Wagner, Markus; Bullock, James M.; Hulmes, Lucy; Hulmes, Sarah; Peyton, Jodey; Amy, Sam R.; Savage, Joanna; Tallowin, Jerry B.; Heard, Matthew S.; Pywell, Richard F.. 2015. **Creation of micro-topographic features: a new tool for introducing specialist species of calcareous grassland to restored sites?** *Applied Vegetation Science*, 19 (1). 89-100.
10.1111/avsc.12198

Creation of micro-topographic features: A new tool for introducing specialist species of calcareous grassland to restored sites?

Markus Wagner[1], James, M. Bullock[1], Lucy Hulmes[1], Sarah Hulmes[1], Jodey Peyton[1], Sam R. Amy[1], Joanna Savage[1], Jerry B. Tallowin[2], Matthew S. Heard[1], Richard F. Pywell[1]

[1]NERC Centre for Ecology & Hydrology, Benson Lane, Wallingford, Oxfordshire OX10 8BB, UK

[2]North Wyke, Rothamsted Research, Okehampton, Devon EX20 2SB, UK

Email addresses:

Markus Wagner: mwagner@ceh.ac.uk; James M. Bullock: jmbul@ceh.ac.uk; Lucy Hulmes: lhu@ceh.ac.uk; Sarah Hulmes: shul@ceh.ac.uk; Jodey Peyton: joyt@ceh.ac.uk; Sam Amy: sariy@ceh.ac.uk; Joanna Savage: joan@ceh.ac.uk; Jerry B. Tallowin: jerry.tallowin@rothamsted.ac.uk; Matthew S. Heard: mshe@ceh.ac.uk; Richard F. Pywell: rfp@ceh.ac.uk

Corresponding author:

M. Wagner (NERC Centre for Ecology & Hydrology, Benson Lane, Wallingford, Oxfordshire OX10 8BB, UK; Fax: ++44 1491 692424; Tel: ++44 1491 692418; E-mail: mwagner@ceh.ac.uk)

Printed journal page estimate: 7040 words (8.5 pages), tables (1.0 pages), figures (2.5 pages), total 12.0 pages.

Abstract

Questions: What types of pre-sowing disturbance are most suitable to establish specialist forbs of calcareous grassland at previously agriculturally improved restored sites? What impact does management regime have on post-establishment abundance-dynamics?

Location: Pegsdon Hills, Bedfordshire, UK

Methods: We set up a 4-yr experiment using a split-plot design to combine pre-sowing disturbance treatments at sub-plot-level (undisturbed control, glyphosate spraying, harrowing, and creation of ridge-and-furrow features) with three post-establishment management regimes applied at main-plot level in years 2-4, involving either summer cutting or summer cattle grazing, and presence or absence of spring sheep grazing, along with autumn cattle grazing in all regimes. After disturbance application, we sowed a seed mixture containing ten specialist species of calcareous grassland. Using quadrat-based methods, we monitored first-year establishment and subsequent dynamics, including reproductive status of species at quadrat level. Initial establishment and subsequent dynamics were analysed separately using linear mixed models.

Results: Initial establishment of sown species was promoted both by harrowing and by ridge-and-furrow creation. While some species were about equally promoted by both, several other species benefited more strongly or exclusively from ridge-and-furrow creation. Effects of disturbance largely persisted in subsequent years, but for some species, different dynamics were observed for harrowed and ridge-and-furrow treatments. *Thymus pulegioides* and *Hippocrepis comosa* gradually achieved higher abundances in the ridge-and-furrow treatment, in which notable levels of bare ground persisted for much longer than in the harrowed treatment. In contrast, *Filipendula vulgaris* and *Pimpinella saxifraga* achieved higher abundance in the harrowed treatment. Sown species tended to reach reproductive stage faster in the ridge-and-furrow treatment than in the harrowed treatment. By the end of the study, management regimes had resulted in few effects on species dynamics.

Conclusions: Establishment of specialist species of calcareous grassland crucially depended on bare-ground creation prior to sowing. Ridge-and-furrow creation resulted in more persistent reduction of competition than the standard practice of harrowing, provided more suitable conditions for low-statured specialist species, and generally enabled faster transition of introduced specialist species to reproductive stage. Our results thus illustrate potential benefits of using more severe disturbance when introducing specialist species of calcareous grassland at restored sites.

Keywords: Bare ground; Calcareous grassland; Ecological restoration; Grazing regimes; Harrowing; Micro-topographic variation; Reproductive stage; Ridge-and-furrow creation; Seedling establishment

Nomenclature: Stace (2010)

Running head: Micro-topographic features in restored grassland

Introduction

Calcareous grasslands are among the oldest (Poschlod & WallisDeVries 2002) and floristically most rich (Wilson et al. 2012) semi-natural ecosystems in Europe. The most species-rich sites are characterised by a long history of continuous management, and associated gradual accumulation of specialist species through natural colonization processes (Aavik et al. 2008). Due to species pool gradients driven by geographic and climatic variation, as well as by variation in management regimes and soil types, calcareous grassland also display high levels of taxonomic diversity at landscape (Newton et al. 2012), regional (Smith 1980; Rodwell 1992), and continental (Willems 1982; Duckworth et al. 2000) scales.

In recent centuries, the extent of calcareous grassland has declined dramatically, due to agricultural intensification, afforestation, and more recently, abandonment (Keymer & Leach 1990; Willems 1990; WallisDeVries et al. 2002). In Great Britain, an estimated 40,600 hectares of lowland calcareous grassland are left, the remaining area being highly fragmented (Bullock et al. 2011). This fragmentation increases the risk of permanent local extinction of specialist species, through a reduction in dispersal processes supporting population persistence and re-establishment processes (Ozinga et al. 2009), and through loss of genetic adaptability (Butaye et al. 2005). Additional efforts are thus required to reduce fragmentation, both by diversification of floristically deficient sites, and by creation of additional high-quality calcareous grassland. To achieve this, an interventionist approach is needed (Walker et al. 2004), as natural regeneration of calcareous grassland tends to be slow even under optimal conditions (Gibson & Brown 1991; Redhead et al. 2014), and outcomes are often poor under less favourable conditions (Fagan et al. 2008).

Two underlying key constraints, seed limitation and microsite limitation, are hampering natural regeneration (Hutchings & Booth 1996a; Bakker & Berendse 1999). To be successful, ecological restoration has to address these constraints. Seed limitation is addressed through active species introduction, often by sowing of species-rich mixtures (Walker et al. 2004; Hedberg & Kotowski 2010; Kiehl et al. 2010). However, restoration sites often are agriculturally improved (Walker et al. 2004), and raised levels of soil fertility result in intense local-scale competitive interactions and increased microsite limitation (Foster 2001; Öster et al. 2009a). As a result, while generalist species well-adapted to these conditions tend to establish reliably, less competitive habitat specialists often fail (Pywell et al. 2003). Although increased microsite limitation can persist for several decades (Öster et al. 2009b), the issue is particularly acute in young restoration sites (Hutchings & Booth 1996b; Öster et al. 2009b; Piqueray et al. 2013). As a result, restoration efforts often fall short, resulting in floristically uniform vegetation not reflecting local or regional distinctiveness and consisting mainly of easy-to-establish generalist species. Such outcomes hamper the aim of restoration to reduce habitat fragmentation and prevent further biodiversity loss.

To alleviate microsite limitation, various strategies can be employed. Rapid-acting techniques to achieve a more lasting reduction of microsite limitation include the removal of fertile topsoil (Edwards et al. 2007; Kiehl & Pfadenhauer 2007; Pywell et al. 2007) or its burial under less fertile subsoil (‘topsoil inversion‘; Glen et al. 2007). However, such methods are labour-intensive and costly (Pywell et al. 2007). Alternatively, ‘phased‘ restoration might be an option, involving introduction of easy-to-establish generalist species first, and of more strongly microsite-limited specialist species later, after fertility has been sufficiently reduced by suitable management (Pywell et al. 2003, 2007). However, decades of targeted management may be required to achieve such reductions in soil fertility (Willems & van Nieuwstadt 1996).

Another strategy is to temporarily reduce competition from extant vegetation to create a window-of-opportunity for species introduction. A standard methodis to create bare ground, e.g. by harrowing, followed by seed sowing (Edwards et al. 2007; Pywell et al. 2007). To allow introduced plants to survive and successfully reproduce in highly productive young restoration sites, suitable management regimes are required to reduce competition by other species and seasonally enhance availability of establishment microsites (Hutchings & Booth 1996b; Hofmann & Isselstein 2004; Pywell et al. 2007). Possible approaches include hay-cutting (Bakker 1989; Coulson et al. 2001) and short periods of heavy grazing at appropriate times of year, especially in spring and autumn (Bullock et al. 1995, 2001; Woodcock et al. 2005). However, grazing in spring might not be an option if restoration objectives include establishment or promotion of yellow-rattle, *Rhinanthus minor* (Mudrák et al. 2014).

Previous work on agriculturally-improved, well-drained calcareous soils has shown that some reduction of competition from extant vegetation may be required to promote establishment of calcareous-grassland specialists (Hutchings & Booth 1996b). However, optimal levels of intervention may vary between species. Some species prefer drier microsites with strongly reduced competition, as e.g. provided by ant hills (King 1977), whereas others, whose seedlings are more vulnerable to desiccation, may prefer more protected microsites (Hutchings & Booth 1996b). Different types of pre-sowing disturbance, differing in intensity, may thus be required to create suitable conditions for the establishment of a wide range of desirable species. Such differences in disturbance intensity also have a temporal component: The stronger a disturbance is initially, the more persistent its effects may be, e.g. in terms of reducing competition from the extant vegetation. In the context of restoring calcareous grassland, this might be an important aspect. Providing reduced levels of competition for a prolonged period might enable newly-established plants to faster accumulate resources and reach flowering stage, thus promoting a quicker transition to forming self-sustaining populations that represent a key benchmark for the success of species introductions (Menges 2008; Godefroid et al. 2011).

Here, we present results from an experimental study in partially restored calcareous grassland deficient in specialist species. We compared effects of different pre-sowing disturbance techniques, covering a range of intensities, on initial establishment and subsequent performance of such species after sowing, and on abiotic and soil conditions. In addition to the standard practice of creating bare soil by harrowing (Hopkins et al. 1999; Hofmann & Isselstein 2004; Pywell et al. 2007), we applied two novel disturbance techniques. To create sheltered microsites, we sprayed with glyphosate, leaving the resulting litter layer in place. To create more persistent bare-ground microsites, we used two-directional ploughing for topsoil inversion and creation of micro-topographic variation. We also examined effects on post-establishment performance of three different management regimes that might be applied in young restorations to reduce competition by a productive sward, including one regime without spring grazing, as e.g. applied in situations where *R. minor* as a desirable species was present. We investigated four questions:

(1) Do specialist species of calcareous grassland differ with respect to pre-sowing disturbance intensity most conducive to their initial establishment and subsequent dynamics?

(2) Do more intense disturbance treatments have longer-lasting effects on abiotic conditions such as bare-ground availability and soil chemistry?

(3) Do more intense disturbance treatments, via a reduction in plant competition, result in larger numbers of plants reaching flowering stage in the short term?

(4) Does sward management regime have species-specific effects on post-establishment performance of introduced specialist species?

Materials and methods

STUDY SITE

In 2008, an experiment was established on ex-arable, species-poor grassland in the Pegsdon Hills, Bedfordshire, south-east England (51° 57’ N, 0°23’W), at the north-eastern end of the Chiltern Hills chalk escarpment. The chosen site is gently sloping, faces SSW and was last under arable cultivation in 1992. A species-poor grass mix was sown in May 1993, and the site was subsequently managed by sheep grazing (A. Fleckney & G. Bellamy, personal communication). At the onset of the experiment in spring 2008, the sward was dominated by the grasses *Arrhenatherum elatius*, *Agrostis stolonifera* and *Poa trivialis*, and the leguminous forbs *Medicago lupulina*, *Trifolium repens* and *Trifolium pratense*, being representative of mid-successional species-poor ex-arable calcareous grassland in southern Britain (Gibson & Brown 1991, Fagan et al. 2008).

EXPERIMENTAL DESIGN

A split-plot design was used to combine three sward management regimes at the main-plot level with four pre-sowing disturbance treatments at the sub-plot level. To this end, in spring 2008, twelve experimental main plots of 75 m × 50 m were set up in four replicate blocks.

Disturbance treatments

At the centre of each main plot, a 35 m × 35 m grid of 5 m × 5m sub-plots was laid out in May 2008, and four disturbance treatments (see Appendix S1 for photos illustrating their creation) were allocated at random to experimental sub-plots separated by guard rows 5 m wide: (1) undisturbed control; (2) band-spraying with glyphosate to kill approximately 50% by cover of the existing sward, leaving all killed plant material in place; (3) power harrowing to create 70-80% bare ground; (4) two-directional ploughing to bury the topsoil and create a 1.5 m wide × 0.4 m high ridge bordered either side by adjacent furrows from ploughing, with the whole structure being oriented in an east-west direction. After ploughing, the resulting soil mounds were ring-rolled to firm the soil and rotavated to create a smooth seed bed for sowing. This treatment resulted in 100% bare ground, thus being the most intense type of disturbance applied in this study. As the resulting structures were somewhat similar in profile to the ridge-and-furrow relic patterns created by a system of ploughing used in medieval times in Britain and in other parts of Europe, we decided to refer to this treatment as ridge-and-furrow treatment.

Prior to application of disturbance treatments, all experimental sub-plots were cut to 5 cm height with a drum mower, and all cut herbage was removed. Sub-plots assigned to the ridge-and-furrow treatments were additionally sprayed with glyphosate. After disturbances were applied, all experimental sub-plots were over-sown on 17 May 2008 using a seed mixture containing nine calcareous grassland species known to perform poorly in restoration (Pywell et al. 2003 and unpublished) and to be slow colonizers of ex-arable sites left to regenerate naturally (Smith 1980; Gibson & Brown 1991). These species were *Campanula glomerata*, *Carex flacca*, *Filipendula vulgaris*, *Helianthemum nummularium*, *Hippocrepis comosa*, *Pimpinella saxifraga*, *Stachys officinalis*, *Succisa pratensis* and *Thymus pulegioides*. In addition, the *Bromopsis erecta*, a signature grass of European calcareous grassland (Smith 1980; Ellenberg 1988) was also included as it was absent from the vegetation. Unlike other species included, *B. erecta* tends to perform relatively well in restoration (Pywell et al. 2003). Prior to sowing, seeds of two species, *H. nummularium* and *H. comosa*, were scarified. Sowing density varied with species, ranging between 30 and 110 seeds m^{-2} (Appendix S2). After sowing, sub-plots were rolled with a ring-roller, and slug pellets (4% metaldehyde) were applied at a rate of 3.5 kg ha^{-1}. During the establishment phase, the whole experiment was uniformly managed by applying a hay-cut at the end of July 2008, followed by autumn cattle grazing.

Management regimes

From 2009 onwards, three management regimes were applied at main-plot level: (1) summer hay cut followed by autumn (aftermath) grazing with cattle; (2) spring grazing with sheep, followed by summer hay cut and autumn grazing with cattle; (3) spring grazing with sheep followed by summer and autumn grazing with cattle. Grazing was managed to achieve sward height targets of 3-5 cm for sheep grazing, and 5-7 cm for cattle grazing. In 2009, summer grazing was carried out between mid-June and mid-July, but in later years this was moved to late August / early September, to encourage flowering of sown species. The summer hay cut was carried out at the end of July, using a drum mower. On the ridge-and-furrow sub-plots, a strimmer was used to accommodate the complex topography. All cut material was dried, turned and removed.

DATA COLLECTION

Plant censuses

Three censuses of sown species were undertaken annually in 2008 and in 2009, and two censuses in 2010 and in 2011. In all years, censuses were carried out in late spring/early summer and in late summer/early autumn. In the first two years, an additional census was carried out in midsummer, to account for potentially more pronounced demographic dynamics expected in the early stages of species introduction. Censuses in each sub-plot were carried out in twelve 0.5 m × 0.5 m quadrats arranged in threes along four staggered parallel transects (Appendix S3). In 2008 and 2009, censuses were carried out as plant counts. By 2010, counting individuals was no longer possible for clonally spreading species, and thus, in 2010 and 2011, we carried out rooted frequency counts, with each 0.5 m × 0.5 m quadrat subdivided in 9 cells. From 2009 to 2011, in addition to plant presence, we also recorded whether at least one individual of each of the sown species in the quadrat had initiated a flowering stem, indicating that plants were not just surviving but reaching reproductive size.

In addition to censuses, from 2009 to 2011, percentage cover of bare ground was surveyed annually in July within two diagonally arranged 1 m × 1 m quadrats per sub-plot.

Soil parameters

To quantify treatment effects surface-soil parameters, samples were collected twice from all sub-plots, in August 2008 and in October 2012. Each time, five cylindrical soil cores (diameter: 25 mm; depth: 75 mm) per sub-plot were collected along a W-shaped transect, pooled and thoroughly mixed to obtain a homogeneous bulk sample. Soil pH was measured in water as 1:2.5 extract. Soil organic matter content was determined via loss-on-ignition at 450°C. Total nitrogen was determined using a Carlo-Erba NA 1500 analyzer (Carlo-Erba, Milan, Italy).

STATISTICAL ANALYSES

First-year establishment

To test for disturbance effects on initial establishment in 2008 of each sown species individually and summed across all specialist forb species (i.e. excluding *B. erecta*), we constructed linear mixed-models, including disturbance as a fixed factor and main plot as random blocking factor. In case of significance, pairwise comparisons using two-sided Tukey tests were carried out to identify differences between disturbance treatments. To ensure normality of residuals and variance homogeneity, all plant count data was square-root-transformed. With one exception, *H. nummularium*, species-level analyses were based on the early September 2008 census, when seedling numbers were generally highest. *H. nummularium* emerged more rapidly, and by September, its seedling numbers had started to drop. Analysis for this species was therefore based on the July census.

Species performance in years 2-4 and abiotic trends

To analyse effects of treatments on performance in years 2 to 4 of each species individually and averaged across specialist forbs, we constructed repeated-measures linear mixed models, including sward management, pre-sowing disturbance, year, and the interactions between these as fixed factors in the model statement, and specifying year as the repeated-measures factor. Blocks and main plots nested within blocks were included as random effects (Schabenberger & Pierce 2002). In case of main-factor significance, and in the absence of significant interactions including a specific main factor (Quinn &Keough 2002), pairwise comparisons between factor levels were carried out using two-sided Tukey tests.

As census methods changed from plant counts to rooted frequencies halfway through the study, for consistency, sown species abundance trends were analysed on the basis of proportional frequency data reflecting the proportion of 0.5 m × 0.5 m quadrats in each sub-plot containing a given species. Data were averaged across censuses in a given year, as we were interested in longer-term trends rather than in seasonal variation.

To investigate trends in plant reproductive status, similar analyses were performed for frequencies based on the proportions of quadrats found to contain individuals having initiated flowering stems. For a given year, a census quadrat was judged to contain such individuals if at least one flowering stem of a given species was observed in at least one census. The extra, middle census in 2009 was not included in this assessment, to ensure comparability across years. Repeated-measures mixed models were also constructed for bare ground data from 2009-2011, based on average values per sub-plot in a given year, and for soil data from 2008 and 2011. To meet requirements of variance homogeneity, all proportional data including bare ground were arcsine-transformed, and soil data were power-transformed. Optimal coefficients for power transformation for each soil parameter were estimated using spread-level-plots as provided in the 'car' package V2.0-12 within R V2.15.1 (R Foundation for Statistical Computing, Vienna, AT).

Analyses of data collected annually between 2009 and 2011 were performed using various alternative covariance structures for the repeated factor, including unstructured, compound symmetric and several autoregressive structures. The most suitable model was selected based on the Akaike Information Criterion (AIC; Akaike 1974). For analyses of soil data, which were gathered only twice, a compound symmetric structure was used by default. All linear mixed models were constructed using SAS 9.1 PROC MIXED (SAS Institute, Cary, NC, US).

Results

SEEDLING ESTABLISHMENT IN RELATION TO DISTURBANCE

Overall seedling establishment of specialist forbs was positively related to disturbance intensity, and decreased in the order ridge-and-furrow > harrowed > sprayed > control (Fig. 1a).

Individually, seven forb species established regularly in at least one disturbance treatment (Fig 1b-i), and so did *B. erecta*. *H. comosa* established very poorly, with just a few seedlings mostly in ridge-and-furrow sub-plots, and *C. flacca* completely failed to establish. In six species, establishment was promoted both by harrowing and by ridge-and-furrow creation. In three of these, *C. glomerata*, *P. saxifraga* and *S. officinalis*, establishment was higher for ridge-and-furrow creation (Fig. 1b,f,g). In *F. vulgaris*, *H. nummularium* and *T. pulegioides*, establishment was roughly equally promoted by harrowing and by ridge-and-furrow creation (Fig. 1c,d,i). *H. nummularium* initially established in large numbers in both the harrowed and ridge-and-furrow treatments, but by the time of the second count in early September 2008, seedling numbers had already started to decline, compared to the first count in early July 2008. In *S. pratensis* and in the additionally sown *B. erecta*, establishment was only promoted significantly by ridge-and-furrow creation (Fig. 1h,j), although for the former, harrowing showed a strong trend (Tukey test for control vs. harrowing: d.f.=33; t = -2.70; P = 0.051). Establishment of any species individually was not promoted by spraying, although a small effect was detected for overall establishment of specialist forbs (Fig. 1a).

ABUNDANCE IN YEARS 2-4

Post-establishment abundance dynamics, as measured by quadrat occupancy, were similar among sown forb species, although to some extent, species responded individually to management regime and pre-sowing disturbance. In general, abundance noticeably increased between 2009 and 2010, and more or less stabilised between 2010 and 2011 (Table 1; Fig. 2a-i). Tukey tests supported this trend for three species in which year had a main effect in the absence of interactions, *S. officinalis*, *S. pratensis*, and *T. pulegioides* (Fig. 2g-i).

Significant interactions of year with disturbance in the remaining forb species (Table 1) reflect the fact that in these species, differences in abundance between years were particularly striking in harrowed and ridge-and-furrow treatments (Fig. 2b-f).

In the majority of sown forb species, post-establishment abundances were similar in the ridge-and-furrow and harrowed treatments, but markedly lower in the sprayed treatment, and very low in the control treatment (Fig. 2). Abundances of *S. pratensis* were very similar in harrowed and ridge-and-furrow treatments (Tukey test: d.f.=99; t = -0.51; P = 0.956), in spite of differences in initial establishment (Fig. 1h). Conversely, post-establishment abundances of *T. pulegioides* were higher in the ridge-and-furrow treatment than in the harrowed treatment (Tukey test: d.f. = 99; t = -2.98; P = 0.018), although no differences were found during initial establishment (Fig. 1i). *F. vulgaris* and to a lesser extent *P. saxifraga*, appeared to be more abundant in the harrowed treatment than in the ridge-and-furrow treatment, particularly in 2010 and 2011. *H. comosa* remained confined to the ridge-and-furrow treatment.

Averaged across sown forbs (Fig. 2a), and individually for *F. vulgaris* (Fig. 2c) and *P. saxifraga* (Fig. 2f), significant interactions between year and management reflected lower abundances over time under the management regime combining spring sheep grazing with summer and autumn cattle grazing, relative to other regimes. In *P. saxifraga*, this was particularly obvious relative to the regime combining summer hay cutting with autumn cattle grazing (Fig. 2f). A significant interaction between pre-sowing disturbance and sward management in *S. officinalis* (Table 1) was due to lower abundances in ridge-and-furrow treatment plots when managed by sheep grazing in spring in combination with cattle grazing in summer and autumn (Fig. 2g).

The grass *B. erecta* increased in abundance between 2010 and 2011 (Fig. 2j), and did so particularly in the harrowed treatment, as reflected by a significant interaction of year with disturbance (Table 1). Dynamics of *B. erecta* were not affected by management regime.

ABUNDANCE OF REPRODUCTIVE-STAGE PLANTS IN YEARS 2-4

Some *T. pulegioides* and *B. erecta* plants reached reproductive stage as early as 2009, but in most species, notable levels of flowering-stem formation were first observed in 2010, and in *S. officinalis* only in 2011 (Fig. 3). For most species individually, and averaged across specialist forbs, significant year × disturbance interactions (Table 1) indicated a larger increase in flowering-stem formation in the ridge-and-furrow treatment than in the harrowed treatment. Significant higher-order interactions of disturbance with management (Table 1), individually for *S. officinalis* and *S. pratensis* as well as averaged across specialist forbs, reflect lower levels of observed flowering-stem formation in ridge-and-furrow sub-plots when managed by sheep grazing in spring in combination with cattle grazing in summer and autumn (Fig. 3a,g,h).

BARE GROUND AND SOIL PARAMETERS

The only disturbance treatment in which notable levels of bare ground persisted beyond the first year was ridge-and-furrow creation (Fig. 4). Whilst levels of bare ground in this treatment steadily decreased between 2009 and 2011, some bare ground persisted until the end of the study in 2011, most notably under the grazing regime involving sheep grazing in spring in combination with cattle grazing in summer and autumn (Fig. 4). In the harrowed treatment, bare ground had already declined to very low levels in 2009, approaching levels in sprayed and control treatments.

Detailed information on the effects of experimental treatments on soil chemical parameters is presented in Appendix S4. Ridge-and-furrow creation reduced surface-soil organic matter content and total nitrogen (Appendix S4). As indicated by significant interactions between year and disturbance, levels of both subsequently remained static in the ridge-and-furrow treatment, but increased in other disturbance treatments (Appendix S4). Disturbance also had a relatively small, but highly significant, effect on surface-soil pH (Appendix S4). Tukey tests indicate that pH was higher in ridge-and-furrow plots than in control and harrowed plots, and marginally higher than in sprayed plots ($P = 0.052$). As suggested by the absence of a treatment × year interaction, this effect persisted throughout the study (Appendix S4).

Discussion

PRE-SOWING DISTURBANCE

Ridge-and-furrow creation promoted initial establishment in a wider range of species than the more conventional practice of harrowing, and several species established better in the former treatment. In addition, plants tended to reach reproductive stage earlier on ridge-and-furrow plots. These effects are likely due to ridge-and-furrow creation involving an initially stronger and at the same time more permanent reduction in competition from unsown species, and are consistent with findings from another study in species-poor chalk grassland, in which severe disturbance by turf stripping similarly worked better than harrowing for introducing species (Edwards et al. 2007).

In our study, ridge-and-furrow creation was the only disturbance to retain appreciable levels of bare ground throughout the study, and to have notable effects on surface-soil chemistry, by reducing organic matter content and total nitrogen, and slightly increasing pH, through replacing top soil with chalky subsurface soil. Compared to ridge-and-furrow creation, harrowing led to faster re-colonization by generalist grassland species from extant vegetation, and triggered transient occurrence mostly in the first year of weed species from the surface-soil seed bank (M. Wagner, personal observation). Such transient emergence may be common in ex-arable situations, and was observed in our study in spite of the site having been managed for 15 years as grassland since its reversion from arable use.

Although ridge-and-furrow creation mostly enabled higher initial establishment, abundances in harrowed sub-plots subsequently caught up in the case of *B. erecta*, *C. glomerata* and *S. pratensis*, and surpassed those in ridge-and-furrow sub-plots in the case of *F. vulgaris* and *P. saxifraga*.

In contrast, two of the lowest-statured species included in our study, *T. pulegioides* and *H. comosa*, tended to establish more permanently in the ridge-and-furrow treatment than in the harrowed treatment. In calcareous grassland, both are associated with south-facing slopes and/or with ant hills (Fearn 1973; Pigott 1955; King 1977; Lakhani & Davis 1982), i.e. with micro-habitats in which levels of abiotic stress are elevated and levels of competition reduced. *H. nummularium*, which is also low-statured and has similar micro-habitat preferences (King 1977; Lakhani & Davis 1982), initially emerged in appreciable numbers both in the harrowed and ridge-and-furrow treatments, but exhibited high seedling mortality, resulting in a marked decline by autumn 2008. This suggests that poor performance of this species in ecological restoration (Pywell et al. 2002, 2003) might partly be due to poor survival, and may not solely be attributable to physical seed dormancy (Thanos et al. 1992). Failure of *C. flacca* to establish in our study and poor establishment of *H. comosa* has likely been due to failure to break seed dormancy, as seed viability was confirmed in germination experiments (Wagner et al. 2011 and unpublished).

MANAGEMENT REGIMES

It can take years before management effects on species abundances become detectable (Bullock et al. 2001; Pywell et al. 2007), and in split-plot designs like ours, statistical power is smaller for main-plot factors than for split-plot factors (Potvin 2001). Accordingly, we found few effects of management regime on sown species abundances in this study. In some species, regimes involving a summer cut tended to boost densities compared to the grazed-only regime. This might suggest that during early restoration on agriculturally improved land, cutting might be effective at limiting competition from more generalist species. However, at least in one species, ecological preference might also have played a role. In *P. saxifraga*, the summer-cut regime without sheep grazing in spring appeared to be more successful than an otherwise similar regime including spring grazing, suggesting genuine ecological preference for cutting. In line with this result, a survey of Swiss mesic calcareous grassland found *P. saxifraga* to be more common in mown sites than in grazed sites (Schläpfer et al. 1998).

IMPLICATIONS FOR RESTORATION

Creation of micro-topographic features is not currently a part of calcareous-grassland restoration in the UK or elsewhere in Europe. Similar features have however been tested in North America in the context of prairie grassland restoration (Biederman & Whisenant 2011; Hough-Snee et al. 2011) and of wetland restoration (Doherty & Zedler 2015), the underlying rationale being that increased topographic heterogeneity provides a wider range of establishment microsites, resulting in more diverse target vegetation. Reasons for the lack of such features in the 'toolbox' for restoring European calcareous grasslands on ex-arable land may include associated labour and cost and the fact that they may be perceived as problematic at sites potentially containing archaeological features. Creation of micro-topographic features in calcareous-grassland restoration may be particularly useful at level sites where topographic variation is largely absent, but may have to be limited to sites where archaeological features are absent. As our results show, grazing, in particular by heavy livestock such as cattle, may have to be carefully managed on such micro-topographic features, to avoid excessive erosion that could negatively affect restoration outcomes. On the other hand, traditionally, calcareous grasslands have often been grazed heavily, and more short-lived species of calcareous grassland depend on relatively high levels of disturbance (Verkaar et al. 1983). Thus, a certain amount of erosion on ridge-and-furrow structures may likely be beneficial.

Only limited recommendations can be made based on our study with respect to management regimes. Optimal regimes will depend on the suite of species to be established, and more work is needed to determine niche preferences of species with respect to cutting vs. grazing in a restoration context, as well as regarding timing of grazing and type of livestock used. However, it is clear that management regimes in early-restoration sites must account for the fact that abiotic conditions on previously agriculturally improved sites markedly differ from those in old calcareous grassland, with levels of competition likely higher in the former. Calcareous-grassland restoration sites with a history of agricultural improvement ideally will have to be managed to limit competition, and create opportunities for seed-set and continued regeneration of target populations. In this context, it could also be beneficial to repeat bare ground creation via targeted disturbance, to promote regeneration of already introduced target species via selfseeding, and, in some instances, to assist with phased introduction of successively more stress-tolerant species.

Acknowledgements

This study was funded by Defra (BD1459) and via National Capability funding from the Natural Environmental Research Council (NEC04924). It was carried out in cooperation with the Wildlife Trust for Bedfordshire, Cambridgeshire and Northamptonshire, and its staff members Graham Bellamy, Andy Fleckney, and Nick Kiddy. Valuable advice was provided by Clare Pinches, Pete Stevens, Steve Peel and Richard Jefferson from Natural England, and by Val Brown. We thank Robert Dunn and lab staff at Rothamsted Research (North Wyke) for soil chemical analyses. Three referees and the co-ordinating editor provided valuable comments on the manuscript.

References

Aavik, T., Ülle, J., Liira, J., Tulva, I. & Zobel, M. 2008. Plant diversity in a calcareous wooded meadow – The significance of management continuity. *Journal of Vegetation Science* 19: 475-484.

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* 19: 716-723.

Bakker, J.P. 1989. *Nature Management by Grazing and Cutting: On the Ecological Significance of Grazing and Cutting Regimes Applied to Restore Former Species-rich Grassland Communities in the Netherlands.* Kluwer, Dordrecht, NL.

Bakker, J.P. & Berendse, F. 1999. Constraints in the restoration of ecological diversity in grassland and heathland communities. *Trends in Ecology and Evolution* 14: 63-68.

Biederman, L.A. & Whisenant, S.G. 2011. Using mounds to create microtopography alters plant community development early in restoration. *Restoration Ecology* 19: 53-61.

Bullock, J.M., Clear Hill, B., Silvertown, J. & Sutton, M. 1995. Gap colonization as a source of grassland community change: effects of gap size and grazing on the rate and mode of colonization by different species. *Oikos* 72: 273-282.

Bullock, J.M., Franklin, J., Stevenson, M.J., Silvertown, J., Coulson, S.J., Gregory, S.J. & Tofts, R. 2001. A plant trait analysis of responses to grazing in a long-term experiment. *Journal of Applied Ecology* 38: 253-267.

Bullock, J.M., Jefferson, R.G., Blackstock, T.H., Pakeman, R.J., Emmett, B.A., Pywell, R.F., Grime, J.P. & Silvertown, J. 2011. Semi-natural grasslands. In: *The UK National Ecosystem Assessment Technical Report*, pp. 161-195. UNEP-WCMC, Cambridge, UK.

Butaye, J., Adriaens, D. & Honnay, O. 2005. Conservation and restoration of calcareous grasslands: a concise review of the effects of fragmentation and management on plant species. *Biotechnologie, Agronomie, Société et Environnement* 9: 111-118.

Coulson, S.J., Bullock, J.M., Stevenson, M.J. & Pywell, R.F. 2001. Colonization of grassland by sown species: dispersal versus microsite limitation in response to management. *Journal of Applied Ecology* 38: 204-216.

Doherty, J.M. & Zedler, J.B. 2015. Increasing substrate heterogeneity as a bet-hedging strategy for restoring wetland vegetation. *Restoration Ecology* 23: 15-25.

Duckworth, J.C., Bunce, R.G.H. & Malloch, A.J.C. 2000. Vegetation-environment relationships in Atlantic European calcareous grasslands. *Journal of Vegetation Science* 11: 15-22.

Edwards, A.R., Mortimer, S.R., Lawson, C.S., Westbury, D.B., Harris, S.J., Woodcock, B.A. & Brown, V.K. 2007. Hay strewing, brush harvesting of seed and soil disturbance as tools for the enhancement of botanical diversity in grasslands. *Biological Conservation* 134: 372-382.

Ellenberg, H. 1988. *Vegetation Ecology of Central Europe.* 4th ed. Cambridge University Press, Cambridge, UK.

Fagan, K.C., Pywell, R.F., Bullock, J.M. & Marrs, R.H. 2008. Do restored calcareous grasslands on former arable fields resemble ancient targets? The effect of time, methods and environment on outcomes. *Journal of Applied Ecology* 45: 1293-1303.

Fearn, G.M. 1973. *Hippocrepis comosa* L. *Journal of Ecology* 61: 915-926.

Foster, B.L. 2001. Constraints on colonization and species richness along a grassland productivity gradient: the role of propagule availability. *Ecology Letters* 4: 530-535.

Gibson, C.W.D. & Brown, V.K. 1991. The nature and rate of development of calcareous grassland in southern Britain. *Biological Conservation* 58: 297-316.

Glen, E., Price, E.A.C., Caporn, S.J.M., Carroll, J.A., Jones, M.L.M., & Scott, R. 2007. A novel technique for restoring species-rich grassland. In: J.J. Hopkins, J.J.; Duncan, A.J.; McCracken, D.I., Peel, S. & Tallowin, J.R.B. (eds.) *High value grassland: providing biodiversity, a clean environment and premium products*, pp. 221-224. Occasional Symposium No. 38. British Grassland Society, Cirencester, UK.

Godefroid, S., Piazza, C., Rossi, G., Buord, S., Stevens, A.-D., Aguraiuja, R., Cowell, C., Weekley, C.W., Vogg, G., (…) & Vanderborght, T. 2011. How successful are plant reintroductions? *Biological Conservation* 144: 672-682.

Hedberg, O. & Kotowski, W. 2010. New nature by sowing? The current state of species introduction in grassland restoration, and the road ahead. *Journal for Nature Conservation* 18:304-308.

Hofmann, M. & Isselstein, J. 2004. Seedling recruitment on agriculturally improved mesic grassland: the influence of disturbance and management schemes. *Applied Vegetation Science* 7: 193-200.

Hopkins, A., Pywell, R.F., Peel, S., Johnson, R.H. & Bowling, P.J. 1999. Enhancement of botanical diversity of permanent grassland and impact on hay production in Environmentally Sensitive Areas in the UK. *Grass and Forage Science* 54: 163-173.

Hough-Snee, N., Long, A.L., Jeroue, L. & Ewing, K. 2011. Mounding alters environmental filters that drive plant community development in a novel grassland. *Ecological Engineering* 37: 1932-1936.

Hutchings, M.J. & Booth, K.D. 1996a. Studies on the feasibility of re-creating chalk grassland vegetation on ex-arable land. I. The potential roles of the seed bank and the seed rain. *Journal of Applied Ecology* 33: 1171-1181.

Hutchings, M.J. & Booth, K.D. 1996b. Studies on the feasibility of re-creating chalk grassland vegetation on ex-arable land. II. Germination and early survivorship of seedlings under different management regimes. *Journal of Applied Ecology* 33: 1182-1190.

Keymer, R.J. & Leach, S.J. 1990. Calcareous grassland – a limited resource in Britain. In: Hillier, S.H., Walton, D.W.H. & Wells, D.A. (eds.) *Calcareous Grasslands: Ecology and Management*, pp. 41-46. Bluntisham, Huntingdon, UK.

Kiehl, K., Kirmer, A., Donath, T.W., Rasran, L. & Hölzel, N. 2010. Species introduction in restoration projects – evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. *Basic and Applied Ecology* 11: 285-299.

Kiehl, K. & Pfadenhauer, J. 2007. Establishment and persistence of target species in newly created calcareous grasslands on former arable fields. *Plant Ecology* 189: 31-48.

King, T.J. 1977. The plant ecology of ant-hills in calcareous grasslands. I. Patterns of species in relation to ant-hills in southern England. *Journal of Ecology* 65: 235-256.

Lakhani, K.H. & Davis, B.N.K. 1982. Multiple regression models of the distribution of *Helianthemum chamaecistus* in relation to aspect and slope at Barnack, England. *Journal of Applied Ecology* 19: 621-629.

Menges, E.S. 2008. Restoration demography and genetics of plants: When is a translocation successful? *Australian Journal of Botany* 56: 187-196.

Mudrák, O., Mládek, J., Blažek, P., Lepš, J., Doležal, J., Nekvapilová, E. & Těšitel, J. 2014. Establishment of hemiparasitic *Rhinanthus* spp. in grassland restoration: lessons learned from sowing experiments. *Applied Vegetation Science* 17: 274-287.

Newton, A.C., Walls, R.M., Golicher, D., Keith, S.A., Diaz, A. & Bullock, J.M. 2012. Structure, composition and dynamics of a calcareous grassland metacommunity over a 70-year interval. *Journal of Ecology* 100: 196-209.

Öster, M., Ask, K., Römermann, C., Tackenberg, O. & Eriksson, O. 2009a. Plant colonization of ex-arable fields from adjacent species-rich grasslands: the importance of dispersal vs. recruitment ability. *Agriculture, Ecosystems and Environment* 130: 93-99.

Öster, M., Ask, K., Cousins, S.A.O. & Eriksson, O. 2009b. Dispersal and establishment limitation reduces the potential for successful restoration of semi-natural grassland communities on former arable fields. *Journal of Applied Ecology* 46: 1266-1274.

Ozinga, W.A. Römermann, C., Bekker, R.M., Prinzing, A., Tamis, W.L.M., Schaminée, J.H.J., Hennekens, S.M., Thompson, K., Poschlod, P., (…) & van Groenendael, J.M. 2009. Dispersal failure contributes to plant losses in NW Europe. *Ecology Letters* 12: 66-74.

Pigott, C.D. 1955. *Thymus* L. *Journal of Ecology* 61: 915-926.

Piqueray, J., Saad, L., Bizoux, J.-P. & Mahy, G. 2013. Why some species cannot colonise restored habitats? The effects of seed and microsite availability. *Journal for Nature Conservation* 21: 189-197.

Poschlod, P. & WallisDeVries, M.F. 2002. The historical and socioeconomic perspective of calcareous grasslands – lessons from the distant and recent past. *Biological Conservation* 104: 361-376.

Potvin, C. 2001. ANOVA: experimental layout and analysis. In: Scheiner, S. & Gurevitch, J. (eds.) *Design and Analysis of Ecological Experiments*, pp. 63-76. Oxford University Press, Oxford, UK.

Pywell, R.F., Bullock, J.M., Hopkins, A., Walker, K.J., Sparks, T.H., Burke, M.J.W. & Peel, S. 2002. Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. *Journal of Applied Ecology* 39: 294-310.

Pywell, R.F., Bullock, J.M., Roy, D.B., Warman, L., Walker, K.J. & Rothery, P. 2003. Plant traits as predictors of performance in ecological restoration. *Journal of Applied Ecology* 40: 65-77.

Pywell, R.F., Bullock, J.M., Tallowin, J.B., Walker, K.J., Warman, E.A. & Masters, G. 2007. Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. *Journal of Applied Ecology* 44: 81-94.

Quinn, G.P. & Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists.* Cambridge University Press, Cambridge, UK.

Redhead, J.W., Sheail, J., Bullock, J.M., Ferreruela, A., Walker, K.J. & Pywell, R.F. 2014. The natural regeneration of calcareous grassland at a landscape scale: 150 years of plant community re-assembly on Salisbury Plain, UK. *Applied Vegetation Science* 17: 408-418.

Rodwell, J.S. 1992. *British Plant Communities. Volume 3: Grasslands and Montane Communities*. Cambridge University Press, Cambridge, UK.

Schabenberger, O. & Pierce, F.J. 2002. *Contemporary Statistical Models for the Plant and Soil Sciences.* CRC Press, London, UK.

Schläpfer, M., Zoller, H. & Körner, C. 1998. Influences of mowing and grazing on plant species composition in calcareous grassland. *Botanica Helvetica* 108: 57-67.

Smith, C.J. 1980. *Ecology of the English Chalk.* Academic Press, London, UK.

Stace, C. 2010. *New Flora of the British Isles*, 3rd ed. Cambridge University Press, Cambridge, UK.

Thanos, C., Georghiou, K., Kadis, C. & Pantazi, C. 1992. Cistaceae: a plant family with hard seeds. *Israel Journal of Botany* 41: 251–263.

Verkaar, H.J., Schenkeveld, A.J. & Brand, J.M. 1983. On the ecology of short-lived forbs in chalk grasslands: micro-site tolerances in relation to vegetation structure. *Vegetatio* 52: 91-102.

Wagner, M., Pywell, R.F., Knopp. T., Bullock, J.M. & Heard, M.S. 2011. The germination niches of grassland species targeted for restoration: effects of seed pre-treatments. *Seed Science Research* 21: 117-131.

Walker, K.J., Stevens, P.A., Stevens, D.P., Mountford, J.O., Manchester, S.J. & Pywell, R.F. 2004. The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. *Biological Conservation* 119: 1-18.

WallisDeVries, M.F., Poschlod, P. & Willems, J.H. 2002. Challenges for the conservation of calcareous grasslands in northwestern Europe: integrating the requirements of flora and fauna. *Biological Conservation* 104: 265-273.

Willems, J.H. 1982. Phytosociological and geographical survey of Mesobromion communities in Western Europe. *Vegetatio* 48: 227-240.

Willems, J.H. 1990. Calcareous grasslands in continental Europe. In: Hillier, S.H., Walton, D.W.H. & Wells, D.A. (eds.) *Calcareous Grasslands: Ecology and Management*, pp. 41-46. Bluntisham, Huntingdon, UK.

Willems, J.H. & van Nieuwstadt, M.G.L. 1996. Long-term after effects of fertilization on above-ground phytomass and species diversity in calcareous grassland. *Journal of Vegetation Science* 7: 177-184.

Wilson, J.B., Peet, R.K., Dengler, J. & Pärtel, M. 2012. Plant species richness: the world records. *Journal of Vegetation Science* 23: 796-802.

Woodcock, B.A., Pywell, R.F., Roy, D.B., Rose, R.J. & Bell, D. 2005. Grazing management of calcareous grasslands and its implications for the conservation of beetle communities. *Biological Conservation* 125: 193-202.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Photos showing disturbance treatments.

Appendix S2. Sowing rates of species experimentally introduced to the Pegsdon Hills field site.

Appendix S3. Placement of census quadrats within experimental sub-plots.

Appendix S4. Soil chemical parameters in 2008 and in 2011 for the top 75mm of surface soil.

Figure 1. Establishment of sown species in relation to the four pre-sowing disturbance treatments. R&F = Ridge-and-furrow creation. Back-transformed means ±SE, along with mixed-model F-values and significance levels (NS: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$). Lower-case superscript letters indicate pair-wise differences between treatments (two-sided Tukey tests, $P < 0.05$).

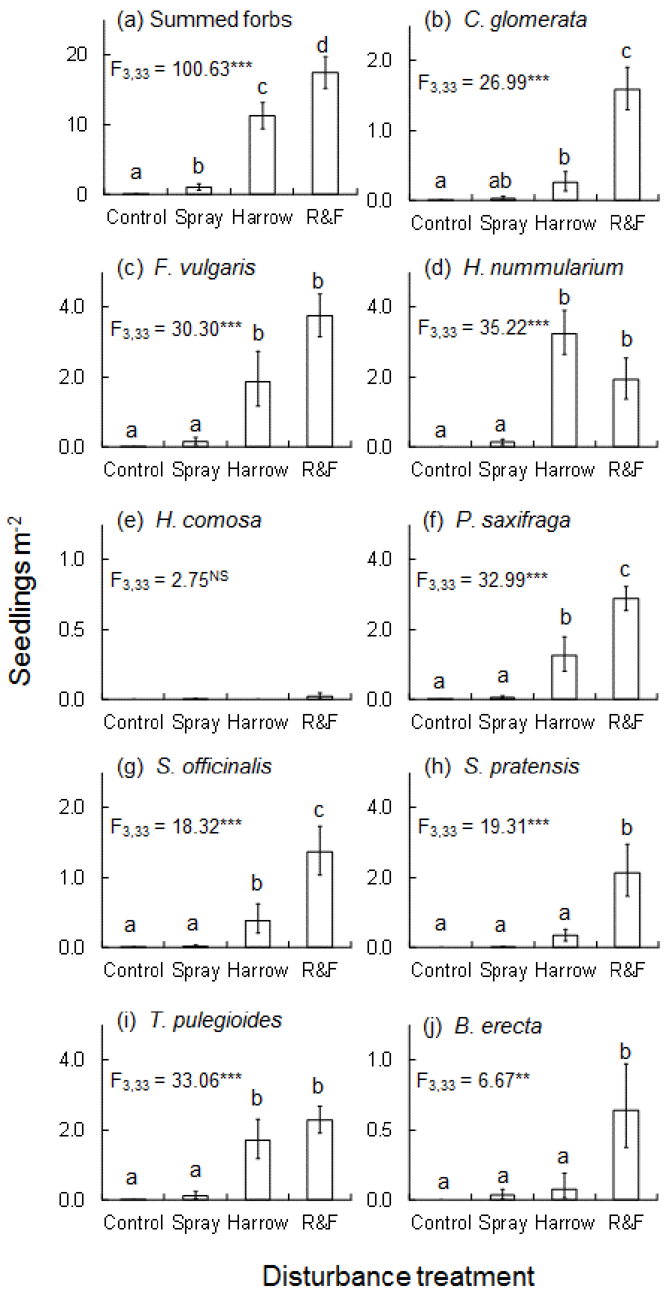

Figure 2. Frequency of occurrence in 0.5 m × 0.5 m quadrats from 2009 to 2011 of sown species, and averaged across specialist forbs. Back-transformed means ±SE.

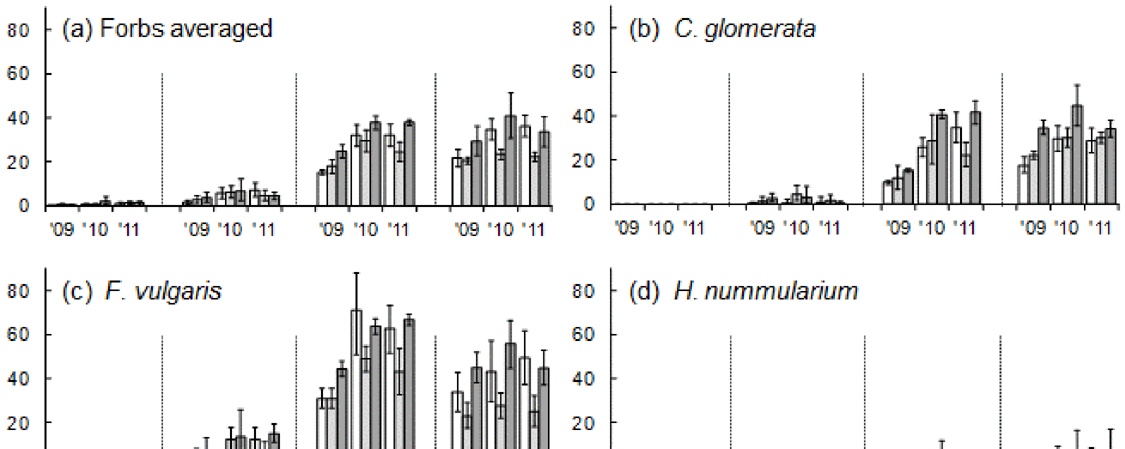

Figure 3. Frequency of occurrence in 0.5 m × 0.5 m quadrats from 2009 to 2011 of reproductive-stage plants of sown species, and averaged across specialist forbs. Back-transformed means ±SE for harrowed and ridge-and-furrow (R&F) treatments.

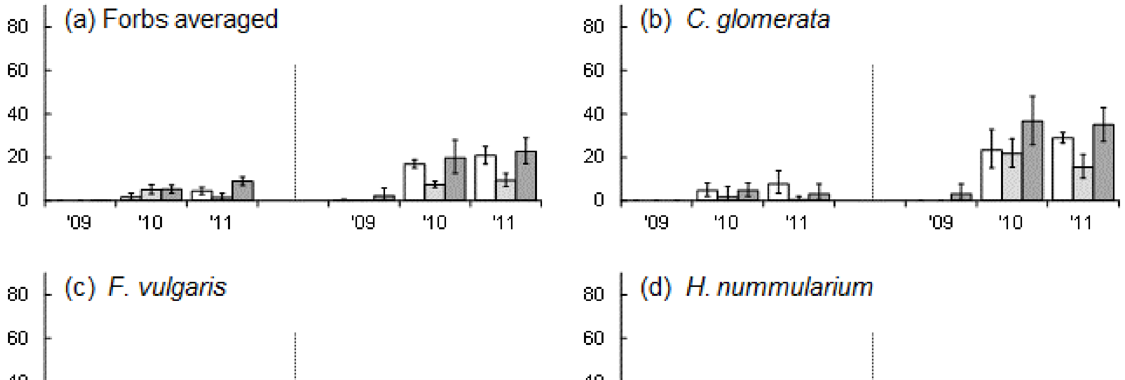

Figure 4. Percentage bare ground from 2009 to 2011, estimated annually in July. Back-transformed means ±SE.

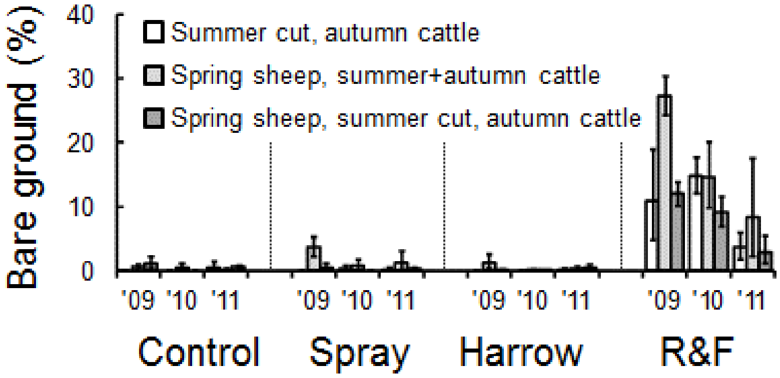

Table 1. Effects of pre-sowing disturbance and management regime from 2009 to 2011 on sown calcareous-grassland species performance and on bare ground. For difficult-to-establish forbs, results are also shown averaged across species. Mixed model F values and significance levels (NS: not significant; *: P < 0.05; **: P < 0.01; ***: P < 0.001) are given.

Effect		Forbs averaged	*C. glomerata*	*F. vulgaris*	*H. nummularium*	*H. comosa*	*P. saxifraga*	*S. officinalis*	*S. pratensis*	*T. pulegioides*	*B. erecta*	Bare ground
Proportional quadrat frequency of plants; percentage bare ground												
Management	F2,6	1.23NS	3.06NS	2.48NS	1.71NS	0.54NS	1.29NS	0.63NS	2.06NS	2.24NS	0.89NS	7.95*
Disturbance	F3,99	120.73***	124.27***	97.71***	6.59***	9.98***	69.19***	55.98***	76.01***	49.65***	48.45***	75.23***
Man × Dist	F6,99	0.92NS	0.96NS	0.74NS	0.73NS	0.47NS	0.69NS	2.69*	0.70NS	0.95NS	1.28NS	1.19NS
Year	F2,99	79.13***	37.60***	21.92***	1.81NS	2.71NS	41.20***	12.60***	11.60***	3.32*	49.56***	3.48*
Year × Man	F4,99	5.99**	1.37NS	2.51*	0.51NS	1.44NS	5.32***	0.33NS	2.24NS	2.02NS	0.30NS	2.47*
Year × Dist	F6,99	3.88***	15.35***	4.53***	2.29*	3.15**	2.83*	0.77NS	1.24NS	1.52NS	5.79***	4.80***
Year × Man × Dist	F12,99	0.87NS	1.20NS	0.80NS	0.88NS	1.46NS	1.17NS	1.83NS	1.56NS	0.77NS	0.70NS	0.70NS
Proportional quadrat frequency of plants initiating flowering-stems												
Management	F2,6	3.85NS	2.82NS	1.76NS	0.50NS	0.51NS	0.37NS	0.38NS	5.68*	3.66NS	1.78NS	
Disturbance	F1,45	20.91***	36.20***	1..59NS*	2.00NS	2.87NS	5.87*	14.51***	9.04**	24.28***	4.66*	
Man × Dist	F2,45	1.03*	1.33NS	0.82NS	0.50NS	0.08NS	1.00NS	3.43*	0.92NS	1.62NS	0.45NS	
Year	F2,45	127.81***	59.36***	12.53***	0.50NS	1.58NS	8.94***	5.64**	76.24***	34.40***	4.11*	
Year × Man	F4,45	2.13NS	1.11NS	0.37NS	1.25NS	1.45NS	0.14NS	0.85NS	6.03***	1.15NS	1.04NS	
Year × Dist	F2,45	8.50***	11.91***	1.43NS	0.50NS	3.17NS	3.44*	3.90*	4.33*	6.18**	4.81**	
Year × Man × Dist	F4,45	2.09NS	0.08NS	1.12NS	1.25NS	1.24NS	1.23NS	1.22NS	2.87*	1.20NS	0.33NS	