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Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints

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Summary

1. Many grasslands in north-west Europe are productive but species-poor communities resulting from intensive agriculture. Reducing the intensity of management under agri-environment schemes has often failed to increase botanical diversity. We investigated biotic and abiotic constraints on diversification by manipulating seed and microsite availability, soil fertility, resource competition, herbivory and deficiencies in the soil microbial community.

2. The effectiveness of 13 restoration treatments was investigated over 4 years in a randomized block experiment established in two productive grasslands in central-east and south-west England.

3. Severe disturbance involving turf removal followed by seed addition was the most effective and reliable means of increasing grassland diversity. Disturbance by multiple harrowing was moderately effective but was enhanced by molluscicide application to reduce seedling herbivory and by sowing the hemiparasite *Rhinanthus* to reduce competition from grasses.

4. Low-level disturbance by grazing or slot-seeding was ineffective in increasing diversity. Inoculation with soil microbial communities from species-rich grasslands had no effect on botanical diversity. Nitrogen and potassium fertilizer addition accelerated off-take of phosphorus in cut herbage but did not cause a reduction in soil phosphorus or increase botanical diversity.

5. Different grazing management regimes had little impact on diversity. This may reflect the constraining effect of the July hay cut on species dispersal and colonization.

6. *Synthesis and applications.* Three alternative approaches to grassland diversification, with different outcomes, are recommended. (i) High intervention deturfing, which would create patches with low competitive conditions for rapid and reliable establishment of the target community. For reasons of cost and practicality this can only be done over small areas but will form source populations for subsequent spread. (ii) Moderate intervention (harrowing or slot-seeding) over large areas, which would establish a limited number of desirable, generalist species that perform well in restoration. This method is low cost and rapid but the increases in biodiversity are less predictable. (iii) Phased restoration, which would complement the above approaches. Productivity and competition are reduced over 3–5 years using *Rhinanthus* or fertilizers to accelerate phosphorus off-take. After this time harrowing and seeding should allow a wide range of more specialist species to establish. However, further research is required to determine the long-term effectiveness of these approaches.

Key-words: competition, disturbance, herbivory, livestock grazing, PLFA, restoration, *Rhinanthus*

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Introduction

Species-poor, structurally uniform and intensively managed permanent pasture is the dominant grassland type in many European countries, including the UK, where it covers 59 073 km^2 (24% of the land area; Fuller *et al*. 2002). There is growing evidence that intensive grassland management has a strongly negative effect on many taxa, including broadleaved plants (Kirkham, Mountford & Wilkins 1996), grasshoppers (van Windergen, van Kreveld & Bongers 1992), butterflies (León-Cortés, Cowley & Thomas 2000) and birds (Vickery *et al*. 2001).

These grasslands are a particularly important component of the UK agri-environment schemes, with more than 4925 km^2 currently under agreement (Carey *et al*. 2002; Carey 2005). However, across Europe the diversification of these grasslands has proved difficult to achieve by the extensification of management practices prescribed by the agri-environment schemes (Kleijn & Sutherland 2003). To understand the reasons for this, it is important to consider the above- and below-ground limitations on ecological restoration (Bakker & Berendse 1999; Walker *et al*. 2004). High residual soil fertility resulting from intensive farming is likely to place severe constraints on the enhancement and long-term maintenance of plant diversity (Marrs 1993). Indeed, eutrophication has emerged as a significant contributor to the loss of botanical diversity in UK over the last 20 years (Dalton & Brand-Hardy 2003) because of the competitive dominance of a few species. Low levels of soil phosphorus appear to be a prerequisite for long-term maintenance of high plant diversity in grasslands (Janssens *et al*. 1998). Highly interventionist approaches, such as topsoil removal, may be the only effective means of reducing soil fertility rapidly in some situations (Tallowin & Smith 2001). It may also be necessary to introduce seed (Pywell *et al*. 2002a) because the assembly of diverse plant communities on intensively managed farmland is often limited by lack of propagules of desirable species, in both the seed bank (Bekker *et al*. 1997; Pywell, Putwain & Webb 1997) and surrounding landscape (Poschlod *et al*. 1998; Bullock *et al*. 2002).

The plant community assembly is further affected by a range of biotic interactions, including competition, predation and soil microbial processes (Brown & Gange 1989; Bullock 2000). The presence of highly competitive species can prevent the colonization of desirable species or even drive established species extinct (Hansson & Fogelfors 1998; Bullock 2000). Competition can be decreased by vertebrate grazing (Bullock *et al*. 2001) and cutting (Coulson *et al*. 2001; Smith *et al*. 2003), which decrease vegetation biomass and create gaps for seedling establishment. Recent research has shown that the introduction of the hemiparasitic annual *Rhinanthus minor* into productive swards can have a similar effect by reducing dominance by competitive species and facilitating establishment of desirable species (Pywell *et al*. 2004b). Herbivory by molluscs can also have a significant effect on community composition by affecting seedling recruitment (Hanley, Fenner & Edwards 1996). Finally, there is increasing evidence that soil microbial communities play a critical role in the regulation and maintenance of plant biodiversity through their control of essential ecosystem functions (Zak *et al*. 2003). The application of mineral fertilizer is generally associated with a shift from fungal-dominated soil microbial communities to those dominated by bacteria (Smith *et al*. 2003). A diversity of mycorrhizal associations is thought to increase plant diversity (van der Heijden *et al*. 1998; Hartnett & Wilson 1999) and so the low rates of mycorrhizal infection in these bacterial-dominated systems may constrain restoration. Increased plant diversity can lead to increased soil fungal biomass (Smith *et al*. 2003) but it is not known whether direct addition of soil fungi could be used to enhance restoration success.

It is likely that a combination of several restoration treatments may be required to overcome the large number of potential constraints on the restoration of biodiversity to productive grasslands. The aim of this study was to develop and test a range of practical solutions to the problem of grassland diversification. In order to achieve this we tested the following non-exclusive hypotheses. (H1) Establishment site limitation: high levels of mechanical and/or biotic disturbance are required to create suitable sites for the initial germination and establishment of desirable plant species. (H2) Dominance by competitive species: competition for space and resources, particularly from agricultural grasses, can be significantly reduced through combinations of cutting, intensive livestock grazing and the introduction of hemiparasitic plants. (H3) Mollusc herbivory: high levels of mollusc herbivory have a strongly detrimental effect on the establishment of desirable plant species. (H4) Soil microbial communities: inoculation with a diverse assemblage of soil microbes accelerates the restoration of botanical diversity. (H5) Soil fertility: reduction of soil phosphorus (P) pools by herbage off-take can be accelerated by the addition of nitrogen (N) and potassium (K) fertilizers. This has beneficial effects on the establishment of desirable plant species.

The success of different combinations of restoration and management treatments was assessed by comparing the plant community composition with that of regional species-rich grassland target communities. The results are discussed in the context of (i) the practicality and effectiveness of the different treatments in restoring and conserving botanical diversity, and (ii) implications for future agri-environment scheme policies aimed at the enhancement and creation of grassland habitats in the wider countryside.

Materials and methods

Study sites

In 1999, identical experiments were established at two productive grassland sites on contrasting soil types: a

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freely drained loam at North Wyke, Devon, in south-west England (50°46′N, 3°54′W), and an impeded clay at Edgcott, Buckinghamshire (Bucks), in central-east England (51°53′N, 1°02′W). The Devon site had a history of relatively high inputs of fertilizer (e.g. 100–180 kg N ha year^{-1} supplemented with 10–24 kg P and 40–65 kg K every 2–3 years) to support silage cutting followed by aftermath sheep grazing. The Bucks site received lower inputs (90 kg N ha year^{-1}) and was grazed by cattle with silage cuts every 2–3 years. Consequently, the Devon site was significantly more fertile, with 40% more total P ($F_{1,174} = 167$, $P < 0{\cdot}001$), 30% more Olsen-extractable P ($F_{1,174} = 42$, $P < 0{\cdot}001$) and 30% more K (K) ($F_{1,174} = 35$, $P < 0{\cdot}001$) and a higher pH ($F_{1,174} = 76$, $P < 0{\cdot}001$). The Bucks site had a longer history of grassland management and therefore had a significantly higher (9%) total N ($F_{1,174} = 16$, $P < 0{\cdot}001$) and C:N ratio ($F_{1,174} = 80$, $P < 0{\cdot}001$). Concentrations of soil nutrients at both sites were several times greater than those of unimproved, species-rich grassland (Pywell *et al*. 2002b). Prior to the experiment vegetation at both sites corresponded to MG7 *Lolio–Cynosuretum* communities (Rodwell 1992) and were relatively species-poor ($5 \pm 0{\cdot}1$ grasses and $1 \pm 0{\cdot}1$ forbs m^{-2} in Devon, and $6 \pm 0{\cdot}1$ grasses and $3 \pm 0{\cdot}1$ forbs m^{-2} in Bucks).

EXPERIMENTAL DESIGN

Restoration treatments

Thirteen treatment combinations, designed to overcome specific constraints on grassland diversification, were applied to large (15 × 15 m) plots at each site in a randomized block design with eight replicates: (1) initial intensive livestock grazing; (2) slot-seeding; (3) harrow; (4) harrow + molluscicide; (5) harrow + soil inoculation; (6) harrow + *Rhinanthus*; (7) harrow + *Rhinanthus* + molluscicide; (8) harrow + *Rhinanthus* + soil inoculation; (9) deturf; (10) deturf + molluscicide; (11) deturf + soil inoculation; (12) cut two or three times in years 1–3 then harrow + seed; (13) N + K fertilizer for years 1–3 to accelerate soil P off-take and manage as treatment 12.

Four main treatments were applied in September 1999 to create a disturbance gradient to investigate H1. The lowest intensity disturbance was intensive autumn sheep grazing (< 4 cm) (treatment 1). Slot-seeding (2) used a drill (Aitchison Seedmatic 3016C tine drill, Aitchison UK, Woodbridge, Suffolk, IP13 OQL) to cut a narrow slit in the soil surface into which seed was dropped (Coulson *et al*. 2001). Harrowing (3–8) created 30–40% bare ground using a rotary power-harrow to a depth of *c.* 5 cm. Deturfing (9–11) removed three equally spaced strips in each plot measuring 15 m long × 1 m wide × 0·05–0·1 m deep using an excavator.

In addition, as harrowing is a more moderate disturbance than deturfing, the hemiparasite *Rhinanthus minor* ssp. *minor* (L.) was sown at a rate of 2·4 kg ha^{-1} to reduce competition further and create an open sward structure (H2). The harrowing or deturf treatments were also combined with other subtreatments to investigate the remaining hypotheses: control of mollusc herbivory (H3) using baited pellets containing 6% w/w metaldyhyde applied at 8 kg ha^{-1} in the autumn and the spring following sowing; inoculation with a diverse assemblage of soil microbes (H4) by broadcasting shredded topsoil from a nearby species-rich grassland onto the harrowed soil surface at a rate of 1·0 L m^{-2} in autumn 1999; and (iii) no subtreatment control. Finally, the practicality of rapid reduction of soil P by accelerating herbage off-take was investigated by adding 250 kg ha^{-1} N fertilizer and 100 kg ha^{-1} K fertilizer per annum from May 1999 combined with cutting two or three times per year (depending on rainfall and temperature) (H5). This was compared with cutting without fertilizer addition. After 2·3 years in September 2001, harrowing followed by seed addition was carried out on treatments 12 and 13 (Tallowin *et al*. 2002). All treatments were accompanied by the hand broadcasting of a seed mixture of desirable species (Pywell *et al*. 2003) to overcome seed limitation, comprising four grasses and 14 forbs (see Table S1 in the supplementary material) sown at 10 kg ha^{-1}, except in the slot-seeding treatment, where 3·3 kg ha^{-1} was sown.

Management regimes

All treatments were managed as traditional hay meadows, with herbage cut and turned in July each year to make hay and allow seed return. Cutting of the *Rhinanthus* plots in Devon was delayed until September 2000 to allow seed set following severe grazing of the flowers by wild deer. Sheep grazing regimes of contrasting intensity were applied to pairs of blocks: (i) extensive management (according to agri-environment scheme guidelines; Defra 2002), with 6–10 wethers ha^{-1} for 6 weeks, to maintain a sward surface height of 6–7 cm; and (ii) intensive ‘restoration management’, with 25–30 wethers ha^{-1} for 10 weeks to maintain a sward height of < 4 cm. The aim of the latter was to provide a further test of H1 and H2 by creating gaps for the germination and spread of desirable species and the control of competitive species (Bullock *et al*. 2001). Target sward heights were maintained by adding and removing stock from each block on the basis of regular sward surface measurements with a sward stick (Stewart, Bourn & Thomas 2001).

DATA COLLECTION

Soil nutrients

In April 1999, prior to the application of the treatments, five bulked soil cores (35 mm diameter × 75 mm deep) were taken at random within each plot. These were analysed for total phosphorus (tot P) using the sodium hydroxide fusion method (Smith & Bain 1982) and Olsen-extractable P (ext P), exchangeable potassium (K), calcium (Ca), magnesium (Mg), sodium (Na) and total nitrogen (%N) and carbon (%C) using standard

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methods (Allen 1974; MAFF 1986). Follow-up soil samples were taken and analysed in July 2001 to determine the effectiveness of the nutrient-reduction treatments 12 and 13. Final samples were taken from all plots in July 2003 to determine changes in soil nutrient status.

Soil microbial communities

In September 1999 five bulked soil cores (35 mm diameter × 75 mm deep) were collected from three locations in two areas of the species-rich grassland donor sites. Identical samples were collected from the productive grassland sites. In July 2003 sampling was repeated on two replicates of the treatment inoculated with a diverse microbial assemblage (5) and the uninoculated treatment (3) at both sites. The ester-linked phospholipid fatty acid composition (PLFA) of the soil was analysed to measure the relative abundance of soil-active fungi and bacteria in nanomoles g^{-1} of dry soil (Frostegård & Bååth 1996).

Herbage productivity

Herbage productivity was estimated in July of each year by cutting a 2·5-m wide strip down the centre of each plot and weighing the vegetation from the central 2-m section. Samples of ≈500 g fresh material were taken from each plot and oven dried at 80 °C to constant weight to determine dry matter yield (DM t ha^{-1}).

Botanical composition

Vegetation composition was recorded in June each year between 1999 and 2003 from five 1 × 1-m quadrats located at random within each plot. Each quadrat was subdivided into 25 cells of 20 × 20 cm. The presence of all vascular plant species was recorded in each cell to derive a quantitative measure of rooted frequency.

STATISTICAL ANALYSIS

The effects of restoration treatment, management, year and interactions were examined at both sites separately for soil, herbage, PLFA and vegetation composition variables using an analysis of variance (ANOVA) model that included block, management and treatment (nested within management) (SAS for Windows version 8; SAS 1990). Each ANOVA had repeated measures by including data from every measurement year (2 years for soil and 4 years for vegetation data). ε-values, for adjustment of degrees of freedom according to the amount by which the population covariance matrix departs from homogeneity, were calculated using the Greenhouse–Geisser method (Greenhouse & Geisser 1959). Tukey's pairwise comparisons were used, where necessary, to determine differences among individual restoration treatments. Vegetation variables included total number of vascular plant species (species richness), number of sown and unsown forb and grass species and cumulative rooted frequency of sown grasses and forbs. Logarithmic or arcsine transformations were undertaken on the richness and frequency data, respectively, to achieve normality of residuals as required.

Five separate ANOVA structures (analyses 1–5) were used to partition the sample variance and address the hypotheses. Analysis 1: the overall effectiveness of all 11 restoration treatments applied in 1999 was compared over time and in conjunction with management regime. Analysis 2, H1: the effects of increasing disturbance intensity (grazing vs. slot-seeding vs. harrowing vs. turf removal) were examined by comparison of treatments 1, 2, 3 and 9 for each year and across years. Treatment × year and management × year interactions were also considered. Analysis 3, H2: the potential for suppressing dominant species by the introduction of the hemiparasite *Rhinanthus* was examined by comparing treatments 6, 7, 8 (*Rhinanthus* addition) with treatments 3, 4, 5 (no *Rhinanthus* addition). The interaction with grazing management intensity provided a further test of this hypothesis. In order to account for the rapid dispersal of *Rhinanthus* between plots after sowing, we did two analyses (Pywell *et al*. 2004b). The first (3a) considered *Rhinanthus* addition as a factor. The second (3b) used the frequency of *Rhinanthus* in the plot as a covariate. Analysis 4, H3, H4: the effects of mollusc herbivory and the manipulation of soil microbial communities by inoculation with topsoil taken from a species-rich grassland were examined by comparing the fully factorial subtreatment combinations of no molluscicide or soil addition, i.e. control (treatments 3, 6, 9), molluscicide addition (4, 7, 10) and soil inoculation (5, 8, 11). Analysis 5, H5: the effectiveness of accelerated P off-take for 2·3 years by N and K fertilizer addition on soil nutrient and vegetation variables was investigated by comparing treatment 13 (P off-take) vs. treatment 12 (no P off-take).

Finally, the overall effectiveness of different treatments was determined by comparing the similarity of the resulting vegetation communities with those described by the British National Vegetation Classification (NVC) (Rodwell 1992) using Tablefit (Hill 1996). Both the percentage fit to the target community (*Cynosurus cristatus–Centaurea nigra* grassland MG5) and the identity of the best-fitting community are reported.

Results

BIOMASS PRODUCTION

Overall, biomass productivity was similar at the two sites for the first 2 years (5–6 DM t ha^{-1}). However, in Bucks there was a significant decline in productivity from 5·19 DM t ha^{-1} in 1999 to 4·55 DM t ha^{-1} by 2003 ($F_{3,63} = 3·4$, $P < 0·05$). In contrast, productivity increased significantly in Devon from 4·89 to 6·49 DM t ha^{-1} ($F_{2,48} = 17·7$, $P < 0·001$). Analysis 2 showed that overall

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deturfing caused a significant (25%) reduction in productivity in Bucks (deturfed 4·05 DM t ha^{-1}; non-deturfed 5·56 DM t ha^{-1}; $F_{3,21}$ = 7·5, P < 0·01), but not in Devon (deturfed 5·84 DM t ha^{-1}; non-deturfed 6·15 DM t ha^{-1}; $F_{3,21}$ = 0·6, P > 0·05). There was no significant effect on productivity at the plot scale of introducing the hemiparasite *Rhinanthus* (analysis 3), either as a factor (3a) or as a covariate (3b), at either site in any year. Similarly, neither mollusc control nor soil inoculation (analysis 4) had any effect on productivity (results not shown). Finally, adding N and K fertilizer to accelerate P off-take significantly increased productivity between 2000 and 2001 (Devon 9·27 DM t ha^{-1}; Bucks 6·01 DM t ha$^{-1)}$ compared with the unfertilized plots (Devon 3·82 DM t ha^{-1}, $F_{2,9}$ = 14, P < 0·01; Bucks 3·77 DM t ha^{-1}, $F_{2,9}$ = 13, P < 0·01) (analysis 5). Significant differences in productivity remained in 2002 (1 year after fertilizer addition ceased) in Bucks but not in Devon.

SOIL NUTRIENTS

Over the 4 years, there were consistent, significant declines in total P, exchangeable Mg, %N and %C between 1999 and 2003 at both sites (see Table S2 in the supplementary material). Overall, there were very few significant effects of management regime or restoration treatment on soil nutrient concentrations or pH (see Table S3 in the supplementary material). Importantly, turf removal had little impact on soil nutrient concentrations after 4 years at both sites (analysis 2). The exceptions were a significant decrease in %N that was consistent at both sites [Devon, deturfed (treatment 9) 0·71, non-deturfed (1, 2, 3) 0·91, $F_{6,21}$ = 3·6, P < 0·05; Bucks, deturfed 0·73, non-deturfed 0·98, $F_{6,21}$ = 4·7, P < 0·01]. Similarly, %C was significantly reduced (Devon, deturfed 0·71, non-deturfed 0·96, $F_{6,21}$ = 4·6, P < 0·05; Bucks, deturfed 0·69, non-deturfed 1·02, $F_{6,21}$ = 4·8, P < 0·01). Finally, the C:N ratio was significantly lower following turf removal in Bucks (deturfed 0·93, non-deturfed 1·04, $F_{6,21}$ = 3·4, P < 0·05) but not in Devon. There was no significant effect of the hemiparasite *Rhinanthus* (analysis 3), mollusc control or soil inoculation (analysis 4) on any soil nutrient measure. The addition of N and K fertilizer in combination with cutting did not result in any significant reduction of soil nutrient concentrations at either site (analysis 5).

SOIL MICROBIAL COMMUNITIES

Total bacterial PLFA and the abundance of the following bacterial fatty acids were all significantly higher in the species-rich grassland donor sites in Devon and Bucks compared with the productive restoration sites: i-C15:0, a-C15:0, C15:0, C16:0, C17:0 ci and C18:1ω7 (Table 1a). Also, fungal PLFA (cis-C18:2ω6) was significantly higher at the Bucks donor site compared with the recipient. However, inoculation with soil from the donor sites had no significant effect on the composition

Table 1. (a) Differences in individual soil phospholipid fatty acids (PLFA) (nmol g^{-1} dry soil) between donor and restoration sites; (b) effects of soil inoculation on PLFA. Nomenclature for the abbreviated fatty acids follows Frostegård, Bååth & Tunlid (1993)

	d.f.	i-C15:0	a-C15:0	C15:0	methyl-C16:0	C16:0	C17:0	C17:0 cyclo	cis C18:1ω7	C19:0 cyclo	Fungal PLFA (cis-C18:2ω6)	Bacterial PLFA	Fungal: bacterial PLFA
(a)													
Devon		176	123	12	75	486	16	33	79	48	84	1011	0·044
Donor		300	219	24	71	768	25	52	113	60	140	1596	0·037
ANOVA F-values	1,23	10***	18***	7·7*	0·1^{NSD}	7·3*	8·9***	4·7*	4·4*	11·8***	0·5^{NSD}	8·7***	0·8^{NSD}
Bucks		263	194	17	133	511	19	68	98	44	113	1339	0·033
Donor		366	511	29	202	829	27	84	179	95	156	2275	0·042
ANOVA F-values	1,23	12***	85***	18***	12***	39***	8·6***	3·2^{NSD}	44***	3·8^{NSD}	32***	48***	2·3^{NSD}
(b)													
Control		229	167	17	112	530	18	50	97	51	105	1242	0·039
Inoculation		209	150	13	96	468	17	51	80	41	91	1108	0·037
ANOVA F-values													
Site	1,3	8·9^{NSD}	2·9^{NSD}	2·4^{NSD}	4·5^{NSD}	0·2^{NSD}	0·8^{NSD}	6·4^{NSD}	7·3^{NSD}	0·5^{NSD}	1·5^{NSD}	8·4^{NSD}	5·4^{NSD}
Block	2,3	8·5^{NSD}	32**	11*	7·8^{NSD}	0·8^{NSD}	6·3^{NSD}	4·2^{NSD}	0·2^{NSD}	39**	0·1^{NSD}	2·1^{NSD}	0·3^{NSD}
Treatment	1,3	3·9^{NSD}	5·9^{NSD}	19*	2·9^{NSD}	1·2^{NSD}	1·7^{NSD}	0·1^{NSD}	0·8^{NSD}	5·0^{NSD}	0·5^{NSD}	3·0^{NSD}	0·8^{NSD}

NSD, no significant difference; *P < 0·05; **P < 0·01; ***P < 0·001.

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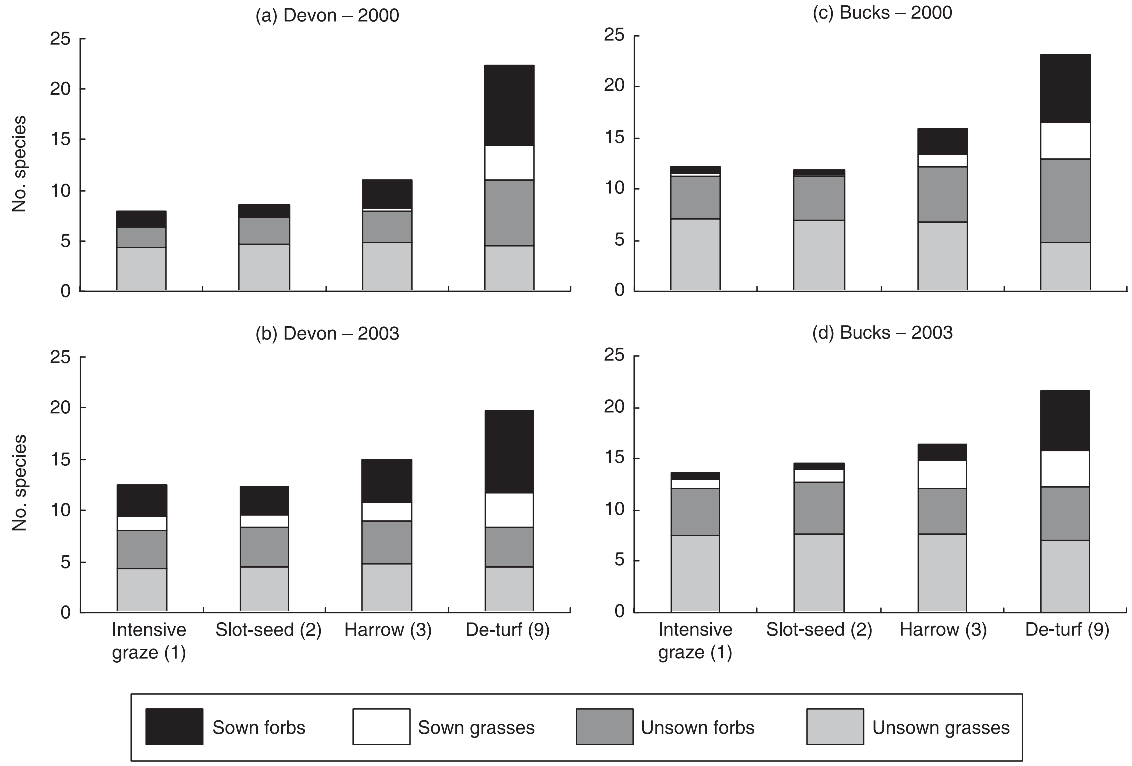

Fig. 1. Effects of disturbance intensity on plant species richness (analysis 2). Only data from the first and last years are shown for clarity.

of the soil microbial communities of the productive grasslands after 4 years (Table 1b).

PLANT SPECIES DIVERSITY AND ABUNDANCE

There were strong and consistent effects of the restoration treatments on plant species richness and abundance at both sites in all years (analysis 1; see Table S4 in the supplementary material). Total species richness and the number and abundance of sown forbs were always highest in the deturfed treatments and lowest in the intensive grazing and slot-seeding treatments. The second highest ranked treatment for these measures was the harrowed + molluscicide treatment in Devon and the harrowed + *Rhinanthus* + molluscicide treatment in Bucks. This effect was extremely consistent across years. The number of unsown forbs was also significantly higher in the deturfed treatments in the first year after sowing. There were highly significant year effects at both sites reflecting the overall increases in plant richness and abundance of sown species with time. The sown grasses showed the greatest increases with time. However, the large number of treatment × year interactions indicated that treatment differences declined over time. Management effects were only significant for unsown species and these were not consistent across sites. Finally, there were weakly significant management × year interactions for sown forbs in Devon and sown grasses in Bucks but the absence of significant ANOVAS for individual years indicated that these management effects were biologically unimportant in this study. The more targeted analyses 2–5 illustrated these effects in greater detail.

Disturbance intensity

The intensity of disturbance prior to sowing had large effects on the richness and abundance of plant species at both sites (analysis 2; Figs 1 and 2). Turf removal resulted in a greater richness of sown forbs (Devon $F_{6,21} = 14$, $P < 0{\cdot}001$; Bucks $F_{6,21} = 90$, $P < 0{\cdot}001$) and sown grasses (Devon $F_{6,21} = 23$, $P < 0{\cdot}001$; Bucks $F_{6,21} = 71$, $P < 0{\cdot}001$) compared with treatments involving less disturbance (Fig. 1; and see Table S4 in the supplementary material). Similarly, the abundance of sown forbs (Devon $F_{6,21} = 18$, $P < 0{\cdot}001$; Bucks $F_{6,21} = 54$, $P < 0{\cdot}001$) and grasses (Devon $F_{6,21} = 29$, $P < 0{\cdot}001$; Bucks $F_{6,21} = 36$, $P < 0{\cdot}001$) was significantly higher in the deturfed treatment compared with all others (Fig. 2; and see Table S4 in the supplementary material). In addition, in the first 2 years following sowing, the deturfed treatment had significantly higher numbers of unsown forbs compared with the other disturbance treatments (Devon $F_{6,21} = 2{\cdot}9$, $P < 0{\cdot}01$; Bucks $F_{6,21} = 4{\cdot}4$, $P < 0{\cdot}05$). Finally, Tukey's multiple comparison tests (see Table S4 in the supplementary material) showed that the number and frequency of sown species was often significantly higher in the harrowed treatments compared with the slot-seeding and intensive grazing treatments in Bucks (Figs 1 and 2).

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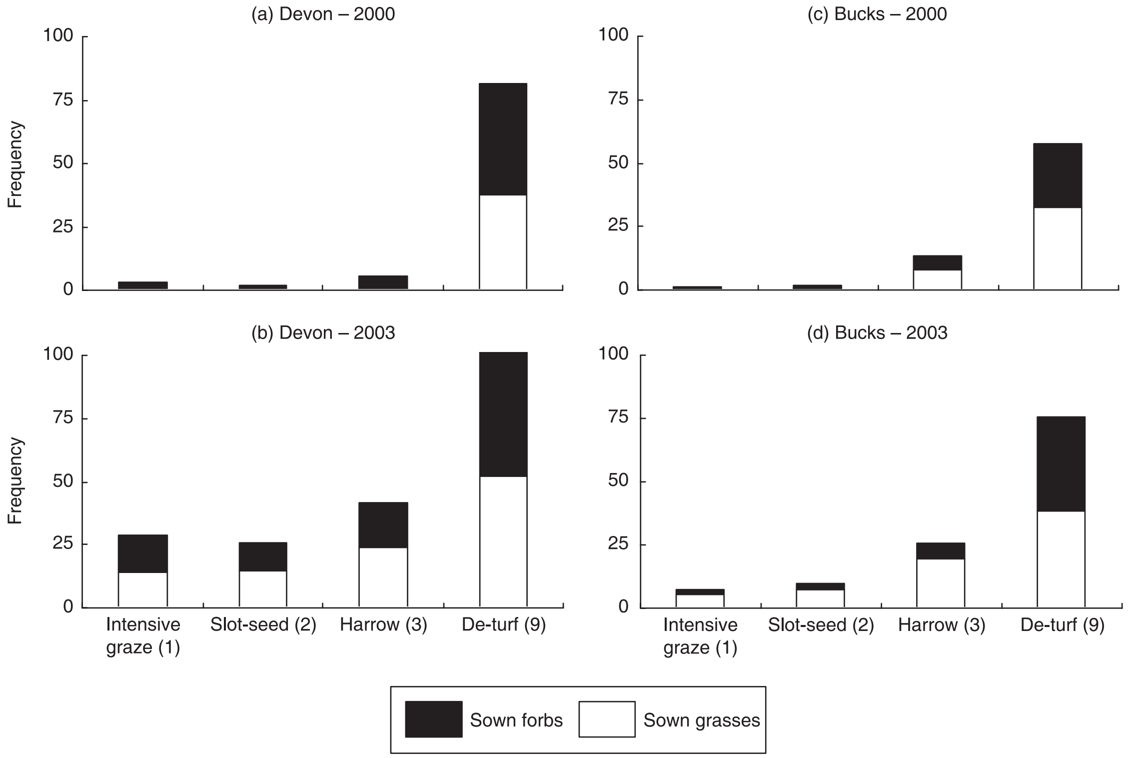

Fig. 2. Effects of disturbance intensity on abundance of sown species (analysis 2). Only data from the first and last years are shown for clarity.

This secondary treatment effect was less apparent in Devon.

Hemiparasitic plants

Plots sown with *Rhinanthus* had a significantly greater frequency of this species compared with those where it was not sown (Table 2). However, the frequency of *Rhinanthus* only increased with time in Bucks. *Rhinanthus* addition as a treatment factor (analysis 3a) was associated with small but significant decreases in the species richness of all components of the vegetation except unsown grasses in Devon, but not in Bucks. However, in Bucks analysis of covariance (analysis 3b) showed there was a significant correlation between high frequencies of *Rhinanthus* in the current year and both higher numbers and frequency of sown forbs and grasses and total species number (excluding *Rhinanthus* from the species count). This effect was most pronounced in the first year after sowing.

Mollusc control and soil inoculation

Mollusc density and activity were estimated on the Bucks harrowed and deturfed treatments, both with (4, 10) and without molluscicide addition (3, 9), for 3 weeks in autumn 1999 and again in spring 2000 (Pywell *et al*. 2004a). Three ceramic tile shelters, each measuring 150 × 150 mm, were placed along the centre line of each plot. Beneath each shelter was a pitfall trap (75 mm diameter × 110 mm deep) containing a preservative solution of 50% propylene glycol. These showed significant reductions in slug densities following molluscicide addition (4·4 ± 1·2 slugs trap^{-1}) compared with no molluscicide ((11·8 ± 2·8), ANOVA $F_{1,9}$ = 19·7, P < 0·01). Overall, there was a significant beneficial effect of this treatment on the number of sown forbs (analysis 4; Figs 3 and 4) (Devon $F_{4,51}$ = 6·6, P < 0·01; Bucks $F_{4,51}$ = 2·8, P < 0·05). Within-year Tukey's tests showed this effect was significant for the first 3 years in Devon and years 2 and 4 in Bucks (see Table S5 in the supplementary material). The abundance of sown forbs was also significantly increased by molluscicide (Devon $F_{4,51}$ = 3·4, P < 0·05; Bucks $F_{4,51}$ = 3·1, P < 0·05). This was significant in the first year in Devon and years 2 and 4 in Bucks. Soil inoculation had no significant effect on the richness and abundance of sown species in any year (see Table S5 in the supplementary material).

Nutrient off-take

Three consecutive seasons (2·3 years) of N and K fertilizer additions to accelerate P off-take resulted in no significant effects on the diversity of the grassland sward (analysis 5; see Table S6 in the supplementary material).

Similarity to the species-rich grassland target communities

After 4 years the restored grassland communities resulting from the lower intervention restoration treatments (1–8) showed the least similarity to the species-rich grassland target community (MG5) (Rodwell 1992),

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Table 2. Effects of *Rhinanthus minor* introduction on plant species richness and abundance (analysis 3). Analysis 3a treats *Rhinanthus* addition as a factor. Analysis 3b uses the frequency of *Rhinanthus* in each plot as a covariate. Only data from the first and last years are shown for clarity. Means with the same letter in the same column are not significantly different ($P > 0.05$).

		(a) Devon								(b) Bucks							
	Year	*Rhinanthus* frequency	Total species	Sown forbs	Sown grasses	Unsown forbs	Unsown grasses	Total freq. sown forbs	Total freq. sown grasses	*Rhinanthus* frequency	Total species	Sown forbs	Sown grasses	Unsown forbs	Unsown grasses	Total freq. sown forbs	Total freq. sown grasses
Rhinanthus addition (6, 7, 8)	2000	2·34b	10·54a	3·41a	0·12a	2·65a	4·36a	7·83a	0·17a	0·12a	16·56a	2·64a	1·49a	5·32a	7·10a	7·19a	0·12a
No *Rhinanthus* addition (3, 4, 5)		0·01a	11·63b	3·54a	0·18a	3·35a	4·56a	7·47a	0·23a	0·00b	16·24a	2·57a	1·25a	5·39a	7·03a	5·55a	0·00b
Rhinanthus addition (6, 7, 8)	2003	1·44b	13·22a	3·52a	1·64a	3·51a	4·55a	15·88a	20·30a	3·56a	16·81a	1·82a	2·67a	4·64a	7·67b	7·37a	3·56a
No *Rhinanthus* addition (3, 4, 5)		0·10a	15·14b	4·37b	1·99b	4·19b	4·59a	19·68a	27·37b	0·05b	17·66a	2·00a	2·88a	4·75a	8·02a	7·60a	0·05b
ANOVA *F*-values	d.f.																
Analysis 3a																	
Management	1,33	2·0^{NSD}	0·1^{NSD}	0·8^{NSD}	0·5^{NSD}	<0·1^{NSD}	1·0^{NSD}	<0·1^{NSD}	<0·1^{NSD}	1·9^{NSD}	0·1^{NSD}	4·5*	0·9^{NSD}	0·7^{NSD}	8·5**	1·7^{NSD}	1·4^{NSD}
Rhinanthus addition	2,33	10***	3·4*	3·7*	4·5*	3·2^{NSD}	0·2^{NSD}	0·7^{NSD}	4·0*	6·5**	2·0^{NSD}	2·0^{NSD}	0·5^{NSD}	1·3^{NSD}	0·2^{NSD}	1·4^{NSD}	<0·1^{NSD}
Year	3,99	4·2*	45***	12***	344***	30***	4·3*	30***	470***	13***	3·9**	5·8**	157***	26***	12***	1·9^{NSD}	69***
Year × Management	3,99	4·6*	0·9^{NSD}	0·6^{NSD}	0·1^{NSD}	1·2^{NSD}	0·1^{NSD}	0·3^{NSD}	0·3^{NSD}	0·7^{NSD}	0·8^{NSD}	0·1^{NSD}/	0·4^{NSD}	1·9^{NSD}	1·6^{NSD}	0·6^{NSD}	1·2^{NSD}
Year × *Rhinanthus*	6,99	3·6**	0·4^{NSD}	0·4^{NSD}	1·3^{NSD}	0·3^{NSD}	0·9^{NSD}	0·3^{NSD}	1·9^{NSD}	6·0***	1·6^{NSD}	1·2^{NSD}	2·4*	1·7^{NSD}	1·6^{NSD}	1·0^{NSD}	1·7^{NSD}
Analysis 3b																	
Rhinanthus frequency																	
2000	1,37	–	2·4^{NSD}	0·7^{NSD}	2·3^{NSD}	2·7^{NSD}	1·0^{NSD}	0·2^{NSD}	1·1^{NSD}	–	17*** +	16*** +	13*** +	2·1^{NSD}	1·4^{NSD}	14*** +	5·3* +
2001	1,37	–	0·1^{NSD}	<0·1^{NSD}	0·8^{NSD}	<0·1^{NSD}	0·1^{NSD}	0·5^{NSD}	1·7^{NSD}	–	<0·1^{NSD}	0·3^{NSD}	6·0* +	2·1^{NSD}	0·3^{NSD}	0·7^{NSD}	0·5^{NSD}
2002	1,37	–	0·4^{NSD}	0·3^{NSD}	<0·1^{NSD}	0·2^{NSD}	0·4^{NSD}	2·5^{NSD}	<0·1^{NSD}	–	6·7* +	7·9* +	2·1^{NSD}	0·8^{NSD}	1·0^{NSD}	12** +	3·8* +
2003	1,37	–	<0·1^{NSD}	<0·1^{NSD}	0·1^{NSD}	0·8^{NSD}	3·5^{NSD}	<0·1^{NSD}	0·8^{NSD}	–	<0·1^{NSD}	1·1^{NSD}	0·4^{NSD}	<0·1^{NSD}	0·4^{NSD}	0·6^{NSD}	<0·1^{NSD}

NSD, no significant difference; *P < 0·05; **P < 0·01; ***P < 0·001.

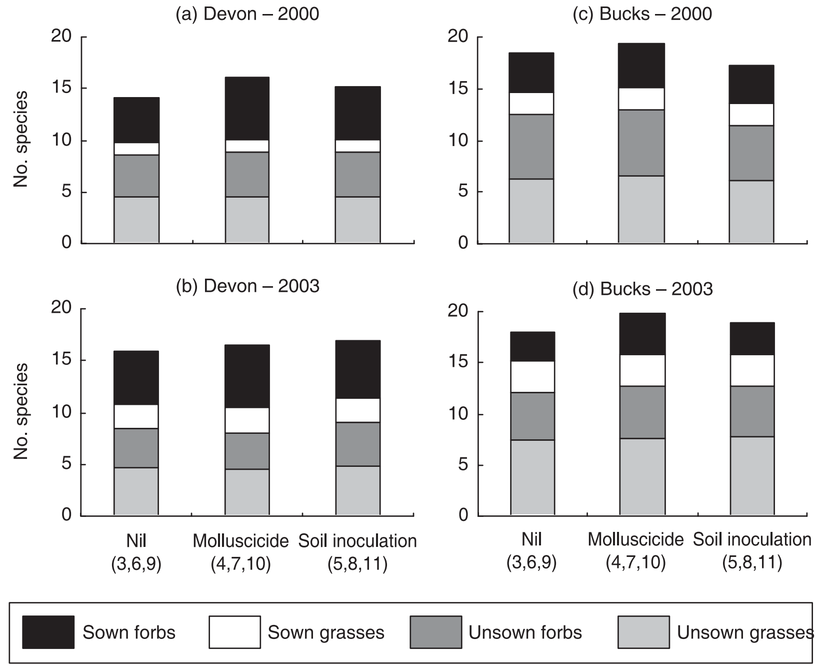

Fig. 3. Effects of mollusc control and soil inoculation on plant species richness (analysis 4). Only data from the first and last years are shown for clarity.

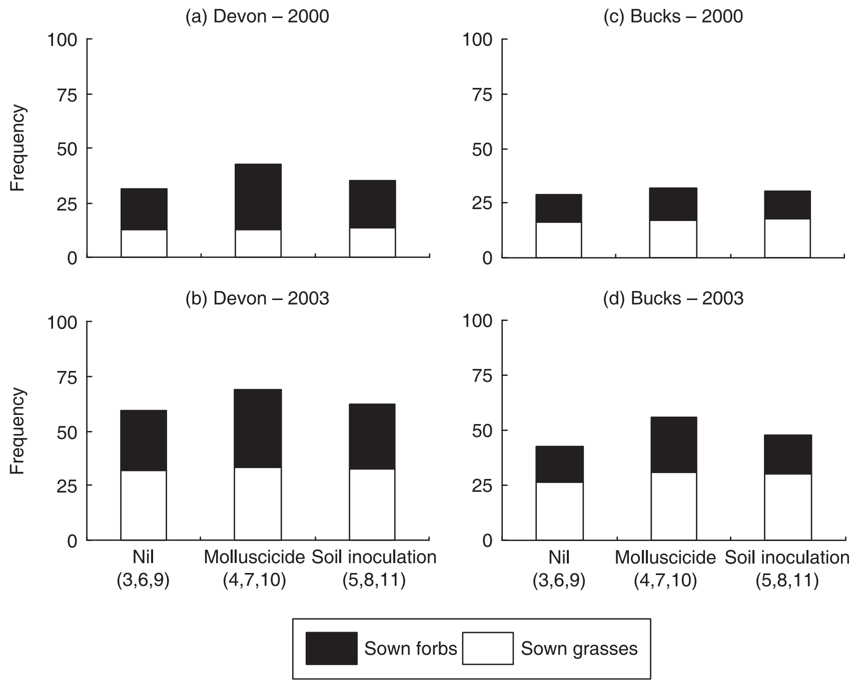

Fig. 4. Effects of mollusc control and soil inoculation on abundance of sown species (analysis 4). Only data from the first and last years are shown for clarity.

with between 29% and 42% similarity in Devon and 32% and 53% in Bucks (Table 3). In both cases, restoration by intensive grazing alone (1) and slot-seeding (2) resulted in vegetation that was amongst the least similar to the target community. All these restored grasslands were most similar to semi-improved MG6 pasture. In contrast, the deturfed treatments at both sites had very close similarity (> 70%) to the target species-rich community (MG5).

Discussion

CAN DISTURBANCE OVERCOME LACK OF ESTABLISHMENT SITES AND COMPETITION?

This study has confirmed that the creation of suitable sites for germination and establishment where intensity of competition remains low is the key limiting factor in the diversification of such productive grasslands (Bullock

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Table 3. Similarity of the restored grassland communities to the National Vegetation Classification target communities in 2003 using Tablefit (Hill 1996)

	Devon			Bucks		
Treatment	% fit to target (MG5)	%best fit	Subcommunity with best fit	% fit to target (MG5)	%best fit	Subcommunity with best fit
1. Intensive grazing	31	56	MG6a	32	70	MG6a
2. Slot-seed	29	56	MG6a	34	71	MG6a
3. Harrow	29	53	MG6a	46	67	MG6a
4. Harrow + molluscicide	36	57	MG6a	53	67	MG6a
5. Harrow + soil	31	55	MG6a	49	67	MG6a
6. Harrow + *Rhinanthus*	35	53	MG6a	47	71	MG6a
7. Harrow + *Rhinanthus* + molluscicide	42	58	MG6a	47	65	MG6a
8. Harrow + *Rhinanthus* + soil	42	65	MG6a	44	65	MG6a
9. Deturf	73	75	MG5a	71	74	MG5a
10. Deturf + molluscicide	72	73	MG5a	77	79	MG5a
11. Deturf + soil	79	81	MG5a	74	76	MG5a
12. Cut 3 times in years 1–3 then harrow + seed	17	60	MG11a	53	70	MG6a
13. N + K fertilizer for nutrient off-take and manage as 12	23	57	MG11a	52	66	MG6a

2000). Considerable disturbance, in the form of turf and topsoil removal, is required to restore rapidly and reliably species-rich communities to these sites (Hopkins *et al*. 1999; Tallowin & Smith 2001; Hofmann & Isselstein 2004). The primary effects of this treatment (9–11) is to eliminate competition from the established sward, reduce competition from the seed bank and create abundant sites for establishment. Despite the removal of 10 cm of topsoil, this treatment had few effects on fertility and particularly the pool of P. This suggests that competition from the established vegetation is perhaps a greater constraining factor on species establishment in the early stages of restoration than high residual soil fertility *per se* (Bullock *et al*. 2001). Further research is required to determine the relative importance of competition and soil fertility on the persistence and regeneration of introduced species in the longer term. Deturfing requires careful targeting to small areas for reasons of cost (deturfing = £2720/€3944 + seed = £800/€1160 per ha), practicality and other environmental impacts. It is perhaps most efficient to utilize deturfing as a means of creating small 'focal' patches of diverse grassland that can provide colonists for larger areas in the later stages of restoration (Pywell *et al*. 2002b).

Intermediate disturbance resulting from multiple harrowing was effective in creating gaps for establishment and was applicable to larger areas on the grounds of practicality and cost (harrowing = £129/187 euros + seed = £800/1160 euros ha^{-1}). However, restoration using this technique is likely to be less immediately effective and will take longer. Moreover, previous studies suggest that more severe mechanical disturbance, in the form of rotary cultivation, is unsuccessful because of increased rates of nutrient mineralization and therefore increased competition (Hopkins *et al*. 1999). Livestock grazing is also an effective means of creating gaps for establishment in low-productivity grasslands (Lavorel *et al*. 1997; Bullock *et al*. 2001). However, this traditional approach to sward diversification, which relies on severe sheep grazing to create gaps, proved to be ineffective in such productive grasslands. It is likely that the gaps created were too small and, in the absence of additional treatments to reduce competition, too short-lived to allow significant seedling recruitment. It is possible that cattle grazing would be more effective because they are heavier and their trampling results in a greater degree of damage and disturbance compared with sheep (Crofts & Jefferson 1999). Indeed, cattle grazing has been shown to result in significant increases in the cover of forbs, as well as flower abundance of key groups such as Fabaceae, compared with sheep grazing (Carvell 2002). Finally, slot-seeding also proved to be an ineffective means of diversifying productive grassland. It is likely that seeds introduced into the shallow slot cut into the soil were rapidly out-competed by the established sward. However, slot-seeding can be highly effective if combined with application of a broad-spectrum herbicide to the slot to reduce competition further (Coulson *et al*. 2001).

CAN HEMIPARASITICS BE USED TO MANIPULATE COMPETITIVE INTERACTIONS AND FACILITATE DIVERSIFICATION?

A number of studies have shown *Rhinanthus* spp. can reduce productivity in semi-natural grassland communities (by between 6% and 73%) and increase the proportion of forbs in the sward relative to grasses (Bullock & Pywell 2005). This has led some authors to suggest that *Rhinanthus* might be a useful tool for the restoration of diverse grasslands by directly reducing the growth of dominant, productive species (Smith *et al*. 2003). Indeed, a recent study has shown that introducing *Rhinanthus minor* to a moderately productive grassland greatly increased the establishment of desirable forbs from seed added 2 years later (Pywell *et al*. 2004b). This

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study has demonstrated that it is possible to establish populations of *Rhinanthus* in productive grasslands by harrowing followed by seeding, while hay cutting greatly facilitates population increase and spread (Coulson *et al.* 2001). The presence of the hemiparasite was associated with increased establishment and persistence of sown species at Bucks. The negative effect of the *Rhinanthus* treatment on diversity at Devon was probably unrelated to the presence of *Rhinanthus* (as indicated by the lack of an effect of *Rhinanthus* frequency in the ANCOVAS) but reflected the delay in cutting of these plots until September in the first year after sowing. It is likely that such prolonged competition for light and resources from the dense sward would have reduced seedling survival in this case, and so this illustrates the importance of slight changes to initial management in determining establishment from sown seed (Lawson, Ford & Mitchley 2004). *Rhinanthus* spp. probably have positive effects on both the recruitment (in gaps) and established phases of less competitive species (Bullock & Pywell 2005).

DOES HERBIVORY INFLUENCE THE OUTCOME OF RESTORATION?

Grazing by molluscs had a greater influence on the outcome of restoration than sheep grazing. Molluscs prefer seedlings to mature plants and they are highly selective, so their influence is likely to be out of proportion to the biomass consumed (Dirzo & Harper 1980). Indeed, grazing by molluscs during the vulnerable seedling stage has marked effects on the seedling recruitment and species composition of gaps in a grasslands (Hanley, Fenner & Edwards 1996; Clear Hill & Silvertown 1997). Molluscicide reduced herbivory and had significant beneficial effects on the richness and abundance of the introduced forbs that were consistent at both sites and lasted for between 2 and 4 years. The greater negative effects of mollusc grazing on forbs compared with grasses reflected differences in their morphology and palatability. However, the positive effects of field-scale molluscicide application on plant communities must be balanced against the potentially damaging effects of the pesticide on non-target species, such as small mammals and birds (Shore *et al.* 1997).

Overall there were very few effects of the contrasting severities of sheep grazing on vegetation composition and the dispersal of sown species (Pywell *et al.* 2004a). One possible explanation is that the July hay cut applied to both management regimes may have limited seed availability for dispersal and colonization by species that otherwise respond positively to increased grazing pressure, such as annuals [e.g. *Trifolium dubium* (Sibth.)], rosette species [e.g. *Hypochaeris radicata* (L.)] and species with seed that is capable of long-distance dispersal [*Leontodon hispidus* (L.)] (McIntyre, Lavorel & Trémont 1995). However, the response of perennial grassland communities to the different grazing intensities has been shown to take a considerable time (> 5 years) to evolve (Bullock *et al.* 2001) and longer term monitoring may therefore be required to elucidate an effect.

CAN SOIL NUTRIENTS BE REDUCED IN PRODUCTIVE GRASSLANDS?

High residual soil fertility is considered to be an important constraining factor on the restoration and maintenance of botanical diversity on productive farmland (Walker *et al.* 2004). Removal of P by continuous cropping is the most practical option available for large, productive sites (Marrs 1993; Pegtel *et al.* 1996). This study has demonstrated that N and K fertilizer additions were effective in accelerating P off-take, and the continuation of this treatment for 5 years at Devon resulted in the removal of a total of 130 kg P ha^{-1} (Pywell *et al.* 2004a). This off-take of P was, however, small in comparison with the large residual pool of soil P. Assuming continued linearity in response, it would take 12·5 years to reduce the total P amount to levels found in unimproved, diverse grassland. A study on arable land in eastern England failed to significantly reduce P and pH after 7 years of continuous cereal cropping with N and K fertilizer additions (Marrs *et al.* 1998). It is therefore not surprising that there was no significant reduction in either total or extractable P and an absence of beneficial effects on the establishment of sown species after just 2·3 years.

IS IT POSSIBLE TO MANIPULATE SOIL MICROBIAL COMMUNITIES FOR RESTORATION?

It is increasingly recognized that soil microbial communities play an important role in the regulation and maintenance of plant biodiversity (van der Heijden *et al.* 1998; Hartnett & Wilson 1999). They may therefore have an important influence on the direction and outcome of restoration. The restoration of plant diversity in an upland hay meadow by seed addition and extensive management over 10 years was associated with an increase in biomass of soil fungi (Smith *et al.* 2003). However, this study has demonstrated that inoculation with topsoil from a species-rich grassland had no beneficial effects on the diversity and abundance of plant species and soil micro-organisms. In a similar study, inoculation of newly abandoned agricultural land with leaf litter from a mature deciduous forest had no affect on the biomass and activity of soil micro-organisms (Hedlund 2002). This may reflect the timing of inoculation, at the early stages of restoration, before populations of key functional plant species (e.g. Fabaceae and the hemiparasite *Rhinanthus*) have built-up (Smith *et al.* 2003), and an unfavourable soil nutrient status. More research is required into the precise relationship between above- and below-ground ecosystem processes and how they may be manipulated to influence the development and maintenance of botanically diverse grassland communities. The differences in microbial

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communities between species-poor and -rich grasslands also need further study. Our donor sites had greater bacterial and fungal biomass than the restoration sites but no difference in the bacteria:fungal ratio. It is also important to consider detrimental effects on donor sites caused by soil removal.

IMPLICATIONS FOR FUTURE RESTORATION PRESCRIPTIONS

This study has allowed a comprehensive comparison of the practicality and effectiveness of a wide range of restoration techniques and three approaches are suggested for increasing the botanical diversity of productive grasslands. Practically, the most appropriate approach will depend on the aims of the restoration, the area to be restored and the funds available. Importantly, further research is required into the long-term persistence and spread of the introduced species under these different restoration regimes.

Restoration of diverse focal patches

This requires costly turf removal and seed addition to create small 'focal' patches of diverse grassland in which all the component species of the target community rapidly establish and persist. With appropriate management, these patches should provide colonists for much larger areas over time. This technique can be applied immediately to highly productive sites to achieve rapidly and reliably diversification at a small scale. However, further research is required to determine the optimum management treatments to accelerate dispersal from the focal patches (Coulson *et al*. 2001).

Restoration of a limited number of generalist species

This requires moderate intervention (harrowing and seeding or slot-seeding) over large areas to establish a small number of desirable, generalist species that perform well in restoration (Pywell *et al*. 2003). The success of this type of restoration can be significantly enhanced by the control of mollusc herbivory. This approach is lower in cost and applicable to large areas but the increases in biodiversity are relatively small in the short term.

Phased restoration

This complements the above approaches (Pywell *et al*. 2002a) either as a precursor to the second method or to facilitate expansion of species from the focal patches in the first method. It involves management to reduce productivity and competition from the established sward by introducing *Rhinanthus* or possibly using N and K fertilizers to accelerate P off-take (although this last method needs further testing). After 3–5 years environmental conditions should be more suitable for establishing a wider range of desirable species following harrowing. This approach is applicable to large areas and provides a useful agronomic return in the early years of restoration. However, it is slower and less certain in achieving the objectives of restoration. The effectiveness of this phased approach to restoration requires further testing.

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Supplementary material

The following supplementary material is available as part of the online article (full text) from http://w.w.w.blackwell-synergy.com.

Table S1. Details of the seed mixtures sown in the experiment.

Table S2. Changes in pH and soil chemistry (kg ha^{-1}) at the two study sites between 1999 and 2003 (analysis 1).

Table S3. Effects of disturbance intensity (analysis 2) on the change ($value^{2003}/value^{1999}$) in soil pH and nutrient concentrations kg ha^{-1} between 1999 and 2003.

Table S4. Effects of restoration treatment on plant species richness and abundance (analysis 1).

Table S5. Effects of mollusc control and soil inoculation on plant species richness and abundance (analysis 4).

Table S6. Effects of nutrient off-take with N and K fertilizer on plant species richness and abundance (analysis 5).

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