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The effectiveness of herbicides for management of tor-grass (*Brachypodium pinnatum s.l.*) in calcareous grassland

Abstract

Calcareous grasslands are highly biodiverse semi-natural habitats. A particular challenge to European calcareous grassland management in recent years has been the increasing dominance of the competitive grass *Brachypodium pinnatum. B. pinnatum* is difficult to control by traditional means but selective herbicides offer a potential alternative.

We trialled five selective herbicides on two levels of *B. pinnatum* cover (sparse and dense) at a UK calcareous grassland site over three years of repeated treatment. We compared the effect of herbicides with a minimal intervention treatment (cutting) and current management practices (cutting and grazing for sparse cover, broad-spectrum glyphosate application for dense cover) on the cover of *B. pinnatum,* key indicator species and the composition of the grassland community.

Areas with initially sparse *B. pinnatum* showed no significant reduction under any herbicide, whilst some herbicides (propyzamide, cycloxydim) showed detrimental impacts on non-target species. Cutting and grazing showed some beneficial effects, despite no significant reduction in *B. pinnatum.*

On areas of dense *B. pinnatum* cover, glyphosate application reduced cover of *B. pinnatum* but led to colonisation by negative indicators or species typical of agricultural situations and disturbed ground. None of the selective herbicides significantly reduced dense *B. pinnatum* cover, and some (propyzamide, tepraloxydim, fluazifop-P-butyl) had significant negative impacts on non-target species.

Our results suggest herbicide treatments, including glyphosate, are unlikely to offer long-term control of *B. pinnatum* on calcareous grasslands. A more promising approach is suggested by the effect of cutting and grazing, although further experimentation is required to determine the most effective regimes.

Keywords

Conservation, control, eradication, expansion, graminicide, grass

1. Introduction

Calcareous grasslands are well known across Europe for their high diversity of plants and large number of rare and threatened species (Poschlod and WallisDeVries 2002). Although some of these grasslands are relicts of open, steppic habitats, the vast majority are 'semi-natural', resulting from forest clearance and centuries of management by low-intensity livestock grazing (Dengler et al. 2014; Poschlod and WallisDeVries 2002; Sheail et al. 1974). As such, the high conservation value of these grasslands depends to a large extent on effective management (WallisDeVries et al. 2002) to prevent succession to scrub and woodland, the development of rank, mesotrophic grassland, and dominance of competitive plant species (either native or introduced). Identifying threats and suitable management methods for mitigating against them is therefore key to the conservation of calcareous grassland biodiversity.

One such threat is the competitive grass *Brachypodium pinnatum* P. Beauv, also known as tor-grass or heath false-brome. *Brachypodium pinnatum* is native to Western Europe and forms part of many typical calcareous grassland communities (Cope and Gray 2009; Robertson and Jefferson 2000). However, the potential for *B. pinnatum* to have negative impacts on the diversity of calcareous grasslands has long been recognised (Bobbink and Willems 1987; Tansley and Adamson 1926; Willems 1978) due to its ability to form dense, exclusive stands and its general unpalatability to grazing livestock (Tansley and Adamson 1926; Wells 1974). During the mid-twentieth century *B. pinnatum* was observed to increase throughout its distribution, dominating greater areas of calcareous grassland (Bobbink and Willems 1987). The predominant drivers of this initial expansion were probably simultaneous reductions in grazing management (Buckland et al. 2001; Corcket et al. 2003; Morris and Duffey 1974) and decreases in rabbit grazing following the introduction of myxomatosis (Morris and Duffey 1974; Natural England 1999), potentially exacerbated by eutrophication from fertiliser addition (Bobbink and Willems 1987) or deposition of atmospheric nitrogen (Stevens et al. 2010; but see Wilson et al. 1995).

A variety of studies have investigated the impacts of *B. pinnatum* dominance on calcareous grassland communities and local environmental variables (Bobbink and Willems 1987; Hurst and John 1999a) and explored potential methods for mitigation, including cutting, grazing and burning (Bobbink and Willems 1993; Buckland et al. 2001; Stampfli and Zeiter 1999). Despite this body of research, there is little consensus on the best way to manage *B. pinnatum* other than to acknowledge that "the expansion of tor-grass is reversible only with great difficulty" (Natural England 1999, p11). Without effective management, *B. pinnatum* is likely to continue to expand on calcareous grasslands, especially since it has been demonstrated to benefit from the milder winters predicted as a result of climate change (Buckland et al. 2001).

Given the apparent difficulty of controlling *B. pinnatum* by traditional means, there is the scope to explore alternative methods, including herbicides. Herbicides are currently used to some extent in the conservation management of many semi-natural grasslands, primarily in the form of broad-spectrum herbicides (e.g. glyphosate) to clear ground prior to reseeding (e.g. Ewing 2002), or as spot treatments to control scrub (e.g. Marrs 1985; Redhead et al. 2012) or dense stands of invasive or otherwise problematic species (Dickens et al. 2016; e.g. Hurst and John 1999b; Milligan et al. 2003). A wide range of selective herbicides also exist to control grasses in agricultural or forestry situations which offer reduced risk to non-target species (Clay et al. 2006; Dixon et al. 2005; Milligan et al. 2003). Whilst the use of herbicides for conservation management can be contentious (Marrs 1985), carefully targeted application could form part of an integrated management regime (Hurst and John 1999b), and form a viable management tool in situations where traditional methods have failed.

In this study, we trialled the use of selective and broad-spectrum herbicides to control *B. pinnatum* at Martin Down, a calcareous grassland site in southern England. We compared the effectiveness of herbicide management with mechanical cutting and with livestock grazing, for two situations: 1) preventing *B. pinnatum* dominance in swards where the species was widespread, but not yet dominant, and 2) restoring areas which had become dominated by *B. pinnatum*. Our aims in both situations were to test the effects of herbicides on: i) *B. pinnatum* cover; ii) the cover of non-target species groups (grasses and forbs) and calcareous grassland indicator species iii) the composition of the grassland community

2. Methods

2.1. Study species

Brachypodium pinnatum is a perennial, long-lived grass with a widespread native distribution across temperate regions of Europe. Although *B. pinnatum* is relatively slow growing compared to other coarse grasses (Ryser and Lambers 1995) it spreads vigorously by extensive, creeping rhizomes. Seed set normally requires cross-pollination and is generally held to be low (but see Buckland et al. 2001), with seeds having low persistence in the seed bank (Cope and Gray 2009).

Brachypodium pinnatum is a complex of taxa, variously considered as species or subspecies (Cope and Gray 2009), consisting in the UK of *B. pinnatum* and *B. rupestre* (Host) Roem. & Schult. This was only comparatively recently recognised and the two are difficult to differentiate in the field, such that many references to *B. pinnatum* on UK calcareous grassland probably refer in fact to *B. rupestre* (Chapman and Stace 2001). It is thus virtually impossible to separate the two species in terms of the existing body of literature on *B. pinnatum* impacts and management. For consistency with previous work we therefore refer throughout to *B. pinnatum* in the broad sense, as a species complex including *B. rupestre*.

2.2. Study site and experimental design

Martin Down is a remnant of the extensive semi-natural grasslands which covered much of southern England prior to widespread losses to ploughing and agricultural improvement during and following the Second World War (Best and Coppock 1962; Fuller 1987; Ridding et al. 2015). At around 340 hectares, it is one of the largest fragments of lowland calcareous grassland in the UK, aside from on the intensively studied military training estates of Salisbury Plain and Porton Down (Redhead et al. 2014; Wells et al. 1976).

Martin Down is located on the Hampshire-Wiltshire border (Fig. 1B, 50.975 N, 1.937 W) and is typical of dry calcareous grasslands in Southern England, being dominated by British National Vegetation Classification (NVC, Rodwell 2006) communities CG2/3/5/7, with patches of deciduous scrub. Soils are calcaric and rendzic leptosols (Cranfield University 2019) with the high pH (~7.8) and organic matter content (~17 %), and low nutrient content (~0.9% total N, ~7.3 mg kg^{-1} extractable P, ~75 mg kg^{-1} K) typically associated with these lowland calcareous grassland communities (Critchley et al. 2002; Ross et al. 2004, authors' unpublished data). The site is topographically varied, with the mid-section (Fig. 1C) gently undulating between 80-100 metres above sea level, and Northern and Southern ends rising to 160 metres. The site receives an annual average of ~800mm rainfall. The surrounding landscape is dominated by intensive arable land and improved pasture. Martin Down is a designated Site of Special Scientific Interest (SSSI, the basic unit of statutory land protection in GB) and a National Nature Reserve (which reinforces its protected status but also recognises value for research, education and public engagement). Martin Down is managed through rotational sheep grazing and cutting/coppicing of scrub. In addition, dense stands of *B. pinnatum* are sprayed with glyphosate in an attempt to reduce their expansion.

We established trial plots at Martin Down in summer 2012 on grassland containing two levels of *B. pinnatum* cover - ‘dense’ and ‘sparse’. Dense cover referred to areas where the sward was already dominated by *B. pinnatum* (mean cover in baseline year =78%), whilst sparse cover referred to grassland areas retaining a species-rich CG2 sward, but with frequent *B. pinnatum* throughout the area (mean cover in baseline year =26%). Example photographs of the two levels are shown in supplementary material, Figure A1. The goals of management differed between levels of *B. pinnatum* cover. In areas of sparse cover, the goal was to prevent *B. pinnatum* from increasing to dominance and, if possible, to reduce it below the threshold for good CG2 grassland condition (<10%, Robertson and Jefferson 2000). In areas where cover was already dense, the goal was to reduce *B. pinnatum* cover to a level which allowed the reestablishment of other calcareous grassland species. In practice, this is likely to effectively require localised eradication (Natural England 1999).

For each level, three experimental blocks were marked out, each consisting of seven plots (each plot =3 m wide x 10 m long). For dense levels of *B. pinnatum*, blocks were spatially separated (100 m – 3 km, Fig. 1C), because dense patches of *B. pinnatum* were less extensive, and intermixed with scrub. For sparse levels of *B. pinnatum*, all three blocks were spatially contiguous in a gridded design for ease of exclusion of livestock and the public from areas where herbicide was applied (Fig. 1D). Within each block, each plot was assigned a different herbicide or management application at random. The location of all plot corners was recorded using a Leica Zeno 20 RTK GPS, allowing accurate (±10 cm) relocation if markers were damaged or removed.

2.3. HERBICIDE APPLICATIONS AND MANAGEMENT

The control experimental management in both sparse and dense areas was a single annual cut in late summer (July-August) with a mechanical mower which removed cut material. This was deemed the minimum level of management acceptable to prevent uninhibited spread of *B. pinnatum*. In dense areas, the current herbicide management strategy of glyphosate application (English Nature 2003; Hurst and John 1999b; Natural England 1999) was used on one plot per block. This was not trialled in areas of sparse *B. pinnatum* cover, as widespread destruction of the species-rich sward along with *B. pinnatum* is not a viable management strategy. Instead, in sparsely covered areas the equivalent current management strategy was to have one plot both cut and grazed immediately afterwards (Green 1972; Hurst 1997). All other plots, including controls, remained ungrazed (except unmanaged rabbit grazing) to negate the risk of livestock exposure to herbicide residues (a condition of the Administrative Trials Permit, see below).

Five herbicides were selected (six including glyphosate, see Table 1) on the basis of their efficacy for controlling perennial grasses in agricultural and/or forestry applications. We obtained an Administrative Trials Permit from the GB Chemicals Regulation Division permitting the application of these herbicides outside their approved field of use. All herbicides were applied evenly across the entire plot using an AZO handheld sprayer with a 3 m horizontal boom, through eight 110° flat fan nozzles. All herbicide applications were made by people trained to the legally required standard, on days of minimal wind and when the ground was not frozen or waterlogged, to reduce risk of drift and runoff, respectively.

Herbicide applications were timed to ensure as close as possible adherence to the recommendations for maximum efficacy given on the product label, given constraints imposed by scheduling of cutting, grazing and public access. All foliar herbicides were recommended to be applied to actively growing leaves, which *B. pinnatum* possesses throughout much of the year in temperate climates (Bobbink et al. 1989). Applications were thus made in autumn to target regrowth after cutting, except in the third year of study, when delays in cutting (due to mechanical issues) resulted in late-spring applications, targeting growth after winter senescence (dates in Table 1). For the residual herbicide (propyzamide) applications were made in winter to maximise potential for root uptake and control of emerging *B. pinnatum* (dates in Table 1). Determining 'optimum' herbicide timings was difficult because the conditions of a naturally-established grassland sward are very different to the products' normal field of use, i.e. controlling arable/forestry weeds which have established in a relatively uniform manner. Naturally-established swards are composed of plants at varying growth stages and thus varying degrees of susceptibly (Clay et al. 2006; Dixon et al. 2005; Milligan et al. 2003). Therefore, whilst our results do not necessarily indicate the susceptibility of *B. pinnatum* to each herbicide under ideal conditions, they show effects under field-realistic conditions that are highly likely to affect the majority of UK protected calcareous grassland sites.

2.4. VEGETATION MONITORING

Vegetation monitoring took place in July-September of 2012 (before all herbicide applications to provide a baseline), 2013, 2014 and 2015. Within each plot, vegetation surveys were conducted by placing five 50 cm × 50 cm quadrats at approximately 2 m intervals (i.e. vertices of a "W"-shaped transect through the plot). Quadrats avoided the outermost 50 cm of each plot, where there was an increased potential for spray drift from neighbouring plots. Within each quadrat, the percentage cover of *B. pinnatum*, all vascular plants and bare ground were recorded. In the first, third and fourth quadrat, all plants were recorded to species level, whilst in the second and fifth quadrat percentage cover was recorded per group level only (grasses and forbs). Recording to species level on only three of the quadrats was a result of limited time and funding for survey.

2.5. DATA ANALYSIS

Sparse and dense plots were analysed separately in all cases because of the differing management goals, treatments, spatial layout and vegetation conditions. For all tests, statistical significance was determined at p <=0.05.

2.5.1. *Univariate analyses*

Total percentage cover for each plant species and grouping was calculated from quadrat data for each of the four years of the study. We calculated total cover of *B. pinnatum,* other grasses, forbs and bare ground for all quadrats. For quadrats giving species-level data (3 per plot), we also calculated summed percentage cover of positive and negative indicator species for calcareous grassland SSSI condition (Robertson and Jefferson 2000), and for species associated with arable land and disturbance (Hill et al. 2004) that may colonise exposed areas following removal of *B. pinnatum* (Hurst 1997; Hurst and John 1999b). See Table A3 for lists of indicator species. We also examined mean Ellenberg-N fertility tolerance values weighted by percentage cover (Ellenberg et al. 1992), as given in PLANTATT (Hill et al. 2004). This allowed analysis of whether species which were removed or colonised following treatment were associated with a particular relative fertility tolerance. This is of interest because *B. pinnatum* stands have been found to increase soil nitrate levels (Hurst and John 1999a), which may encourage fertility tolerant species (Hurst and John 1999a) and/or discourage fertility intolerant calcareous grassland species (Natural England 1999). All response variables were averaged across quadrats per plot per year to avoid pseudoreplication.

These variables (% cover of *B. pinnatum*, other grasses, forbs, bare ground, positive indicators, negative indicators, arable indicators and Ellenberg-N weighted) were analysed using linear mixed effects models (LMEs) with repeated measures, using the *lme4* (Bates et al. 2015), *nlme* (Pinheiro et al. 2017) and *lmerTest* (Kuznetsova et al. 2017) packages of the R statistical software (v3.4.0, R Core Team 2017). An initial set of LMEs modelled the response against treatment, year and the interactions between them, with block as a random effect. The null hypothesis in these models is that treatment and year have no effect. A second set of LMEs modelled the difference in the response per plot from the baseline year (2012) against treatments, with block and year as random effects. These models have the null hypothesis that treatments do not differ in their change from the baseline year once variation between blocks and years is accounted for. In preliminary analysis we tested for the effect of temporal autocorrelation due to repeated measures of the same plot over subsequent years but inclusion of a correlation structure was found not to improve model fit substantially (ΔAIC <2).

2.5.2. *Multivariate analyses*

To analyse the effect of treatments on the vegetation community, we used partial constrained correspondence analysis (CCA), implemented in the *vegan* R package (Oksanen et al. 2017). For this we used data from all quadrats with species-level data, again averaging cover across quadrats per plot per year. We excluded cover of *B. pinnatum* itself from multivariate analyses, because this was the target of experimental manipulations and would otherwise bias the results (Lepš and Šmilauer 2003). We also excluded species found only in a single plot to reduce bias from rare species. We then conducted CCA for each year independently, firstly to confirm that communities did not differ between treatments in the baseline year (2012) and, subsequently, to examine the effect on the plant community post-treatment. These analyses used block and plot as conditioning variables. We also analysed all three post-baseline years (2013, 2014, 2015) in a single CCA to explore the effect of treatments across the three years once interannual variation was partialled out, by including year as a conditioning variable. For all CCA analyses we used permutation tests (n =1000) to assess the significance of constraints, with permutations constrained within blocks.

3. Results

3.1. SPARSE *B. PINNATUM*: IMPACTS ON PERCENTAGE COVER

For plots with initially sparse *B. pinnatum* cover, few percentage cover variables appeared affected by treatment (Fig 2). Percentage cover of *B. pinnatum* was not significantly reduced in comparison to control plots by any of the herbicides, or the cut-and-graze treatment (Table 2). The only significant result, in terms of *B. pinnatum* cover, was a positive interaction term between treatment and year for propyzamide in 2014 (Table A1). This reflects the greatly increased cover of *B. pinnatum* on these plots in this year, despite the initially promising reduction seen in 2013 (Fig 2) and the obvious visual impact of this herbicide (Fig A2A). The latter is explained by the fact that propyzamide significantly reduced cover of other, non-target grasses (e.g. *Bromopsis erecta, Festuca ovina, F. rubra, Trisetum flavescens* Table A3), with significant interaction terms in 2013 and 2015 (Table A1) and a significant effect of this treatment on difference from the baseline year (Table 2). However, propyzamide did not significantly affect the cover of forbs or positive indicators. When comparing change from the baseline year, treatment with cycloxydim resulted in a reduced cover of positive indicators. The cut-and-graze treatment appeared to show a reduced forb cover compared to controls (Table 2). Total percentage cover weighted by Ellenberg-N showed a significant impact of year, generally increasing over 2013 and 2014 and then decreasing in 2015, although not to 2012 levels (Fig 2, Table A1). Negative indicators and species typical of arable land were so rarely found on sparsely covered plots that there were insufficient data for analysis.

3.2. SPARSE *B. PINNATUM*: IMPACTS ON THE GRASSLAND COMMUNITY

Sparsely covered plots had a mean 29 species per plot in the baseline year (derived from the three quadrats where plants were identified to species level), across all treatments, and 28 species per plot across all subsequent years. The number of species recorded across all sparsely covered plots was 69 in 2012 and 89 across subsequent years (mean per year =68).

Permutation tests following CCA showed a significant year effect in 2015 and when all three post-baseline years were analysed together with the effect of year partialled out (Table 3). From the CCA plots, much of this effect appears due to high CCA scores on axis-1 for propyzamide plots (associated with at least one negative indicator species) and low scores for cut-and-grazed plots (Fig 3A). The latter may be due to several grasses (Fig 3B) which were most abundant on cut-and-grazed plots (e.g. *Cynosurus cristatus*, *Phleum bertolonii*, *Agrostis capillaris*, *Bromopsis erecta, Briza media* Table A3). Total proportion of variance explained by the constraining variables was relatively low (0.12) compared to conditioning variables (0.24).

3.3. DENSE *B. PINNATUM*: IMPACTS ON PERCENTAGE COVER

On densely covered plots treated with glyphosate, percentage cover of *B. pinnatum* showed a significant decline from the baseline year (Fig 2, Table 2) and a significant interaction with year for 2013, 2014 and 2015 (Table A2). These results indicate that glyphosate treatment consistently reduced cover of the target species, with mean cover of *B. pinnatum* on glyphosate plots being 8% after the baseline year, compared to 66% on control plots. Glyphosate also significantly increased cover of bare ground and forbs (Table 2). However, the forb species that colonised these plots following the reduction in *B. pinnatum* were associated with arable land and disturbance, more fertility tolerant (leading to higher Ellenberg-N weighted percentage cover) and included negative indicator species (Table 2). Typical examples found almost entirely on initially densely covered plots following glyphosate treatment were *Galium aparine*, *Myosotis arvensis* and *Senecio jacobaea* (Table A3).

As on sparsely covered plots, treatment with propyzamide significantly reduced the cover of non-target grasses, but not *B. pinnatum* (Fig 2, Table 2). Forb cover increased after treatment with propyzamide but, as with glyphosate, these appear to have been mostly negative indicators or fertility tolerant species (e.g. *Cirsium vulgare*, *Sonchus asper*, *Convolvulus arvensis,* Table A3). Treatment with tepraloxydim appeared to increase *B. pinnatum* cover relative to controls, as well as reducing the number of positive indicator species on densely covered plots (Table 2).

3.4. DENSE *B. PINNATUM*: IMPACTS ON THE GRASSLAND COMMUNITY

Plant communities with dense B*. pinnatum* cover were less species rich, averaging 14 species per plot in 2012 and 15 species per plot across the subsequent years and treatments. However, there was a greater degree of variation in species composition between plots, especially following treatment, with 71 species recorded in 2012, rising to 114 species across subsequent years (mean per year =79).

CCA of densely covered plots showed a significant effect of treatment in 2013 and 2015, and when all three post-baseline years were analysed together (Table 3). Total variance explained by treatment was generally higher than in sparsely covered plots (Table 3). From Figure 3C, these results appear to be driven by radical changes on glyphosate-treated plots, with low scores on CCA axis-1, associated with species associated with arable and disturbed land (Fig. 3D). Propyzamide treated plots clustered with low CCA axis-2 scores, associated to some extent with negative indicators and with reduced cover of most grasses. Despite not showing any significant differences in univariate tests, plots treated with fluazifop-P-butyl showed a distinct grouping with high CCA axis-2 scores (Fig. 3C). Species associated with particularly high axis-2 scores included *Lotus corniculatus, Origanum vulgare* and *Potentilla reptans* which tended to be present on fluazifop-P-butyl plots and absent or very low in cover on other treatments (Table 3).

4. **Discussion**

4.1. PREVENTING B. PINNATUM DOMINANCE ON AREAS OF CURRENTLY SPARSE COVER

The use of selective herbicides to control *B. pinnatum* in situations where it has not yet achieved dominance but is scattered throughout the sward does not appear promising. Percentage cover of *B. pinnatum* was not significantly reduced in comparison to control plots by any of the herbicides, whilst some showed detrimental impacts on non-target grasses (propyzamide) or positive indicators (cycloxydim). Although none of the treatments with initially sparse cover showed an increase in *B. pinnatum* to the levels of dominance seen on dense plots over the timescale of our experiment, none showed decreases to the <10% cover deemed indicative of good condition for CG2/3 grasslands (Robertson and Jefferson 2000). Propyzamide, the only residual herbicide, and the only selective herbicide with a different mode of action (Table 1), showed an initially promising reduction in *B. pinnatum* cover in 2013 but this did not translate to a longer term reduction. This was in spite of repeated treatment, illustrative of the importance of multi-year trials (Milligan et al. 2003). As acknowledged in Section 2.3, some of the lack of effect of herbicides on *B. pinnatum* may be due to the far from ideal conditions for herbicide application that result from a diverse calcareous grassland sward. As well as variation in growth stages, vegetation is of non-uniform height and there is abundant litter, both of which prevent spray from reaching leaves (foliar herbicides) or the soil (residual herbicides). High concentrations of organic matter in the soil, typical of calcareous grasslands, are known to reduce the effectiveness of propyzamide in the formulation we applied. Therefore species which are susceptible in controlled experiments or agricultural situations may well respond differently in grassland conservation situations (Clay et al. 2006; Dixon et al. 2005). It may also be that *B. pinnatum* is particularly resilient to herbicide management due to its extensive network of rhizomes which may escape aboveground herbicide effects if translocation is not optimal, leading to rapid regeneration. Other problematic, rhizomatous species have been found to be difficult to control with herbicides unless the regime explicitly accounts for the linkage between above and belowground tissues (Jones et al. 2018).

The cut-and-graze treatment appeared to show some beneficial effects on sparsely covered plots. Although it did not significantly reduce *B. pinnatum* cover, there was some evidence that it resulted in a different grassland community, with a lesser increase in forbs and an increase in some grasses typical of dry, unimproved grasslands (*Cynosurus cristatus*, *Bromopsis erecta, Briza media*). There is some evidence that appropriately timed cutting regimes can reduce *B. pinnatum* cover (Bobbink and Willems 1993; Green 1972; Hurst 1997), but these need to be more frequent than the single, annual cut used here to have a lasting effect. The additional effect of grazing and trampling after cutting is likely to further reduce the ability of *B. pinnatum* to compete with faster growing, stress-tolerant calcareous grassland species (Natural England 1999; Wilson et al. 1995), especially where *B. pinnatum* is not yet dominant so that there are seeds in the seedbank and the potential for short distance recolonisation (Buckland et al. 2001; Stampfli and Zeiter 1999). Although our study was primarily focussed at exploring the potential for herbicide management, our results suggest that evaluating the impacts of different cutting and grazing regimes on preventing *B. pinnatum* dominance would be a useful avenue for further study. Because of the conditions imposed by the Administrative Trials Permit we were unable to investigate herbicides and grazing in combination. Whilst such approaches might be potentially interesting, they are unlikely to be practical since this would require extensive research into the risks associated with livestock grazing on treated areas.

4.2. RESTORING GRASSLAND ON AREAS OF DENSE *B. PINNATUM* COVER

The current management strategy of glyphosate application appears to be largely counterproductive, confirming Hurst and John (1999b). Although cover of *B. pinnatum* was reduced, the species which recolonised the resulting bare ground were not desirable calcareous grassland species. Whilst some calcareous grassland species can colonise disturbed ground quite rapidly (Redhead et al. 2014), our results showed that glyphosate treated plots were instead associated with an assortment of ruderal species typical of eutrophic-mesotrophic conditions. This is probably because dense *B. pinnatum* stands increase soil nitrate levels (Hurst and John 1999a), capturing nutrients via an extensive rhizome system and releasing them into the soil via decay of the large amounts of litter produced (Bobbink et al. 1989; Canals et al. 2016). Our results suggest that the likely end result of repeated glyphosate application to dense *B. pinnatum* is a community dominated by those few species which can rapidly recolonise disturbed ground and are tolerant of high soil fertility, typically arable weeds (e.g. *Galium aparine*, *Myosotis arvensis, Sonchus asper*, *Convolvulus arvensis*) and species of improved and mesotrophic grasslands (e.g. *Cirsium vulgare*, *Senecio jacobaea, Anthriscus sylvestris*). Without repeat application, the most likely result is a rapid reinvasion of *B. pinnatum* (Hurst and John 1999b). In many respects, glyphosate application is akin to burning, destroying the sward and leaving nutrient-rich bare ground. Likewise burning has been found to be ineffective at controlling *B. pinnatum* (Canals et al. 2014; Kahmen et al. 2002; Moser and Wohlgemuth 2006; Tansley and Adamson 1926). Although we do not rule out spot-spraying of small (<1 m^2) patches of dense *B. pinnatum* (which we did not directly examine), the wider risks associated with the use of glyphosate are also under particular scrutiny at present (Myers et al. 2016), so basing control strategies on this herbicide may be short-sighted given uncertainty over its future regulation.

Of the selective herbicides, none were successful as a potential tool for eradication of dense *B. pinnatum* cover. Whist some herbicides appeared to drive the community in particular directions (most notably propyzamide and fluazifop-P-butyl), presumably due to differences in product formulation, modes of action and interspecific differences in susceptibility to the active ingredients, none made a significant difference to *B. pinnatum* which might counter the significant negative impacts on other grasses (propyzamide) or on non-target species (tepraloxydim and fluazifop-P-butyl). Despite propyzamide being a broad-spectrum graminicide with the ability to control several coarse, rhizomatous grasses in agricultural and forestry situations (Clay et al. 2006; Dixon et al. 2005), it did not significantly reduce cover of *B. pinnatum,* probably because of the aforementioned shielding of the soil by *B. pinnatum* litter (Clay et al. 2006) and high levels of soil organic matter. On densely covered plots this problem was exacerbated as cutting had only limited effectiveness, leaving large quantities of low-growing leaves and litter. It therefore appears that the propyzamide treatment of dense *B. pinnatum* is likely to merely thin out any remaining other grasses, allowing less desirable species to invade in the resultant space.

Given the ineffectiveness of selective herbicides and the counterproductive effects of glyphosate, there seem to be few options for the restoration of densely covered areas. Some ability to reduce dense *B. pinnatum* cover has been demonstrated in sub-Mediterranean systems using heavy livestock grazing (Catorci et al. 2014; Catorci et al. 2012), although these require careful consideration of stocking density and animal welfare issues (Catorci et al. 2014; Scocco et al. 2013). Repeated cutting and removal of *B. pinnatum* and litter has also demonstrated reductions in *B. pinnatum* cover and increased cover of forbs (Bobbink and Willems 1993). The removal of rhizomes and accumulated nutrients via localised topsoil stripping (Natural England 1999) is also a potential route for further investigation and there are existing management techniques for ex-arable land which could be used to follow up such measures and increase the likelihood of successful restoration (Fagan et al. 2008; Kiehl et al. 2006; Pywell et al. 2002).

4.3. CONCLUSIONS

Brachypodium pinnatum is well placed to benefit from climate change (Buckland et al. 2001) and increases in soil nutrients from fertiliser use or atmospheric deposition (Canals et al. 2014; Canals et al. 2016; Green 1972). A robust management strategy for *B. pinnatum* is therefore vital if we wish to conserve our remaining calcareous grasslands. Our results suggest that currently available herbicides are unlikely to form a key part of such a management strategy under the conditions imposed by conservation management on UK protected calcareous grasslands. We detected few beneficial effects which would offset the considerable problems which widespread application of herbicides to calcareous grasslands is likely to offer. These range from practical concerns, such as how to apply herbicide effectively on rough or steep terrain, to potential effects on non-target plants and animals (Marshall 2001). There is also a challenge in presenting herbicide management as a viable tool for conservation management. Although selective herbicides can offer the opportunity to control problematic species on conservation sites in ways which would be difficult to achieve otherwise (Marshall 2001; Milligan et al. 2003), there is increasing public and political perception of the risks associated with pesticide usage (Freedman 1990; Myers et al. 2016; Peterson 2000) and pressure towards increased regulation (Skevas et al. 2013). Treating large areas with herbicides on publically accessible nature reserves may therefore be deeply unpopular even if beneficial effects were clear (Marrs 1985). A more promising avenue for *B. pinnatum* management is offered by studies demonstrating positive impacts of targeted cutting and grazing, although further experimentation is required to determine the most effective regimes.

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Data statement

All data from this study are freely accessible under license from the NERC Environmental Information Data Centre (Redhead et al. 2019, DOI: 10.5285/43095c2d-b959-4216-8362-0fb18deed1e6).

References

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1-48.

Best, R.H., Coppock, J.T., 1962. The changing use of land in Britain. Faber and Faber, London.

Bobbink, R., den Dubbelden, K., Willems, J.H., 1989. Seasonal dynamics of phytomass and nutrients in chalk grassland. Oikos 55, 216-224.

Bobbink, R., Willems, J., 1993. Restoration management of abandoned chalk grassland in the Netherlands. Biodivers. Conserv. 2, 616-626.

Bobbink, R., Willems, J.H., 1987. Increasing dominance of *Brachypodium pinnatum* (L.) beauv. in chalk grasslands: A threat to a species-rich ecosystem. Biol. Conserv. 40, 301-314.

Buckland, S.M., Thompson, K., Hodgson, J.G., Grime, J.P., 2001. Grassland invasions: effects of manipulations of climate and management. J. Appl. Ecol. 38, 301-309.

Canals, R.-M., Pedro, J., Rupérez, E., San-Emeterio, L., 2014. Nutrient pulses after prescribed winter fires and preferential patterns of N uptake may contribute to the expansion of *Brachypodium pinnatum* (L.) P. Beauv. in highland grasslands. Applied Vegetation Science 17, 419-428.

Canals, R., Durán, M., San Emeterio, L., Múgica, L., 2016. Success of *Brachypodium pinnatum* in oligotrophic grasslands: seasonal allocation patterns of nutrients and metals, The multiple roles of grassland in the European bioeconomy. Proceedings of the 26th General Meeting of the European Grassland Federation, Trondheim, Norway, 4-8 September 2016. pp. 110-112. NIBIO.

Catorci, A., Antolini, E., Tardella, F.M., Scocco, P., 2014. Assessment of interaction between sheep and poorly palatable grass: a key tool for grassland management and restoration. Journal of Plant Interactions 9, 112-121.

Catorci, A., Gatti, R., Cesaretti, S., 2012. Effect of sheep and horse grazing on species and functional composition of sub-Mediterranean grasslands. Applied Vegetation Science 15, 459-469.

Chapman, M.A., Stace, C.A., 2001. Tor-grass is not *Brachypodium pinnatum*! BSBI News 87:74.

Clay, D.V., Dixon, F.L., Willoughby, I., 2006. Efficacy of graminicides on grass weed species of forestry. Crop Protect. 25, 1039-1050.

Cope, T.A., Gray, A.J. eds., 2009. Grasses of the British Isles. Botanical Society of the British Isles.

Corcket, E., Liancourt, P., Callaway, R., Michalet, R., 2003. The relative importance of competition for two dominant grass species as affected by environmental manipulations in the field. Ecoscience 10, 186-194.

Cranfield University, 2019. The Soils Guide, available: www.landis.org.uk. Cranfield University, UK.

Critchley, C.N.R., Chambers, B.J., Fowbert, J.A., Sanderson, R.A., Bhogal, A., Rose, S.C., 2002. Association between lowland grassland plant communities and soil properties. Biol. Conserv. 105, 199-215.

Dengler, J., Janišová, M., Török, P., Wellstein, C., 2014. Biodiversity of Palaearctic grasslands: a synthesis. Agric., Ecosyst. Environ. 182, 1-14.

Dickens, S.J.M., Mangla, S., Preston, K.L., Suding, K.N., 2016. Embracing variability: environmental dependence and plant community context in ecological restoration. Restor. Ecol. 24, 119-127.

Dixon, F., Clay, D., Willoughby, I., 2005. The relative efficacy of herbicides for the control of Deschampsia flexuosa (L.) Trin. in woodland establishment in the UK. Forestry 78, 229-238.

Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulißen, D., 1992. Zeigerwerte von pflanzen in Mitteleuropa.

English Nature, 2003. The herbicide handbook: Guidance on the use of herbicides on nature conservation sites. English Nature in association with FACT, Perterborough, UK.

Ewing, K., 2002. Effects of initial site treatments on early growth and three-year survival of Idaho fescue. Restor. Ecol. 10, 282-288.

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

Freedman, B., 1990. Controversy over the use of herbicides in forestry, with particular reference to glyphosate usage. Environmental Carcinogenesis Reviews 8, 277-286.

Fuller, R.M., 1987. The changing extent and conservation interest of lowland grasslands in England and Wales: a review of grassland surveys 1930–1984. Biol. Conserv. 40, 281-300.

Green, B.H., 1972. The relevance of seral eutrophication and plant competition to the management of successional communities. Biol. Conserv. 4, 378-384.

Hill, M.O., Preston, C.D., Roy, D., 2004. PLANTATT-attributes of British and Irish plants: status, size, life history, geography and habitats. Centre for Ecology & Hydrology.

Hurst, A., 1997. Community dominance: An investigation into the competitive mechanisms of *Brachypodium pinnatum* and possible methods of reducing its dominance on ancient chalk grassland. University of Sussex.

Hurst, A., John, E., 1999a. The biotic and abiotic changes associated with *Brachypodium pinnatum* dominance in chalk grassland in south-east England. Biol. Conserv. 88, 75-84.

Hurst, A., John, E., 1999b. The effectiveness of glyphosate for controlling *Brachypodium pinnatum* in chalk grassland. Biol. Conserv. 89, 261-265.

Jones, D., Bruce, G., Fowler, M.S., Law-Cooper, R., Graham, I., Abel, A., Street-Perrott, F.A., Eastwood, D., 2018. Optimising physiochemical control of invasive Japanese knotweed. Biol. Invasions 20, 2091–2105

Kahmen, S., Poschlod, P., Schreiber, K.-F., 2002. Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. Biol. Conserv. 104, 319-328.

Kiehl, K., Thormann, A., Pfadenhauer, J., 2006. Evaluation of initial restoration measures during the restoration of calcareous grasslands on former arable fields. Restor. Ecol. 14, 148-156.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. Journal of Statistical Software 82.

Lepš, J., Šmilauer, P., 2003. Multivariate analysis of ecological data using CANOCO. Cambridge university press.

Marrs, R.H., 1985. The effects of potential bracken and scrub control herbicides on lowland Calluna and grass heath communities in East Anglia, UK. Biol. Conserv. 32, 13-32.

Marshall, E., 2001. Biodiversity, herbicides and non-target plants, Brighton Crop Protection Conference Weeds. pp. 855-862.

Milligan, A.L., Putwain, P.D., Marrs, R.H., 2003. A field assessment of the role of selective herbicides in the restoration of British moorland dominated by Molinia. Biol. Conserv. 109, 369-379.

Morris, M.G., Duffey, E., 1974. Zoological characteristics and interest of the grassland ecosystem, In Grassland Ecology and Wildlife Management. eds E. Duffey, M.G. Morris, J. Sheail, L.K. Ward, D.A. Wells, T.C.E. Wells. Chapman & Hall, London.

Moser, B., Wohlgemuth, T., 2006. Which plant species dominate early post-fire vegetation in the Central Alps, and why? For. Ecol. Manage. 234, S174.

Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., Hansen, M., Landrigan, P.J., Lanphear, B.P., Mesnage, R., Vandenberg, L.N., Vom Saal, F.S., Welshons, W.V., Benbrook, C.M., 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environmental health : a global access science source 15, 19-19.

Natural England, 1999. Chapter 10: Grassland restoration, In The Lowland Grassland Management Handbook (2nd edition). eds A. Crofts, R.G. Jefferson. English Nature/The Wildlife Trusts.

Oksanen, J., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, M., Wagner, H., 2017. vegan: community ecology package. R package. version 2.4-5.

Peterson, R., 2000. Public perceptions of agricultural biotechnology and pesticides: Recent understandings and implications for risk communication. Am. Entomol. 46, 8-16.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2017. Linear and nonlinear mixed effects models, R package version 3.1.

Poschlod, P., WallisDeVries, M.F., 2002. The historical and socioeconomic perspective of calcareous grasslands—lessons from the distant and recent past. Biol. Conserv. 104, 361-376.

Pywell, R.F., Bullock, J.M., Hopkins, A., Walker, K.J., Sparks, T.H., Burke, M.J., Peel, S., 2002. Restoration of species-rich grassland on arable land: assessing the limiting processes using a multi-site experiment. J. Appl. Ecol. 39, 294-309.

R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Redhead, J., Cuevas-Gonzales, M., Smith, G., Gerard, F., Pywell, R., 2012. Assessing the effectiveness of scrub management at the landscape scale using rapid field assessment and remote sensing. J. Environ. Manage. 97, 102-108.

Redhead, J., Nowakowski, M., Ridding, L., Wagner, M., Hulmes, S., Hulmes, L., Amy, S., McDonald, R., Mitschunas, N., Nuttall, P., Peyton, J., Pywell, R., 2019. Vegetation survey of *Brachypodium pinnatum* herbicide control trials at the Martin Down National Nature Reserve (2012-2015). NERC Environmental Information Data Centre, https://doi.org/10.5285/43095c2d-b959-4216-8362-0fb18deed1e6.

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

Ridding, L.E., Redhead, J.W., Pywell, R.F., 2015. Fate of semi-natural grassland in England between 1960 and 2013: A test of national conservation policy. Global Ecology and Conservation 4, 516-525.

Robertson, H.J., Jefferson, R.G., 2000. Monitoring the conditon of lowland grassland SSSIs: I, English Nature's rapid assessment method. English Nature's rapid assessment method. I. English Nature.

Rodwell, J.S., 2006. National Vegetation Classification: Users' Handbook. Joint nature conservation committee.

Ross, S., Bealey, C., Cox, J., 2004. Validation Network Project Lowland calcareous grasslands: Bromus erectus (CG3) grassland. English Nature Research Reports ENRR609.

Rowland, C.S., Morton, R.D., Carrasco, L., McShane, G., O'Neil, A.W., Wood, C.M., 2017. Land Cover Map 2015 (25m raster, GB). NERC Environmental Information Data Centre.

Ryser, P., Lambers, H., 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. Plant Soil 170, 251-265.

Scocco, P., Mercati, F., Brusaferro, A., Ceccarelli, P., Belardinelli, C., Malfatti, A., 2013. Keratinisation degree of rumen epithelium and body condition score in sheep grazing on *Brachypodium rupestre*. Veterinaria italiana 49, 211-217.

Sheail, J., Wells, T.C.E., Wells, D.A., Morris, M.G., 1974. Grasslands and their history, In Grassland Ecology and Wildlife Management. eds E. Duffey, M.G. Morris, J. Sheail, L.K. Ward, D.A. Wells, T.C.E. Wells. Chapman & Hall, London.

Skevas, T., Oude Lansink, A.G.J.M., Stefanou, S.E., 2013. Designing the emerging EU pesticide policy: A literature review. NJAS - Wageningen Journal of Life Sciences 64-65, 95-103.

Stampfli, A., Zeiter, M., 1999. Plant species decline due to abandonment of meadows cannot easily be reversed by mowing. A case study from the southern Alps. Journal of Vegetation Science 10, 151-164.

Stevens, C.J., Thompson, K., Grime, J.P., Long, C.J., Gowing, D.J., 2010. Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition. Funct. Ecol. 24, 478-484.

Tansley, A., Adamson, R., 1926. Studies of the vegetation of the English Chalk: IV. A preliminary survey of the Chalk Grasslands of the Sussex Downs. J. Ecol. 14, 1-32.

WallisDeVries, M.F., Poschlod, P., Willems, J.H., 2002. Challenges for the conservation of calcareous grasslands in northwestern Europe: integrating the requirements of flora and fauna. Elsevier.

Wells, T., Sheail, J., Ball, D., Ward, L., 1976. Ecological studies on the Porton Ranges: relationships between vegetation, soils and land-use history. The Journal of Ecology, 589-626.

Wells, T.C.E., 1974. Management and grazing, In Grassland Ecology and Wildlife Management. eds E. Duffey, M.G. Morris, J. Sheail, L.K. Ward, D.A. Wells, T.C.E. Wells. Chapman & Hall, London.

Willems, J., 1978. Observations on north-west European limestone grassland communities: phytosociological and ecological notes on chalk grasslands of southern England, In Plant Species and Plant Communities. pp. 11-20. Springer.

Wilson, E.J., Wells, T.C.E., Sparks, T.H., 1995. Are calcareous grasslands in the UK under threat from nitrogen deposition?--an experimental determination of a critical load. J. Ecol. 83, 823-832.

Tables

Table 1. Herbicides applied to trial plots. Because formulations and concentrations differ between products containing the same active ingredients, the table gives the active ingredient alongside the registered name of the product used in this experiment, along with the Ministerially Approved Pesticide Product (MAPP) number, concentration of the active ingredient and application rate to enable precise identification of what was applied. The table also gives detail on application method (foliar vs residual) and timing (pre- or post- emergence), mode of action (according to the Weed Science Society of America classification) and specificity. The final three columns give application dates (dd/mm/yy) for the three years of the study. Herbicides are ordered in increasing predicted order of herbicidal effect (four selective graminicides < one selective broadleaf + gramincide < one broad-spectrum herbicide). Note that glyphosate was only applied to plots on areas with dense *Brachypodium pinnatum* cover.

Active ingredient	**Product name**	**MAPP number**	**Active conc. (g l^{-1})**	**Rate (l ha^{-1})**	**Application**	**WSSA Group**	**Specificity**	**Application dates**		
								Year 1	**Year 2**	**Year 3**
Propaquizafop	Falcon	16459	100	1.25	Foliar, post-em.	1	Graminicide	10/10/12	04/11/13	01/06/15
Tepraloxydim	Aramo	10608	50	1.50	Foliar, post-em.	1	Graminicide	10/10/12	04/11/13	01/06/15
Fluazifop-P-butyl	Fusilade Max	11519	125	1.50	Foliar, post-em.	1	Graminicide	10/10/12	04/11/13	01/06/15
Cycloxydim	Laser	17339	200	2.00	Foliar, post-em.	1	Graminicide	10/10/12	04/11/13	01/06/15
Propyzamide	Kerb Flo	13716	400	2.10	Residual, pre- and post-em.	3	Graminicide and selective broadleaf	08/01/13	10/01/14	22/12/14
Glyphosate	Touchdown Quattro	10608	360	5.00	Foliar, post-em.	9	Broad-spectrum	10/10/12	04/11/13	01/06/15

WSSA Groups: 1 = ACCase Inhibitors, 3 = Microtubule Assembly Inhibitors, 9 = EPSP synthase inhibitors

Table 2 Results of linear mixed models for percentage cover variables recorded on trial plots on areas of sparse and dense *Brachypodium pinnatum* cover (separate models). All models included treatment as a fixed factor, with a random effect of experimental block and year. Herbicide treatments are named by their active ingredient (see Table 1 for details, including the product name)

		Sparse							Dense						
Variable (% cover)	**Coefficient**	Intercept	Cut-and-graze	Tepraloxydim	Propaquizafop	Fluazifop-P-butyl	Cycloxydim	Propyzamide	Intercept	Glyphosate	Tepraloxydim	Propaquizafop	Fluazifop-P-butyl	Cycloxydim	Propyzamide
B. pinnatum	**Coef.**	0.36	11.60	-3.95	-5.58	-2.70	11.02	11.75	-10.22	-69.84	16.24	-0.44	1.76	-7.56	-3.04
	t	0.05	1.53	-0.51	-0.74	-0.35	1.46	1.50	-1.68	-8.86	2.06	-0.06	0.22	-0.96	-0.39
	p	0.958	0.132	0.615	0.465	0.731	0.152	0.139	0.109	<0.001***	0.044*	0.955	0.825	0.342	0.701
Other grasses	**Coef.**	-18.35	2.32	7.11	5.09	-13.41	0.52	-27.55	14.95	-4.73	-10.61	-2.76	-0.46	-7.68	-14.15
	t	-3.05	0.33	0.98	0.72	-1.85	0.07	-3.80	2.74	-0.85	-1.92	-0.50	-0.08	-1.39	-2.56
	p	0.010**	0.743	0.332	0.473	0.070	0.941	<0.001***	0.025*	0.397	0.061	0.620	0.934	0.171	0.014*
Forbs	**Coef.**	29.16	-15.56	-9.85	-2.25	-0.22	-9.23	8.58	6.35	27.39	-3.08	6.79	9.77	10.49	13.09
	t	4.53	-2.14	-1.32	-0.31	-0.03	-1.27	1.15	0.98	5.42	-0.61	1.35	1.93	2.08	2.59
	p	0.001***	0.037*	0.194	0.758	0.976	0.209	0.257	0.364	<0.001***	0.545	0.184	0.058	0.043*	0.012
Positive indictors	**Coef.**	16.94	-10.17	-10.12	-3.29	-12.17	-15.31	-2.75	2.77	0.49	-5.84	0.69	-1.17	-2.87	2.03
	t	1.92	-1.52	-1.46	-0.49	-1.76	-2.28	-0.40	1.12	0.19	-2.25	0.27	-0.45	-1.11	0.78
	p	0.109	0.136	0.150	0.626	0.085	0.027*	0.693	0.286	0.851	0.028*	0.790	0.653	0.273	0.438
Negative indicators	**Coef.**	Insufficient data - did not occur on more than a few plots							-0.22	2.84	0.82	1.41	0.54	-0.37	2.10
	t								-0.25	2.78	0.80	1.38	0.53	-0.36	2.06
	p								0.811	0.008**	0.427	0.174	0.597	0.719	0.045*
N-weighted	**Coef.**	0.82	0.16	-0.23	-0.37	-0.68	-0.35	-0.07	0.51	1.76	-0.18	-0.11	0.00	-0.14	-0.09
	t	3.19	0.84	-1.18	-1.94	-3.42	-1.82	-0.37	2.29	9.49	-0.95	-0.60	0.02	-0.77	-0.47
	p	0.037*	0.403	0.243	0.058	0.001***	0.074	0.709	0.068	<0.001***	0.345	0.548	0.983	0.447	0.641
Arable indicators	**Coef.**	Insufficient data - did not occur on more than a few plots							1.86	17.64	-1.00	-0.13	0.63	-0.71	1.82
	t								0.63	5.72	-0.32	-0.04	0.20	-0.23	0.59
	p								0.542	<0.001***	0.748	0.967	0.840	0.818	0.558
Bare ground	**Coef.**	0.94	0.73	1.11	0.28	-0.03	0.32	-0.34	-6.77	22.89	-6.35	-2.98	1.79	2.73	2.63
	t	0.81	0.60	0.89	0.23	-0.02	0.26	-0.27	-1.65	5.33	-1.48	-0.69	0.42	0.63	0.61
	p	0.446	0.550	0.379	0.816	0.980	0.795	0.787	0.144	<0.001***	0.145	0.490	0.678	0.528	0.542

* p <=0.05, ** p <=0.01, *** p <=0.001

Table 3 Results of permutation tests on the effect of treatment following canonical correspondence analysis (CCA) of plant communities before (2012) and following (2013, 2014, 2015) *Brachypodium pinnatum* management trials. Also given are the proportions of variance explained by constraining and conditioning variables. Results are given for both sparse and dense levels of *B. pinnatum* cover, for all years independently and for the post-baseline years combined. In all cases treatment was the constraining variable and experimental block and plot were conditioning variables, along with year in the multi-year analysis.

		Proportion of variance		**Treatment**		
	Year	**Conditional**	**Constrained**	**χ^2**	**F**	**p**
Sparse	2012	0.18	0.27	0.31	0.89	0.697
	2013	0.21	0.32	0.53	1.27	0.061
	2014	0.19	0.39	0.47	1.22	0.139
	2015	0.19	0.35	0.39	1.40	0.011*
	All post-baseline	0.24	0.12	0.22	1.44	0.001***
Dense	2012	0.26	0.27	1.15	1.05	0.306
	2013	0.24	0.34	1.59	1.47	0.005**
	2014	0.25	0.31	1.17	1.28	0.143
	2015	0.21	0.33	1.44	1.31	0.010*
	All post-baseline	0.19	0.14	0.90	1.84	0.001***

* p <=0.05, ** p <=0.01, *** p <=0.001

Figure captions

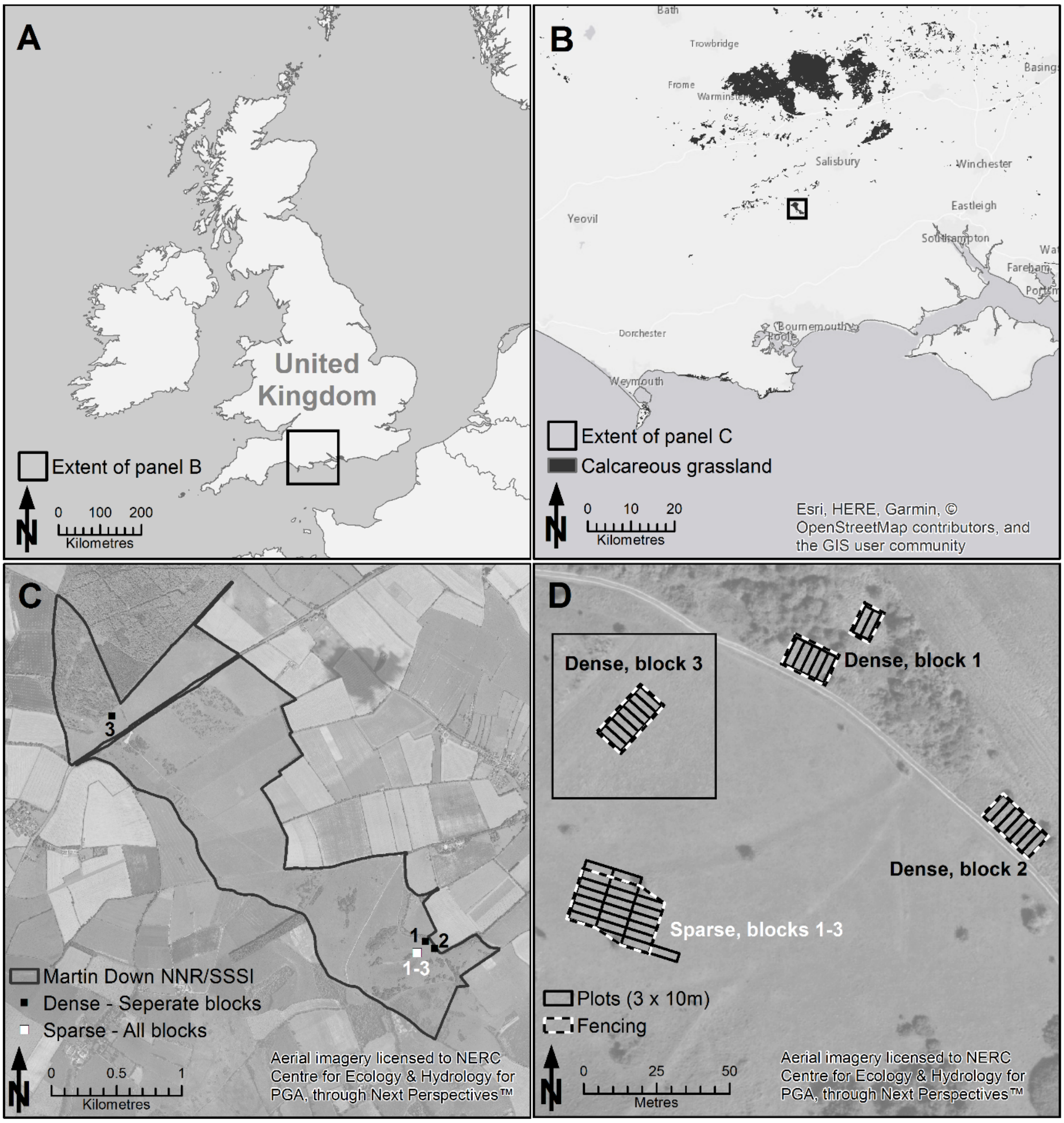

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Fig. 1 A) Map of UK showing context of lowland landscapes around Martin Down, B) Location of Martin Down in southern UK, showing extent of calcareous grassland from CEH Land Cover Map 2015 (Rowland et al. 2017) C) Extent of Martin Down as determined by the boundary of the National Nature Reserve and Site of Special Scientific Interest, showing the location of *Brachypodium pinnatum* trial plots on areas of dense (three spatially separated, blocks) and sparse (three spatially contiguous blocks) *B. pinnatum* cover. D) Layout of 3 x10m plots within blocks. The locations of herbicide treatments were randomised within blocks, with the exception that on sparsely covered blocks the cut and grazed treatment was always placed outside the fencing to allow grazing.

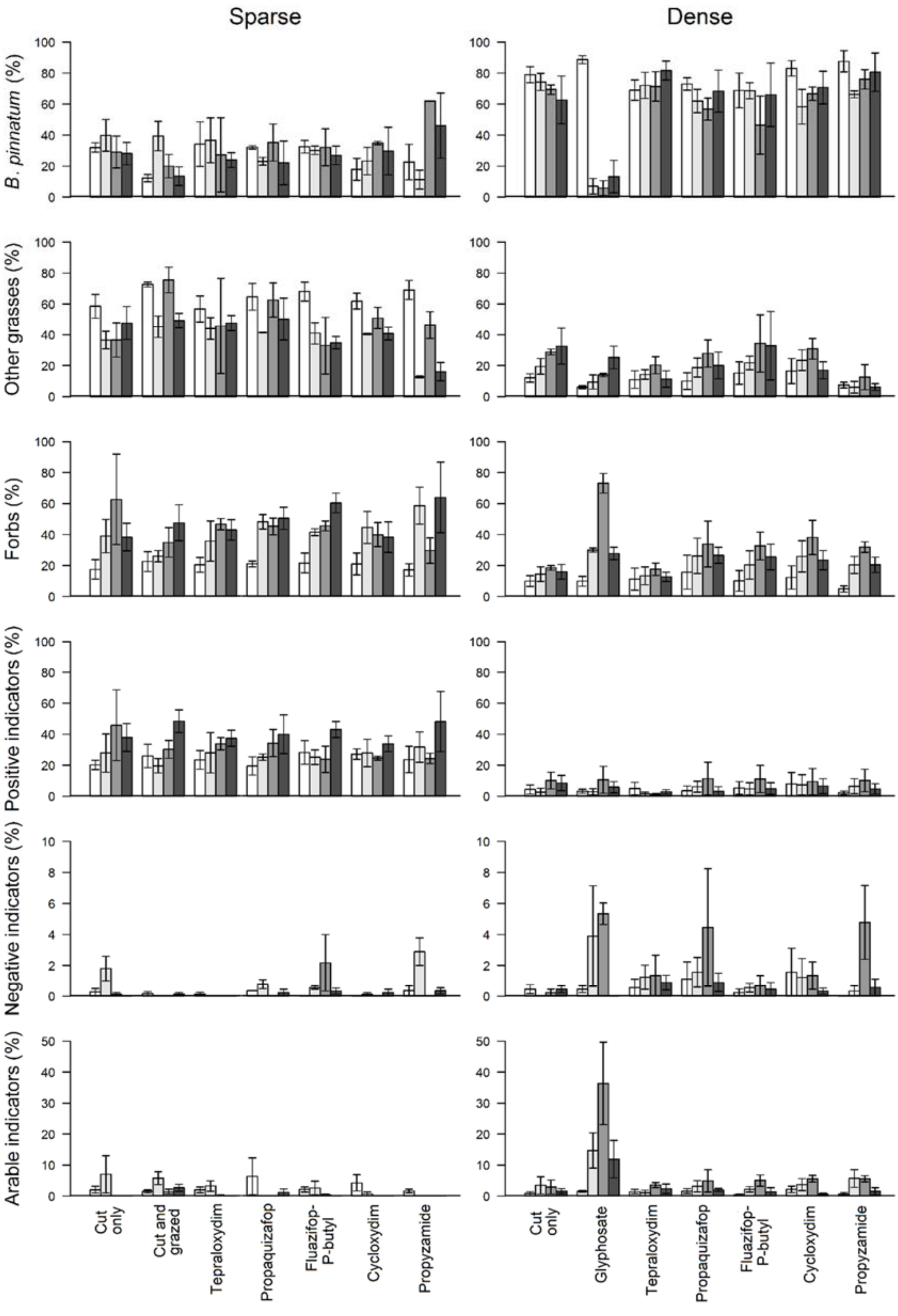

Fig. 2 Bar plots showing percentage cover of *Brachypodium pinnatum* and of plant groupings (grasses, forbs and indicator species), by treatment and year (white bars =2012, light grey bars =2013, mid grey bars =2014, dark grey bars =2015). Errors bars are ± one standard error.

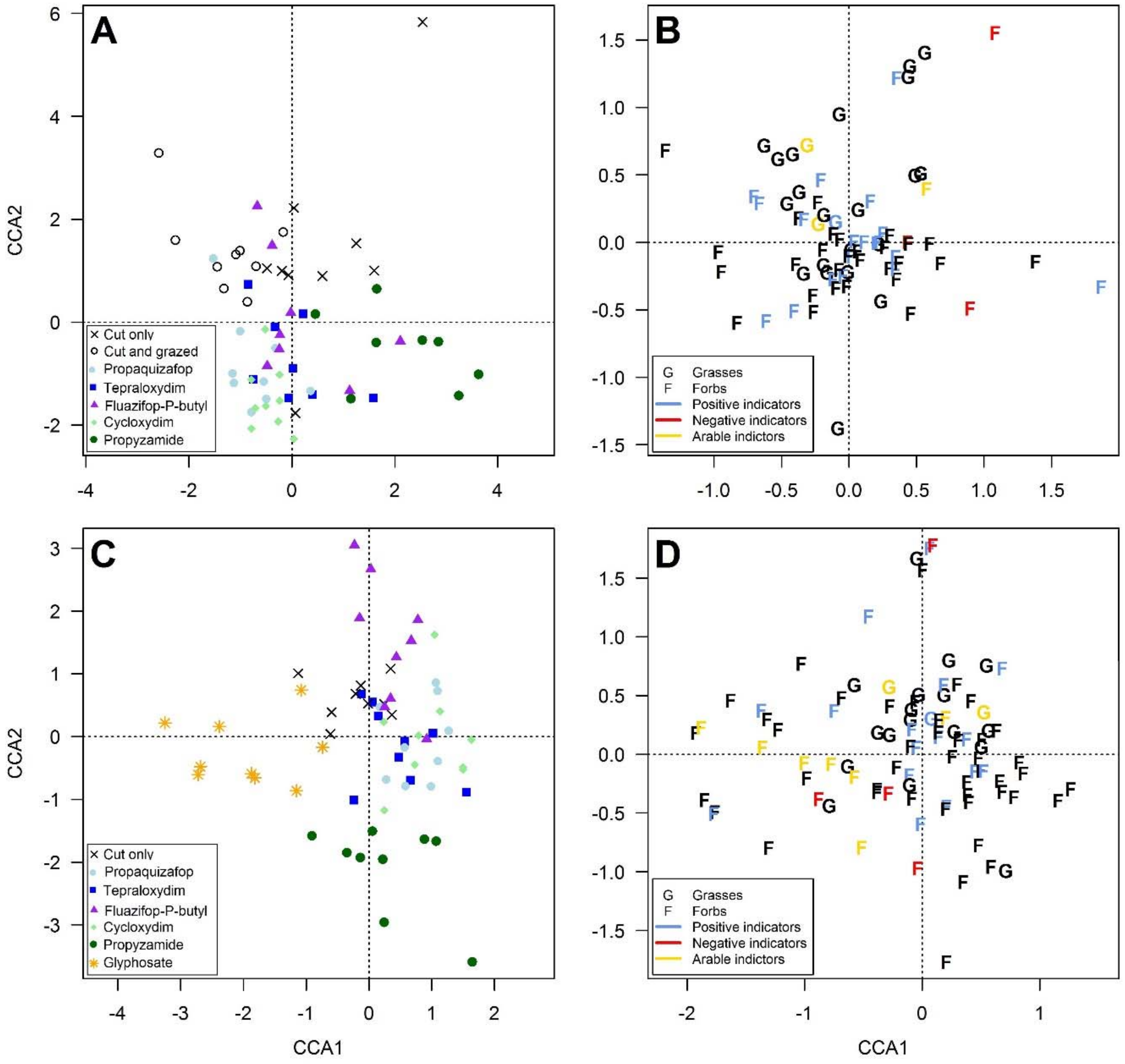

Fig. 3 Plots of first two CCA axes**,** following CCA of plant communities for all post-baseline years (excluding *B. pinnatum*) on plots with sparse (A, B) and dense (C,D) initial *B. pinnatum* cover, with treatment as constraining variable and year, experimental block and plot as conditioning variables. Panels A and C show treatment plots, coloured and symbolised by treatment (see legends on figures and Table 1 for herbicide active ingredients), whilst panels B and D show plant species coloured and symbolised by plant groupings used in univariate analyses (grasses, forbs, positive, negative and arable indicators, see legends on figures and Table S3).