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Methods of enhancing botanical diversity within field margins of intensively managed grassland: a seven year field experiment

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Summary

1. Increased intensification has led to well-documented declines in the flora associated with agricultural grasslands. Manipulation of field margins for biodiversity enhancement in arable systems has been extensively investigated. However, there is a paucity of corresponding long-term research within intensively managed grasslands.

2. We investigated a combination of establishment and management methods to enhance botanical diversity of field margins in intensively managed grassland systems. The method of field margin establishment was investigated by fencing, natural regeneration by rotavation, or seeding with a wildflower mixture. Subsequent sward management by either grazing or mowing was tested at three margin widths. Success of establishment was addressed in terms of persistence of species richness, plant community composition and incidence of noxious weeds.

3. Seeding with a wildflower mixture was the most successful establishment treatment to enhance plant species richness and this effect persisted throughout the seven years of the experiment ($\bar{x} = 16.08 \pm 0.50$ s.e. plant species richness per 1x3 m^2 quadrat). Fenced and rotavated treatments contained significantly fewer plant species per quadrat ($\bar{x} = 8.01 \pm 0.36$ s.e., $\bar{x} = 9.57 \pm 0.39$ s.e. respectively).

4. Grazing led to a modest increase in species richness in fenced and rotavated plots compared to the mowing treatment, but had no effect in seeded plots. Grazing also led to an increased frequency and cover of competitive grasses in the seeded treatment.

5. While margin width was not found to significantly influence species richness, increased herb cover and reduced abundance of noxious weeds was recorded within the wider, seeded margins.

6. *Synthesis and applications* Choice of establishment method and subsequent management of grassland field margins significantly affected their conservation value.

Botanical diversity of margins within intensively managed pasture can be enhanced by sowing wildflower seed mixtures. This diversity can be maintained over time through appropriate management. Minimal change management approaches currently adopted in many agri-environment schemes, such as cessation of nutrient inputs and/or fencing, did not produce field margin swards of conservation value.

Key words: wildflower seed mixture, plant diversity, margin width, grazing, hay cutting, pasture, natural regeneration

Introduction

Changes in grassland management, such as increased use of inorganic fertiliser, increased stocking rates, frequency of sward reseeding with species monocultures and a move from hay to silage production, have led to dramatic decreases in the biodiversity associated with agricultural grasslands (Frame 2000, Blackstock *et al.* 1999). These losses have affected all aspects of farmland biodiversity, including plants (Vickery *et al.* 2001, Stehlik *et al.* 2007), invertebrates (Benton *et al.* 2002; Fenner & Palmer 1998), and birds (Donald *et al.* 2001 & 2006).

Many floral and faunal species would have restricted ranges or be absent altogether from intensively farmed land were it not for field boundary and field margin habitats (Marshall & Moonen 2002). Changes in field margin management, such as sowing wildflower mixtures or reducing of pesticide inputs, have been shown to increase farmland biodiversity within arable ecosystems (Asteraki *et al*. 2004; Critchley *et al.* 2006). However, corresponding methods of field margin enhancement within grassland systems largely remain unexplored (but see Haysom *et al.* 2004; Cole *et al.* 2007; Sheridan *et al.* 2008). We know of no studies that have specifically addressed the long-term development of botanically diverse grassland field margin swards.

Grassland conservation research primarily focuses on reduction of management intensity over entire fields or larger areas. While these aims are important, they do little to address the decline of diversity associated with improved grasslands. Furthermore, uptake of agri-environmental measures in intensive areas has been low (Hynes *et al.* 2008). Intensive farms may be more likely to participate in conservation efforts if these are focused on contained, well defined areas, and therefore do not interfere with overall farm production levels.

The creation of new habitats within intensively managed agricultural land can promote beneficial organisms for biological control, foster ecological resilience by increasing local alpha diversity (Duelli & Obrist 2003) and can act as 'island' refuges facilitating the movement of species between patches of semi-natural habitats (Albrecht 2010). Furthermore, field margin diversity may be particularly important for the maintenance of higher trophic level species, particularly farmland birds (Marshall & Moonen 2002).

High soil nutrient levels are often associated with reduced botanical diversity and the dominance of a few, highly competitive species in grasslands (Kleijn *et. al* 2009). Decreasing soil fertility may lead to swards of conservation value, but results vary and largely depend on soil type and previous management (Smith *et al*. 2000; Warren, Christal &Wilson 2002).

Natural regeneration is the only method of field margin establishment which preserves the local flora. If this method is employed then the potential diversity of the field margin is a function of the seeds coming from local sites or from the seed bank (Asteraki et al. 2004). Some studies suggest that natural regeneration of field margin vegetation promotes local invertebrate taxa equally as well as sown margins (Thomas & Marshall 1999, Anderson & Purvis 2008).

However, the success of grassland restoration is often seed-limited (Bakker & Berendse 1999) as the lower botanical diversity associated with intensively managed grasslands generally results in a less diverse soil seed bank. Most 'desirable' grassland species have significantly shorter seed longevity than arable and ruderal species (Bossuyt & Hermy 2003), with only a few common species producing large, persistent seed banks (Bekker et al. 1997)

Reintroduction of botanical diversity, through the use of seed mixtures, has been successful in the restoration of both arable (Martin & Wilsey 2006) and intensively managed grasslands (Jefferson 2005). However, this technique has not been applied to grassland field margins (but see Hovd 2008).

Temporal persistence of floral diversity within sown arable field margins is a difficulty which has been attributed to lack of disturbance (Pywell *et al.* 2006). Within grassland systems, grazing herbivores can potentially increase levels of disturbance. However, success is largely dependent on intensity of grazing (Bullock *et al.* 1994), herbivore type (Vickery *et al.* 2001) and timing of grazing (Smith & Rushton 1994).

This study investigated whether botanical diversity within field margins on intensively managed lowland grasslands can be enhanced by: (1) method of establishment, (2) modification of management, and (3) increased margin width. Success of these methods, individually and in combination, is assessed in relation to: species richness, species persistence, abundance of undesirable species, and the stability of the plant communities established. Results are discussed in the context of the practicality and effectiveness of different treatments in restoring and managing botanical diversity, and the implications for future agri-environment policies which focus on the creation and enhancement of grassland field margin habitats.

Materials and methods

Site location & description

The experiment was undertaken on a lowland dairy farm at the Teagasc Research Centre, Johnstown Castle, Co. Wexford (52°17'N, 6°30'W). The site is situated on clay-loam soil. All hedgerows were removed in the 1970's and paddocks separated by electric wire. The area was sown with a mid-season yielding variety of *Lolium perenne* approximately 4 years before the experiment commenced. Paddocks were grazed at a stocking rate of between 2.4-2.8 livestock units ha^{-1} by a Friesian dairy herd on a 21 day rotation and cut for silage in alternate years. Between 200-375 kg ha^{-1} nitrogen (N), 0-50 kg ha^{-1} phosphorous (P) and 0-75 kg ha^{-1} potassium (K) were applied annually to the swards adjacent to the experimental plots from 2002-2008.

Experimental Design

A stratified randomised factorial split-plot field margin experiment was established in spring 2002. Nine 90m long strips of grass sward were fenced off from the surrounding paddocks. One of three field margin widths (1.5. 2.5, and 3.5m) was randomly assigned to each strip to provide three replicates of each width (see Fig. S1 in Supporting Information).

Three establishment treatments investigated were: (1) fenced off from the main part of the sward ('fenced'), (2) rotavated and allowed to regenerate naturally from the seed bank ('rotavated'), and (3) rotavated and seeded with a grass and wildflower mixture ('seeded'). Vegetation was removed prior to rotavation and reseeding, using a glyphosate herbicide at recommended application rates. The seed mixture contained 10 grass and 31 herb species (Table 1) and the mixture was sown at a rate of 2.5g m^{-2}. Control plots consisted of existing pasture vegetation which were grazed but had no subsequent application of nutrients or herbicide. Each 90m strip was divided into three sections and an establishment method was randomly allocated to each 30m section. Fencing was used to exclude grazing from all treatment plots during the establishment period from February 2002 to June 2003.

All establishment treatment plots were mown and the clippings removed in September 2002. During June 2003, plots were split and half of each (randomly selected) grazed on a 21-day rotation basis in conjunction with the main sward ('grazed' treatment). The ungrazed portion of each plot was mown annually in September and all vegetation clippings removed ('mown' treatment). Nutrient and pesticide inputs were excluded from all plots over the duration of the experiment, although grazed plots, including the controls, received dung and urine inputs from the cattle.

Botanical Sampling

Botanical data were collected using permanent, nested quadrats. Two, four and six 1 x 3m quadrats were taken from the 1.5, 2.5 and 3.5m wide margins respectively (see Fig. S2). Presence/absence data were recorded for the entire 1 x 3m quadrat. Percentage cover of each species in the central $1m^2$ was visually estimated according to the Braun-Blanquet scale (Braun-Blanquet, Fuller & Conrad 1932). To avoid edge effects, a 9m long strip between treatments was not sampled. Plots were sampled in July of 2002, 2003, 2007, and 2008. Here, data from 2003, 2007 and 2008 were analysed while 2002 was treated as an establishment period (see Sheridan *et al*. 2008). Species were identified according to Stace (1997).

Soil sampling

Soil samples, consisting of twenty pooled 10cm depth cores, were taken from each plot in February 2003 and 2008. Samples were analysed to investigate levels of Morgan's available P, K, and Mg (Jackson 1958, Murphy & Reilly 1962).

Data analyses

A repeated measures analysis of total species richness per 1 x 3m quadrat was undertaken, using GLIMMIX (SAS 9.1.3) and a spatial covariance matrix, to account for permanency of quadrat location. Effects of establishment treatment, grazing, width, and time on species richness were investigated with all interactions between factors included in the full model. Initial maximal models were refined by the sequential removal of all non-significant terms. Minimal adequate models were identified by a process of assessment before and after the removal of terms using Akaike's Information Criterion (AIC) (Akaike 1974). All tests of significance were at the $p < 0.05$ level.

For each plot species turnover (which is equivalent to 1 – Sørensen's similarity index) between two years was calculated as:

$$t = \frac{b_i + c_j}{S_i + S_j}$$

where b_i = the number of species present in a plot that are unique to year *i*; c_j = the number of species present in a plot that are unique to year j; S_i = the total number of species present in a plot in year *i*; and S_j = the total number of species present in a plot in year *j* (Magurran 2004).

Plant community dynamics were investigated further by dividing species into three groups: 1) weed species (species listed on the Irish Noxious Species Act 1936 and including *Senecio jacobaea*, *Rumex obtusifolius*, *R. crispus*, *Cirsium arvense* and *C. vulgare*); 2) herbaceous species (excluding weeds) and 3) grass species (monocotyledonous species). Species richness and changes in abundance were analysed for each of these groups using a nonparametric factorial analysis as in Brunner & Puri (2001) using Proc Mixed (SAS 9.1.1) with repeated measures and a spatial covariance matrix. This method was used as the data were zero inflated and could not be analysed using parametric methods.

Multivariate analysis using CANOCO 4.5 was used to investigate the main effects of establishment treatment, grazing, width and their interactions, on the plant community composition. A partial Redundancy Analysis (RDA) was performed using species percentage cover data that were averaged across quadrat sub-samples within each replicate plot and centred by species, with the Monte Carlo permutation test (reduced model, 9999 permutations restricted to six split-plots, freely exchangeable whole-plots, no permutation at the split plot level). Partial RDA was used as plots had a homogeneous composition and showed linear species responses (Leps & Smilauer 2003).

In addition, differences in end-point vegetation composition for each establishment treatment x grazing management were tested in six separate RDA analyses of the null hypothesis that treatment *X* differed from treatment *Y*. Significance tests were performed by Monte Carlo permutation tests after 9999 unrestricted permutations, as only one environmental variable (treatment) was considered.

A Principal Response Curve (PRC) graphically demonstrated the change in plant species composition for each establishment treatment x grazing management interaction, over time, with the grazed control as the reference zero line. PRCs were based on partial RDA with time as a co-variable and treatment x time interactions set as environmental variables. Within these analyses, the average temporal trend was removed by treating it as a continuous covariate. The PRC diagrams correspond to the first and second RDA axes with species highly correlated to each axis displayed to the right of the diagram.

Results

A total of 76 higher plant species were recorded during the experiment. This included 50 herb, 17 grass, five woody, three rush, and one sedge species. A summary of species recorded with > 5% frequency can be seen in Table 1. For simplicity, only data from 2003 and 2008 are presented. In 2003, 16 of the 31 herb species and all of the 10 grass species included in the seed mixture were recorded. In 2008 the number of seeded herbs had reduced to 11 while all 10 grass species persisted (Table 1). A full list of species recorded within each treatment can be seen in the supporting information (Table S1).

Effect of establishment method

Method of field margin establishment had the greatest influence on botanical species richness ($p < 0.0001$, Table 2, Table 3 Fig. 1a), with an increase from 4.5 species per quadrat in control plots to 15.3 species per quadrat in the seeded plots in 2003. This increased species richness within seeded plots was maintained over the full experimental period with seeded plots having the greatest species richness, followed by rotavated plots, and then fenced plots ($p < 0.0001$). Species richness of herbs showed a similar trend ($p < 0.0001$, Fig 1b). Total species richness also increased within the rotavated and fenced treatments; however, species richness was always significantly higher in rotavated plots than in fenced ones. This increase in species richness may be partially attributed to the movement of species from the seeded plots. For example, *Cynosurus cristatus* and *Alopecurus pratensis* were not present in the fenced and rotavated treatments during 2003, but were recorded there in 2008 (Table 1).

Herb cover was also greatest in seeded plots during all sampling years ($p <0.0001$, Fig. 1c). There was a significant establishment treatment x time interaction ($p < 0.0001$, Table 3). The fenced and rotavated plots showed significant increases in herb cover from 2003 to 2008 ($p < 0.0001$ and $p = 0.0003$ respectively), while seeded plots did not.

Effect of grazing

The interaction between grazing and establishment treatment was significant for total species richness ($p = 0.0017$, Fig. 1a, Table 2). In seeded plots, grazing had no significant impact on species richness, while in fenced and rotavated plots grazing significantly increased species richness. Grazing increased the frequency of competitive grass species (*Lolium perenne* and *Poa trivialis*) in rotavated and seeded plots (Table 1).

Effect of field margin width

There was a significant interaction between width and establishment treatment (Table 2, Fig.2a), with 2.5m rotavated plots having higher species richness than 1.5m and 3.5m rotavated plots ($p =0.013$ and $p = 0.044$ respectively). Seeded plots showed a trend of increasing species richness with width, although this was not significant. Fenced plots showed an opposite trend, with species richness decreasing over increasing margin widths, i.e. species richness was significantly greater in the 1.5m than in the 3.5m plots ($p = 0.046$). A significant interaction was found between herb cover and plot width, ($p = 0.04$, Fig. 2b, Table 3), with 3.5m seeded margins containing a higher herb cover than 1.5m margins ($p = 0.031$).

Noxious weeds

Cover of noxious weed was significantly greater in rotavated plots compared to seeded ($p = <0.0001$) or fenced plots ($p <0.0001$; Fig 1d, Table 3) and generally decreased over time (Fig. 1d). This can largely be attributed to reductions in cover of *Rumex* species and *C. vulgare* over time (Table 1). Grazing did not significantly influence weed cover, however, the interaction between margin width and establishment treatment was significant ($p = 0.002$, Table 2). In seeded plots, weed cover decreased as width increased ($p < 0.05$; Fig. 2c).

Species turnover

There was a significant interaction between establishment treatment and turnover period (Table 4, $p < 0.0001$). Relative species turnover was significantly higher in the seeded and rotavated plots during the initial period compared with fenced plots (Fig. 3). This was primarily due to the loss of ruderal species in these plots following the establishment period. There was also a significant interaction between grazing and turnover period ($p = 0.012$, Table 4). Grazing did not affect turnover in the establishment period from 2002 to 2003 (Table 4). However grazing caused a significantly increased species turnover in the short term from 2007 to 2008 and in long-term from 2003 to 2008. Turnover from 2007 to 2008 was similar in all plots (c. 20% per year) with the exception of those which were seeded and mown, where it was significantly lower, c. 12% per year and this indicated the most stable plant community (Table 4).

Plant community composition

The Monte Carlo test showed a significant effect of establishment treatment, width, and grazing, as well as an interaction between these factors on the plant species composition (Table 6). By 2008 most plant communities were significantly different from each other, with the exception of those in the 'rotavated & mown' treatment and the 'mown' treatment, which had converged over time (Table 5).

The principal response curve for the first RDA axis showed a clear distinction in plant community structure between the seeded & mown treatments compared with all others (Fig. 4a). These plots also displayed stability in community composition over time (as also indicated by turnover), while the composition of all other treatment plots moved towards the grazed control situation (zero line). The principal response curve for the second RDA axis (Fig. 4b) primarily showed the effect of grazing, with community composition of the grazed plots clustering near the control (zero line), while the mown plots clustered together.

Soil

Grazing led to an increase in available soil magnesium and potassium compared to the mown plots, but had no effect on soil phosphate levels in 2008(see Table S2 & Table S3).

Discussion

Establishment method

This study demonstrated that use of seed mixtures produced the highest species richness and herbaceous cover in experimental field margins over the seven year experiment. Within this intensively managed grassland system the seed bank and rain were not sufficient to improve species richness over the duration of the experiment. These findings concur with many studies on grassland restoration which have found that restoration tends to be propagule-limited (Bakker & Berendse 1999, Pywell *et al*. 2002, Martin & Wilsey 2006) and therefore requires the addition of seed to increase species richness. Although species richness increased within rotavated and fenced plots over time, this may have been due to the migration of seeded species into adjacent plots through wind dispersal or while plots were being cut (through the movement of clippings).

The use of a seed mixture was also found to increase herbaceous cover and decrease noxious weed cover. Rotavation gave rise to problems with noxious weeds and there was little successful herb establishment from the seed bank. These results support our assertion that use of a seed mixture is appropriate when margins have been degraded by intensification and there are limited seed resources locally. However, introduction of seed mixtures may not be appropriate in extensively managed grasslands or where appropriate species are found locally in the landscape. Under such conditions seed rain may be sufficient to enhance margin diversity when coupled with appropriate management, such as reduced nutrient inputs and moderate grazing and/or mowing. These methods have been used successfully in some grassland restoration projects where the management history was less intensive (Walker *et al.* 2004).

Field margin management

Plant community composition was significantly affected by subsequent management of the field margin. Paddock grazing practiced at this site, and which is the norm within intensively managed pastoral systems in general, results in low and uniform sward height with few species capable of setting seed (Vickery *et al.* 2001). Over the experimental period, the plant community composition of all the grazed plots became more similar to the control plots, with increased frequency of competitive grass species. On the other hand, plots which were mown once annually were subject to lower levels of disturbance. Where soil nutrient status is high, this lack of disturbance can lead to low levels of seedling recruitment, as established vegetation can quickly out-compete seedlings for light (Hautier, Niklaus & Hector 2009).

Under these experimental conditions mowing led to a more stable sward community composition, with lower species turnover rates than were recorded in grazed plots. It is likely that a disturbance level between these extremes may have promoted enhanced seedling recruitment and sward stability. Hay meadows usually have both mowing and grazing management. Seedling emergence is enhanced by manual soil disturbance (to simulate aftermath grazing) after mowing (Hellström *et al*. 2009). The implications for plant biodiversity rest in the minor alterations in management, such as the timing and frequency of both mowing and grazing (Coulson *et al.* 2001). There is a lack of data comparing the effectiveness of mowing versus grazing in the restoration of biodiversity in grasslands (Pykala 2000). Increases in biodiversity may be achieved through the manipulation of disturbance through the timing, intensity and frequency of grazing, to produce more micro-sites for seedling germination (Bullock 1994). The use of grazing to manipulate disturbance rates may be more appropriate than mowing in pasture field margins, as herbivores are readily available and it simplifies management for farmers. Making management options more practical attracts farmers to AES and may lead to wider participation (Morris, Mills, & Crawford 2000).

Margin width

Wider margins, with their increased area, should theoretically lead to decreased extinction risk and thus higher species richness (MacArther & Wilson 1967).While width seemed less important than seeding and sward management in determining species richness of plots it appeared to be an essential factor in the successful establishment of sown plant communities. Wider margins facilitated increased cover of herb species within sown margins, while reducing the dominance of noxious perennial weeds. According to Joshi (2006), specialist species tend to be lost when grassland patch size is small whereas generalist plant species remain constant. The retention of less competitive perennial herb species, and thus the conservation quality of seeded grassland field margins, may be determined by margin width.

Within the Irish context, field sizes are relatively small ($\bar{x}$ = 3.93 ha.) and over 70% of the fields are smaller than 4 ha. (Deverell, McDonnell & Devlin 2009). Therefore, the establishment of a 6m margin, a width that is generally recommended in many agri-environment schemes, along all field edges would be inappropriate as it could constitute 12%

of the average field area. A more rational approach would be to dedicate a percentage of the productive area (for example 1-4%) of the farm to seeded and expanded margins.

Implications for enhancing field margins within intensive grasslands

With investment in agri-environment schemes costing €3.7 billion annually in the EU (OECD 2004), new policy measures must show clearly identifiable and measurable biodiversity benefits. This research shows that there are more efficient measures for the enhancement of plant diversity within grassland systems than current policy reflects.

Cessation of nutrient inputs alone was not sufficient to restore plant diversity. While this is essential to ensure further diversity is not lost, it is of potentially limited value in terms of enhancing botanical diversity over an agri-environment contact period (see Sheridan, Finn & O'Donovan 2009).

Our results imply that under intensive grazing systems, plant diversity can be enhanced through the introduction of seed mixtures and this diversity can be maintained over time. Wider margins allow better establishment of herbaceous cover and should be used when creating seeded field margins. When designing seed mixtures, appropriate species should be chosen, ideally targeting suitable grassland, geographical and soil types. The use of a single standard mixture may lead to homogeneity within field margins and therefore might not promote diversity at the wider landscape scale. The use of seed which is of local provenance should be adopted to ensure that local genotypes are not depleted (Walker *et al*., 2004).

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Supporting Information

Fig. S1. Diagram of experimental design.

Fig. S2. Diagram of botanical sampling.

Table S1. Presence/absence of species recorded in all years.

Table S2. Mean soil nutrient levels.

Table S3. Effects of establishment treatment, grazing and width on soil nutrient levels.

Table 1. Summary of (a) changes in the frequency of plant species (with frequencies >5%) in permanent field margin quadrats between 2003 and 2008, ranked by most abundant species and (b) mean percentage cover of the ten most frequent species in 2008. M = Mown, C = grazed control, R+M = rotavated & mown, R+G = rotavated & grazed, S+M = seeded & mown, S+G = seeded & grazed. Categories: '--' is <-50%, '-' is -50% to -11%, '0' is -10% to +10%, '+' is +11% to 50%, '++' is >50%, blank spaces are absent from both years, * denotes species with cover <1%.

	(a) % frequency change 2003 to 2008						(b) Mean % cover in 2008					
	M	C	R+M	R+G	S+M	S+G	M	C	R+M	R+G	S+M	S+G
Agrostis spp†	0	0	0	0	0	0	29	21	16	21	16	20
Holcus lanatus†	+	+	+	-	0	0	32	26	27	24	12	18
Lolium perenne	-	0	0	0	-	++	1	33		15		14
Rumex spp.	0	0	+	+	-	-			4	3		
Ranunculus repens	+	+	+	0	+	+	5	2	11	8	6	
Rumex acetosa†		+	-		+	-			1		4	2
Cynosurus cristatus†	0	+		0	-	0					2	4
Holcus mollis	+	0	0	+	+	0	21	1	17			
Poa trivialis	0	++	0	0	+	++		5		7		2
Cirsium arvense	+	+	-	-	+	+	4	1	8	4		
Dactylis glomerata†	+	0	0	0	+	+	4	2				
Anthoxanthum odoratum†			+		0	-					3	
Arrhenatherum elatius†	+		0		+	-	4		11		23	
Plantago lanceolata†		0		+	0	+					7	14
Phleum pratense†			0		--	-						
Daucus carota†		0	0	0	--	-						
Cerastium fontanum	0	++	+	+	0	++		1		1		1
Alopecurus pratensis†		0	0	0	+	0					6	
Leucanthemum vulgare†					-	0						1
Epilobium spp		+	0	0	-	-						
Senecio jacobaea		-	0	+	+	+			2	1		
Trifolium repens		+	+	0	0	+		2		9		9
Festuca rubra†					+	+					4	
Juncus spp.			0	0	+	+			*			
Taraxacum agg. †		-		0	0	0						
Filipendula ulmaria †					+	+						
Poa annua		+		++		+						
Urtica dioica			0	+	0							
Prunella vulgaris†					0	+						
Veronica serpyllifolia		+		0		+						
Juncus bufonius	+			0		+	*					
Cirsium vulgare			0	+	0	0						
Ranunculus acris†					+	0						
Lychnis flos-cuculi†					0	+						
Digitalis purpurea†					-	-						
Elytrigia repens	+		-		+		*					
Alopecurus geniculatus		+				+						
Achillea millefolium†						0						
Centaurea nigra†					+							
Lotus corniculatus			0			0						
Quercus spp.	0				0							
Trifolium pratense						-						

Species included in the seed mixture are denoted by †. Other species included within the seed mixture are: *Alliaria petiolata, Angelica sylvestris, Anthyllis vulneraria, Arctium minus, Capsella bursa-pastoris, Dipsacus fullonum, Eupatorium cannabinum, Galium verum, Leontodon hispidus, Lythrum salicaria, Medicago lupulina, Origanum vulgare, Pedicularis palustris, Primula veris, Pulicaria dysenterica, Rhinanthus minor, Silene vulgaris, Succisa pratensis, and Vicia cracca.*

Table 2. Effects of treatment, year, grazing and width and the interactions of these factors, on total species richness of the experimental plots over three sampling periods (2003, 2007 and 2008) calculated using GLIMMIX.

		Total species richness	
	DF	***F***	***P***
Establishment treatment	(2, 68)	366.89	***
Grazing	(1, 76)	68.15	***
Grazing x est. treatment	(2, 76)	6.93	**
Width	(2, 68)	1.23	n.s.
Width x est. treatment	(4, 68)	2.83	*
Year x est. treatment	(4, 68)	9.18	***
Year x grazing	(2, 76)	9.24	***

* *P* < 0.05., ** *P* < 0.01., *** *P* < 0.001.

Table 3. Effects of treatment, year, grazing and width and the interactions of these factors, on the herb species richness, herb percentage cover, and weed percentage cover of the experimental plots over three sampling periods (2003, 2007 and 2008) calculated using nonparametric methods.

	Herb species richness		**Herb percentage cover**		**Weed percentage cover**	
	F	***P***	***F***	***P***	***F***	***P***
Year	21.80	***	19.19	***	14.56	***
Establishment treatment	217.36	***	126.32	***	19.76	***
Grazing	41.28	***	4.79	*	1.93	n.s.
Grazing x est. treatment	2.41	n.s.	0.42	n.s.	0.01	n.s.
Width	0.33	n.s.	1.53	n.s.	2.7	n.s.
Width x est. treatment	3.02	n.s.	2.89	*	6.09	**
Width x grazing	0.00	n.s.	1.64	n.s.	3.7	*
Width x grazing x est. treatment	0.28	n.s.	0.28	n.s.	0.82	n.s.
Year x est. treatment	12.61	***	12.62	***	4.49	**
Year x grazing	24.93	***	4.52	*	1.15	n.s.
Year x Width	0.89	n.s.	0.71	n.s.	2.52	*

* *P* < 0.05., ** *P* < 0.01., *** *P* < 0.001.

Table 4. Effects of treatment, year, grazing and width and the interactions of these factors, on species turnover rates of the experimental plots over the establishment period (year 1- 2) and the experimental end point (year 5-6) and long-term duration (year 2-6).

	Species turnover rate		
	DF	***F***	***P***
Establishment treatment	(2, 36)	6.92	**
Grazing	(1, 36)	9.58	**
Grazing x est. treatment	(2, 36)	1.52	n.s.
Width	(2, 36)	2.26	n.s.
Width x est. treatment	(4, 36)	1.42	n.s.
Width x grazing	(2, 36)	0.67	n.s.
Width x grazing x est. treatment	(4, 36)	0.54	n.s.
Year x est. treatment	(4, 72)	16.05	***
Year x grazing	(2, 72)	4.69	n.s.
Year x Width	(4, 72)	0.85	n.s.
Year x grazing x est. treatment	(4, 72)	2.25	n.s.
Year x width x est. treatment	(8, 72)	0.97	n.s.
Year x width x grazing x est. treatment	(12, 72)	0.80	n.s.

* *P* < 0.05., ** *P* < 0.01., *** *P* < 0.001.

Table 5. F-values and significance of six separate Monte Carlo tests for the null hypothesis that species composition (measured as percentage cover using the Braun-Blanquet scale) is the same in comparison to each other in 2008. M = Mown, C = grazed control, R+M = rotavated & mown, R+G = rotavated & grazed, S+M = seeded & mown, S+G = seeded & grazed.

	F	*P*
M vs R+M	1.728	ns
M vs S+M	10.616	***
R+M vs S+M	5.718	**
C vs R+G	3.134	**
C vs S+G	5.827	***
R+G vs S+G	5.827	***

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

Table 6. F-values and significance of six separate Monte Carlo tests for the null hypothesis that species composition is effected by specific treatments and their interactions in 2008.

	F	P
Establishment treatment	14.797	***
Grazing	14.797	***
Width	1.605	ns
Grazing x est. treatment	1.999	**
Width x est. treatment	0.790	ns
Width x grazing x est. treatment	0.94	ns

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

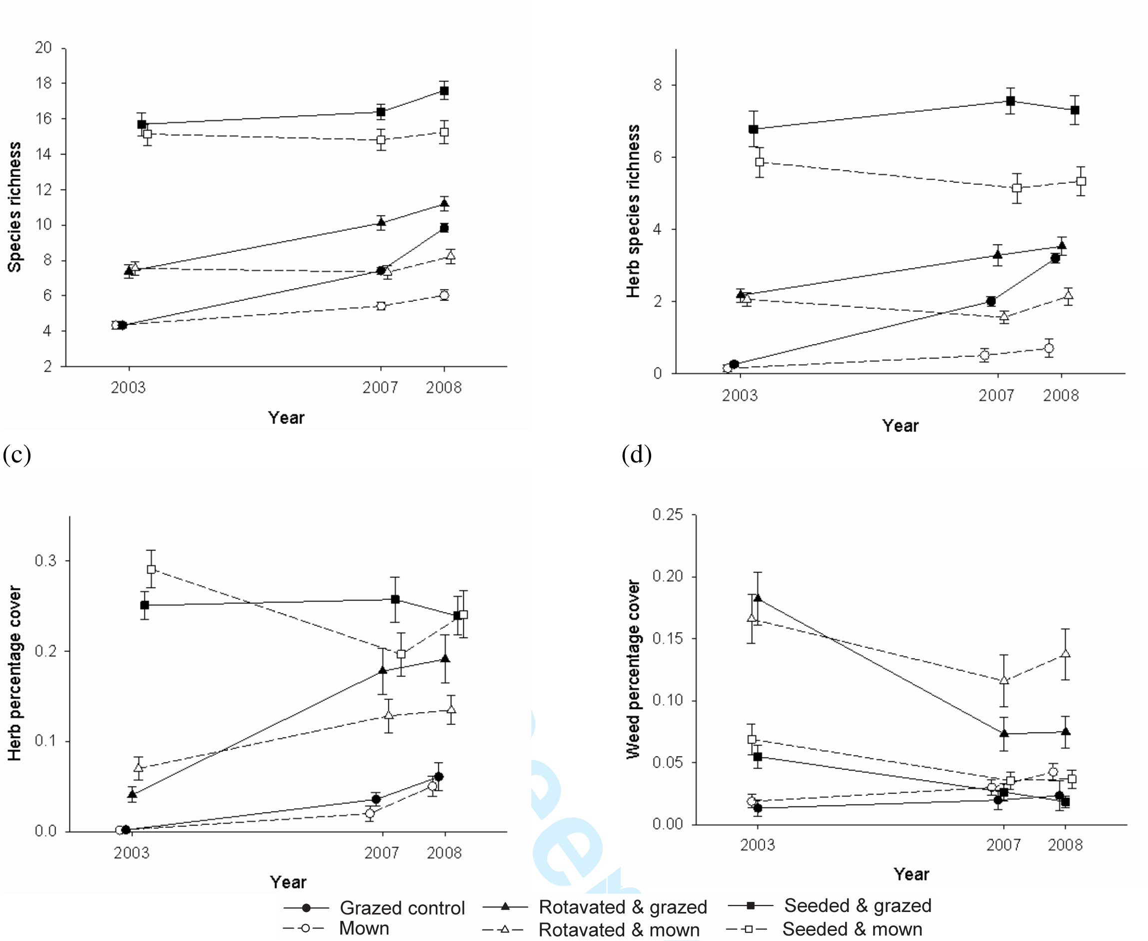
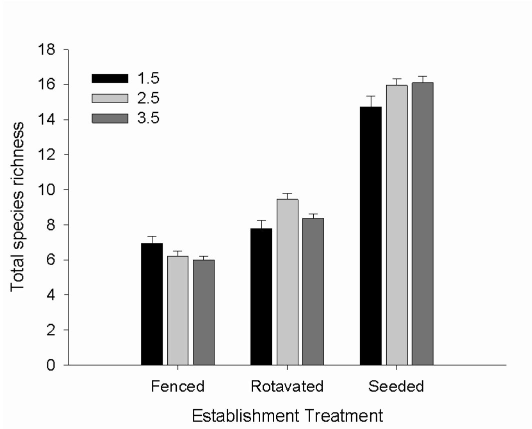
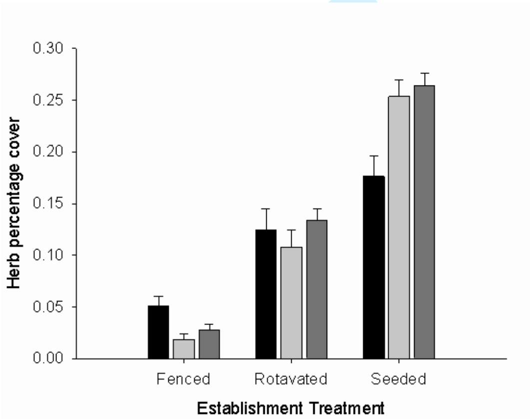


Fig.1. Year-to-year changes in mean (a) total species richness, (b) herb species richness, (c) herb cover, and (d) weed cover with error bars denoting SE (n = 36).

(a)



(b)



(c)

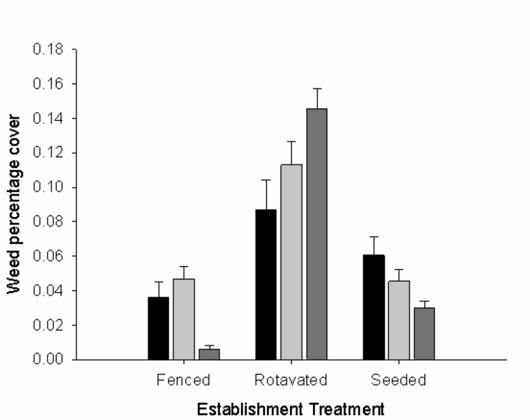


Fig. 2. Responses of (a) total species richness (mean species per quadrat ± SE), (b) herb percentage cover (mean cover per quadrat ± SE), and (c) weed percentage cover (mean cover per quadrat ± SE) to establishment treatment and margin widths: 1.5 (black) 2.5 (light grey) 3.5 (dark grey) (n = 36, 72, 108 for 1.5, 2.5 & 3.5m widths respectively).

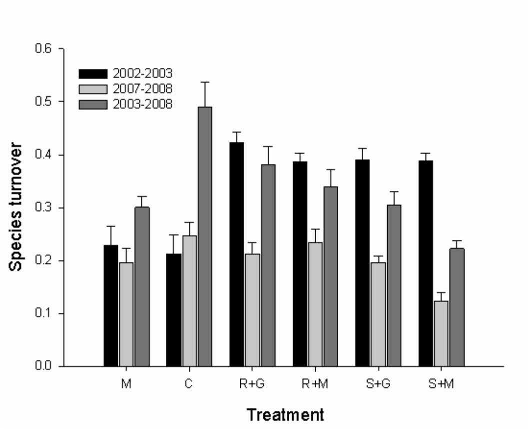


Fig. 3. Species turnover rates (mean turnover per quadrat ± SE) within initial establishment period 2002-2003 (black), endpoint 2007-2008 (light-grey) and long-term turnover from 2003 to 2008 (dark-grey) of experiment within each establishment treatment x grazing split-plot (n = 36). M = Mown, C = grazed control, R+M = rotavated & mown, R+G = rotavated & grazed, S+M = seeded & mown, S+G = seeded & grazed.

(a) (b)

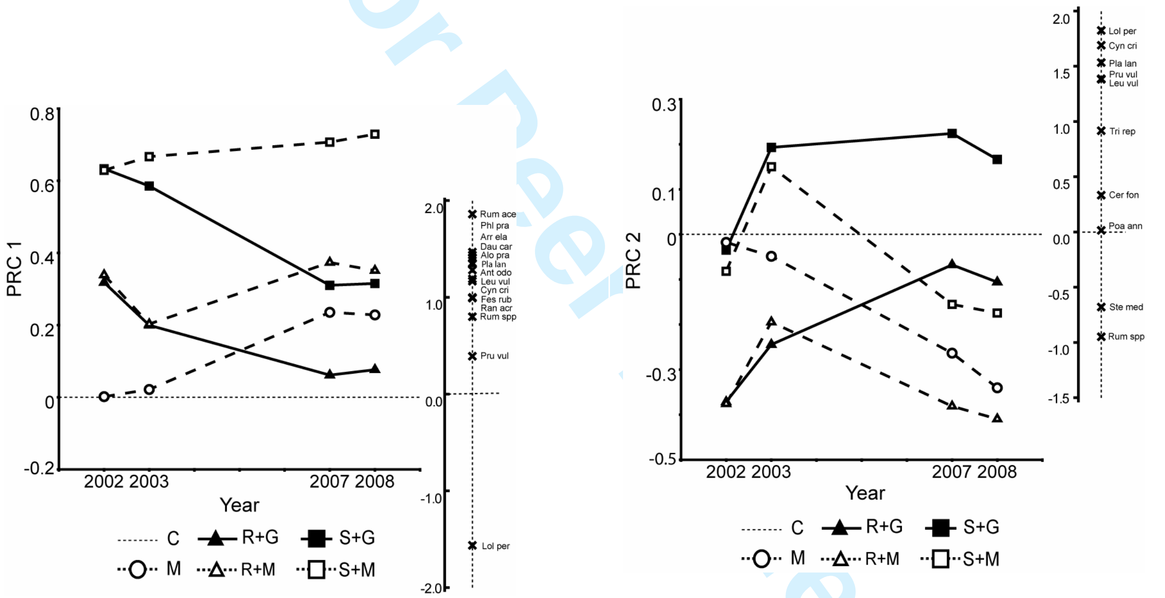


Fig. 4. Principal response curves (PRC) corresponding to the first (a) and second (b) partial RDA axis for plant community data (as percentage cover using the Braun Blanquet scale) change over time versus the grazed control, the zero line, with interactions between the treatments and time acting as environmental variables and sampling time indicators as co-variables. The one-dimensional diagram on the right shows the species scores on the RDA axis. Species which are highly associated with each axis are shown on the right of each panel.

Fig. S1. Diagram of experimental design and plot locations (not to scale).

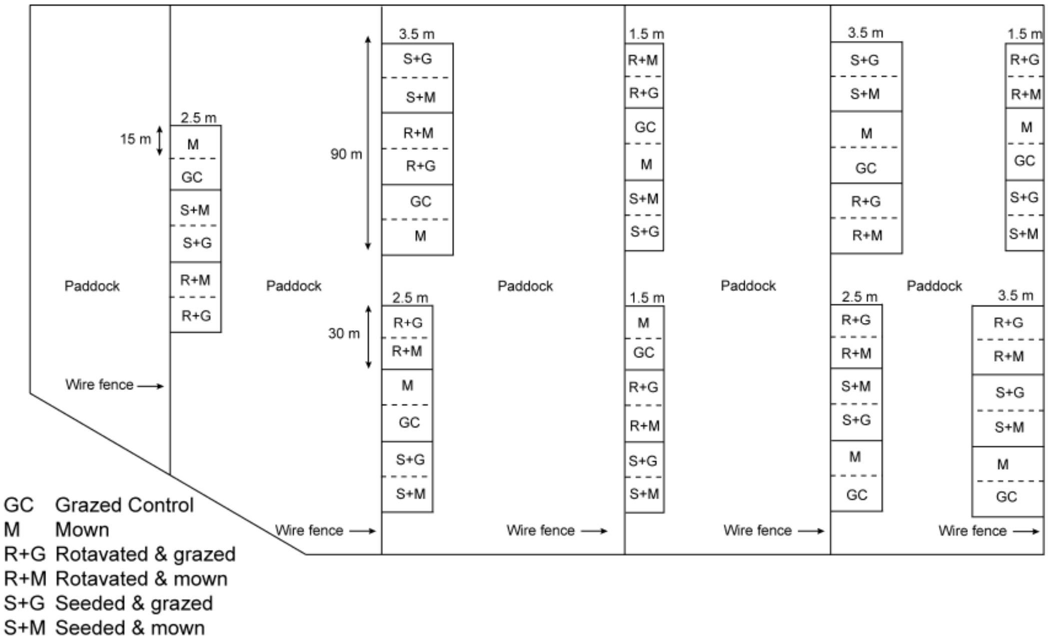


Fig. S2. Diagram of botanical sampling, quadrat sampling for 3.5m margin width *a*, *b*, & *c* quadrats were sampled, at 2.5m width *a* & *b* quadrats were sampled, in 1.5m width *a* quadrats were sampled (not to scale).

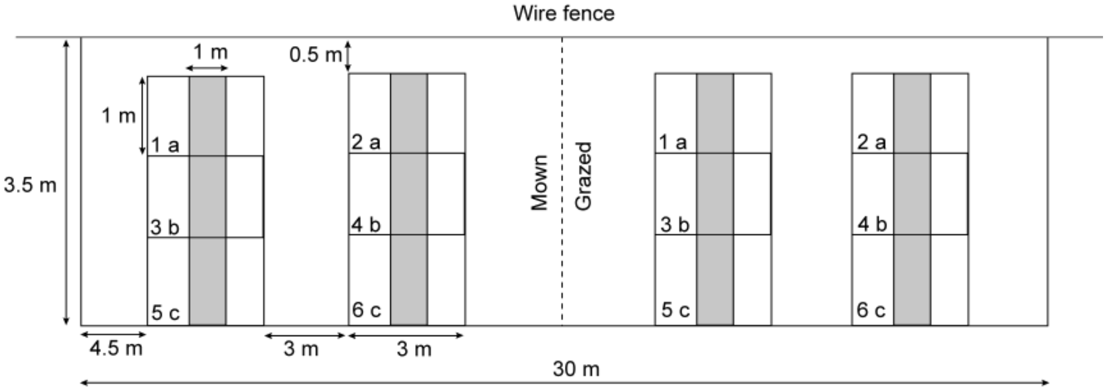


Table S1. List of presence/absence of all species recorded within experimental plots in 2002, 2003, 2007, and 2008.

	Mown				Grazed Control				Seeded & Mown				Seeded & Grazed				Rotavated & Mown				Rotavated & grazed			
Year	02	03	07	08	02	03	07	08	02	03	07	08	02	03	07	08	02	03	07	08	02	03	07	08
Acer spp												x												x
Achillea millefolium									x				x	x	x	x								
Agrostis gigantea									x															
Agrostis spp	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Alopecurus geniculatus								x				x		x		x		x				x		
Alopecurus pratensis				x	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x		x
Anagallis arvensis									x				x	x			x				x			
Angelica sylvestris										x														
Anthoxanthum odoratum					x		x		x	x	x	x	x	x	x	x		x	x	x		x	x	x
Arctium minus									x	x			x	x										
Arrhenatherum elatius	x		x	x					x	x	x	x	x	x	x		x	x	x	x	x	x	x	
Bellis perennis							x									x							x	x
Capsella bursa-pastoris									x				x											
Carex ovalis																				x				x
Centaurea nigra										x	x	x		x	x	x						x		
Cerastium fontanum			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Chenopodium album									x				x											
Cirsium arvense	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Cirsium vulgare	x						x	x	x	x			x	x	x	x	x	x		x	x	x	x	x
Cynosurus cristatus			x	x			x	x	x	x	x	x	x	x	x	x		x	x	x			x	x
Dactylis glomerata	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x
Daucus carota			x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x			x	x
Digitalis purpurea									x	x			x	x										
Elytrigia repens			x	x								x							x	x				x
Epilobium spp	x	x	x	x	x		x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Festuca rubra							x			x	x	x		x	x	x		x	x	x		x	x	x
Filipendula ulmaria										x	x	x		x	x	x			x	x				
Gnaphalium uliginosum									x				x				x				x			
Hedera helix																				x				
Heracleum sphondylium														x										
Holcus lanatus	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Holcus mollis	x	x	x	x	x	x		x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Isolepis setacea																x								x
Juncus bufonius	x				x			x	x				x			x	x				x			x
Juncus spp								x		x	x	x				x		x	x	x		x	x	x
Leucanthemum vulgare							x	x	x	x	x	x	x	x	x	x		x		x		x		
Lolium perenne	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Lotus spp									x	x			x	x		x			x	x				
Lychnis flos-cuculi										x	x	x		x	x	x							x	
Matricaria discoidea									x				x				x				x			
Matricaria recutita									x				x								x			
Medicago lupulina									x				x	x										
Papaver rhoeas									x				x											
Persicaria maculata									x				x				x				x			
Phleum pratense					x				x	x	x	x	x	x	x	x	x	x	x	x	x		x	
Plantago lanceolata							x	x	x	x	x	x	x	x	x	x		x				x	x	x
Plantago major													x			x					x			
Poa annua				x			x	x	x		x		x		x	x	x		x		x		x	x
Poa pratensis	x												x				x				x			
Poa trivialis	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Polygonum aviculare																	x							

Potentilla anglica																				x				
Prunella vulgaris							x	x		x	x	x		x	x	x	x	x					x	x
Quercus spp	x		x	x				x			x	x							x	x				
Ranunculus acris							x			x	x	x		x	x	x								
Ranunculus repens	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Rubus fruticosus																	x	x				x		
Rumex acetosa			x	x			x	x	x	x	x	x	x	x	x	x		x	x	x		x	x	x
Rumex acetosella												x		x		x	x	x						
Rumex spp	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Sagina procumbens								x								x								
Salix spp																		x				x		
Senecio jacobaea		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Senecio vulgaris													x				x				x			
Silene vulgaris									x				x											
Sonchus asper									x				x				x				x			
Sonchus oleraceus									x				x				x		x		x	x	x	
Spergula arvensis									x				x				x				x			
Stellaria graminea											x	x												
Stellaria media						x	x	x	x		x	x	x		x		x		x	x	x		x	x
Succisa pratensis														x										
Taraxacum spp		x			x		x	x	x	x	x	x	x	x	x	x	x			x	x	x	x	x
Trifolium pratense									x	x			x	x										
Trifolium repens	x		x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Urtica dioica					x	x			x	x	x	x	x	x			x	x	x	x	x	x	x	x
Veronica serpyllifolia					x			x				x	x	x	x	x	x				x	x	x	x

Table S2. Mean soil nutrient levels for phosphate (P), potassium (K), and magnesium (Mg) and standard error for establishment year (2002) before grazing commenced and final year (2008). Treatments are within main sward (F), mown (M), grazed control (C), rotavated and mown (S+M), rotavated and grazed (R+G), seeded and mown (S+M), seeded and grazed (S+G).

Year	2002			2008					
Treatment	Fenced	Seeded	Rotavated	M	C	S+M	S+G	R+M	R+G
P	4.12	3.66	3.76	3.54	4.84	4.28	4.9	3.88	4.43
SE P	0.27	0.25	0.37	0.23	0.35	0.42	0.28	0.3	0.3
K	64.23	94.22	69.67	58.56	145.56	67.73	131.88	63.62	127.4
SE K	4.77	13.31	5.99	7.19	13.61	4.98	19.46	10.34	16.25
Mg	202.88	193.89	178.33	162.56	188.53	165.39	178.4	171.27	178.7
SE Mg	11.93	13.42	7.4	4.99	9.13	11.13	9.03	8.33	4.87

Table S3. Effects of establishment treatment, grazing and width on Morgan's soil phosphate (P), potassium (K), and magnesium (Mg) of the experimental plots in 2008.

		P		**K**		**Mg**	
		F	*P*	*F*	*P*	*F*	*P*
Establishment treatment	(2, 40)	0.24	n.s..	1.26	n.s.	0.4	n.s.
Width	(2, 40)	0.45	n.s.	22.82	***	5.61	**
Grazing	(1, 40)	2.11	n.s.	665.45	***	18.55	***
Est. treat x width	(4, 40)	0.15	n.s.	16.73	***	1.33	n.s.
Est. treat x grazing	(2, 40)	0.19	n.s.	7.61	**	2.28	n.s.
Grazing x width	(2, 40)	0.21	n.s.	5.1	*	0.38	n.s.

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