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The natural regeneration of calcareous grassland at a landscape scale: 150 years of plant community re-assembly on Salisbury Plain, UK

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Abstract

Questions: What is the timescale for natural regeneration of calcareous grassland? Is this timescale the same for individual plant species, plant community composition and functional traits?

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

Methods: We investigated the rate of natural regeneration of species-rich calcareous grassland across a 20 000 hectare landscape. We combined a large scale botanical survey with historic land use data (6 - 150 years before present) and examined differences between grasslands age classes in the occurrence of individual plant species, floristic community composition and community functional traits.

Results: Many species showed a significant association with grasslands over 100 years in age. These included the majority of those defined elsewhere as calcareous grassland indicators, although some such appeared on grasslands <10 years in age. Community composition showed increasing similarity to the oldest grasslands with increased grassland age, with the exception of very recently ex-agricultural grasslands. Most functional traits showed clear trends with grassland age, with dispersal ability differing most strongly between recent and older grasslands, whilst soil fertility and pH tolerance were more influential over longer timescales.

Conclusions: Even in a well connected landscape, re-assembly of a community resembling ancient grassland in terms of functional traits and community composition takes over a century, although changes at the level of individual species may occur much earlier. These findings confirm the uniqueness of ancient calcareous grassland. They also suggest that the targets of re-establishment efforts should be adjusted to account for the likely timescale of full community reassembly.

Key Words:

Agri environment, chalk grassland, chronosequence, GIS, historic landuse, indicator species, restoration.

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

Running head: Natural regeneration on Salisbury Plain, UK

Introduction

Calcareous grassland has great conservation value across Europe due to its high floral and faunal diversity and large number of associated rare and threatened species (Poschold & WallisDeVries 2002). However, calcareous grassland has also undergone one of the most significant declines of any European grassland habitat since the mid-twentieth century. Intensification of agriculture has destroyed or degraded many calcareous grasslands through ploughing, fertilizer input and the sowing of crops (Fuller 1987; Van Dijk 1991; Poschlod & WallisDeVries 2002). Much of what remains is now highly fragmented and vulnerable to further degradation from scrub encroachment (Redhead et al. 2012), over- or under-grazing (Poschlod &WallisDeVries 2002) and the increased risk of species extinction that isolation brings (Matthies et al. 2004). Whilst the maintenance and protection of remnant calcareous grasslands is a vital part of conservation, many countries have also set targets for the re-establishment of calcareous grasslands on areas where the land use is, or has recently been, primarily agricultural (Fagan et al. 2008). Strategies to achieve these targets include the use of options within agri-environment schemes which aim to restore agricultural land to semi-natural grassland, using a variety of techniques, including natural regeneration, reduction of soil fertility and the sowing and management of calcareous grassland species (Walker et al. 2004).

If the re-establishment of calcareous grasslands on ex-agricultural land is to be successful (and thus cost effective) it is important to have information on how best to monitor the progress of restoration, how long it is likely to take, and the most effective restoration methods to employ. Whilst a range of studies have contributed towards answering these questions experimentally (Gibson & Brown 1991;Wells et al. 1994; Stevenson et al. 1995; Pywell et al. 2002, 2003; Kiehl et al. 2006; Fagan et al. 2008) results have been varied. Some authors have suggested that restoration schemes on ex-agricultural land have yet to prove their effectiveness, given the severe biotic and abiotic constraints imposed on re-assembly by modern agricultural methods (Dobson et al. 1997; Walker et al. 2004). However, many studies have necessarily focussed on the comparatively short timescales over which grassland restoration projects have been carried out, ranging from four years

(Pywell et al. 2002) to 60 years (Fagan et al. 2008). Observed rates of regeneration and restoration are also confounded by the small area and comparative isolation of agri-environment sites, which might be expected to act as a constraint by inhibiting colonisation and establishment of calcareous grassland plants (Gibson &Brown 1991; Bullock et al. 2002; Matthies et al. 2004; Butaye et al. 2005; Ozinga et al. 2009). In addition to this, there is little information on the 'natural' rate of plant community reassembly in landscapes offering good conditions for dispersal, colonisation and establishment (Karlick & Poschlod 2009) despite some authors advocating 'natural regeneration' as an effective strategy (Fagan et al. 2008). This lack of information has often made it difficult to set appropriate targets, which are essential for interpreting the results of restoration efforts (McCoy & Mushinsky 2002; Butaye et al. 2005).

Here we define natural regeneration to mean the re-establishment of a calcareous grassland floristic community following the cessation of agricultural practices, in the absence of active restoration management (Fagan et al. 2008). Monitoring the progress of natural regeneration can be achieved using a variety of measures. These include the presence or abundance of a set of calcareous grassland 'indicator' species selected as a proxy for communities of varying degrees of quality (e.g. Robertson & Jefferson 2000). Whilst this approach has the benefit of simplicity and speed, some studies have suggested that indicator species do not necessarily signal more lasting shifts in total species richness, grassland community composition, which should be monitored directly (Gibson & Brown 1991; Zobel et al. 1996; Pywell et al. 2003; Walker et al. 2004; Fagan et al. 2008). A further approach is to assess changes in life-history traits of the plant community. Using 'functional' traits may give greater insight into the underlying mechanisms which determine community composition under different conditions (Pywell et al. 2003; Marini et al. 2012), especially when analysed in conjunction with data on species composition (Kachergis et al. 2013), and also facilitates predictions about the likely fate of regenerating communities (Kahmen et al. 2002).

This study used a variety of response variables, including individual species presence, community composition and life-history traits to investigate plant community reassembly in a naturally regenerating grassland landscape over a well characterised 150 year period, with the intention of contributing to a baseline against which to compare the results of restoration experiments.

Methods

Study Site

The studied landscape, the Defence Training Estate Salisbury Plain (DTE SP, Wiltshire, UK) is unique in Western Europe in its extent and minimal fragmentation of calcareous grassland (Toynton & Ash 2002). The present-day extent of DTE SP is the result of land purchases made by the War Office (now the UK Ministry of Defence), beginning in the late 1890s and spanning the first half of the twentieth century. The restrictions of access imposed by military training and unexploded ordinance have limited the extent of intensive cultivation and grazing. There are thus large areas which have been free from significant agricultural influences (aside from extensive grazing) for over 150 years. There are also widespread areas which were cultivated more recently but then abandoned after military purchase. There are also instances of what was once unimproved grassland (i.e. lacking application of agricultural fertilizers and pesticides) becoming intensively used and managed in the present. All these grassland ages form a largely continuous mosaic, covering around 38 000 hectares in total, such that the DTE may represent a best case scenario for natural regeneration in a favourable landscape.

Some 14 000 hectares of DTE SP are currently high quality, species-rich calcareous grassland, mostly variants of CG3 *Bromus erectus* [*Bromopsis erectus*] grassland as defined by the British National Vegetation Classification (NVC, Rodwell 1992). The remainder is typified by other grassland types including MG1 *Arrhenatherum elatius* grasslands with gradations towards improved MG7 *Lolium perenne* leys. Current management practices include extensive grazing (for full details see Woodcock et al. 2005). There is wide variation in the present and historic extent and intensity of disturbance from military vehicles (Hirst et al. 2003), burning (Iliffe et al. 2000) and encroachment of scrub species, most notably *Crategaus monogyna*, *Prunus spinosa* and, more locally, *Ulex europaeus*

(Redhead et al. 2012). As is common in the analysis of historical data, where many such processes go unrecorded (Sheail 1980), data is lacking on the present and historic extents of these variables. However, there is no evidence of systematic bias in these variables in relation to grassland age on DTE SP.

Generating a Land Use History Map

In the absence of historic records of floral communities we used a chronosequence approach (Pickett 1989; Foster and Tilman 2004) based on historical land use data sets. The extent of unimproved grassland on DTE SP has been mapped on various occasions. Suitable land use data were available for six time periods: 1840s, 1880s, 1930s, 1967, 1985 and 1996. The earliest two datasets were derived from tithe maps and first edition Ordnance Survey maps, respectively (Sheail 1980). Data for the 1930s were drawn from the Dudley-Stamp Land Utilisation Survey (Stamp 1931, 1948; see Hooftman and Bullock 2011 for a description). The latter datasets were based on three grassland surveys (Wells 1967; Porley 1986; Walker & Pywell 2000). Where maps were not already in digital form each map was georeferenced and digitized. Datasets prior to and including 1930 mapped all areas of non-arable grazing land and natural grassland. Post 1930 maps refined this to map only species-rich, non-improved grasslands (i.e. those not showing any signs of agricultural management beyond traditional grazing or hay cutting). Data for the adjacent county of Dorset (Hooftman and Bullock 2011) and for Britain as a whole (Fuller 1987) suggest that all mapped grasslands were agriculturally unimproved prior to the late 1930s. It is important to note that even those grasslands for which there is no evidence of cultivation in this study are likely to have some history of agricultural influence, with archaeological evidence of such up to the post-medieval period (Postan 1973). However, the available historic data is insufficiently spatially explicit to provide a meaningful sample of grasslands uncultivated since medieval times.

Land use maps were overlain to form a composite map of land use history from 1840 to 1996 (Figure 1). Coverage was limited to the western and central areas of DTE SP, giving a total area of around

20 000 Ha with full historic land use data. The varied land use histories were divided into four categories, the first three of which represent the minimum age of unimproved grassland: Old = grassland since 1840 to 1880 (≈136 years old) , Mid = grassland since 1930-1967 (≈50 years old) , Recent = grassland since 1985 to 1996 (≈6 years old). The fourth category, Lost, denotes areas which were once unimproved grassland but had been degraded or lost to improvement by 1996, mostly by conversion to re-sown grass leys. Georeferencing, digitising and analysis of digital maps was performed in ArcMAP (v9.3.1 © 2011, ESRI Inc., Redlands, CA, USA)

Fig.1

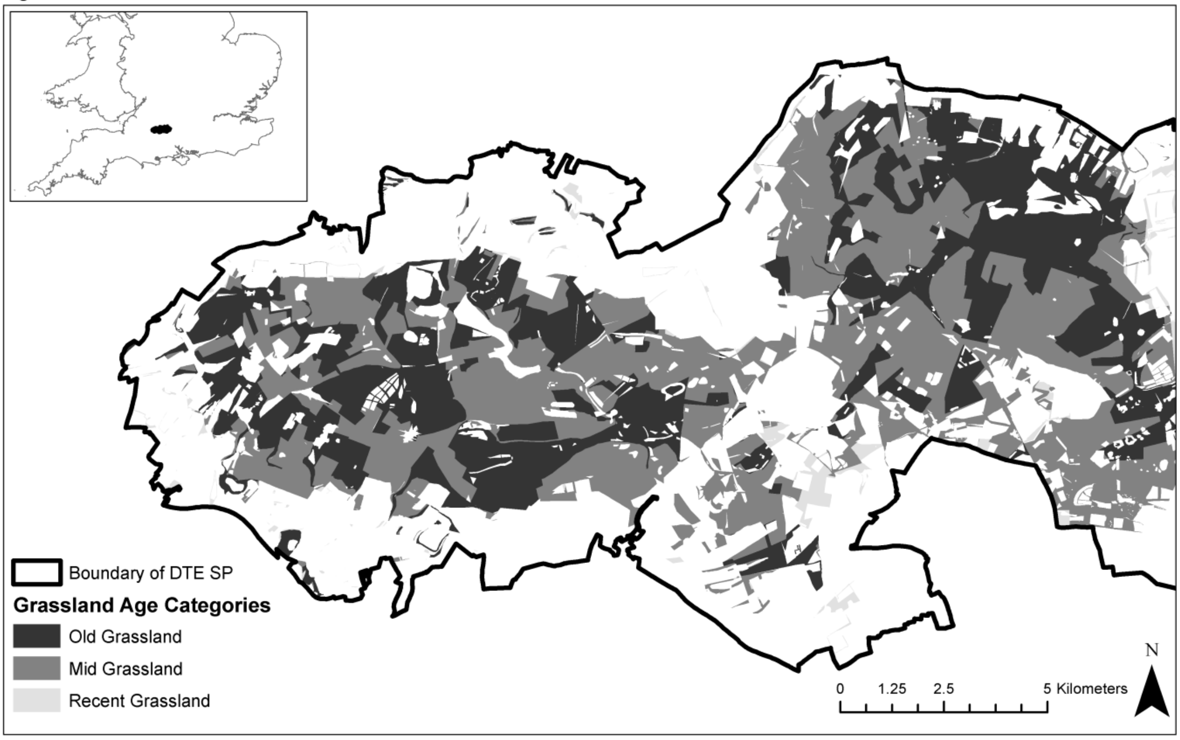

Fig.1 Map of estimated grassland age on western and central Defence Training Estate, Salisbury Plain (DTE SP). Derived from overlay of historic landuse maps. Unshaded areas within the DTE are those lost to improvement, lacking historic data or those which are not grassland (forest, built up, etc). Inset map shows location of DTE SP in southern UK.

Floristic Data

Between 1996 and 1997 all vegetation communities present on DTE SP were mapped in the field and their composition described using the NVC methodology (Rodwell 1992). For each discrete community the cover of vascular plant species was recorded from 2 × 2 m quadrats to aid assignation to an NVC community (Walker & Pywell 2000). However, these quadrats were limited in number and thus did not give an accurate record of total species richness or occurrence of all species. Thus an estimate of abundance for all plants present was made from a walk-over survey, using the DAFOR scale (Kershaw 1985). The mapped plant communities were captured as digital polygons and overlain with the land use map. As NVC polygons and land use history polygons did not overlap exactly, analyses were restricted to NVC polygons over one hectare in area and having at least 75% overlap with a single land use history polygon, giving 1352 polygons for analysis (484 Old, 658 Mid, 101 Recent, 109 Lost). Lists of species presence, DAFOR abundance and associated floral life-history traits were compiled for each polygon. Traits were selected on the basis of having information readily available for the majority of species present in the study, and of having a clear potential to characterise ecological function, namely the ability of a species to colonise, compete and persist in the landscape concerned (functional traits, Violle et al. 2007). Traits were measured on either a continuous scale (e.g. height) or as ordinal 'scores' (e.g. dispersal ability, seedbank persistence). Colonisation ability was represented by a dispersal ability score. Traits reflecting persistence ability included seedbank longevity, clonal spread and plant longevity. Traits representing competitive ability were leaf area and typical summer plant height. Also included in the analyses were measures of ecological performance which, whilst arguably not traits *sensu* Violle et al. (2007), are meaningful proxies for multiple traits which affect the ability of species to survive in the conditions of recently ex-agricultural land (Pywell et al. 2003). These were Ellenberg (Ellenberg et al. 1991) tolerance values for light, soil reactivity (expressed as difference from neutral to correct for skew towards the acid range, Preston et al. 2003) and soil fertility. Descriptions of all these traits, including values and sources, are given in the Supplementary Material (Table S1).

STATISTICAL ANALYSIS

To investigate how individual species presence related to grassland age, Chi-squared tests were used to examine whether presence fitted a null hypothesis of distribution at random across age categories. In order for the assumptions of the test to be met, if a species generated expected values of less than five in any category, the Recent and Lost categories were combined. If expected values remained below five, the species was excluded from analysis. Species showing significant ($p < 0.05$) results were grouped according to which land use history categories showed higher than expected values and contributed the greatest proportion of the significant Chi-squared statistic. Although conducting a large number of independent tests we did not apply a sequential Bonferroni correction but instead examined effect sizes and whether the observed differences were ecologically explicable, following Moran (2003).

Differences in grassland community composition between NVC polygons were analysed by calculating Euclidean distances (*ED*) for all pairwise comparisons. Euclidean distance is defined as:

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

where ED_{jk} = Euclidean distance between samples *j* and *k*; X_{ij} = abundance of individuals of species *i* in sample *j*; X_{ik} = abundance of individuals of species *i* in sample *k* (Krebs 1999).

The DAFOR scale on which species were recorded across the NVC polygon was converted to a numeric representation (Dominant = 5, Abundant = 4, Frequent = 3, Occasional = 2, Rare = 1, Present = 0.1) to provide a measure of relative abundance. Differences in *ED* between the different age categories were then analysed by one-way Analysis of Variance (ANOVA).

The weighted mean of each trait was calculated for each NVC polygon, with weightings derived from the numeric representation of the DAFOR scale. Differences in traits between grassland age categories were analysed using analyses of covariance (ANCOVA). Two covariates were included. Firstly, the area of the NVC polygon, in order to account for the potential for larger polygons to support more, and rarer, species (Matthies et al. 2004). Secondly, the proximity of each NVC polygon to the nearest old grassland, as distance from established calcareous grassland has previously been found to strongly affect the rate of community reassembly (Matthies et al. 2004, Fagan et al. 2008). Variables giving significant results from ANCOVA were then included in a Discriminant Function Analysis (DFA), which classified grasslands to age class using the linear combinations of traits (discriminant functions, DFs) which best differentiate between categories. Analyses were performed in R (R Development Core Team 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria) utilising the MASS package (Venables, W.N. & Ripley, B.D. 2002. Modern Applied Statistics with S, 4th ed. Springer, New York).

Results

Species Presence Data

Of over 450 plant species recorded on DTE SP in the NVC survey, 193 species met the criteria for analysis, and 77% of these (149 species) showed a significant ($p < 0.05$) associations with grassland age-classes (Table 1; full species lists are available in Supplementary Material, Table S2). For eight species, small effect sizes or ecologically inexplicable results indicated a possible Type I error, the relationship with grassland age was designated as 'unclear' and the results are not reported further.

Table 1. Presence of individual species in response to grassland age category. The number of species showing higher than expected presence for each combination of classes, as determined by Chi 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

Forty-eight species showed a significant association with unimproved grasslands over 100 years in age (Table 1). These included some species entirely restricted to ancient chalk and limestone grassland in the UK (e.g. *Campanula glomerata*, *Carex humilis*, *Picris heiracoides*, *Thesium humifusum*) but the majority are confined to species-rich communities on infertile soils across a broader pH range (e.g. *Anthyllis vulneraria, Filipendula vulgaris, Poterium sanguisorba, Serratula tinctoria*). This group also included species more often associated with disturbed ground (e.g. *Myosotis arvensis, Reseda lutea, Sinapis arvensis, Sonchus asper*). Species associated with both old and mid age grasslands included many typical chalk downland species (e.g. *Asperula cynanchica, Cirsium acaule, Viola hirta*) whereas those associated with mid or mid and recent age grasslands included many more species that occur across a broader range of ecological conditions and habitats (e.g. *Centaurea scabiosa, Cynosurus cristatus, Lathyrus pratensis, Leontodon hispidus*) or more disturbed conditions on calcareous soils (e.g. *Chaenorhinum minus, Linum bienne, Onobrychis viciifolia, Senecio erucifolius*). Species associated with recent, recent and lost or lost grasslands were almost entirely species of eutrophic, often agricultural, habitats, including intensively managed arable land (e.g. *Anisantha sterilis, Convolvulus arvensis, Galium aparine,, Papaver rhoeas, Veronica persica*) or improved pasture (e.g. *Cirsium* spp., *Rumex* spp., *Taraxacum* spp.).

Of particular interest are the species designated as indicators of calcareous grassland condition by Robertson & Jefferson (2000), from degraded (negative indicators) to good (positive indicators). All six negative indicator species (*Cirsium arvense*, *Cirsium vulgare*, *Rumex crispus*, *Rumex obtusifolius, Senecio jacobaea*, *Urtica dioica*) were significantly more common on recent grasslands and grasslands lost to improvement. Of 24 positive indicator species with sufficient sample size for analysis, 13 were associated with old grasslands and a further four with both old and medium age grasslands (Supplementary Material S2).

Grassland Community Composition

The analysis of *ED* between plant communities (Fig. 2) showed that differences showed generally clear trends along the age gradient (a larger *ED* indicates a bigger difference in community composition). This was indicated by increasing *ED* when comparing old grasslands against increasingly younger sites (one-way ANOVA, F = 1777.7 p <0.001); and decreasing *ED* when comparing lost grasslands against grasslands of increasing age (one-way ANOVA, F = 2671.2, p <0.001).

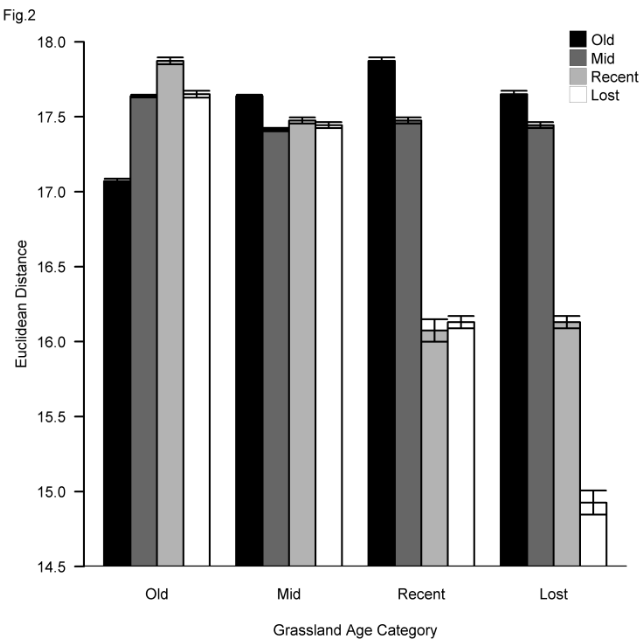

Fig.2 Bar plot of mean Euclidean distance between grassland communities of four different age categories. Also shown are mean Euclidean distances between grassland communities within the same age category. Age classes are arranged on the x-axis in order of decreasing grassland age. Capped lines represent ± one standard error.

Differences were greatest overall between the old and recent categories, with mid age grasslands roughly equidistant between the two. The values of *ED* within each age class indicated variation among communities of the same age, a measure of β diversity (Newton et al. 2012). For such comparisons within age classes, mid-age grasslands showed the highest within-class *ED*, followed by old, then recent and lost grasslands (Fig. 2). This suggests variation in community composition increases from the early to middle stages of reversion and then decreases somewhat as grasslands age. Communities within the lost grassland category showed the lowest mean *ED* value, suggesting that grasslands lost to agricultural improvement have communities which are comparatively homogenous across DTE SP.

COMMUNITY FUNCTIONAL TRAITS

The grassland age categories showed significant differences in all nine traits ($p < 0.01$), with many traits also showing a clear trend with grassland age (Fig. 3). Even in this landscape of virtually continuous grassland, many traits also varied with the two covariates of polygon area and isolation (Table 2).

Table 2. Results of analyses of covariance (ANCOVA) for 9 plant functional traits. Polygon area and distance from nearest ancient grassland (Isolation) are covariates and grassland age category is the factor. Also shown are standardised coefficients from discriminant function analysis (DFA), for each of the three discriminant functions (DFs).

	Isolation			Polygon Area			Age Category			Discriminant Functions		
Trait	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

Dispersal ability, Ellenberg fertility score, seedbank longevity and clonal spread all declined with increasing grassland age. Soil pH showed the reverse trend, with older grasslands supporting species which prefer soils further from neutral pH. Other traits showed a trend across the three grassland ages but had intermediate values on lost grassland (plant height, leaf area) or values which were notably high or low for a single category (high plant longevity on old grasslands, low light tolerance on recent grasslands).

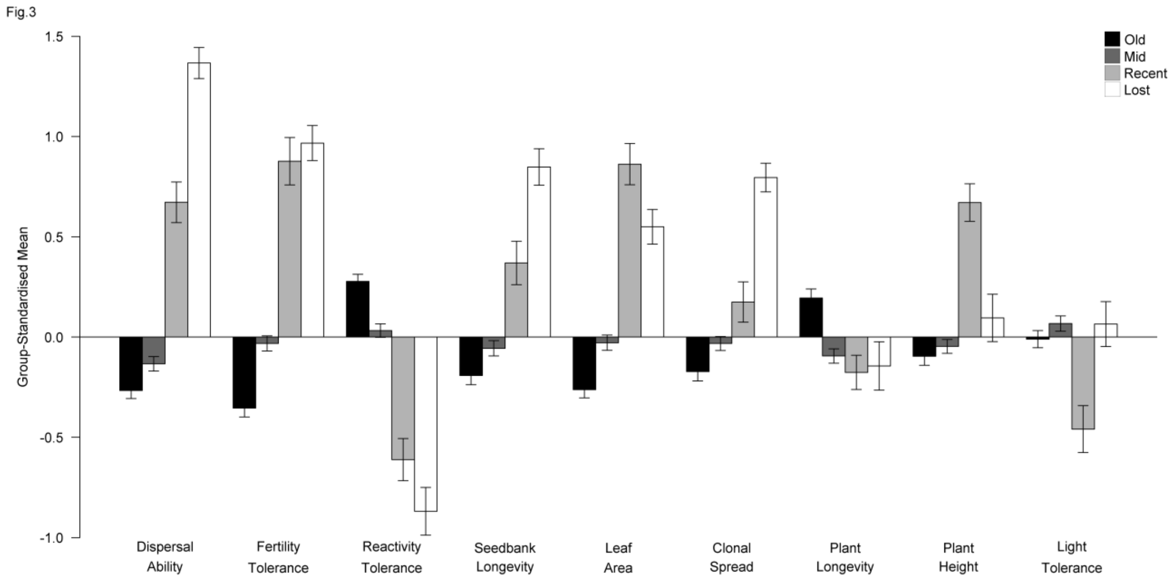

Fig.3 Bar plot of group-standardised means of plant functional traits between communities occurring on grassland of four different ages classes. Capped lines represent ± one standard error. Traits are weighted by approximate plant abundance (0.1 = present, 1 = rare, 2 = occasional, 3 = frequent, 4= abundant, 5 = dominant) and scaled for display on the same axis. Traits are ordered along the x axis in order of increasing differentiation between classes, according to the results of ANCOVA on each trait with area and isolation as covariates.

Discriminant function analysis gave a classification accuracy (proportion classified correctly) of 0.614, although classification accuracy varied between age classes (Table 3). The most common misclassifications (as proportions of classifications made) assigned mid age grassland to the old or recent classes, or recent grassland to the lost or mid age classes (Table 3).

Table 3. Results from classification into grassland age categories by discriminant function analysis (DFA) based on 15 plant functional traits. Values shown are proportion of true class assigned to each 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

Most separation between groups was achieved by the first two discriminant functions, which together accounted for 0.804 of between class variance. Using the standardised coefficients (Table 2) and differences between the group standardised means (Fig. 4), DF1 was most influenced by dispersal ability and fertility tolerance (which distinguish well between old-mid grasslands and recent-lost grasslands), along with plant height (which distinguishes recent from all other grasslands). DF2 was influenced by fertility tolerance, clonal spread and seedbank longevity, which further distinguish old and lost grasslands from mid and recent grasslands respectively. Thus older grasslands tend to be characterised by immobile, perennial species with transient seedbanks and low clonal spread, whilst more recent or improved grasslands are dominated by annuals with high mobility, both in terms of dispersal and clonal spread, and high competitive ability. The species of old grassland are also intolerant of high soil fertility and prefer a more basic soil pH. Recent grasslands also differ from old, mid and lost grasslands in supporting more tall, shade tolerant species.

Discussion

Many previous studies have documented changes in plant communities over several decades following reversion from arable to grassland on calcareous soils (Wells et al. 1976; Gibson & Brown 1991; Walker et al. 2004; Fagan et al. 2008; Karlik & Poschlod 2009). The results of this study strongly suggest that even within an extensive, unfragmented chalk grassland landscape, natural regeneration continues to alter the plant community over more than 100 years in a manner detectable at the species, community and functional trait levels.

Individual Species and Community Composition

The oldest grasslands were highly distinctive at a species level, with a large proportion of distinctive species, and in composition, with the highest mean *ED* from any other age class. The majority of species associated with the oldest grasslands have a well known affinity with long established chalk grasslands (e.g. *Carex humilis*, *Cirsium tuberosum*, *Thesium humifusum*) or that are slow to colonise restored sites (e.g. *Succisa pratensis,* Herben et al. 2006). Interestingly the eight species which were less abundant in old grasslands than in all other age categories (*Bellis perennis, Convolvulus arvensis, Elytrigia repens, Lolium perenne, Taraxacum agg., Orobanche minor* and its host *Trifolium repens, Sisymbrium officinale*) are all species of sown leys. Either the oldest grasslands are unsuitable for these species or it takes up to 100 years for these species to 'die out' on previously agricultural sites. Alternatively, the oldest grasslands may be the only sites where some of these species have not been deliberately introduced, as *Lolium perenne* and *Trifolium repens* are known to have been were sown prior to land abandonment during the agricultural depression of the late 1870s, in the hope that this would speed conversion into productive pasture (Royal Commission on Agriculture 1894). Other species associated with mid age grasslands (e.g. *Cynosurus cristatus, Onobrychis viciifolia*) are also likely to be indicators of an agricultural history, where the agriculture concerned occurred 50 to 150 years ago and involved little or no introduction of artificial fertilisers, as has been demonstrated in other landscapes (Karlik &Poschlod 2009).

The occurrence of many species shared between mid age and old grasslands suggests that natural regeneration can make substantial progress towards an ancient grassland assemblage within 50 years. However, the high *ED* among grasslands within the mid age class also suggests that after 50 years grasslands vary widely. The rate of community re-assembly in its early to mid stages is likely to be highly variable and heavily dependent upon stochastic processes such as local grazing pressure, soil disturbance, fertility and seed dispersal (Gibson & Brown 1991; Matthies et al. 2004; Butaye et al. 2005).

It is clear that some calcareous grassland species can colonise ex-arable land in less than 10 years, as shown by the few species which were more common in both recent and mid age grasslands than lost grasslands (e.g. *Orobanche elatior* and its host *Centaurea scabiosa, Leontodon hispidus*). Although other studies have demonstrated similar increases in some calcareous grassland indicators within ten years (Zobel et al. 1996; Pywell et al. 2002; Fagan et al. 2008), these are comparatively minor changes compared with re-assembly of an entire ancient grassland community. Such findings most likely demonstrate the rapid colonisation ability of a small subset of calcareous grassland species with good dispersal abilities or persistent seedbanks (Zobel et al. 1996; Matthies et al. 2004) and the very depauperate nature of control sites such as arable fields (Pywell et al. 2002; Fagan et al. 2008; Walker et al. 2004) or scrub forest (Zobel et al. 1996) to which restoration trajectories are often compared. Indeed, most species of recent grasslands in this study (e.g. *Anthriscus sylvestris, Arrhenatherum eliatus, Galium album, Heracleum sphondyllium*) were more typical of tall unmanaged mesotrophic grassland, characteristic of roadside verges and neglected agricultural land, as well as ex-arable areas up to 50 years in age on other dry, calcareous grasslands (Wells et al. 1976; Karlik & Poschlod 2009). Several studies have interpreted such findings as a tendency of restored calcareous grassland to 'stall' at a stable but relatively species-poor stage (Pywell et al. 2002, 2003; Fagan et al. 2008). From this study it is apparent that even in an extensive, well-connected landscape, such species are prevalent for up to 50 years.

The results from the species and community data generally concur, lending support to the notion of using groups of species as indicators of trajectories towards (or away from) communities resembling ancient grassland. However, great care must be taken in selecting suitable species. Whilst many positive indicators (*sensu* Robertson & Jefferson 2002) did appear to have a genuine affinity for the oldest grasslands, several appeared to colonise ex-agricultural sites comparatively quickly or even showed no response to grassland age. These latter species (e.g. *Bromopsis erectus, Galium verum, Leontodon saxatilis, Lotus corniculatus*) have also been shown to be ubiquitous across other grassland landscapes (Wells et al. 1976; Karlik & Poschlod 2009). Where connectivity is high (as on DTE SP), these species are likely to be able colonise relatively rapidly and have some ability to persist even on degraded or improved grasslands, with the result that they were amongst the commonest species in the dataset, present in over 45% of all polygons analysed. Negative indicators appeared successful in detecting either recently re-established or agriculturally improved grassland, but whilst their presence on calcareous grassland may be a reliable indicator of degradation their absence is not necessarily indicative of long established or good quality calcareous grassland. Even the rarest species are unlikely to be responding directly to grassland age. Wells et al. (1976) found *Thesium humifusum* to be associated not just with grassland over 100 years in age but particularly with remnants of grassland on ancient banks and tracks. *Carex humilis,* although associated with the oldest grasslands in this study, can rapidly colonise suitable areas created by anthropogenic disturbance, such as gardens and road verges within modern housing estates built on chalk downland (Wells et al. 1976; K.J. Walker, pers. obs.). Thus regarding the presence or absence of any one species as a certain indicator of ancient grassland is inadvisable.

Functional Traits

Even on DTE SP, an extensive landscape of well connected grasslands, with a high potential for seed movement by grazing livestock and military vehicles, dispersal ability remained highly influential in separating grassland communities of different ages. This suggests that many species of ancient grasslands are severely limited in dispersal ability, and thus have a very low probability of reaching isolated sites unaided (Pywell et al. 2003; Violle et al. 2007; Ozinga et al. 2009; Marini et al. 2012).

Previous studies have demonstrated that traits determining colonisation ability are most influential at the earlier stages of grassland regeneration on ex-arable land, with traits associated with tolerance of local conditions becoming increasingly important over time (Thompson et al. 2001;

Pywell et al. 2003). Consequently, the greatest differences in dispersal ability were between lost, recent and mid age grasslands. Between mid age and old grasslands however, the species assemblage shows greater differences in traits relating to tolerance of local conditions (i.e. Ellenberg fertility and soil pH), which in turn lead to changes in competitive ability (as seen by decreases in leaf area and plant height) as conditions allow stress tolerant but less competitive species to establish. Such species tend to favour stable conditions allowing a long lifespan, unlike the species which typify lost and recent grasslands, which have shorter lifespans but can establish rapidly and compete well under high nutrient levels (Pywell et al. 2003; Fagan et al. 2008). Thus, the slow decline in soil fertility and associated rise in soil pH (McClean et al. 2011) after cessation of intensive agriculture are likely to be the major drivers of changes in the plant community over longer timescales (Pywell et al. 2003;Walker & Preston 2006; Fagan et al. 2008, Walker et al. 2009; Ozinga et al. 2009; McClean et al. 2011).

Implications for Conservation and Restoration Of Chalk Grassland

That community reassembly in a well connected, expansive and predominantly unimproved landscape such as DTE SP takes over a century confirms the high conservation value of existing ancient grasslands. They support uniquely large and diverse communities and, moreover, are difficult if not impossible to replace once lost. Regeneration following cessation of agricultural use appears to progress comparatively rapidly (<10 years) when measured at the species level, with recruitment of those species which good dispersers can take advantage of reductions in soil fertility. Increases in diversity and species richness are therefore rapidly achievable if the site and subsequent restoration management techniques are carefully selected. However, it takes considerably longer (up to 100 years) before community composition and traits begin to show a consistent similarity to ancient grassland (Zobel et al. 1996; Pywell et al. 2003; Walker et al. 2004). This supports previous assertions that small scale restoration projects, such as agri-environment re-establishment, are unlikely to achieve the complete re-assembly of ancient types via natural regeneration within human lifespans (Gibson & Brown 1991, Walker et al. 2004). Consequently, there is clearly a need for more realistic targets and measures of restoration success, as well as incentives to promote management techniques such as species introduction by seed which might accelerate regeneration, especially given the apparently strong limiting effect of dispersal ability in the first 10 – 50 years. Where such restoration management produces detectable similarities to ancient grasslands in community or trait measures this may well indicate that regeneration has been enhanced beyond the rate of natural regeneration, and potentially represents the start of a trajectory towards an ancient grassland community. Although many management techniques remain contentious and have given mixed results in experimental trials (Pywell et al. 2002; Kiehl et al. 2006; Fagan et al. 2008) the growing body of evidence, including this study, should contribute to informing the design and implementation of restoration management methods, and the monitoring of their effects.

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