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1 **The natural regeneration of calcareous grassland at a landscape scale: 150 years of plant**

2 **community re-assembly on Salisbury Plain, UK**

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10

11 **Abstract**

12 **Questions:** What is the timescale for natural regeneration of calcareous grassland? Is this timescale

13 the same for individual plant species, plant community composition and functional traits?

14 **Location:** Defence Training Estate Salisbury Plain, Wiltshire, UK.

15 **Methods:** We investigated the rate of natural regeneration of species-rich calcareous grassland

16 across a 20 000 hectare landscape. We combined a large scale botanical survey with historic land use

17 data (6 - 150 years before present) and examined differences between grasslands age classes in the

18 occurrence of individual plant species, floristic community composition and community functional

19 traits.

20 **Results:** Many species showed a significant association with grasslands over 100 years in age. These

21 included the majority of those defined elsewhere as calcareous grassland indicators, although some

22 such appeared on grasslands <10 years in age. Community composition showed increasing similarity

23 to the oldest grasslands with increased grassland age, with the exception of very recently ex-

24 agricultural grasslands. Most functional traits showed clear trends with grassland age, with dispersal

25   ability differing most strongly between recent and older grasslands, whilst soil fertility and pH

26   tolerance were more influential over longer timescales.

27   **Conclusions:** Even in a well connected landscape, re-assembly of a community resembling ancient

28   grassland in terms of functional traits and community composition takes over a century, although

29   changes at the level of individual species may occur much earlier. These findings confirm the

30   uniqueness of ancient calcareous grassland. They also suggest that the targets of re-establishment

31   efforts should be adjusted to account for the likely timescale of full community reassembly.

32

33   **Key Words:**

34   Agri environment, chalk grassland, chronosequence, GIS, historic landuse, indicator species,

35   restoration.

36

37   **Nomenclature:** Stace, C. 2010. New Flora of the British Isles. 3rd Edition. Cambridge University Press

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39   **Running head:** Natural regeneration on Salisbury Plain, UK

40   **Introduction**

41   Calcareous grassland has great conservation value across Europe due to its high floral and faunal

42   diversity and large number of associated rare and threatened species (Poschold & WallisDeVries

43   2002). However, calcareous grassland has also undergone one of the most significant declines of

44   any European grassland habitat since the mid-twentieth century. Intensification of agriculture has

45   destroyed or degraded many calcareous grasslands through ploughing, fertilizer input and the

46   sowing of crops (Fuller 1987; Van Dijk 1991; Poschlod & WallisDeVries 2002). Much of what remains

47   is now highly fragmented and vulnerable to further degradation from scrub encroachment (Redhead

48   et al. 2012), over- or under-grazing (Poschlod &WallisDeVries 2002) and the increased risk of species

49   extinction that isolation brings (Matthies et al. 2004). Whilst the maintenance and protection of

50   remnant calcareous grasslands is a vital part of conservation, many countries have also set targets

51   for the re-establishment of calcareous grasslands on areas where the land use is, or has recently

52   been, primarily agricultural (Fagan et al. 2008). Strategies to achieve these targets include the use of

53   options within agri-environment schemes which aim to restore agricultural land to semi-natural

54   grassland, using a variety of techniques, including natural regeneration, reduction of soil fertility and

55   the sowing and management of calcareous grassland species (Walker et al. 2004).

56   If the re-establishment of calcareous grasslands on ex-agricultural land is to be successful (and

57   thus cost effective) it is important to have information on how best to monitor the progress of

58   restoration, how long it is likely to take, and the most effective restoration methods to employ.

59   Whilst a range of studies have contributed towards answering these questions experimentally

60   (Gibson & Brown 1991;Wells et al. 1994; Stevenson et al. 1995; Pywell et al. 2002, 2003; Kiehl et al.

61   2006; Fagan et al. 2008) results have been varied. Some authors have suggested that restoration

62   schemes on ex-agricultural land have yet to prove their effectiveness, given the severe biotic and

63   abiotic constraints imposed on re-assembly by modern agricultural methods (Dobson et al. 1997;

64   Walker et al. 2004). However, many studies have necessarily focussed on the comparatively short

65   timescales over which grassland restoration projects have been carried out, ranging from four years

66 (Pywell et al. 2002) to 60 years (Fagan et al. 2008). Observed rates of regeneration and restoration

67 are also confounded by the small area and comparative isolation of agri-environment sites, which

68 might be expected to act as a constraint by inhibiting colonisation and establishment of calcareous

69 grassland plants (Gibson &Brown 1991; Bullock et al. 2002; Matthies et al. 2004; Butaye et al. 2005;

70 Ozinga et al. 2009).   In addition to this, there is little information on the 'natural' rate of plant

71 community reassembly in landscapes offering good conditions for dispersal, colonisation and

72 establishment (Karlick & Poschlod 2009) despite some authors advocating 'natural regeneration' as

73 an effective strategy (Fagan et al. 2008). This lack of information has often made it difficult to set

74 appropriate targets, which are essential for interpreting the results of restoration efforts (McCoy &

75 Mushinsky 2002; Butaye et al. 2005).

76 Here we define natural regeneration to mean the re-establishment of a calcareous grassland

77 floristic community following the cessation of agricultural practices, in the absence of active

78 restoration management (Fagan et al. 2008). Monitoring the progress of natural regeneration can be

79 achieved using a variety of measures. These include the presence or abundance of a set of

80 calcareous grassland 'indicator' species selected as a proxy for communities of varying degrees of

81 quality (e.g. Robertson & Jefferson 2000). Whilst this approach has the benefit of simplicity and

82 speed, some studies have suggested that indicator species do not necessarily signal more lasting

83 shifts in total species richness, grassland community composition, which should be monitored

84 directly (Gibson & Brown 1991; Zobel et al. 1996; Pywell et al. 2003; Walker et al. 2004; Fagan et al.

85 2008). A further approach is to assess changes in life-history traits of the plant community. Using

86 'functional' traits may give greater insight into the underlying mechanisms which determine

87 community composition under different conditions (Pywell et al. 2003; Marini et al. 2012), especially

88 when analysed in conjunction with data on species composition (Kachergis et al. 2013), and also

89 facilitates predictions about the likely fate of regenerating communities (Kahmen et al. 2002).

90 This study used a variety of response variables, including individual species presence, community

91 composition and life-history traits to investigate plant community reassembly in a naturally

4

92 regenerating grassland landscape over a well characterised 150 year period, with the intention of

93 contributing to a baseline against which to compare the results of restoration experiments.

94 **Methods**

95 STUDY SITE

96 The studied landscape, the Defence Training Estate Salisbury Plain (DTE SP, Wiltshire, UK) is unique

97 in Western Europe in its extent and minimal fragmentation of calcareous grassland (Toynton & Ash

98 2002). The present-day extent of DTE SP is the result of land purchases made by the War Office (now

99 the UK Ministry of Defence), beginning in the late 1890s and spanning the first half of the twentieth

100 century. The restrictions of access imposed by military training and unexploded ordinance have

101 limited the extent of intensive cultivation and grazing. There are thus large areas which have been

102 free from significant agricultural influences (aside from extensive grazing) for over 150 years. There

103 are also widespread areas which were cultivated more recently but then abandoned after military

104 purchase. There are also instances of what was once unimproved grassland (i.e. lacking application

105 of agricultural fertilizers and pesticides) becoming intensively used and managed in the present. All

106 these grassland ages form a largely continuous mosaic, covering around 38 000 hectares in total,

107 such that the DTE may represent a best case scenario for natural regeneration in a favourable

108 landscape.

109   Some 14 000 hectares of DTE SP are currently high quality, species-rich calcareous grassland,

110 mostly variants of CG3 *Bromus erectus* [*Bromopsis erectus*] grassland as defined by the British

111 National Vegetation Classification (NVC, Rodwell 1992). The remainder is typified by other grassland

112 types including MG1 *Arrhenatherum elatius* grasslands with gradations towards improved MG7

113 *Lolium perenne* leys. Current management practices include extensive grazing (for full details see

114 Woodcock et al. 2005). There is wide variation in the present and historic extent and intensity of

115 disturbance from military vehicles (Hirst et al. 2003), burning (Iliffe et al. 2000) and encroachment of

116 scrub species, most notably *Crategaus monogyna*, *Prunus spinosa* and, more locally, *Ulex europaeus*

117   (Redhead et al. 2012). As is common in the analysis of historical data, where many such processes

118   go unrecorded (Sheail 1980), data is lacking on the present and historic extents of these variables.

119   However, there is no evidence of systematic bias in these variables in relation to grassland age on

120   DTE SP.

121   GENERATING A LAND USE HISTORY MAP

122   In the absence of historic records of floral communities we used a chronosequence approach

123   (Pickett 1989; Foster and Tilman 2004) based on historical land use data sets. The extent of

124   unimproved grassland on DTE SP has been mapped on various occasions. Suitable land use data

125   were available for six time periods: 1840s, 1880s, 1930s, 1967, 1985 and 1996. The earliest two

126   datasets were derived from tithe maps and first edition Ordnance Survey maps, respectively (Sheail

127   1980). Data for the 1930s were drawn from the Dudley-Stamp Land Utilisation Survey (Stamp 1931,

128   1948; see Hooftman and Bullock 2011 for a description). The latter datasets were based on three

129   grassland surveys (Wells 1967; Porley 1986; Walker & Pywell 2000). Where maps were not already in

130   digital form each map was georeferenced and digitized. Datasets prior to and including 1930

131   mapped all areas of non-arable grazing land and natural grassland. Post 1930 maps refined this to

132   map only species-rich, non-improved grasslands (i.e. those not showing any signs of agricultural

133   management beyond traditional grazing or hay cutting). Data for the adjacent county of Dorset

134   (Hooftman and Bullock 2011) and for Britain as a whole (Fuller 1987) suggest that all mapped

135   grasslands were agriculturally unimproved prior to the late 1930s. It is important to note that even

136   those grasslands for which there is no evidence of cultivation in this study are likely to have some

137   history of agricultural influence, with archaeological evidence of such up to the post-medieval period

138   (Postan 1973). However, the available historic data is insufficiently spatially explicit to provide a

139   meaningful sample of grasslands uncultivated since medieval times.

140   Land use maps were overlain to form a composite map of land use history from 1840 to 1996 (Figure

141   1). Coverage was limited to the western and central areas of DTE SP, giving a total area of around

142   20 000 Ha with full historic land use data.   The varied land use histories were divided into four

143   categories, the first three of which represent the minimum age of unimproved grassland: Old =

144   grassland since 1840 to 1880 (≈136 years old) , Mid = grassland since 1930-1967 (≈50 years old) ,

145   Recent = grassland since 1985 to 1996 (≈6 years old). The fourth category, Lost, denotes areas which

146   were once unimproved grassland but had been degraded or lost to improvement by 1996, mostly by

147   conversion to re-sown grass leys. Georeferencing, digitising and analysis of digital maps was

148   performed in ArcMAP (v9.3.1 © 2011, ESRI Inc., Redlands, CA, USA)

Fig.1
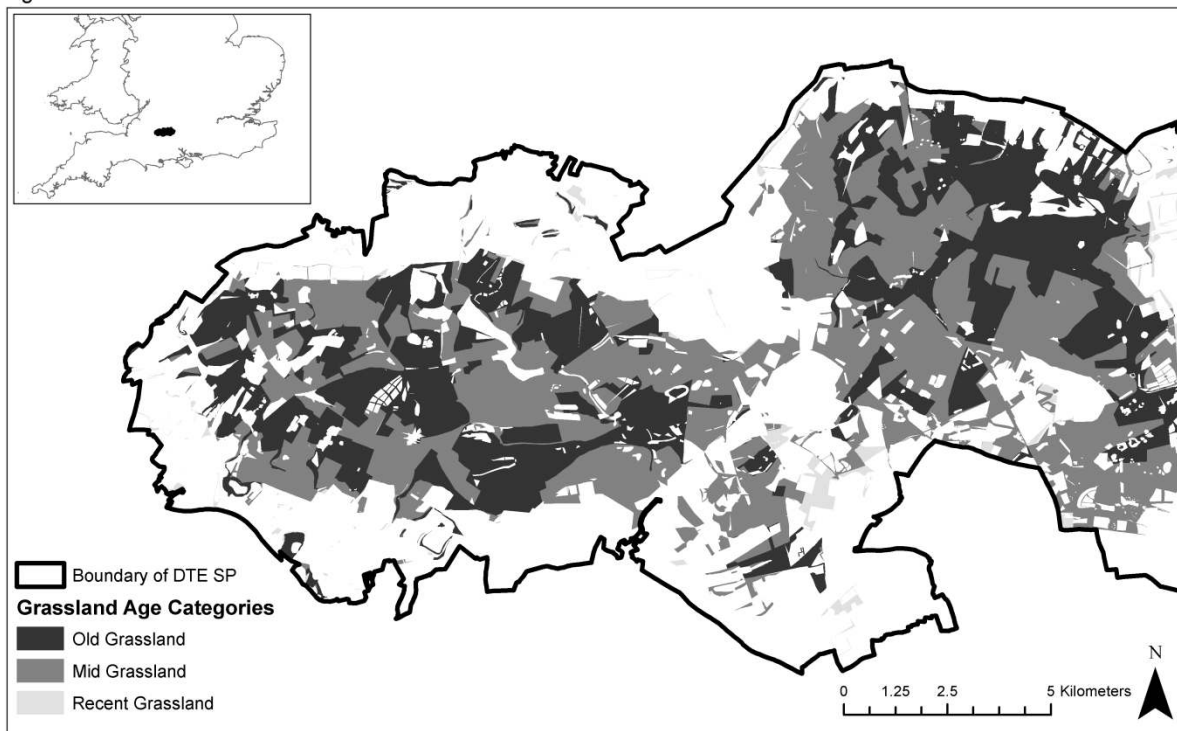
149
150   **Fig.1** Map of estimated grassland age on western and central Defence Training Estate, Salisbury

151   Plain (DTE SP). Derived from overlay of historic landuse maps. Unshaded areas within the DTE are

152   those lost to improvement, lacking historic data or those which are not grassland (forest, built up,

153   etc). Inset map shows location of DTE SP in southern UK.

154   FLORISTIC DATA

155   Between 1996 and 1997 all vegetation communities present on DTE SP were mapped in the field and

156   their composition described using the NVC methodology (Rodwell 1992). For each discrete

157   community the cover of vascular plant species was recorded from 2 × 2 m quadrats to aid

7

158   assignation to an NVC community (Walker & Pywell 2000). However, these quadrats were limited in

159   number and thus did not give an accurate record of total species richness or occurrence of all

160   species. Thus an estimate of abundance for all plants present was made from a walk-over survey,

161   using the DAFOR scale (Kershaw 1985). The mapped plant communities were captured as digital

162   polygons and overlain with the land use map. As NVC polygons and land use history polygons did

163   not overlap exactly, analyses were restricted to NVC polygons over one hectare in area and having at

164   least 75% overlap with a single land use history polygon, giving 1352 polygons for analysis (484 Old,

165   658 Mid, 101 Recent, 109 Lost). Lists of species presence, DAFOR abundance and associated floral

166   life-history traits were compiled for each polygon. Traits were selected on the basis of having

167   information readily available for the majority of species present in the study, and of having a clear

168   potential to characterise ecological function, namely the ability of a species to colonise, compete

169   and persist in the landscape concerned (functional traits, Violle et al. 2007).   Traits were measured

170   on either a continuous scale (e.g. height) or as ordinal 'scores' (e.g. dispersal ability, seedbank

171   persistence). Colonisation ability was represented by a dispersal ability score. Traits reflecting

172   persistence ability included seedbank longevity, clonal spread and plant longevity. Traits

173   representing competitive ability were leaf area and typical summer plant height. Also included in

174   the analyses were measures of ecological performance which, whilst arguably not traits *sensu* Violle

175   et al. (2007), are meaningful proxies for multiple traits which affect the ability of species to survive in

176   the conditions of recently ex-agricultural land (Pywell et al. 2003). These were Ellenberg (Ellenberg

177   et al. 1991) tolerance values for light, soil reactivity (expressed as difference from neutral to correct

178   for skew towards the acid range, Preston et al. 2003) and soil fertility. Descriptions of all these traits,

179   including values and sources, are given in the Supplementary Material (Table S1).

180   STATISTICAL ANALYSIS

181   To investigate how individual species presence related to grassland age, Chi-squared tests were used

182   to examine whether presence fitted a null hypothesis of distribution at random across age

183   categories. In order for the assumptions of the test to be met, if a species generated expected

184 values of less than five in any category, the Recent and Lost categories were combined. If expected

185 values remained below five, the species was excluded from analysis. Species showing significant (p <

186 0.05) results were grouped according to which land use history categories showed higher than

187 expected values and contributed the greatest proportion of the significant Chi-squared statistic.

188 Although conducting a large number of independent tests we did not apply a sequential Bonferroni

189 correction but instead examined effect sizes and whether the observed differences were ecologically

190 explicable, following Moran (2003).

191   Differences in grassland community composition between NVC polygons were analysed by

192 calculating Euclidean distances (*ED*) for all pairwise comparisons. Euclidean distance is defined as:

193
$$ED_{jk} = \sqrt{\sum \left(X_{ij} - X_{ik} \right)^2}$$
(Equation 1)

194   where ED_{jk} = Euclidean distance between samples *j* and *k*; X_{ij} = abundance of individuals of species *i*

195 in sample *j*; X_{ik} = abundance of individuals of species *i* in sample *k* (Krebs 1999).

196   The DAFOR scale on which species were recorded across the NVC polygon was converted to a

197 numeric representation (Dominant = 5, Abundant = 4, Frequent = 3, Occasional = 2, Rare = 1, Present

198 = 0.1) to provide a measure of relative abundance. Differences in *ED* between the different age

199 categories were then analysed by one-way Analysis of Variance (ANOVA).

200   The weighted mean of each trait was calculated for each NVC polygon, with weightings derived

201 from the numeric representation of the DAFOR scale. Differences in traits between grassland age

202 categories were analysed using analyses of covariance (ANCOVA). Two covariates were included.

203 Firstly, the area of the NVC polygon, in order to account for the potential for larger polygons to

204 support more, and rarer, species (Matthies et al. 2004). Secondly, the proximity of each NVC

205 polygon to the nearest old grassland, as distance from established calcareous grassland has

206 previously been found to strongly affect the rate of community reassembly (Matthies et al. 2004,

207 Fagan et al. 2008).   Variables giving significant results from ANCOVA were then included in a

208 Discriminant Function Analysis (DFA), which classified grasslands to age class using the linear

209 combinations of traits (discriminant functions, DFs) which best differentiate between categories.

210 Analyses were performed in R (R Development Core Team 2008. R: A language and environment for

211 statistical computing. R Foundation for Statistical Computing,Vienna, Austria) utilising the MASS

212 package (Venables, W.N. & Ripley, B.D. 2002. Modern Applied Statistics with S, 4th ed. Springer,

213 New York).

214 **Results**

215 SPECIES PRESENCE DATA

216 Of over 450 plant species recorded on DTE SP in the NVC survey, 193 species met the criteria for

217 analysis, and 77% of these (149 species) showed a significant (p <0.05) associations with grassland

218 age-classes (Table 1; full species lists are available in Supplementary Material, Table S2). For eight

219 species, small effect sizes or ecologically inexplicable results indicated a possible Type I error, the

220 relationship with grassland age was designated as 'unclear' and the results are not reported further.

221 **Table 1.** Presence of individual species in response to grassland age category. The number of species

222 showing higher than expected presence for each combination of classes, as determined by Chi

223 squared analysis are given along with the associated approximate age range in years.

| Grassland Age Classes	Species	Age of Semi-natural Grassland
Old	48	> 100 years
Old and Mid	11	> 60 years
Mid	26	30-60 years
Mid and Recent	11	< 60 years
Mid, Recent and Lost	8	< 100 years
Recent	7	< 10 years
Recent and Lost	22	< 10 and improved/degraded
Lost	5	Improved/degraded

224

225   Forty-eight species showed a significant association with unimproved grasslands over 100 years

226   in age (Table 1). These included some species entirely restricted to ancient chalk and limestone

227   grassland in the UK (e.g. *Campanula glomerata*, *Carex humilis*, *Picris heiracoides*, *Thesium*

228   *humifusum*) but the majority are confined to species-rich communities on infertile soils across a

229   broader pH range (e.g. *Anthyllis vulneraria, Filipendula vulgaris, Poterium sanguisorba, Serratula*

230   *tinctoria*). This group also included species more often associated with disturbed ground (e.g.

231   *Myosotis arvensis, Reseda lutea, Sinapis arvensis, Sonchus asper*). Species associated with both old

232   and mid age grasslands included many typical chalk downland species (e.g. *Asperula cynanchica,*

233   *Cirsium acaule, Viola hirta*) whereas those associated with mid or mid and recent age grasslands

234   included many more species that occur across a broader range of ecological conditions and habitats

235   (e.g. *Centaurea scabiosa, Cynosurus cristatus, Lathyrus pratensis, Leontodon hispidus*) or more

236   disturbed conditions on calcareous soils (e.g. *Chaenorhinum minus, Linum bienne, Onobrychis*

237   *viciifolia, Senecio erucifolius*). Species associated with recent, recent and lost or lost grasslands were

238   almost entirely species of eutrophic, often agricultural, habitats, including intensively managed

239   arable land (e.g. *Anisantha sterilis, Convolvulus arvensis, Galium aparine,, Papaver rhoeas, Veronica*

240   *persica*) or improved pasture (e.g. *Cirsium* spp., *Rumex* spp., *Taraxacum* spp.).

241   Of particular interest are the species designated as indicators of calcareous grassland condition

242   by Robertson & Jefferson (2000), from degraded (negative indicators) to good (positive indicators).

243   All six negative indicator species (*Cirsium arvense*, *Cirsium vulgare*, *Rumex crispus*, *Rumex*

244   *obtusifolius, Senecio jacobaea*, *Urtica dioica*) were significantly more common on recent grasslands

245   and grasslands lost to improvement. Of 24 positive indicator species with sufficient sample size for

246   analysis, 13 were associated with old grasslands and a further four with both old and medium age

247   grasslands (Supplementary Material S2).

248   GRASSLAND COMMUNITY COMPOSITION

249   The analysis of *ED* between plant communities (Fig. 2) showed that differences showed generally

250   clear trends along the age gradient (a larger *ED* indicates a bigger difference in community

251   composition). This was indicated by increasing *ED* when comparing old grasslands against

252   increasingly younger sites (one-way ANOVA, F = 1777.7 p <0.001); and decreasing *ED* when

253   comparing lost grasslands against grasslands of increasing age (one-way ANOVA, F = 2671.2, p

254   <0.001).

Fig.2

255
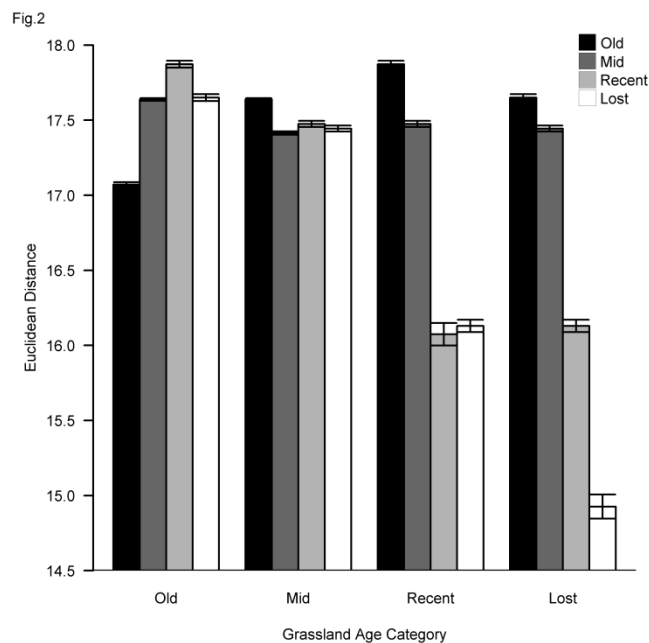
256   **Fig.2** Bar plot of mean Euclidean distance between grassland communities of four different age

257   categories. Also shown are mean Euclidean distances between grassland communities within the

258   same age category. Age classes are arranged on the x-axis in order of decreasing grassland age.

259   Capped lines represent ± one standard error.

260   Differences were greatest overall between the old and recent categories, with mid age grasslands

261   roughly equidistant between the two. The values of *ED* within each age class indicated variation

262   among communities of the same age, a measure of β diversity (Newton et al. 2012). For such

263   comparisons within age classes, mid-age grasslands showed the highest within-class *ED*, followed by

264   old, then recent and lost grasslands (Fig. 2). This suggests variation in community composition

265   increases from the early to middle stages of reversion and then decreases somewhat as grasslands

266 age. Communities within the lost grassland category showed the lowest mean *ED* value, suggesting

267 that grasslands lost to agricultural improvement have communities which are comparatively

268 homogenous across DTE SP.

269 COMMUNITY FUNCTIONAL TRAITS

270 The grassland age categories showed significant differences in all nine traits (p < 0.01), with many

271 traits also showing a clear trend with grassland age (Fig. 3). Even in this landscape of virtually

272 continuous grassland, many traits also varied with the two covariates of polygon area and isolation

273 (Table 2).

274 **Table 2.** Results of analyses of covariance (ANCOVA) for 9 plant functional traits. Polygon area and

275 distance from nearest ancient grassland (Isolation) are covariates and grassland age category is the

276 factor. Also shown are standardised coefficients from discriminant function analysis (DFA), for each

277 of the three discriminant functions (DFs).

Trait	Isolation			Polygon Area			Age Category			Discriminant Functions		
	F	p		F	p		F	p		DF1	DF2	DF3
Ellenberg Fertility	90.376	<0.001	**	13.454	<0.001	**	72.478	<0.001	**	-1.183	2.221	1.479
Height	4.970	0.026	*	5.167	0.023	*	15.135	<0.001	**	0.895	0.200	-1.075
Dispersal Ability	87.049	<0.001	**	18.742	<0.001	**	110.175	<0.001	**	-0.551	-0.410	-1.124
Ellenberg Light	2.354	0.125		19.442	<0.001	**	6.448	<0.001	**	-0.418	-0.283	0.218
Leaf Area	76.514	<0.001	**	17.861	<0.001	**	33.125	<0.001	**	-0.367	-0.457	0.437
Ellenberg Difference from Neutrality	47.783	<0.001	**	15.647	<0.001	**	46.076	<0.001	**	-0.315	0.159	0.434
Clonal Spread	28.891	<0.001	**	24.062	<0.001	**	27.160	<0.001	**	-0.118	-1.063	0.675
Seedbank Longevity	2.380	0.123		1.089	0.297		40.149	<0.001	**	-0.099	-0.720	-0.451
Plant Longevity	0.834	0.361		22.158	<0.001	**	16.574	<0.001	**	0.046	0.241	-0.611

[a] * Denotes significance at p = 0.05, ** Denotes significance at p = 0.01

278   Dispersal ability, Ellenberg fertility score, seedbank longevity and clonal spread all declined with

279   increasing grassland age. Soil pH showed the reverse trend, with older grasslands supporting species

280   which prefer soils further from neutral pH.   Other traits showed a trend across the three grassland

281   ages but had intermediate values on lost grassland (plant height, leaf area) or values which were

282   notably high or low for a single category (high plant longevity on old grasslands, low light tolerance

283   on recent grasslands).
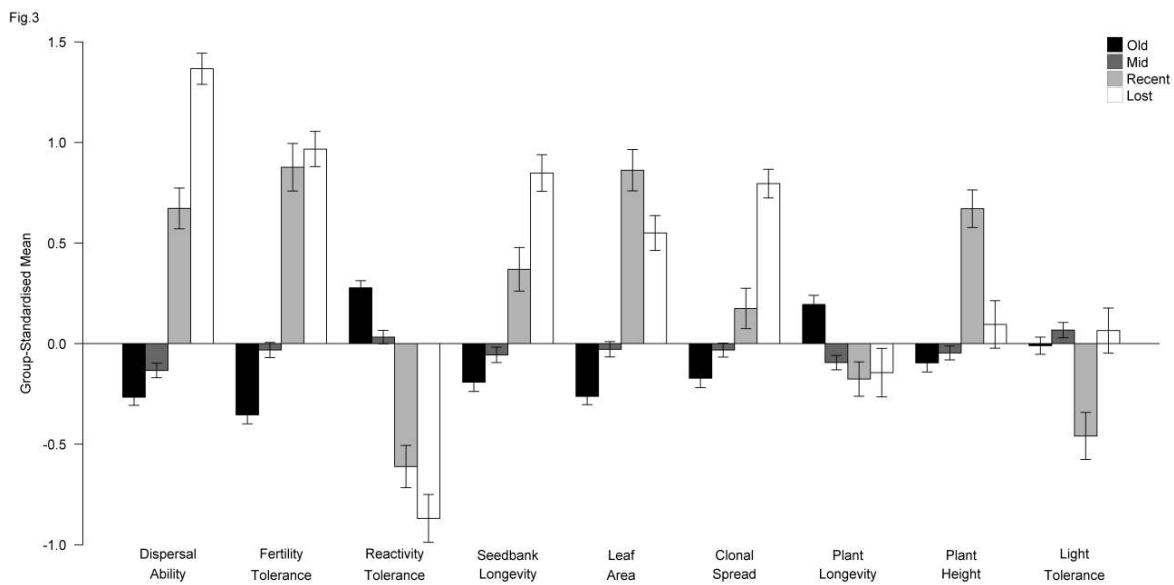
284
285   **Fig.3** Bar plot of group-standardised means of plant functional traits between communities occurring

286   on grassland of four different ages classes. Capped lines represent ± one standard error. Traits are

287   weighted by approximate plant abundance (0.1 = present, 1 = rare, 2 = occasional, 3 = frequent, 4=

288   abundant, 5 = dominant) and scaled for display on the same axis. Traits are ordered along the x axis

289   in order of increasing differentiation between classes, according to the results of ANCOVA on each

290   trait with area and isolation as covariates.

291   Discriminant function analysis gave a classification accuracy (proportion classified correctly) of

292   0.614, although classification accuracy varied between age classes (Table 3). The most common

293   misclassifications (as proportions of classifications made) assigned mid age grassland to the old or

294   recent classes, or recent grassland to the lost or mid age classes (Table 3).

14

295   **Table 3.** Results from classification into grassland age categories by discriminant function analysis

296   (DFA) based on 15 plant functional traits. Values shown are proportion of true class assigned to each

297   class by DFA. Grey shaded values indicate correct assignments to class.

		True Class			
		Old	Medium	Recent	Lost
Assigned Class	Old	0.698	0.196	0.030	0.174
	Medium	0.153	0.574	0.253	0.110
	Recent	0.087	0.157	0.475	0.110
	Lost	0.062	0.073	0.242	0.606

298   Most separation between groups was achieved by the first two discriminant functions, which

299   together accounted for 0.804 of between class variance. Using the standardised coefficients (Table

300   2) and differences between the group standardised means (Fig. 4), DF1 was most influenced by

301   dispersal ability and fertility tolerance (which distinguish well between old-mid grasslands and

302   recent-lost grasslands), along with plant height (which distinguishes recent from all other

303   grasslands). DF2 was influenced by fertility tolerance, clonal spread and seedbank longevity, which

304   further distinguish old and lost grasslands from mid and recent grasslands respectively. Thus older

305   grasslands tend to be characterised by immobile, perennial species with transient seedbanks and

306   low clonal spread, whilst more recent or improved grasslands are dominated by annuals with high

307   mobility, both in terms of dispersal and clonal spread, and high competitive ability. The species of

308   old grassland are also intolerant of high soil fertility and prefer a more basic soil pH. Recent

309   grasslands also differ from old, mid and lost grasslands in supporting more tall, shade tolerant

310   species.

311   **Discussion**

312   Many previous studies have documented changes in plant communities over several decades

313   following reversion from arable to grassland on calcareous soils (Wells et al. 1976; Gibson & Brown

314   1991; Walker et al. 2004; Fagan et al. 2008; Karlik & Poschlod 2009). The results of this study

315 strongly suggest that even within an extensive, unfragmented chalk grassland landscape, natural

316 regeneration continues to alter the plant community over more than 100 years in a manner

317 detectable at the species, community and functional trait levels.

318 INDIVIDUAL SPECIES AND COMMUNITY COMPOSITION

319 The oldest grasslands were highly distinctive at a species level, with a large proportion of distinctive

320 species, and in composition, with the highest mean *ED* from any other age class. The majority of

321 species associated with the oldest grasslands have a well known affinity with long established chalk

322 grasslands (e.g. *Carex humilis*, *Cirsium tuberosum*, *Thesium humifusum*) or that are slow to colonise

323 restored sites (e.g. *Succisa pratensis,* Herben et al. 2006). Interestingly the eight species which were

324 less abundant in old grasslands than in all other age categories (*Bellis perennis, Convolvulus arvensis,*

325 *Elytrigia repens, Lolium perenne, Taraxacum agg., Orobanche minor* and its host *Trifolium repens,*

326 *Sisymbrium officinale*) are all species of sown leys. Either the oldest grasslands are unsuitable for

327 these species or it takes up to 100 years for these species to 'die out' on previously agricultural sites.

328 Alternatively, the oldest grasslands may be the only sites where some of these species have not

329 been deliberately introduced, as *Lolium perenne* and *Trifolium repens* are known to have been were

330 sown prior to land abandonment during the agricultural depression of the late 1870s, in the hope

331 that this would speed conversion into productive pasture (Royal Commission on Agriculture 1894).

332 Other species associated with mid age grasslands (e.g. *Cynosurus cristatus, Onobrychis viciifolia*) are

333 also likely to be indicators of an agricultural history, where the agriculture concerned occurred 50 to

334 150 years ago and involved little or no introduction of artificial fertilisers, as has been demonstrated

335 in other landscapes (Karlik &Poschlod 2009).

336   The occurrence of many species shared between mid age and old grasslands suggests that natural

337 regeneration can make substantial progress towards an ancient grassland assemblage within 50

338 years. However, the high *ED* among grasslands within the mid age class also suggests that after 50

339 years grasslands vary widely. The rate of community re-assembly in its early to mid stages is likely to

340   be highly variable and heavily dependent upon stochastic processes such as local grazing pressure,

341   soil disturbance, fertility and seed dispersal (Gibson & Brown 1991; Matthies et al. 2004; Butaye et

342   al. 2005).

343   It is clear that some calcareous grassland species can colonise ex-arable land in less than 10 years,

344   as shown by the few species which were more common in both recent and mid age grasslands than

345   lost grasslands (e.g. *Orobanche elatior* and its host *Centaurea scabiosa, Leontodon hispidus*).

346   Although other studies have demonstrated similar increases in some calcareous grassland indicators

347   within ten years (Zobel et al. 1996; Pywell et al. 2002; Fagan et al. 2008), these are comparatively

348   minor changes compared with re-assembly of an entire ancient grassland community. Such findings

349   most likely demonstrate the rapid colonisation ability of a small subset of calcareous grassland

350   species with good dispersal abilities or persistent seedbanks (Zobel et al. 1996; Matthies et al. 2004)

351   and the very depauperate nature of control sites such as arable fields (Pywell et al. 2002; Fagan et al.

352   2008; Walker et al. 2004) or scrub forest (Zobel et al. 1996) to which restoration trajectories are

353   often compared. Indeed, most species of recent grasslands in this study (e.g. *Anthriscus sylvestris,*

354   *Arrhenatherum eliatus, Galium album, Heracleum sphondyllium*) were more typical of tall

355   unmanaged mesotrophic grassland, characteristic of roadside verges and neglected agricultural land,

356   as well as ex-arable areas up to 50 years in age on other dry, calcareous grasslands (Wells et al.

357   1976; Karlik & Poschlod 2009). Several studies have interpreted such findings as a tendency of

358   restored calcareous grassland to 'stall' at a stable but relatively species-poor stage (Pywell et al.

359   2002, 2003; Fagan et al. 2008). From this study it is apparent that even in an extensive, well-

360   connected landscape, such species are prevalent for up to 50 years.

361   The results from the species and community data generally concur, lending support to the notion

362   of using groups of species as indicators of trajectories towards (or away from) communities

363   resembling ancient grassland. However, great care must be taken in selecting suitable species.

364   Whilst many positive indicators (*sensu* Robertson & Jefferson 2002) did appear to have a genuine

365   affinity for the oldest grasslands, several appeared to colonise ex-agricultural sites comparatively

366 quickly or even showed no response to grassland age. These latter species (e.g. *Bromopsis erectus,*

367 *Galium verum, Leontodon saxatilis, Lotus corniculatus*) have also been shown to be ubiquitous across

368 other grassland landscapes (Wells et al. 1976; Karlik & Poschlod 2009). Where connectivity is high (as

369 on DTE SP), these species are likely to be able colonise relatively rapidly and have some ability to

370 persist even on degraded or improved grasslands, with the result that they were amongst the

371 commonest species in the dataset, present in over 45% of all polygons analysed. Negative indicators

372 appeared successful in detecting either recently re-established or agriculturally improved grassland,

373 but whilst their presence on calcareous grassland may be a reliable indicator of degradation their

374 absence is not necessarily indicative of long established or good quality calcareous grassland. Even

375 the rarest species are unlikely to be responding directly to grassland age. Wells et al. (1976) found

376 *Thesium humifusum* to be associated not just with grassland over 100 years in age but particularly

377 with remnants of grassland on ancient banks and tracks. *Carex humilis,* although associated with the

378 oldest grasslands in this study, can rapidly colonise suitable areas created by anthropogenic

379 disturbance, such as gardens and road verges within modern housing estates built on chalk

380 downland (Wells et al. 1976; K.J. Walker, pers. obs.). Thus regarding the presence or absence of any

381 one species as a certain indicator of ancient grassland is inadvisable.

382 FUNCTIONAL TRAITS

383 Even on DTE SP, an extensive landscape of well connected grasslands, with a high potential for seed

384 movement by grazing livestock and military vehicles, dispersal ability remained highly influential in

385 separating grassland communities of different ages. This suggests that many species of ancient

386 grasslands are severely limited in dispersal ability, and thus have a very low probability of reaching

387 isolated sites unaided (Pywell et al. 2003; Violle et al. 2007; Ozinga et al. 2009; Marini et al. 2012).

388   Previous studies have demonstrated that traits determining colonisation ability are most

389 influential at the earlier stages of grassland regeneration on ex-arable land, with traits associated

390 with tolerance of local conditions becoming increasingly important over time (Thompson et al. 2001;

391   Pywell et al. 2003). Consequently, the greatest differences in dispersal ability were between lost,

392   recent and mid age grasslands. Between mid age and old grasslands however, the species

393   assemblage shows greater differences in traits relating to tolerance of local conditions (i.e. Ellenberg

394   fertility and soil pH), which in turn lead to changes in competitive ability (as seen by decreases in leaf

395   area and plant height) as conditions allow stress tolerant but less competitive species to establish.

396   Such species tend to favour stable conditions allowing a long lifespan, unlike the species which typify

397   lost and recent grasslands, which have shorter lifespans but can establish rapidly and compete well

398   under high nutrient levels (Pywell et al. 2003; Fagan et al. 2008). Thus, the slow decline in soil

399   fertility and associated rise in soil pH (McClean et al. 2011) after cessation of intensive agriculture

400   are likely to be the major drivers of changes in the plant community over longer timescales (Pywell

401   et al. 2003;Walker & Preston 2006; Fagan et al. 2008, Walker et al. 2009; Ozinga et al. 2009;

402   McClean et al. 2011).

403   IMPLICATIONS FOR CONSERVATION AND RESTORATION OF CHALK GRASSLAND

404   That community reassembly in a well connected, expansive and predominantly unimproved

405   landscape such as DTE SP takes over a century confirms the high conservation value of existing

406   ancient grasslands. They support uniquely large and diverse communities and, moreover, are

407   difficult if not impossible to replace once lost. Regeneration following cessation of agricultural use

408   appears to progress comparatively rapidly (<10 years) when measured at the species level, with

409   recruitment of those species which good dispersers can take advantage of reductions in soil fertility.

410   Increases in diversity and species richness are therefore rapidly achievable if the site and subsequent

411   restoration management techniques are carefully selected. However, it takes considerably longer

412   (up to 100 years) before community composition and traits begin to show a consistent similarity to

413   ancient grassland (Zobel et al. 1996; Pywell et al. 2003; Walker et al. 2004). This supports previous

414   assertions that small scale restoration projects, such as agri-environment re-establishment, are

415   unlikely to achieve the complete re-assembly of ancient types via natural regeneration within human

416   lifespans (Gibson & Brown 1991, Walker et al. 2004). Consequently, there is clearly a need for more

417   realistic targets and measures of restoration success, as well as incentives to promote management

418   techniques such as species introduction by seed which might accelerate regeneration, especially

419   given the apparently strong limiting effect of dispersal ability in the first 10 – 50 years. Where such

420   restoration management produces detectable similarities to ancient grasslands in community or trait

421   measures this may well indicate that regeneration has been enhanced beyond the rate of natural

422   regeneration, and potentially represents the start of a trajectory towards an ancient grassland

423   community. Although many management techniques remain contentious and have given mixed

424   results in experimental trials (Pywell et al. 2002; Kiehl et al. 2006; Fagan et al. 2008) the growing

425   body of evidence, including this study, should contribute to informing the design and

426   implementation of restoration management methods, and the monitoring of their effects.

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