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

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22 total 12.0 pages.

23

24 Abstract

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

28 Location: Pegsdon Hills, Bedfordshire, UK

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

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

47 furrow treatment than in the harrowed treatment. By the end of the study, management regimes  
48 had resulted in few effects on species dynamics.

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

56

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

60

61 Nomenclature: Stace (2010)

62

63 Running head: Micro-topographic features in restored grassland

64

65 Introduction

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

74 In recent centuries, the extent of calcareous grassland has declined dramatically, due to  
75 agricultural intensification, afforestation, and more recently, abandonment (Keymer & Leach  
76 1990; Willems 1990; WallisDeVries et al. 2002). In Great Britain, an estimated 40,600 hectares  
77 of lowland calcareous grassland are left, the remaining area being highly fragmented (Bullock et  
78 al. 2011). This fragmentation increases the risk of permanent local extinction of specialist  
79 species, through a reduction in dispersal processes supporting population persistence and re-  
80 establishment processes (Ozinga et al. 2009), and through loss of genetic adaptability (Butaye et  
81 al. 2005). Additional efforts are thus required to reduce fragmentation, both by diversification of  
82 floristically deficient sites, and by creation of additional high-quality calcareous grassland. To  
83 achieve this, an interventionist approach is needed (Walker et al. 2004), as natural regeneration  
84 of calcareous grassland tends to be slow even under optimal conditions (Gibson & Brown 1991;  
85 Redhead et al. 2014), and outcomes are often poor under less favourable conditions (Fagan et al.  
86 2008).

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

102           To alleviate microsite limitation, various strategies can be employed. Rapid-acting  
103 techniques to achieve a more lasting reduction of microsite limitation include the removal of  
104 fertile topsoil (Edwards et al. 2007; Kiehl & Pfadenhauer 2007; Pywell et al. 2007) or its burial  
105 under less fertile subsoil ('topsoil inversion'; Glen et al. 2007). However, such methods are  
106 labour-intensive and costly (Pywell et al. 2007). Alternatively, 'phased' restoration might be an  
107 option, involving introduction of easy-to-establish generalist species first, and of more strongly  
108 microsite-limited specialist species later, after fertility has been sufficiently reduced by suitable

109 management (Pywell et al. 2003, 2007). However, decades of targeted management may be  
110 required to achieve such reductions in soil fertility (Willems & van Nieuwstadt 1996).

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

122 Previous work on agriculturally-improved, well-drained calcareous soils has shown that  
123 some reduction of competition from extant vegetation may be required to promote establishment  
124 of calcareous-grassland specialists (Hutchings & Booth 1996b). However, optimal levels of  
125 intervention may vary between species. Some species prefer drier microsites with strongly  
126 reduced competition, as e.g. provided by ant hills (King 1977), whereas others, whose seedlings  
127 are more vulnerable to desiccation, may prefer more protected microsites (Hutchings & Booth  
128 1996b). Different types of pre-sowing disturbance, differing in intensity, may thus be required to  
129 create suitable conditions for the establishment of a wide range of desirable species. Such  
130 differences in disturbance intensity also have a temporal component: The stronger a disturbance  
131 is initially, the more persistent its effects may be, e.g. in terms of reducing competition from the

132 extant vegetation. In the context of restoring calcareous grassland, this might be an important  
133 aspect. Providing reduced levels of competition for a prolonged period might enable newly-  
134 established plants to faster accumulate resources and reach flowering stage, thus promoting a  
135 quicker transition to forming self-sustaining populations that represent a key benchmark for the  
136 success of species introductions (Menges 2008; Godefroid et al. 2011).

137         Here, we present results from an experimental study in partially restored calcareous  
138 grassland deficient in specialist species. We compared effects of different pre-sowing  
139 disturbance techniques, covering a range of intensities, on initial establishment and subsequent  
140 performance of such species after sowing, and on abiotic and soil conditions. In addition to the  
141 standard practice of creating bare soil by harrowing (Hopkins et al. 1999; Hofmann & Isselstein  
142 2004; Pywell et al. 2007), we applied two novel disturbance techniques. To create sheltered  
143 microsites, we sprayed with glyphosate, leaving the resulting litter layer in place. To create more  
144 persistent bare-ground microsites, we used two-directional ploughing for topsoil inversion and  
145 creation of micro-topographic variation. We also examined effects on post-establishment  
146 performance of three different management regimes that might be applied in young restorations  
147 to reduce competition by a productive sward, including one regime without spring grazing, as  
148 e.g. applied in situations where *R. minor* as a desirable species was present. We investigated four  
149 questions:

- 150 (1) Do specialist species of calcareous grassland differ with respect to pre-sowing disturbance
- 151 intensity most conducive to their initial establishment and subsequent dynamics?
- 152 (2) Do more intense disturbance treatments have longer-lasting effects on abiotic conditions such
- 153 as bare-ground availability and soil chemistry?



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

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

158

159 Materials and methods

160 STUDY SITE

161 In 2008, an experiment was established on ex-arable, species-poor grassland in the Pegsdon  
162 Hills, Bedfordshire, south-east England (51° 57' N, 0°23'W), at the north-eastern end of the  
163 Chiltern Hills chalk escarpment. The chosen site is gently sloping, faces SSW and was last under  
164 arable cultivation in 1992. A species-poor grass mix was sown in May 1993, and the site was  
165 subsequently managed by sheep grazing (A. Fleckney & G. Bellamy, personal communication).

166 At the onset of the experiment in spring 2008, the sward was dominated by the grasses

167 *Arrhenatherum elatius*, *Agrostis stolonifera* and *Poa trivialis*, and the leguminous forbs

168 *Medicago lupulina*, *Trifolium repens* and *Trifolium pratense*, being representative of mid-

169 successional species-poor ex-arable calcareous grassland in southern Britain (Gibson & Brown

170 1991, Fagan et al. 2008).

171 EXPERIMENTAL DESIGN

172 A split-plot design was used to combine three sward management regimes at the main-plot level

173 with four pre-sowing disturbance treatments at the sub-plot level. To this end, in spring 2008,

174 twelve experimental main plots of 75 m × 50 m were set up in four replicate blocks.

175 *Disturbance treatments*

176 At the centre of each main plot, a 35 m × 35 m grid of 5 m × 5m sub-plots was laid out in May  
177 2008, and four disturbance treatments (see Appendix S1 for photos illustrating their creation)  
178 were allocated at random to experimental sub-plots separated by guard rows 5 m wide: (1)  
179 undisturbed control; (2) band-spraying with glyphosate to kill approximately 50% by cover of  
180 the existing sward, leaving all killed plant material in place; (3) power harrowing to create 70-  
181 80% bare ground; (4) two-directional ploughing to bury the topsoil and create a 1.5 m wide × 0.4  
182 m high ridge bordered either side by adjacent furrows from ploughing, with the whole structure  
183 being oriented in an east-west direction. After ploughing, the resulting soil mounds were ring-  
184 rolled to firm the soil and rotavated to create a smooth seed bed for sowing. This treatment  
185 resulted in 100% bare ground, thus being the most intense type of disturbance applied in this  
186 study. As the resulting structures were somewhat similar in profile to the ridge-and-furrow relic  
187 patterns created by a system of ploughing used in medieval times in Britain and in other parts of  
188 Europe, we decided to refer to this treatment as ridge-and-furrow treatment.

189 Prior to application of disturbance treatments, all experimental sub-plots were cut to 5 cm  
190 height with a drum mower, and all cut herbage was removed. Sub-plots assigned to the ridge-  
191 and-furrow treatments were additionally sprayed with glyphosate. After disturbances were  
192 applied, all experimental sub-plots were over-sown on 17 May 2008 using a seed mixture  
193 containing nine calcareous grassland species known to perform poorly in restoration (Pywell et  
194 al. 2003 and unpublished) and to be slow colonizers of ex-arable sites left to regenerate naturally  
195 (Smith 1980; Gibson & Brown 1991). These species were *Campanula glomerata*, *Carex flacca*,  
196 *Filipendula vulgaris*, *Helianthemum nummularium*, *Hippocrepis comosa*, *Pimpinella saxifraga*,  
197 *Stachys officinalis*, *Succisa pratensis* and *Thymus pulegioides*. In addition, the *Bromopsis erecta*,  
198 a signature grass of European calcareous grassland (Smith 1980; Ellenberg 1988) was also

199 included as it was absent from the vegetation. Unlike other species included, *B. erecta* tends to  
200 perform relatively well in restoration (Pywell et al. 2003). Prior to sowing, seeds of two species,  
201 *H. nummularium* and *H. comosa*, were scarified. Sowing density varied with species, ranging  
202 between 30 and 110 seeds m<sup>-2</sup> (Appendix S2). After sowing, sub-plots were rolled with a ring-  
203 roller, and slug pellets (4% metaldehyde) were applied at a rate of 3.5 kg ha<sup>-1</sup>. During the  
204 establishment phase, the whole experiment was uniformly managed by applying a hay-cut at the  
205 end of July 2008, followed by autumn cattle grazing.

#### 206 *Management regimes*

207 From 2009 onwards, three management regimes were applied at main-plot level: (1) summer hay  
208 cut followed by autumn (aftermath) grazing with cattle; (2) spring grazing with sheep, followed  
209 by summer hay cut and autumn grazing with cattle; (3) spring grazing with sheep followed by  
210 summer and autumn grazing with cattle. Grazing was managed to achieve sward height targets of  
211 3-5 cm for sheep grazing, and 5-7 cm for cattle grazing. In 2009, summer grazing was carried out  
212 between mid-June and mid-July, but in later years this was moved to late August / early  
213 September, to encourage flowering of sown species. The summer hay cut was carried out at the  
214 end of July, using a drum mower. On the ridge-and-furrow sub-plots, a strimmer was used to  
215 accommodate the complex topography. All cut material was dried, turned and removed.

216

#### 217 DATA COLLECTION

##### 218 *Plant censuses*

219 Three censuses of sown species were undertaken annually in 2008 and in 2009, and two censuses  
220 in 2010 and in 2011. In all years, censuses were carried out in late spring/early summer and in  
221 late summer/early autumn. In the first two years, an additional census was carried out in mid-

222 summer, to account for potentially more pronounced demographic dynamics expected in the  
223 early stages of species introduction. Censuses in each sub-plot were carried out in twelve 0.5 m ×  
224 0.5 m quadrats arranged in threes along four staggered parallel transects (Appendix S3). In 2008  
225 and 2009, censuses were carried out as plant counts. By 2010, counting individuals was no  
226 longer possible for clonally spreading species, and thus, in 2010 and 2011, we carried out rooted  
227 frequency counts, with each 0.5 m × 0.5 m quadrat subdivided in 9 cells. From 2009 to 2011, in  
228 addition to plant presence, we also recorded whether at least one individual of each of the sown  
229 species in the quadrat had initiated a flowering stem, indicating that plants were not just  
230 surviving but reaching reproductive size.

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

### 233 *Soil parameters*

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

240

## 241 STATISTICAL ANALYSES

### 242 *First-year establishment*

243 To test for disturbance effects on initial establishment in 2008 of each sown species individually  
244 and summed across all specialist forb species (i.e. excluding *B. erecta*), we constructed linear

245 mixed-models, including disturbance as a fixed factor and main plot as random blocking factor.  
246 In case of significance, pairwise comparisons using two-sided Tukey tests were carried out to  
247 identify differences between disturbance treatments. To ensure normality of residuals and  
248 variance homogeneity, all plant count data was square-root-transformed. With one exception, *H.*  
249 *nummularium*, species-level analyses were based on the early September 2008 census, when  
250 seedling numbers were generally highest. *H. nummularium* emerged more rapidly, and by  
251 September, its seedling numbers had started to drop. Analysis for this species was therefore  
252 based on the July census.

253

#### 254 *Species performance in years 2-4 and abiotic trends*

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

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

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

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

286

287 Results

288 SEEDLING ESTABLISHMENT IN RELATION TO DISTURBANCE

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

291 Individually, seven forb species established regularly in at least one disturbance treatment (Fig  
292 1b-i), and so did *B. erecta*. *H. comosa* established very poorly, with just a few seedlings mostly  
293 in ridge-and-furrow sub-plots, and *C. flacca* completely failed to establish. In six species,  
294 establishment was promoted both by harrowing and by ridge-and-furrow creation. In three of  
295 these, *C. glomerata*, *P. saxifraga* and *S. officinalis*, establishment was higher for ridge-and-  
296 furrow creation (Fig. 1b,f,g). In *F. vulgaris*, *H. nummularium* and *T. pulegioides*, establishment  
297 was roughly equally promoted by harrowing and by ridge-and-furrow creation (Fig. 1c,d,i). *H.*  
298 *nummularium* initially established in large numbers in both the harrowed and ridge-and-furrow  
299 treatments, but by the time of the second count in early September 2008, seedling numbers had  
300 already started to decline, compared to the first count in early July 2008. In *S. pratensis* and in  
301 the additionally sown *B. erecta*, establishment was only promoted significantly by ridge-and-  
302 furrow creation (Fig. 1h,j), although for the former, harrowing showed a strong trend (Tukey test  
303 for control vs. harrowing: d.f.=33;  $t = -2.70$ ;  $P = 0.051$ ). Establishment of any species  
304 individually was not promoted by spraying, although a small effect was detected for overall  
305 establishment of specialist forbs (Fig. 1a).

306

#### 307 ABUNDANCE IN YEARS 2-4

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

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

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

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



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

339

#### 340 ABUNDANCE OF REPRODUCTIVE-STAGE PLANTS IN YEARS 2-4

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

350

#### 351 BARE GROUND AND SOIL PARAMETERS

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

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

368

369 Discussion

370 PRE-SOWING DISTURBANCE

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

379 In our study, ridge-and-furrow creation was the only disturbance to retain appreciable  
380 levels of bare ground throughout the study, and to have notable effects on surface-soil chemistry,  
381 by reducing organic matter content and total nitrogen, and slightly increasing pH, through

382 replacing top soil with chalky subsurface soil. Compared to ridge-and-furrow creation,  
383 harrowing led to faster re-colonization by generalist grassland species from extant vegetation,  
384 and triggered transient occurrence mostly in the first year of weed species from the surface-soil  
385 seed bank (M. Wagner, personal observation). Such transient emergence may be common in ex-  
386 arable situations, and was observed in our study in spite of the site having been managed for 15  
387 years as grassland since its reversion from arable use.

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

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

404 dormancy, as seed viability was confirmed in germination experiments (Wagner et al. 2011 and  
405 unpublished).

406

#### 407 MANAGEMENT REGIMES

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

420

#### 421 IMPLICATIONS FOR RESTORATION

422 Creation of micro-topographic features is not currently a part of calcareous-grassland restoration  
423 in the UK or elsewhere in Europe. Similar features have however been tested in North America  
424 in the context of prairie grassland restoration (Biederman & Whisenant 2011; Hough-Snee et al.  
425 2011) and of wetland restoration (Doherty & Zedler 2015), the underlying rationale being that  
426 increased topographic heterogeneity provides a wider range of establishment microsites,

427 resulting in more diverse target vegetation. Reasons for the lack of such features in the ‘toolbox’  
428 for restoring European calcareous grasslands on ex-arable land may include associated labour  
429 and cost and the fact that they may be perceived as problematic at sites potentially containing  
430 archaeological features. Creation of micro-topographic features in calcareous-grassland  
431 restoration may be particularly useful at level sites where topographic variation is largely absent,  
432 but may have to be limited to sites where archaeological features are absent. As our results show,  
433 grazing, in particular by heavy livestock such as cattle, may have to be carefully managed on  
434 such micro-topographic features, to avoid excessive erosion that could negatively affect  
435 restoration outcomes. On the other hand, traditionally, calcareous grasslands have often been  
436 grazed heavily, and more short-lived species of calcareous grassland depend on relatively high  
437 levels of disturbance (Verkaar et al. 1983). Thus, a certain amount of erosion on ridge-and-  
438 furrow structures may likely be beneficial.

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

450 seeding, and, in some instances, to assist with phased introduction of successively more stress-  
451 tolerant species.

452

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462

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624 Supporting Information

625 Additional Supporting Information may be found in the online version

626 of this article.

627 Appendix S1. Photos showing disturbance treatments.

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

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

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

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

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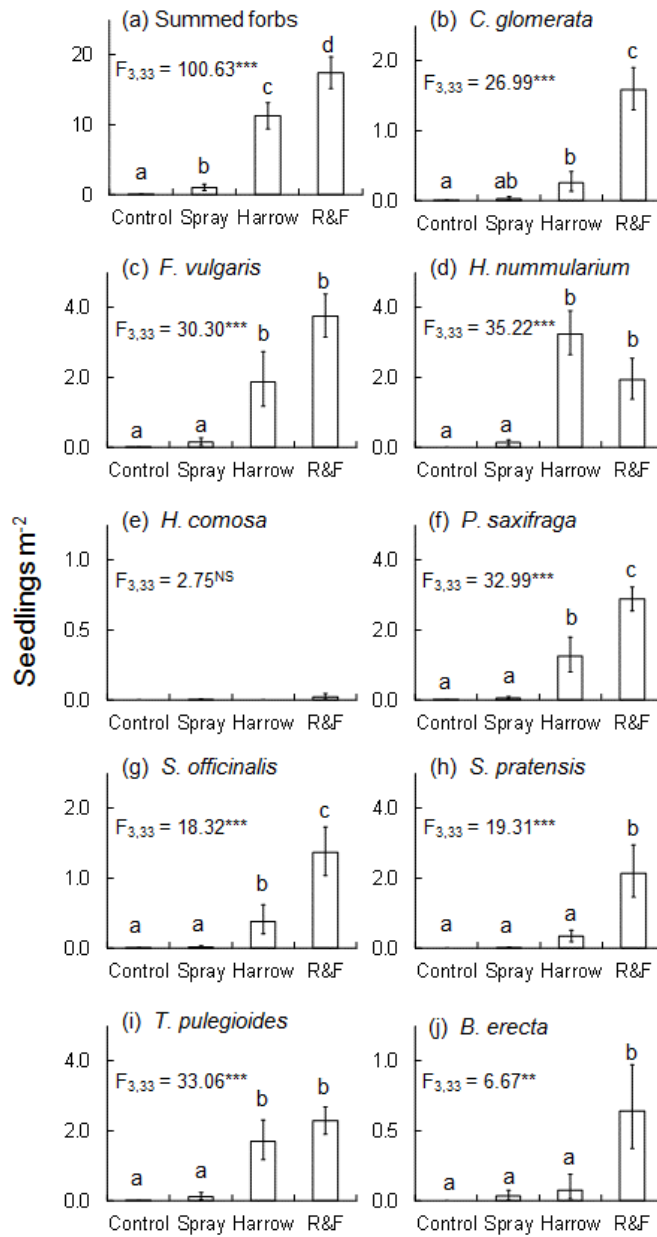


Figure 2.

occurrence in

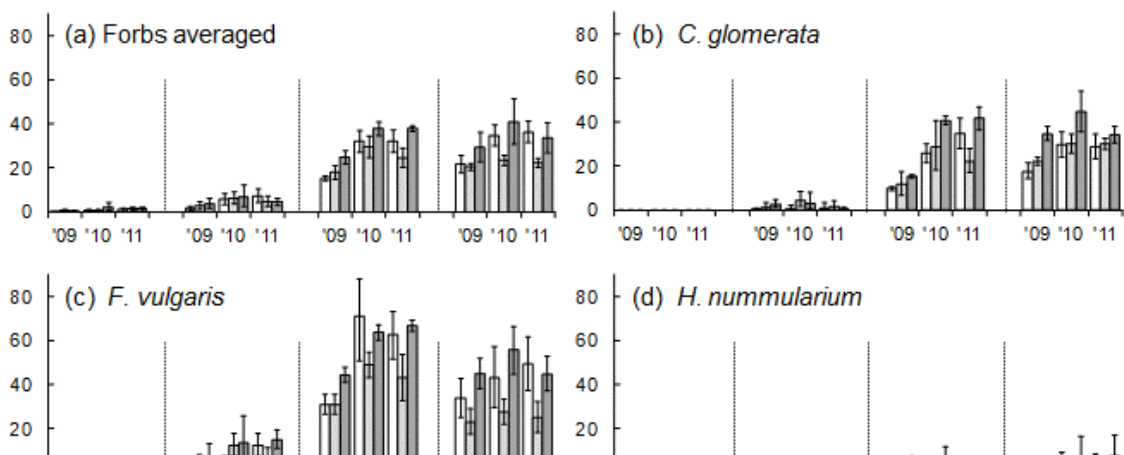
Disturbance treatment

Frequency of

0.5 m × 0.5 m

quadrats from 2009 to 2011 of sown species, and averaged across specialist forbs. Back-

transformed means ±SE.

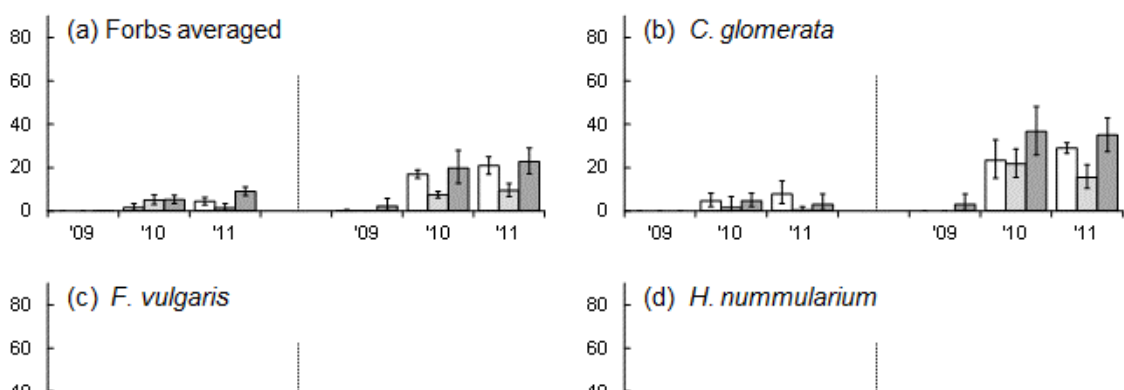




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694 Figure 3. Frequency of occurrence in 0.5 m × 0.5 m quadrats from 2009 to 2011 of reproductive-  
695 stage plants of sown species, and averaged across specialist forbs. Back-transformed means ±SE  
696 for harrowed and ridge-and-furrow (R&F) treatments.

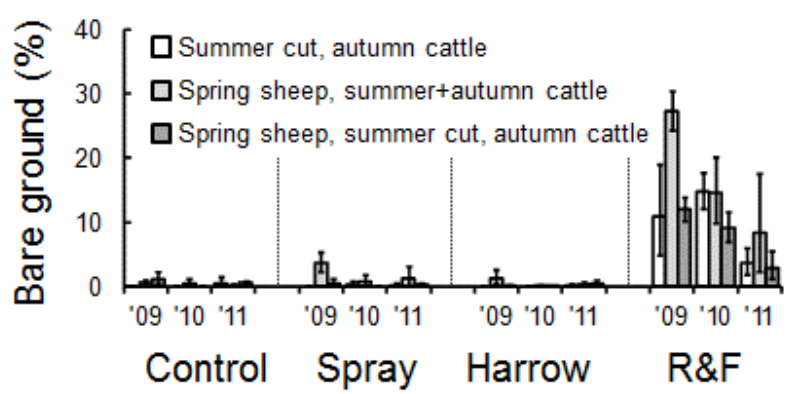
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717 Figure 4. Percentage bare ground from 2009 to 2011, estimated annually in July. Back-  
718 transformed means  $\pm$ SE.

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

Effect		Forbs averaged	<i>C. glomerata</i>	<i>F. vulgaris</i>	<i>H. nummularium</i>	<i>H. comosa</i>	<i>P. saxifraga</i>	<i>S. officinalis</i>	<i>S. pratensis</i>	<i>T. pulegioides</i>	<i>B. erecta</i>	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	

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