08	0.11	0.029	0.157
	3	4.60	0.21	0.37	0.208	0.340
	Mean 2006	1.94	0.12	0.19	0.08	0.20
Cow	**Annual mean 1986–2006**	4.68	0.24	0.36	0.08	0.37
	SE	0.285	0.013	0.016	0.013	0.047
	Range: from	1.31	0.06	0.07	0.02	0.03
	to	7.08	0.40	0.52	0.25	0.79
	2006					
	Cut					
	1	5.9	0.24	0.39	0.076	0.929
	2	8.8	0.19	0.43	0.136	0.669
	3	1.5	0.06	0.13	0.033	0.190
	Mean 2006	5.4	0.16	0.32	0.08	0.60

Table 2 Mean DM yield at each cut and total for 2006 (t DM ha^{-1})

Cut		Control		Slurry type	Slurry rate ($m^3 ha^{-1}$)			Slurry rate
		CU	CF		50	100	200	Mean
1,2,&3		3.14	10.56	Pig	7.47	11.12	16.22	11.60
				Cow	10.08	15.80	18.23	14.70
				Mean	8.78	13.46	17.22	
1		0.80	3.98	Pig	2.58	3.41	5.17	3.72
				Cow	4.57	5.86	5.67	5.37
				Mean	3.59	4.63	5.42	
2		1.48	3.20	Pig	2.25	3.08	4.35	3.22
				Cow	2.93	4.95	5.66	4.51
				Mean	2.59	4.01	5.00	
3		0.86	3.38	Pig	2.64	4.64	6.70	4.66
				Cow	2.56	5.00	6.89	4.82
				Mean	2.60	4.82	6.80	
	Cuts 1,2&3		Cut 1		Cut 2		Cut 3	
Treatment	LSD	Prob.	LSD	Prob.	LSD	Prob.	LSD	Prob.
Control	0.735	***	0.485	***	0.483	***	0.319	***
Type (T)	0.435	***	0.280	***	0.279	***	0.184	NS
Rate (R)	0.533	***	0.343	***	0.341	***	0.226	***
T×R	0.753	***	0.485	***	0.483	**	0.319	NS

**$P<0.01$, ***$P<0.001$

closest of the yields of the three rates of cow slurry at each cut and for the year to CF was Cow50. The comparison was more variable for pig slurry, with yield for the year and at cut 2 from Pig100 being closest of the pig slurry treatments to CF while Pig200 and Pig50 were closest to CF at harvests 1 and 3, respectively.

Botanical composition

Botanical composition differed markedly between treatments (Table 3). Averaged over the year, harvested herbage in cow slurry treatments had a significantly greater proportion of meadow grasses and significantly less Yorkshire fog (*Holcus lanatus*) and red fescue (*Festuca rubra*) than that in pig slurry treatments. Increasing slurry rate had a positive effect on creeping bent, meadow grasses and couch grass (*Agropyron repens*) and a negative effect on the content of perennial ryegrass, Yorkshire fog, common bent (*Agrostis tenuis*), timothy grass (*Phleum pratense*) and red fescue. None of the interactions between slurry type and rate was significant for annual botanical composition. For the principal species CF had, in general, similar contents to the 50 m^3 slurry rate treatment while botanical composition of CU was quite different to any of the other treatments, having a particularly high content of Yorkshire fog and common bent rather than creeping bent and meadow grasses (Table 3). It also had a higher content of red fescue and white clover than CF.

Other than for CU, contribution of the three principal species to total DM in 2006 was similar to that in 1981 (Table 3). In CU, the content of creeping bent and meadow grasses was much lower in 2006 than 1981 while the principal species at the earlier assessment, in addition to perennial ryegrass, were common bent and Yorkshire fog.

At successive cuts meadow grasses and, to a lesser extent, perennial ryegrass content declined while creeping bent content increased, especially at the higher slurry rates (Fig. 1 and Table 5). However, botanical composition at each harvest reflected the annual composition with similar trends caused by the main treatments. Significant interactions between slurry type and rate in cut 2 for creeping bent and meadow grasses were due to a greater response in content of the species to increasing application rate

Table 3 Annual mean proportion of principal plant species (weighted according to contribution of DM yield at each cut to annual DM yield) in harvested DM (Statistical analyses were carried out on square root transformed data (sq) when distribution of original data was skewed); key: Lp *Lolium perenne*, As *Agrostis stolonifera*, Po *Poa* spp., Hl *Holcus lanatus*, At *Agrostis tenuis*, Dg *Dactylis glomerata*, Pp *Phleum pratense*, Ar *Agropyron repens*, Fr *Festuca rubra*, and Tr *Trifolium repens*. Weighted mean values for Lp, As and Po in bold are from 1981 for comparison

Species	Control (C)		Type (T)		Rate (R)			LSD/Sig.		
	CU	CF	Pig	Cow	50	100	200	C	T	R
Lp	0.227	0.311	0.203	0.230	0.384	0.217	0.060	0.118NS	0.068NS	0.084***
	0.192	**0.417**	**0.205**	**0.248**	**0.383**	**0.294**	**0.070**			
As	0.003	0.286	0.422	0.400	0.221	0.473	0.540	0.150***	0.086NS	0.036***
	0.284	**0.350**	**0.468**	**0.488**	**0.270**	**0.429**	**0.639**			
Po	0.007	0.079	0.114	0.175	0.091	0.148	0.195	0.051***	0.029***	0.036***
	0.096	**0.085**	**0.230**	**0.186**	**0.152**	**0.200**	**0.252**			
Hl	0.226	0.032	0.037	0.014	0.063	0.015	0			
sqHl	0.473	0.177	0.154	0.084	0.240	0.110	0.007	0.073***	0.042**	0.052***
At	0.344	0.074	0.037	0.020	0.080	0.006	0.001			
sqAt	0.582	0.259	0.129	0.091	0.262	0.058	0.010	0.111***	0.064NS	0.078***
Dg	0.001	0.113	0.046	0.031	0.044	0.045	0.027			
sqDg	0.026	0.311	0.192	0.147	0.208	0.172	0.129	0.197*	0.114NS	0.139NS
Pp	0.023	0.008	0.021	0.012	0.044	0.006	0			
sqPp	0.142	0.071	0.089	0.073	0.204	0.039	0	0.082NS	0.047NS	0.058***
Ar	0.004	0.067	0.087	0.107	0.030	0.090	0.171	0.108NS	0.062NS	0.076**
Fr	0.086	0.021	0.021	0.005	0.033	0.006	0			
sqFr	0.276	0.118	0.109	0.049	0.166	0.067	0.004	0.099***	0.057*	0.070***
Tr	0.048	0.002	0.004	0.001	0.006	0.001	0.001			
sqTr	0.203	0.040	0.042	0.023	0.065	0.021	0.011	0.072***	0.041NS	0.051NS

*$P<0.05$, **$P<0.01$, ***$P<0.001$

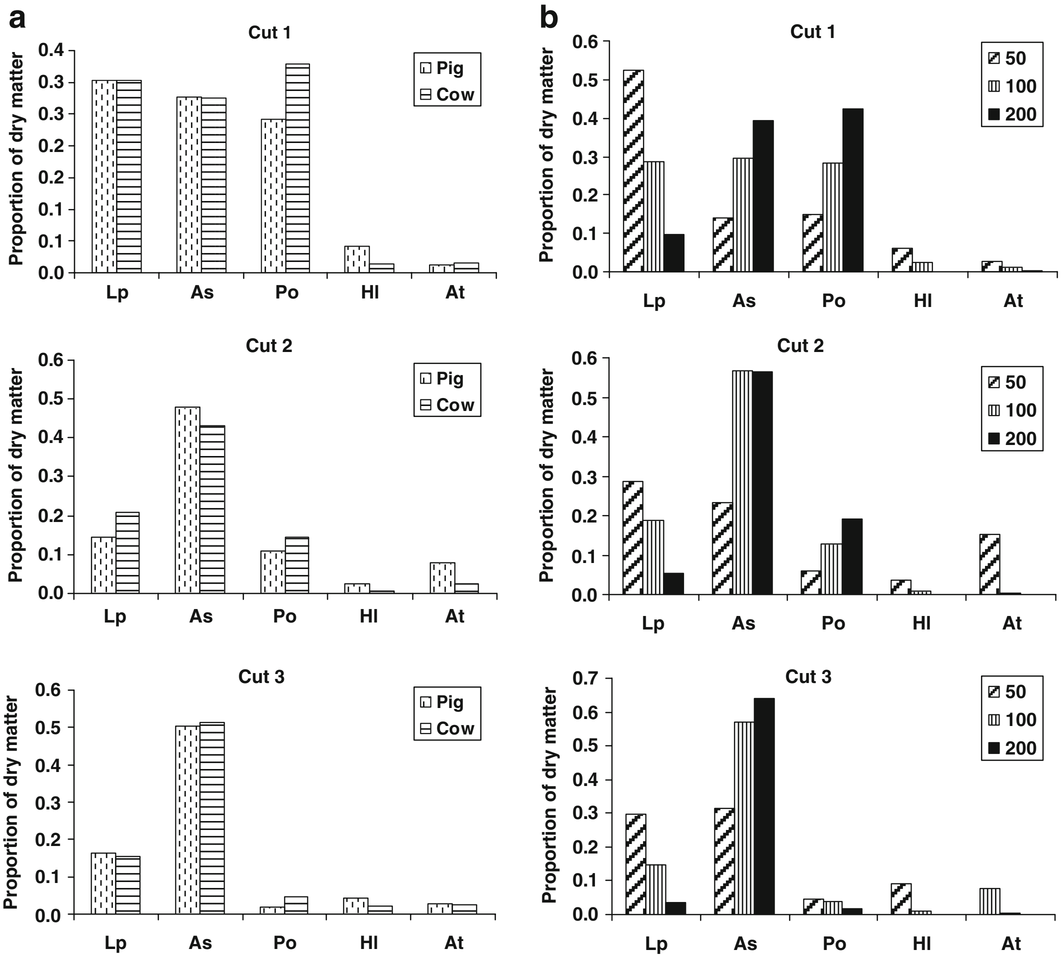

Fig. 1 Mean proportion of species in harvested DM at each cut in (**a**) pig and cow slurry (mean values of rate) and (**b**) slurry rate treatments (mean values of slurry type): Lp *Lolium perenne*, As *Agrostis stolonifera*, Po *Poa* spp., Hl *Holcus lanatus*, At *Agrostis tenuis*

of pig than cow slurry (Table 5). A particularly high content of common bent in at the lowest pig slurry rate at cut 2 almost resulted in a significant interaction.

Nitrogen and mineral nutrients

Annual weighted concentrations of potassium and copper were significantly higher in herbage harvested from plots treated with cow than pig slurry while those of phosphorus, calcium, magnesium, and sodium content were significantly higher in herbage from pig than cow slurry treated plots (Table 4). A significant interaction between slurry type and rate was due to a significantly higher concentration of P in herbage in Pig100 than Cow100 (data not presented).

Increasing slurry rate had a significantly positive effect on annual content of N, K and Cu (linear component $P<0.001$) and a negative effect on Ca and Na. Mean N content of herbage from all of the slurry treatments did not differ significantly from that of either of the controls. The analysis of variance model restricted comparison of the means for the two control treatments to the mean of all of the treatments in the factorial. The test was therefore used to make a judgment about the similarity of either of the controls to any of the mean values for slurry rate. The CF treatment had significantly higher content of P and K than CU but significantly lower content of Mg, S and Na. The fertilized control had a similar P content to the mean of the slurry treatments, a similar content of K and Cu concentration to the lowest slurry rate and

Table 4 Mean annual concentration (mg g^{-1}) of nutrients in DM of harvested herbage in 2006 in total herbage calculated from weighted concentrations at each of the three harvests

Element	Control (C)		Type (T)		Rate ($m^3 ha^{-1}$) (R)			LSD/Sig.		
	CU	CF	Pig	Cow	50	100	200	C	T	R
N	16.4	18.3	17.4	18.0	17.0	17.1	19.1	1.65	0.95	1.17**
P	2.57	3.32	3.60	3.40	3.43	3.54	3.53	0.25***	0.19*	0.24*
K	14.7	22.6	19.8	31.3	23.5	24.5	28.6	2.22***	1.28***	1.57***
Ca	6.74	5.55	5.20	3.69	4.99	4.44	3.91	0.74***	0.43***	0.52**
Mg	1.52	1.14	1.70	1.58	1.68	1.63	1.61	0.12***	0.07**	0.09
Na	2.99	2.12	2.29	0.75	1.85	1.45	1.27	0.53***	0.40***	0.49
S	2.18	1.46	2.07	2.14	2.15	2.09	2.07	0.155***	0.118***	0.144
Cu	5.73	6.08	7.70	10.84	7.38	9.27	11.16	0.971***	0.734***	0.899***

*$P<0.05$, **$P<0.01$, ***$P<0.001$

significantly lower concentration of Mg and S than the lowest slurry rate. Calcium concentration was higher in CF than in any of the slurry rate treatments and Na concentration was significantly higher in CF than the two higher rates of slurry (Table 4).

The effect of slurry type on mineral content of the principal plant species was generally similar to the effect on the total herbage (Table 6a). Concentrations of S and Cu in perennial ryegrass and Ca in creeping bent were not affected by slurry rate while Ca concentration in perennial ryegrass and meadow grasses was reduced by increasing slurry rate; in perennial ryegrass Ca concentration was reduced by one third by increasing slurry application from 50 to 200 m^3 ha^{-1}. Significant interactions between slurry type and rate were mainly due to high concentrations of N in perennial ryegrass and *Poa* spp. and P in *Poa* spp. at Cow200, Na declining with increasing rate of pig slurry in creeping bent and meadow grasses and Cu increasing particularly steeply in creeping bent and meadow grasses with increase in cow slurry (Table 6b).

Nitrogen content in perennial ryegrass in CU was particularly low (Table 6). As Yorkshire fog and common bent replaced creeping bent and meadow grasses, respectively, in CU as principal species they were considered separately and not included in the statistical analyses. However, their nutrient contents are included in Table 6a for comparison with the corresponding principal species in CF. Contents of N, P, K and Ca were lower and Mg higher in Yorkshire fog than creeping bent and concentration of P was lower and Mg and S higher in common bent than meadow grasses.

Slurry, at any of the three rates, produced significantly higher concentrations of Mg and S in all three principal species and Na and Cu in meadow grasses than in CF. All slurry rates gave significantly lower concentrations of Ca in all three principal species and Na in perennial ryegrass than in those species in CF (averaged over the two slurry types). Concentrations of other nutrients in CF fell significantly within the range of concentrations in slurry treated swards.

Table 5 (a) Significance of differences between means of proportion of species at each cut in Fig. 1 (means for Hl and At transformed as square root before analyses) and **(b)** significant interactions including that for common bent. Mean values for At are untransformed

(a)	Lp	As	Po	Hl	At
Cut 1					
Type	NS	NS	**	**	NS
Rate	***	**	***	***	*
Type×Rate	NS	NS	NS	NS	NS
Cut 2					
Type	NS	NS	*	NS	*
Rate	***	***	***	**	***
Type×Rate	NS	**	**	NS	(P=0.051)
Cut 3					
Type	NS	NS	**	NS	NS
Rate	***	**	*	***	***
Type×Rate	NS	NS	NS	NS	NS
(b)	Type	Rate $m^3 ha^{-1}$			
		50	100	200	
As Cut 2	Pig	0.18	0.61	0.65	
	Cow	0.28	0.53	0.48	
Po Cut 2	Pig	0.03	0.09	0.21	
	Cow	0.09	0.16	0.17	
At Cut 2	Pig	0.23	0.01	0	
	Cow	0.07	0	0	

*$P<0.05$, **$P<0.01$, ***$P<0.001$

Table 6 a) Weighted mean concentration (mg g^{-1}) of nutrients in DM of harvested herbage in first two cuts in 2006 in species fractions (*Agrostis tenuis* and *Holcus lanatus* replaced *Agrostis stolonifera* and *Poa* species as two of the principal species fractions in CU; although not included in the analysis of variance, their nutrient contents are included in parenthesis for comparison to the other treatments; sq denotes square root transformation) b) Means of significant interactions (Arithmetic mean values of N content in Lp are in parenthesis)

Fraction	Element	Control (C)		Type (T)		Rate ($m^3 ha^{-1}$) (R)			LSD/Sig.			
		CU	CF	Pig	Cow	50	100	200	C	T	R	T x R
a)												
Lp	N	11.7	14.4	12.5	14.8	12.5	12.8	15.6				
	sqN	10.8	12.0	11.2	12.1	11.2	11.3	12.4	0.95*	0.55**	0.67**	0.95*
	P	1.82	2.35	2.54	2.49	2.31	2.42	2.83	0.333***	0.192NS	0.236***	0.333NS
	K	11.8	13.1	12.0	21.5	14.4	15.8	20.0	3.06***	1.77***	2.16***	3.06NS
	Ca	4.22	5.16	3.78	2.83	3.90	3.36	2.65	0.604***	0.349***	0.427***	0.604NS
	Mg	0.93	0.80	1.09	1.18	1.09	1.12	1.21	0.160*	0.093NS	0.113*	0.160NS
	Na	2.54	3.17	3.15	0.86	2.38	2.21	1.42	0.648***	0.374***	0.458**	0.648**
	S	1.50	0.99	1.48	1.62	1.54	1.50	1.61	0.196***	0.113*	0.139NS	0.196NS
	Cu	3.83	4.73	4.00	5.11	4.00	4.44	5.22	1.572NS	0.907*	1.111NS	1.572NS
As	N	(17.4)	20.5	16.4	18.8	16.9	16.6	19.3	2.21**	1.27**	1.56**	2.21NS
	P	(2.02)	2.77	2.64	2.66	2.57	2.60	2.79	0.239NS	0.138NS	0.169*	0.239NS
	K	(10.7)	17.9	14.5	21.5	16.4	16.9	20.6	1.90NS	1.09***	1.34***	1.90NS
	Ca	(3.62)	4.33	4.32	3.17	3.59	3.78	3.85	0.471**	0.272***	0.333NS	0.471NS
	Mg	(0.95)	0.93	1.26	1.26	1.15	1.25	1.38	0.162***	0.094NS	0.115**	0.162NS
	Na	(1.59)	1.13	1.43	0.61	0.99	1.04	1.03	0.198NS	0.114***	0.140NS	0.198***
	S	(1.50)	1.29	1.84	1.86	1.74	1.84	1.97	0.168***	0.097NS	0.119**	0.168NS
	Cu	(4.64)	6.17	6.11	8.88	5.75	6.12	7.61	0.824NS	0.476**	0.583***	0.824NS
Po	N	(18.9)	18.2	15.3	17.5	15.8	15.5	17.9	1.72*	1.00***	1.22**	1.72***
	P	(2.37)	2.88	2.70	2.83	2.82	2.60	2.87	0.315NS	0.182NS	0.223*	0.315*
	K	(21.0)	20.4	17.6	25.0	19.7	20.6	23.6	2.40NS	1.39***	1.70***	2.40NS
	Ca	(4.94)	4.97	4.25	3.09	4.07	3.69	3.26	0.516***	0.298***	0.365**	0.516NS
	Mg	(1.19)	1.03	1.32	1.38	1.30	1.34	1.41	0.117***	0.068NS	0.083*	0.117NS
	Na	(2.51)	0.71	0.86	0.45	0.78	0.61	0.57	0.246NS	0.142***	0.174*	0.246**
	S	(2.07)	1.30	1.91	2.11	1.75	2.00	2.28	0.224***	0.129**	0.158***	0.224NS
	Cu	(4.66)	5.69	6.32	7.56	6.34	6.57	7.90	0.856**	0.494***	0.605***	0.856**

b)

	Type	Rate ($m^3 ha^{-1}$)		
		50	100	200
Lp sqN	Pig	11.1 (12.4)	11.0 (12.0)	11.5 (13.2)
	Cow	11.3 (12.7)	11.7 (13.6)	13.4 (18.0)
Po N	Pig	16.2	14.7	14.9
	Cow	15.5	16.2	20.8
Po P	Pig	2.86	2.60	2.64
	Cow	2.78	2.61	3.11
Lp Na	Pig	3.85	3.58	2.02
	Cow	0.91	0.85	0.83
As Na	Pig	1.58	1.45	1.25
	Cow	0.41	0.62	0.80
Po Na	Pig	1.18	0.82	0.57

Table 6 (continued)

Fraction	Element	Control (C)		Type (T)		Rate ($m^3 ha^{-1}$) (R)			LSD/Sig.			
		CU	CF	Pig	Cow	50	100	200	C	T	R	T x R
					Cow		0.39		0.40		0.57	
			Po Cu		Pig		6.36		6.76		5.84	
					Cow		6.32		7.31		9.05	

*$P<0.05$, **$P<0.01$, ***$P<0.001$

Relationship between N and P offtake in principal species and total herbage

Relationships were established between N and P offtake by principal species fractions in harvested herbage and total offtake at the first two cuts in slurry treatments (Fig. 2). While response of N and P offtake of creeping bent and meadow grasses to increasing total offtake was positive (linearly for creeping bent and exponential for meadow grasses), there was no clear response of perennial ryegrass offtake of N and P to increasing total offtake. The exponential relationship for meadow grasses was due to a slow response when total offtake was low but at higher levels its offtake was similar to that of creeping bent. The CF treatment had a total offtake of 123 kg N ha^{-1} with 38, 38 and 15 kg N ha^{-1} removed by perennial ryegrass, creeping bent and meadow grasses, respectively, with corresponding offtakes for P (total 21 kg P ha^{-1}) of 6, 5 and 2, respectively.

Discussion

As the field experiment described here is long term, treatment effects need to take into account management imposed during the preceding 37 years. Characteristics of the slurries have changed in that time. During the first 16 years reported by Christie (1987), pig slurry applied had about 3.5 times the dry matter content of the mean of that applied since then although nutrient content has differed considerably less. While pig slurry had generally a higher nutrient content than cow slurry in the first 16 years of the experiment, 41% more ammonium N, 64% more total N, 60% more P and 146% more K have been applied in cow than pig slurry in the subsequent 21 years. The corresponding higher amounts of nutrients in cow slurry in 2006 were 33, 68, 3 and 200 % more than in pig slurry, respectively. These changes in nutrient concentration in the two slurry types over the duration of the experiment are reflected in the higher annual herbage dry matter produced in cow than pig slurry treatments in the latter 21 years (Table 7), of which the yields in 2006 are typical.

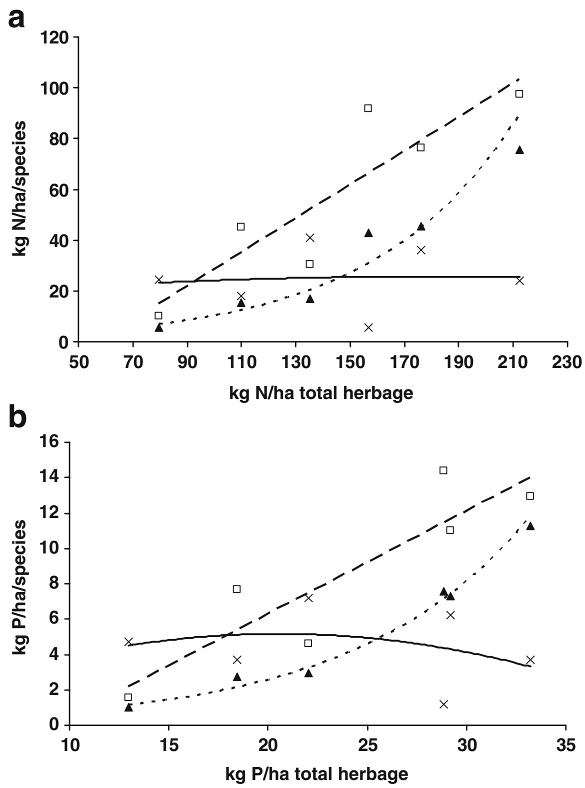

Fig. 2 Relationship between concentration of element in total harvested herbage in slurry treatments at first two harvests and in each of the three grass fractions i.e. perennial ryegrass (x), creeping bent (□) and meadow grasses (▲) for (**a**) N and (**b**) P. Trend line equations respectively for perennial ryegrass, creeping bent and meadow grasses for Fig. 2 (a) are $y=-0.00022x+0.0874x+17.85$ ($r^2=0.01$), $y=0.665x-37.8$ ($r^2=0.79$), $y=1.527e^{0.019x}$ ($r^2=0.94$) and Fig. 2 (b) are $y=-0.015+0.473x-0.31$ ($r^2=0.10$), $y=0.584x-5.40$ ($r^2=0.80$), $y=0.261e^{0.115x}$ ($r^2=0.98$)

Table 7 Mean annual DM yields (with standard errors) from 1970 to 1985 (*Christie 1987) and 1986 to 2006 (Unpublished) (t DM/ha/yr)

Treatment	Mean annual DM yield	
	1970 to 1985*	1986 to 2006
CU	6.4±0.68	3.3±0.24
CF	12.1±0.35	11.6±0.37
PIG50	10.2±0.43	7.9±0.42
PIG100	13.4±0.64	13.1±0.58
PIG200	14.8±0.63	16.7±0.72
COW50	9.3±0.63	9.4±0.38
COW100	12.2±0.44	15.1±0.52
COW200	14.2±0.59	16.7±0.69

The highest rate of slurry applied is well in excess of the amount which would be applied in practice. However the treatment provides evidence of the problems which could occur when excessively high rates of slurry are applied regularly in the long term. For example, in 2006 P offtake was 35 kg P ha^{-1} in the 200 m^{3}ha^{-1} treatment but 165 kg P ha^{-1}, averaged over the two slurry types, were applied, i.e. 21% recovery, in contrast to 21 kg P ha^{-1} offtake when 50 m^3 ha^{-1} slurry supplied 41 kg ha^{-1} P (42 % recovery). Corresponding recoveries in this experiment calculated for 1973 to 1985, averaged over the two slurry types, were 28 and 43 % for 200 and 50 m^3 ha^{-1}, respectively (Christie 1987).

With the exception of CU, the three principal species fractions in this study, i.e. perennial ryegrass, creeping bent and meadow grasses, assessed 36 years after the commencement of the experiment, were the same as those reported by Christie (1987) to be present in all treatments 25 years earlier and is typical of ageing perennial ryegrass swards (Forbes et al. 1980; Smith and Allcock 1985; Hopkins et al. 1988). The sward botanical composition in CF was similar to that of plots receiving the lower rates of slurry. The proportions of perennial ryegrass declined and the content of creeping bent increased with increasing slurry application rate, a trend similar to that found by Christie (1987). Although three of the ten main species fractions differed significantly between the two slurry types, the differences were relatively small. Application of cow slurry resulted in a higher content of meadow grasses and a lower content of Yorkshire fog and red fescue. These differences and a particularly high content of common bent in herbage at the lowest rate of pig slurry indicate the relatively low fertility status of the Pig50 treatment.

The high content of perennial ryegrass at the first cut is to be expected as the variety sown was an early heading type and this would give it a competitive advantage at that time of year (Spedding and Diekmahns 1972). The rapidly declining content of meadow grasses through the season reflects the high content of rough stalked meadow grass (*Poa trivialis*) which, in monoculture, produces very low yields of DM in late summer and autumn (Haggar 1976).

There is some contradiction between the lower nutrient status of pig slurry and the apparently greater sensitivity of botanical content to the increasing rate of pig than cow slurry. Despite low fertility and infrequent cutting, two major factors associated with deterioration in content of perennial ryegrass in sown grassland (Peeters 2004), perennial ryegrass contributed almost 40% to total annual DM yield after 36 years of a three-cut management and low nutrient supply in the lowest slurry application rate treatments, a similar contribution of perennial ryegrass to that recorded by Christie (1987) in the low slurry rate treatment 12 years after the experiment commenced.

Surprisingly, the CF treatment did not have significantly higher perennial ryegrass content than CU. Nevertheless, in addition to DM production, a further difference between CF and CU was the replacement of creeping bent with common bent in CU and a higher content of Yorkshire fog and lower content of meadow grasses in CU than CF. Common bent occurs widely in grassland on impoverished soils and can be an indicator of low P conditions (Peeters 2004). Yorkshire fog is capable of growing under low soil fertility (Watt 1978) and has a higher DM production potential at low N than perennial ryegrass (Frame 1991). In contrast, rough stalked meadow-grass, a major component of the meadow grass fraction, is associated with grassland on fertile soils (Peeters 2004). This marked difference in botanical composition between CU and the other treatments was not detected in the analysis carried out in 1982 (Christie 1987). Possibly, during that analysis, the relatively subtle differences between the two bent species were not detected or, alternatively, the botanical change took place subsequently.

Although white clover content was low in 2006, content in previous years may have been higher as it can fluctuate from year to year (Frame et al. 1998). White clover is the most likely source of N to the low slurry, in addition to N in slurry, and CU plots, and so, especially in the second regrowth period, this may have masked the effect of fertilizer or slurry N due to nitrogen fixation and the high N content in its herbage (Frame et al. 1998). Although more N has been applied in the cow than pig slurry treatments, the mean N content in herbage between the two slurry types did not differ. While the mean P content of both slurries was similar for 2006 and had been higher on average for cow than pig slurry since 1985, the slight but significantly higher P content in herbage of pig than cow slurry treatments suggests that less P in pig slurry may be in organic form than in cow slurry although Christie (1987) considered that the two slurries had similar P availability. None of the major species had significantly more P when treated with pig than cow slurry. The higher content of K in herbage in cow than pig slurry treatments reflected the higher content in the slurry applied. In addition, herbage from pig slurry treatments had lower Ca, Mg and Na contents than herbage in cow slurry treatments. The particularly high content of K in herbage in cow slurry treatments (averaged over slurry rate treatments) would have reduced uptake of the other three cations, resulting in Mg content in particular falling below the minimum requirement for perennial ryegrass growth and for the diet of a high producing dairy cow (McKenzie and Jacobs 2002). The content of Ca in herbage from cow slurry treatments was just above the minimum requirement for a dairy cow diet. Copper, included in pig and cow diets to increase feed utilization efficiency and control dysentery, was higher in cow slurries possibly due to a higher content in the slurry, related to its higher DM content.

The response of each of the principal species fractions to slurry application generally followed the content in total herbage. However, while Ca content in creeping bent remained relatively insensitive to increasing slurry rate, it declined particularly steeply in response to slurry rate in perennial ryegrass. In slurry application trials in Finland, in some circumstances Ca content in herbage declined as the availability of K increased (Mattila et al. 2003). Low Na content in the cow slurry treated herbage would also have been caused by the high content of K in that slurry (Kopittke and Menzies 2005). Magnesium content was generally low in all principal species fractions. While magnesium would be expected to increase with increasing N fertility (Adams 1984; Whitehead 1995; McKenzie and Jacobs 2002) its response to increasing slurry rate was generally low; again this may be explained by high K content in the slurry interacting with uptake of other cations. Increasing cation imbalance in the soil due to high rates of slurry application may impact on the physical characteristics of the soil which in turn can also affect cation availability and uptake (Murphy et al. 2005).

Perennial ryegrass is associated with high fertility conditions (Peeters 2004) but the response of its offtake of two of the most important nutrients in grassland to their increasing availability (measured as total herbage uptake) is poor. Limitations in Ca uptake and possibly other cations have already been discussed as factors imposing disadvantages on the competitive ability of perennial ryegrass; however creeping bent, the most aggressive species towards perennial ryegrass in slurry-treated swards, might have other competitive attributes. Howe and Snaydon (1986) consider that creeping bent is at a competitive advantage in ageing swards due to its ability to spread vegetatively. The increase in content of stoloniferous/rhizomatous grasses suggests that the smothering effect of slurry dry matter may have been implicated in the decline of perennial ryegrass. The content of creeping bent and couch grass increased in response to slurry application and are considered to be ‘guerrilla species’ in that they creep into and colonise gaps in vegetation due their ability to produce rhizomes or stolons (Amiaud et al. 2008). Potentially this could confer advantages over tussock type grasses when herbage is covered in solid matter as a result of treatment with high rates of slurry. These characteristics of the invading species may have contributed to the positive, albeit small, response of DM to the highest slurry rate despite the increasing nutrient imbalance brought about by high rates of slurry.

Although the annual application of mineral fertilizer and animal slurry for 36 years to the grassland altered the botanical composition, the annual DM yields were not depressed by high rates of slurry application. Frame (1991) has shown that the highest yielding of the so-called secondary grasses (those not usually sown but ingressing with sward age) can out-yield perennial ryegrass at low fertilizer application rates (up to 200 kg N ha^{-1}). The data from this study also support the conclusion drawn by Collins and

Murphy (1979) from their survey data of grassland in Ireland that soil fertility is more important than botanical composition in determining the productivity of grassland. However, nutritive value needs also to be taken into account in assessing value of herbage for animal production. Most of the secondary grasses have lower digestibility than the commonly sown perennial ryegrass (Frame 1991). Perennial ryegrass is generally easier to ensile than species such as common bent due mainly to the higher water soluble carbohydrate content of the former (Wilson and Collins 1980). Benefits to beef production in feeding perennial ryegrass silage compared to silage made from similarly managed permanent grassland with a diverse botanical content was associated with the higher digestibility and better preservation of perennial ryegrass during ensiling (Keating and O'Kiely 2000). Therefore animal production from herbage, either grazed or conserved, may not reflect these high yields of harvested herbage.

Conclusions

With few exceptions, the botanical composition has remained remarkably stable in slurry treated swards over the 24 years between botanical assessments. Undoubtedly, herbage quality of swards receiving consistently high rates of slurry is reduced due to the decline in perennial ryegrass content which cannot be explained solely on account of the swards ageing. The inability of perennial ryegrass to take up the nutrients supplied in high rates of slurry application, irrespective of slurry type, is a major limitation in grassland production, especially when efficiency in nutrient use in grassland is so important economically and environmentally. Decline in some nutrients below the requirement of perennial ryegrass, e.g. Ca, due to physical or chemical factors associated with increasing slurry content or direct physical impact on the species, such as smothering, are indicated as possible causes in this study. Further work is required to confirm these possibilities and to find ways of ameliorating these adverse effects.

Acknowledgments We thank the Stapledon Memorial Trust, British Grassland Society, for a Travelling Fellowship awarded to WJL. We also thank Elizabeth Anne Wasson, Aaron Carrick and Margaret Wallace for excellent technical assistance.

References

Adams SN (1984) Some effects of lime, nitrogen, and soluble and insoluble phosphate on the yield and mineral composition of established grassland. J Agric Sci Camb 102:219–226. doi:10.1017/S0021859600041666

Amiaud B, Touzard B, Bonis A, Bouzille JB (2008) After grazing exclusion, is there any modification of strategy for guerrilla species: *Elymus repens* (L.) Gould and *Agrostis stolonifera* (L.)? Plant Ecol 197:107–117. doi:10.1007/s11258-007-9364-z

Anderson R, Wu YX (2001) Phosphorus quantity-intensity relationships and agronomic measures of P in surface layers of soil from a long-term slurry experiment. Chemosphere 42:161–170. doi:10.1016/S0045-6535(00)00121-1

Christie P (1987) Some long-term effects of slurry on grassland. J Agric Sci Camb 108:529–541. doi:10.1017/S0021859600079910

Christie P, Beattie JAM (1989) Grassland soil microbial biomass and accumulation of potential toxic metals from long-term slurry application. J Appl Ecol 26:597–612. doi:10.2307/2404085

Christie P, Beattie JAM (1992) Vesicular-arbuscular mycorrhiza infection in cut grassland following long-term slurry application. Soil Biol Biochem 24:325–330. doi:10.1016/0038-0717(92)90192-Z

Collins DP, Murphy WE (1979) Effect of sward composition on animal output. In: Charles AH, Haggar RJ (eds) Change in sward composition and productivity. Occasional Symposium No 10. British Grassland Society, Hurley, pp 93–100

Forbes TJ, Dibb C, Green JO, Hopkins A, Peel S (1980) Factors affecting the productivity of permanent grassland. A national farm survey. Grassland Research Institute and Agricultural Development and Advisory Service, Hurley

Frame J (1989) Herbage productivity of a range of grass species under a silage cutting regime with high fertilizer nitrogen application. Grass Forage Sci 44:267–276. doi:10.1111/j.1365-2494.1989.tb02164.x

Frame J (1991) Herbage production and quality of a range of secondary grass species at five rates of fertilizer nitrogen application. Grass Forage Sci 46:139–151. doi:10.1111/j.1365-2494.1991.tb02216.x

Frame J, Charlton JFL, Laidlaw AS (1998) Temperate forage legumes. CAB International, Wallingford

Haggar RJ (1976) The seasonal productivity, quality and response to nitrogen of four indigenous grasses compared with *Lolium perenne*. J Br Grassl Soc 31:197–207

Hao XY, Chang C (2002) Effect of 25 annual cattle manure applications on soluble and exchangeable cations in soil. Soil Sci 167:126–134. doi:10.1097/00010694-200202000-00005

Haynes RJ, Naidu R (1998) Influence of lime, fertilizer and manure applications on soil organic matter and soil physical conditions: a review. Nutr Cycl Agroecosyst 51:123–137. doi:10.1023/A:1009738307837

Hopkins A, Wainwright J, Murray PJ, Bowling PJ, Webb M (1988) 1986 survey of upland grassland in England and Wales: changes in age structure, and botanical composition since 1970–72 in relation to grassland management and physical features. Grass Forage Sci 43:185–198. doi:10.1111/j.1365-2494.1988.tb01887.x

Hopkins A, Adamson AH, Bowling PJ (1994) Response of permanent and reseeded grassland to fertilizer nitrogen. 2. Effects on concentrations of Ca, Mg, K, Na, S, P, Mn, Zn, Cu, Co and Mo in herbage at a range of sites. Grass Forage Sci 49:9–20. doi:10.1111/j.1365-2494.1994.tb01971.x

Howe CD, Snaydon RW (1986) Factors affecting the performance of seedlings and ramets of invading grasses in an established ryegrass sward. J Appl Ecol 23:139–146. doi:10.2307/2403087

Keating T, O'Kiely P (2000) Comparison of old permanent grassland, *Lolium perenne* and *Lolium multiflorum* swards grown for silage. 1. Effects on beef production per hectare. Ir J Agric Food Res 39:1–24

Kopittke PM, Menzies NW (2005) Effect of pH and Na induced Ca deficiency. Plant Soil 269:119–129. doi:10.1007/s11104-004-0395-0

Mattila PK, Joki-Tokola E, Tanni R (2003) Effect of treatment and application technique of cattle slurry on its utilization by ley: 2. Recovery of nitrogen and composition of herbage yield. Nutr Cycl Agroecosyst 65:231–242. doi:10.1023/A:1022671321636

McKenzie FR, Jacobs JL (2002) Effects of application of nitrogen fertilizer on concentrations of P, K, S, Ca, Mg, Na, Cl, Mn, Fe, Cu and Zn in perennial ryegrass/white clover pastures in south-western Victoria, Australia. Grass Forage Sci 57:48–53. doi:10.1046/j.1365-2494.2002.00300.x

Murphy PNC, Stevens RJ, Christie P (2005) Long-term application of animal slurries to grassland alters soil cation balance. Soil Use Manage 21:240–244. doi:10.1079/SUM2005317

Peeters A (2004) Wild and sown grasses. Food and Agriculture Organization of the United Nations, Rome

Smith A, Allcock PJ (1985) Influence of age and year of growth on the botanical composition and productivity of swards. J Agric Sci Camb 105:299–325. doi:10.1017/S0021859600056379

Spedding CRW, Diekmahns EC (1972) Grasses and legumes in British agriculture. Commonwealth Agricultural Bureaux, Farnham Royal, UK

Watt TA (1978) The biology of *Holcus lanatus* L. (Yorkshire Fog) and its significance in grassland. Herb Abs 48:195–204

Whitehead DC (1995) Legumes: biological nitrogen fixation and interaction with grasses. In: Whitehead DC (ed) Grassland nitrogen, chapter 3. Biddles Ltd, Guildford, UK

Whitehead DC, Jones LHP, Barnes RJ (1978) The influence of fertilizer N plus K on N, S and other mineral elements in perennial ryegrass at a range of sites. J Sci Food Agric 21:1–11. doi:10.1002/jsfa.2740290102

Wilson RK, Collins CP (1980) Chemical composition of silages made from different grass genera. Ir J Agric Food Res 19:75–84