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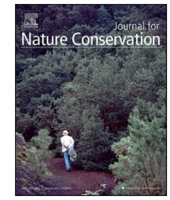

Practical methods for the control of tor-grass (*Brachypodium pinnatum s.l.*) and the restoration of calcareous grassland

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A R T I C L E I N F O

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A B S T R A C T

Calcareous grasslands are sites of high conservation value across Western Europe; however, they are increasingly threatened by the dominance of a native competitive grass, *Brachypodium pinnatum,* which reduces the diversity of the grassland. Despite this, there is no clear consensus on the most effective method for controlling *B. pinnatum* and restoring the grassland community. We established two experiments at a calcareous grassland of high nature conservation value in the UK, i) a herbicide spraying experiment with seeding and ii) a seasonal cut-and-graze experiment, to investigate the potential for reducing dense *B. pinnatum* cover and preventing further expansion of sparse cover, respectively. We examined the effect of different herbicide and cut-and-graze treatments on *B. pinnatum* cover, and on the species richness and diversity of the grassland over three consecutive years. Herbicide spraying reduced the cover of *B. pinnatum,* though two spray applications led to a greater reduction longer-term. Species richness and diversity initially declined with the herbicide spray, however this recovered rapidly to levels higher than before spraying commenced. Seeding the spray plots was beneficial for the establishment of *Bromopsis erecta* and potentially reduced the likelihood of re-colonisation by *B. pinnatum* and undesirable arable species. The cut-and-graze experiment also showed promising potential in terms of controlling the spread of *B. pinnatum*. Compared with a single cut in the spring or autumn, cutting and grazing twice, in both spring and autumn was found to reduce the cover of *B. pinnatum*, whilst also increasing species richness and diversity. Further monitoring is needed to determine the long-term effectiveness of this management treatment.

1. Introduction

Calcareous grasslands are sites of high conservation value across Western Europe. They support a high richness and diversity of plant and animal species (Wilson et al., 2012), many of which are rare or threatened (Webb et al., 2010). They are considered to be semi-natural habitats that would, in the absence of human intervention, follow natural succession into scrub and woodland (Poschlod and Wallis de Vries, 2002). Effective management is therefore required, usually through grazing but also by burning or cutting. During the twentieth century the extent of calcareous grassland declined considerably in Europe (Fuller, 1987; Polus et al., 2007; Ridding et al., 2015). This was largely attributed to agricultural intensification which led to many calcareous grasslands being tilled, fertilised and/or sown with crop or forage grass species, whilst declines in traditional livestock grazing practices led to the abandonment of much of the remainder (Fuller, 1987; Poschlod and Wallis de Vries, 2002).

The condition of remaining calcareous grasslands has also deteriorated over time, with reports of declines in species richness and diversity (Bennie et al., 2006; Ridding et al., 2020), and increases in competitive grasses (Hawes et al., 2018; Stroh et al., 2017). One such grass, *Brachypodium pinnatum*, which is native to Western Europe, also known as tor-grass or heath false-brome is an increasing threat to calcareous grasslands. *B. pinnatum* is a complex species, which is difficult to reliably differentiate from *B. rupestre* in the UK and has only recently been separated (Cope and Gray, 2009), hence we refer to *B. pinnatum* in the broad sense. The perennial grass can quickly spread and dominate surrounding vegetation leading to species-poor swards and a decline in

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grassland diversity (Bobbink and Willems, 1987; Hurst and John, 1999a). It spreads vigorously by long, creeping rhizomes which initiate and grow in the spring, before penetrating the soil surface in the autumn, where single over-wintering tillers are produced (Hall, 1971). The increase in *B. pinnatum* over the mid-twentieth century is predominately attributed to the decline in traditional grazing management, as well as the decline in rabbit grazing following myxomatosis in the early 1950s (Thomas, 1960). This is potentially exacerbated by eutrophication from fertiliser addition or atmospheric nitrogen deposition (Bobbink, 1991; Bobbink et al., 1988), however, this is still debated since the majority of evidence originates from the Netherlands, where levels of nitrogen deposition are higher than in many other European countries (Wilson et al., 1995). Furthermore, *B. pinnatum* is likely to expand with climate change (Buckland et al., 2001), thus the need to control this competitive grass and prevent further degradation of calcareous grassland is of high conservation importance.

Several strategies have been trialled for controlling *B. pinnatum*, including cutting, burning, grazing and herbicides (Bobbink and Willems, 1991, 1993; Farmer and Baxter, 1998; Redhead et al., 2019; Schläpfer, 1997), with goals ranging from reducing existing cover in densely infested areas, to preventing further spread in sparsely infested ones. The results of such studies have been mixed, with no clear consensus on the most effective method for managing this species. *B. pinnatum* has been shown to be typically unpalatable, particularly to sheep. Evidence from site managers regarding the success of sheep grazing for controlling *B. pinnatum* is variable (Farmer and Baxter, 1998). Some report summer sheep grazing actually created ideal conditions for *B. pinnatum* to spread, whilst others noted some success with winter grazing. However, few studies compare the effectiveness of grazing within different seasons under experimental conditions. Farmer and Baxter (1998) suggested that sheep grazing may be useful for maintaining current levels of *B. pinnatum*, however cattle grazing or more severe methods are likely to be required where there are large dense areas of *B. pinnatum*. One such method is the use of herbicides (Hurst and John, 1999b). Redhead et al. (2019) examined the use of several selective and broad-spectrum herbicides on both sparse and dense patches of *B. pinnatum*. Most herbicides showed little effect on sparse *B. pinnatum* patches and although dense *B. pinnatum* patches were initially reduced by glyphosate, the plants that colonised these areas were indicative of poor habitat condition (i.e. species more typical of arable or ruderal situations) rather than those associated with calcareous grassland. Baba et al. (2012) suggested that measures needed to go beyond just limiting the dominance of *B. pinnatum* and that the habitat could be restored by sowing calcareous grassland species.

In this study we establish two experiments, with the first aiming to reduce *B. pinnatum* cover, whilst the second aims to control the expansion of *B. pinnatum* at Martin Down, a calcareous grassland in southern England. To reduce dense stands of *B. pinnatum*, we compare the effectiveness of additional glyphosate herbicide spraying, combined with the cutting and seeding of typical calcareous grassland species to restore the grassland community. In a complementary study, to control sparse stands of *B. pinnatum* and prevent further degradation of the grassland, we contrast cutting and grazing undertaken in the spring, autumn and both seasons. For both the herbicide spray and the cut-and-graze experiment, we aim to examine the effect on i) the cover of *B. pinnatum,* ii) species richness, iii) species diversity and iv) the cover of typical calcareous grassland species. For the herbicide experiment we evaluate additional parameters, to assess the effectiveness of the seeding for restoring the calcareous grassland community: i) cover of all seeded species, ii) cover of seeded forbs only, iii) cover of *Bromoposis erecta* (dominant species seeded) and iv) cover of typical arable species.

2. Methods

2.1. Study site

Martin Down is located on the borders of Hampshire, Wiltshire and Dorset in southern England (50.975 N, 1.937 W) (Fig. 1). The majority of the 336 ha site consists of calcareous grassland, but smaller areas of broadleaved woodland, chalk heath and mosaics of scrub are also present. Following Salisbury Plain and Porton Down, it is one of the largest expanses of lowland calcareous grassland in the UK (Gibbons, 1989). Several types of calcareous grassland are present, but the site is largely dominated by British National Vegetation Classification (NVC) (Rodwell, 1992) community CG3 (*Bromopsis erecta* grassland) with some smaller areas of CG2 (*Festuca-Avenula* grassland) (both phytosociological alliances - *Bromion erecti*) (Rodwell et al., 2007). Soils are calcaric and rendzic leptosols (Cranfield University, 2023) with a pH of about 7.8. The average annual maximum temperature is 14.7 °C and the average annual rainfall is 896.46 (data from nearest station at Fontwell Magna, 1991–2020) (Met Office, 2023).

The site has been managed jointly by Natural England (public body advising on the natural environment in England, formerly the Nature Conservancy Council) and Hampshire County Council since it was acquired in 1978. The site is managed by sheep grazing within compartments controlled by temporary electric fences and cutting or coppicing of scrub. Martin Down was designated as a Site of Special Scientific Interest (SSSI, the basic unit of statutory land protection in Great Britain) in 1987 and is further protected through designation as a National Nature Reserve.

We established two different field experiments at Martin Down in 2019, which investigate the use of two different management strategies, to address 1) the potential for reducing dense *B. pinnatum* cover and 2) preventing further expansion of sparse cover (see Figure S1 for examples of *B. pinnatum* cover). The location and design of each experiment is therefore partly driven by the distribution of dense and sparse stands of *B. pinnatum* at Martin Down. For reducing dense stands of *B. pinnatum*, a more severe method is needed, hence we establish a herbicide spraying experiment with seeding (experiment 1), whilst the seasonal cut-and-graze experiment (experiment 2) aims to prevent further expansion in areas of sparse cover of *B. pinnatum*. The experiments examine practical methods for management of *B. pinnatum* on a nature reserve with the aim of enhancing current management. Experimental controls are therefore based on business-as-usual management scenarios, since it is unlikely that stands of *B. pinnatum* would be unmanaged over long periods.

2.2. Experiment 1: Control of B. pinnatum with additional herbicide spraying and seeding

For the herbicide spraying experiment, we set up three experimental blocks over dense patches of *B. pinnatum* (Fig. 1, Figure S1). Each block consisted of four plots, each measuring 8 m x 5 m that were marked out using a Leica Zeno 20 RTK dGPS (±3 cm). This allowed accurate relocation if markers were damaged or removed between years. Four treatments were randomly applied within each block: 1) spray and seed (SP1R1), 2) spray and no seed (SP1R0), 3) spray twice and seed (SP2R1) and 4) spray twice and no seeding (SP2R0). The first treatment (SP1R0) was similar to the usual management within this compartment at Martin Down, and is therefore considered a control. The whole area of all the plots were first sprayed with Roundup Biactive glyphosate (360 g l^{-1} active ingredient) at a 1:67 dilution on the 19th September 2019 using an adjustable nozzle set to a fine spray on a lance from a quadbike. A cut and removal of dead material then followed in April 2020 before the growing season. For plots which required a second spray (SP2R1 and SP2R0) this was carried out on 23rd June 2020, using Rodeo glyphosate with an active ingredient of 360 g l^{-1} at a rate of 5 l ha^{-1} using orange 110° T jets at 3 bar pressure. All herbicide applications were made by

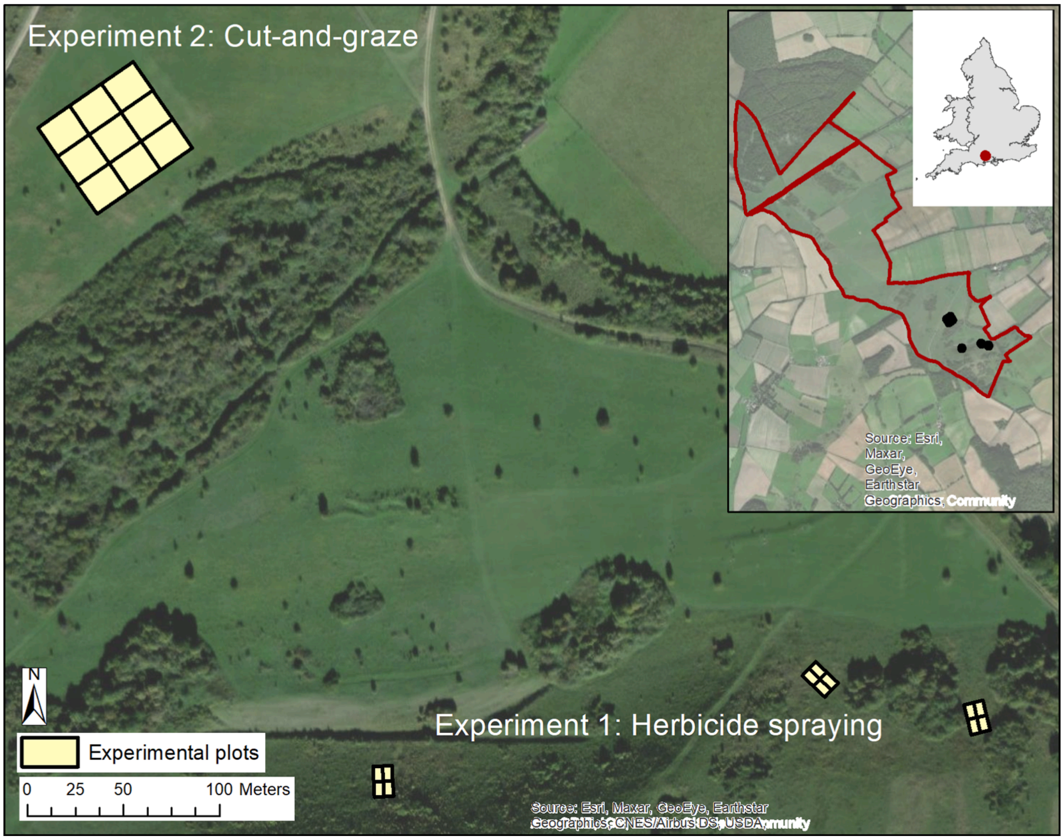

Fig. 1. The location of the two experiments at Martin Down (red), southern England. The seasonal cut-and-graze experiment (experiment 2) consisted of nine plots (each 20 m x 18 m) and the herbicide spray experiment with seeding (experiment 1) consisted of three blocks with four plots (each 8 m x 5 m). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

people trained to the legally required standard, on days of minimal wind to reduce risk of drift. Another cut and removal took place in September 2020, as well as seeding on the treatments SP2R1 and SP1R1.

Seeding was applied at a rate of 160 g of seed per plot (40 m^2), using seeds that were hand-collected throughout the 2020 season at Martin Down. The seed mixture included 80 % *Bromopsis erecta* and 20 % forbs (see Table 1). Forbs that were identified as positive indicators for CG2/3 grassland were targeted (JNCC, 2004), as well as typical calcareous grassland species that were easily collected from the study site. Common ragwort (*Senecio jacobaea*) was cut using a strimmer across plots in July 2021, with the aim of reducing seeding, since the species is listed as an injurious weed under the Weeds Act 1959 and its spread must be prevented (DEFRA, 2004). Strimming the flower heads only, allowed the species to be recorded during the vegetation monitoring. All plots were winter grazed with sheep throughout the experiment, which is the usual practice within this compartment at Martin Down.

Table 1
Seeds collected from Martin Down which were sown in September 2020 for the herbicide spray experiment.

Species	Weight per plot (g)
Agrimonia eupatoria	15
Anthyllis vulneraria	5
Briza media	1.24
Bromopsis erecta	130
Cirsium acaule	3
Filipendula vulgaris	1.5
Galium verum	0.04
Helianthemum nummularium	0.33
Leontodon hispidus	1.9
Linum catharticum	0.12
Lotus corniculatus	0.31
Primula veris	0.48
Rhinanthus minor	1.13
Sanguisorba minor	1.6
Scabiosa columbaria	0.5

2.3. *Experiment 2: Control of B. pinnatum with seasonal cutting and grazing*

A large plot was established for the cutting and grazing experiment, consisting of nine individual plots each measuring 20 m x 18 m (Fig. 1, Figure S1). This coincided with the cover of *B. pinnatum*, however, this was much patchier within the individual plots and was considered less dense compared with the herbicide experiment, where *B. pinnatum* stands covered the majority of the plot (see Figure S1 for comparison). One large plot was established for this experiment rather than three blocks as per the first experiment, because the sparse patches of *B. pinnatum* were located in one large area, whereas the dense stands were in three separate locations (Fig. 1). Three replicates of the three treatments were applied: 1) autumn cut-and-graze, 2) spring cut-and-graze and 3) both spring and autumn cut-and-graze. The autumn cut and removal of material took place in early September, with sheep grazing commencing 3–4 weeks after the cutting, whereas the spring cut and removal took place in April with sheep grazing 3–4 weeks after. The seasons were selected based on the initial growth of *B. pinnatum* rhizomes (spring) and the period where the rhizomes penetrate the soil (autumn). The cutting and removal are important for eliminating the larger unpalatable stands of *B. pinnatum*, whilst the sheep are important for eating the fresh regrowth after cutting. Sheep usually grazed the plots for a month equating to 1300–1400 sheep days. Due to practicalities of setting up fencing for sheep grazing, the cut-and-graze experiment was not fully randomised (see Figure S2), however this is controlled for in the analyses (see Data analysis). Similarly, the business-as-usual management within this compartment at Martin Down was autumn grazing (similar to treatment 1).

2.4. Vegetation monitoring

Vegetation monitoring was carried out in early September of 2019 (before all treatments were applied to provide a baseline), and post treatment in late August/early September of 2020, 2021 and 2022. For the herbicide experiment, vegetation surveys were conducted within each plot by placing five 50 cm × 50 cm quadrats at approximately 1 m intervals (i.e. vertices of a "W"-shaped transect through the plot) as seen in Redhead et al. (2019) (see Figure S3). Quadrats avoided the outermost 50 cm of each plot, where there was an increased potential for spray drift from neighbouring plots. Whilst the quadrat locations themselves were not recorded with a dGPS, the edges of the plots were, thus we have high confidence that the quadrat locations were largely consistent between years. Within each quadrat, the percentage cover of *B. pinnatum*, all other vascular plants and bare ground were recorded. Since the plots were larger and the cover of *B. pinnatum* was more variable in the cut-and-graze experiment, an extra two quadrats were recorded on the edge of each plot (avoiding the outermost 2 m), alongside five quadrats recorded within a diamond formation each 5 m apart in the centre (Figure S3). Nomenclature follows Stace (2010).

2.5. Data analysis

The herbicide experiment and the cut-and-graze experiment were analysed separately using R v4.1.2 (R Core Team, 2022). The response variables investigated to assess the effectiveness of the experiments were i) the cover *B. pinnatum,* ii) species richness, iii) species diversity, iv) the cover of typical calcareous grassland species, v) cover of seeded species, vi) cover of seeded forbs, vii) cover of *Br. erecta* and viii) cover of typical arable species. The latter four response variables were determined for the herbicide experiment only, since these related to the success of the seeded species (or colonising species). We evaluated seeded forbs and *Br. erecta* separately, since *Br. erecta* comprised the largest element of the seed mixture. Cover values for each variable were weighted by the total cover within each quadrat (e.g. cover of *Br. erecta*/total cover of vascular plants in quadrat). We classified species as typically calcareous grassland or arable species using the habitat preferences of (Hill et al., 2004). Species were included if they were associated with the relevant habitat (calcareous grassland or arable) and any other habitat (see Table S1). *B. pinnatum* was excluded from the list of calcareous grassland species since it was the target of the experiment and would otherwise be uninformative. Species diversity was calculated as the inverse Simpson's diversity index using the vegan R package (Oksanen et al., 2007).

The effect of the treatments on species richness and diversity were analysed using generalised linear mixed models (GLMMs) for both the herbicide and the cut-and-graze experiment. A Poisson error structure was used for species richness, whilst a Gaussian structure was used for diversity (Zuur et al., 2009) using the lme4 R package (Bates et al., 2014). The cover variables were divided by 100 and modelled using mixed effects beta regression in the glmTMB R package (Brooks et al., 2017). Beta regression is increasingly being used for proportional data constrained between zero and one (Douma and Weedon, 2019; Geissinger et al., 2022). However, as the beta distribution is restricted to data bounded by 0 or 1 and cannot accommodate 0 or 1 values, we rescaled the cover variables using the following equation (Smithson and Verkuilen, 2006), since some of our response variables contained zeros:

$$x_i^o = \frac{x_i(n-1) + 0.5}{n} \quad (1)$$

Where x_i is the observed value and n is the sample size. We ran two variations of the GLMMs and beta regression models for both the herbicide spray and the cut-and-graze experiment separately. The first set of GLMMs and beta regression models included treatment and year as an interaction, to understand if treatment and year were having an effect on any of the response variables. Quadrat (where vegetation was recorded) was included as a random effect for the cut-and-graze experiment, whereas quadrat nested within block was included as a random effect for the herbicide experiment, to account for the variation in the block locations. The second set of GLMMs and beta regression models, modelled the difference in the response from the baseline year (2019, quadrats recorded before the experiment commenced). Treatment and the baseline year (2019) were modelled as fixed effects, whilst the year and quadrat (nested within block for the herbicide experiment) were included as random effects, to understand whether treatments change from the baseline year once the variation from year and block are accounted for. The baseline year values were included in the model to correct for the artefact of the so-called "regression to the mean", where change scores tend to be negatively related to their baseline values (Mazalla and Diekmann, 2022). The "spray once and no seeding" treatment (SP1R0) and "autumn cut-and-graze" were used as the baseline for comparison for the spray and the cut-and-graze experiments, respectively, since this was most similar to the business-as-usual management applied before the experiment commenced. Model assumptions were checked with diagnostic plots and statistical tests using the DHARMa R package (Hartig, 2022).

As previously mentioned, the cut-and-graze experiment was not fully randomised. To account for the spatial structure of the experiment, we used principal coordinate analysis of neighbour matrices (PCNM) analysis to generate spatial eigenvectors for the quadrats to use as predictors in the cut-and-graze models described above (Dray et al., 2006). First, a pair-wise geographic Euclidean distance matrix was generated, which was consequently used to create a truncated connectivity matrix, using the minimum spanning tree algorithm to determine the threshold distance. The ME function in the spdep R package (Bivand, 2022; Bivand and Wong, 2018) was applied to the connectivity matrix to identify the most important eigenvectors that reduce spatial dependence, based on a permutation bootstrap test on Moran's I for the residuals (see Fletcher and Fortin, 2018). The resulting spatial eigenvectors were then included in the GLMMs and beta regression models described above as a covariate.

3. Results

3.1. Experiment 1: Control of B. pinnatum with additional herbicide spraying and seeding

The cover of *B. pinnatum* declined across all four herbicide spray treatments since the baseline in 2019 (Fig. 2A, Table S2). There was strong evidence for this decline for the treatments which involved two spray applications compared with one spray (Table 2), as indicated by the low p-values (p-values are interpreted on a continuous scale, as a measure of compatibility with the null hypothesis, see Hurlbert et al. (2019) and Rafi and Greenland (2020)). Though the cover of *B. pinnatum* remained similar regardless of whether seeding occurred after the two sprays (Fig. 2A). Species richness dropped considerably in 2020 for plots that were sprayed twice, however, by the end of the experiment species richness had recovered to be similar to that of the baseline (SP1R0: 2019 = 16.1 ± 1.2 (mean per 0.25 m^2 ± SE), 2022 = 16.1 ± 1.1; SP1R1: 2019 = 16.7 ± 1.3, 2022 = 16.7 ± 1.2, SP2R0: 2019 = 14.7 ± 1.0, 2022 = 14.9 ± 0.62, SP2R1: 2019 = 12.7 ± 0.99, 2022 = 15.8 ± 1.1) (Fig. 2C). Species diversity was more variable between years but overall increased slightly in 2021 and 2022 across all spray treatments compared to the baseline (Fig. 2E, Figure S4), though there was no evidence of any consistent difference between treatments (Table 2). The cover of calcareous grassland species was higher in all three years compared with the baseline across all four treatments, however, the difference was small between treatments in 2021 and 2022 (Fig. 2G).

The additional parameters that were explored to assess the

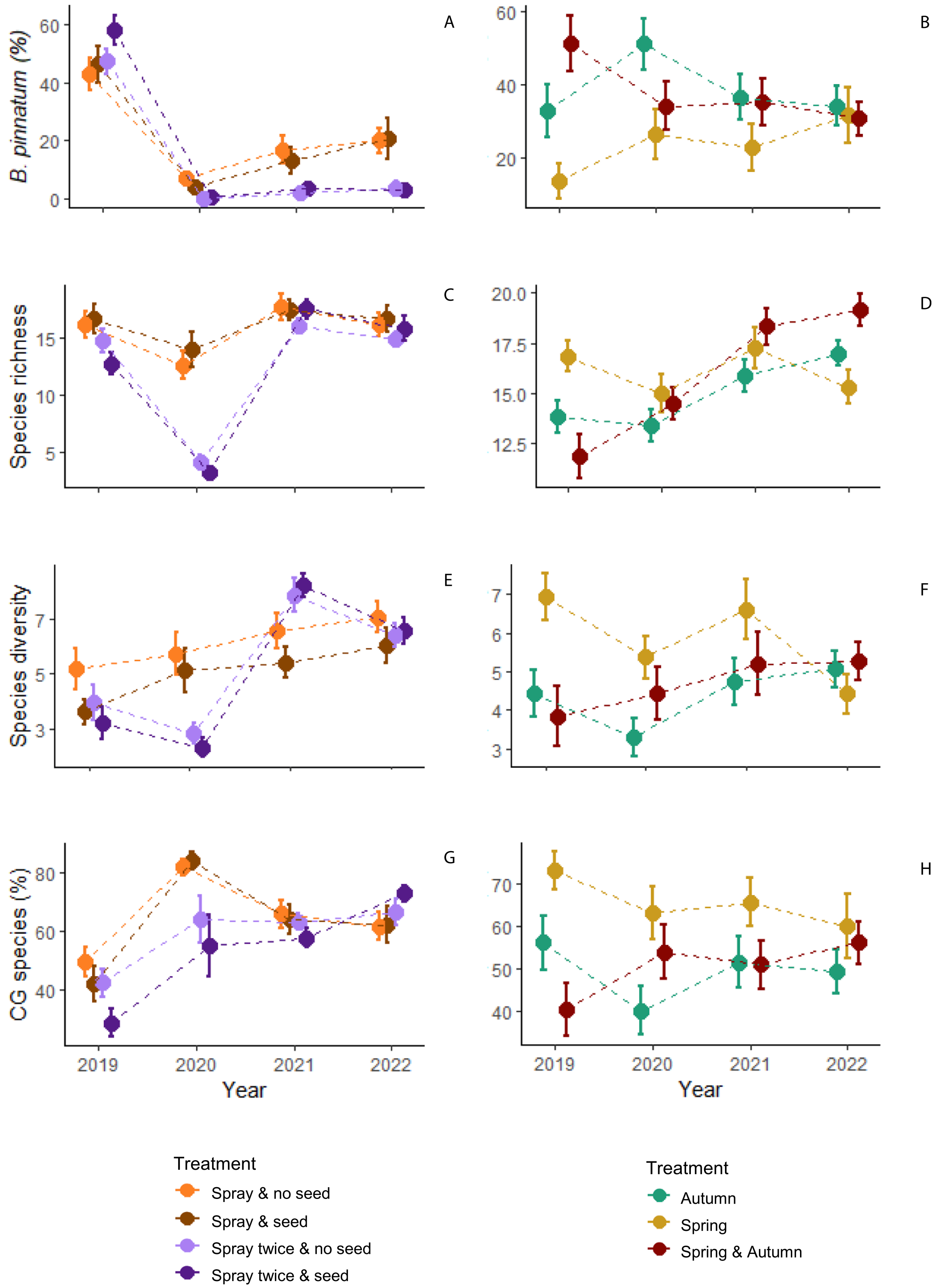

Fig. 2. Mean (±SE) cover of *Brachypodium pinnatum* (A, B), species richness (C, D), species diversity (E, F) and cover of calcareous grasslands (CG) species (G, H) within each of the treatments for the herbicide (left) and cut-and-graze (right) experiment at Martin Down between 2019 (baseline) and 2022 (see Figure S4 for the change from the baseline).

effectiveness of the seeding were variable (Fig. 3). The years 2021 and 2022 were of particular interest following the sowing of species in 2020. The total cover of all of the seeded species types was the highest in the SP2R1 treatment, however in 2021 the cover of seeded species was actually higher in the once-sprayed treatment which had not been seeded. When focusing solely on the seeded forbs (i.e. not *Briza media* or *Br. erecta*) the cover was higher for the unseeded treatments for both once-sprayed and twice-sprayed plots, though this was also the case during the baseline. On the other hand, the cover of *Br. erecta*, the most dominant seed in the seed mixture, increased considerably in the two seeded treatments, particularly in 2022 (Fig. 3B, Table S2). There was evidence for this effect for the SP2R1 treatment (Table 2). This suggests that the patterns seen with all seeded species are largely driven by the cover of *Br. erecta*. The cover of arable species was greatest across all treatments in 2020, particularly for the plots which had just been sprayed for a second time, as expected. In the following years, plots

Table 2
Results of generalised linear mixed models (species richness (exponentiated) and diversity) and mixed effects beta regression models (cover of *Brachypodium pinnatum* (logit scale), cover of calcareous grassland species (logit scale), cover of seeded species (logit scale), cover of seeded forbs (logit scale), cover of *Bromopsis erecta* (logit scale) and cover of arable species (logit scale)) for the herbicide experiment at Martin Down. All models included treatment and the baseline (2019) as fixed factors, with a random effect of year and quadrat nested in experimental block. The model coefficients for the respective baseline variables (_2019) are ecologically uninterpretable, as they are correlated with the error terms, and will therefore not be discussed.

Model	Explanatory variable	Coefficient	SE	Z value	p-value
Species richness	(Intercept)	13.41	0.22	11.78	<0.001
	Spray & seed	1.03	0.05	0.61	0.544
	Spray twice & no seed	0.76	0.06	−4.67	<0.001
	Spray twice & seed	0.80	0.06	−3.66	<0.001
	Species richness 2019	1.01	0.01	1.06	0.288
Diversity	(Intercept)	6.13	1.14	5.39	0.005
	Spray & seed	−0.84	0.51	−1.63	0.105
	Spray twice & no seed	−0.68	0.51	−1.34	0.184
	Spray twice & seed	−0.63	0.53	−1.20	0.230
	Diversity 2019	0.06	0.09	0.69	0.489
Cover of *B. pinnatum*	(Intercept)	−2.79	0.40	−6.91	<0.001
	Spray & seed	−0.32	0.26	−1.21	0.228
	Spray twice & no seed	−1.42	0.29	−4.93	<0.001
	Spray twice & seed	−1.48	0.30	−4.96	<0.001
	B. pinnatum 2019	0.02	0.01	3.15	0.002
Cover of calcareous grassland species	(Intercept)	0.28	0.30	0.96	0.338
	Spray & seed	0.05	0.26	0.19	0.853
	Spray twice & no seed	−0.17	0.26	−0.66	0.507
	Spray twice & seed	−0.20	0.27	−0.73	0.463
	CG species 2019	0.01	0.00	2.08	0.037
Cover of seeded species	(Intercept)	−0.83	0.25	−3.37	0.001
	Spray & seed	−0.32	0.21	−1.52	0.128
	Spray twice & no seed	−0.34	0.21	−1.57	0.116
	Spray twice & seed	−0.13	0.21	−0.60	0.547
	Seeded species 2019	0.01	0.01	1.53	0.126
Cover of seeded forbs	(Intercept)	−0.96	0.28	−3.47	0.001
	Spray & seed	−0.45	0.23	−1.99	0.046
	Spray twice & no seed	−0.32	0.23	−1.42	0.155
	Spray twice & seed	−0.59	0.23	−2.53	0.012
	Seeded forbs 2019	0.02	0.01	1.55	0.122
Cover of *Br. erecta*	(Intercept)	−3.59	0.38	−9.52	<0.001
	Spray & seed	0.58	0.21	2.82	0.005
	Spray twice & no seed	0.06	0.20	0.28	0.781
	Spray twice & seed	1.54	0.21	7.38	<0.001
	Br. erecta 2019	0.00	0.01	−0.23	0.815

Table 2 (*continued*)

Model	Explanatory variable	Coefficient	SE	Z value	p-value
Cover of arable species	(Intercept)	−2.54	0.20	−12.49	<0.001
	Spray & seed	0.02	0.22	0.11	0.912
	Spray twice & no seed	0.34	0.22	1.55	0.121
	Spray twice & seed	0.06	0.22	0.28	0.781
	Arable species 2019	0.06	0.04	1.44	0.150

which were not seeded have a greater cover of arable species compared with their equivalent treatment which had been seeded. However, there was no evidence for these effects (Table 2).

3.2. Experiment 2: Control of B. pinnatum with seasonal cutting and grazing

The cover of *B. pinnatum* was inconsistent across the three treatments during the baseline of the cut-and-graze experiment (Fig. 2B). The cover of *B. pinnatum* was variable throughout the duration, however there was strong evidence for a decline in *B. pinnatum* with cut-and-graze in the "spring and autumn treatment" plots (Table 3) and an interaction with year in 2020, 2021 and 2022 (Table S4). This pattern was also evident without the inclusion of spatial eigenvectors in the model (Table S3, Table S5). With the exception of 2020, the cover of *B. pinnatum* remained largely consistent throughout the experiment with cut-and-graze in the autumn only (Fig. 2B, Figure S4). Spring and autumn cut-and-graze resulted in an increase in species richness and diversity over time, with strong and moderate evidence, respectively (Table 3). The cover of calcareous grassland species varied across time and treatments. The cover was higher across all years for the spring only treatment, with the highest cover recorded in the baseline (Fig. 2H). There was strong evidence for an increase in calcareous grassland species cover for the "spring and autumn treatment", with evidence of an interaction with year in 2020, 2021 and 2022 (Table S4). There was strong evidence for the effect of the spatial eigenvectors within each of the models (Table 3), suggesting that there may be some underlying spatial patterns. However, the main differences in effect sizes and p-values were seen for the spring only treatment (Table S3).

4. Discussion

4.1. Controlling dense B. pinnatum with additional herbicide spraying and seeding

The additional application of glyphosate showed potential as a management treatment for reducing the cover of dense *B. pinnatum* stands on Martin Down. All spray treatments reduced the cover of *B. pinnatum* by at least half of that observed during the baseline in 2019. There was stronger evidence for this reduction with the two treatments that were sprayed twice, with *B. pinnatum* cover less than 4 % in the two subsequent years, a decline of 44–57 % since the baseline. This is below the 10 % threshold required for good condition status of CG3 grassland communities as part of the Common Standards Framework (JNCC, 2004). Hurst and John (1999b) assessed the effectiveness of glyphosate for controlling *B. pinnatum* at two chalk grasslands in Sussex. They found that after two years following the treatment, *B. pinnatum* formed ca. 25 % and 10 % of the total vegetation cover at their two study sites, respectively, which is similar to the business-as-usual treatment (once-sprayed) in our experiment (17–21 % in 2021 and 2022).

Our results suggest that a follow-up spray the next year is beneficial for targeting additional *B. pinnatum* patches that may re-establish following the first round of spraying. In 2022, two years after the

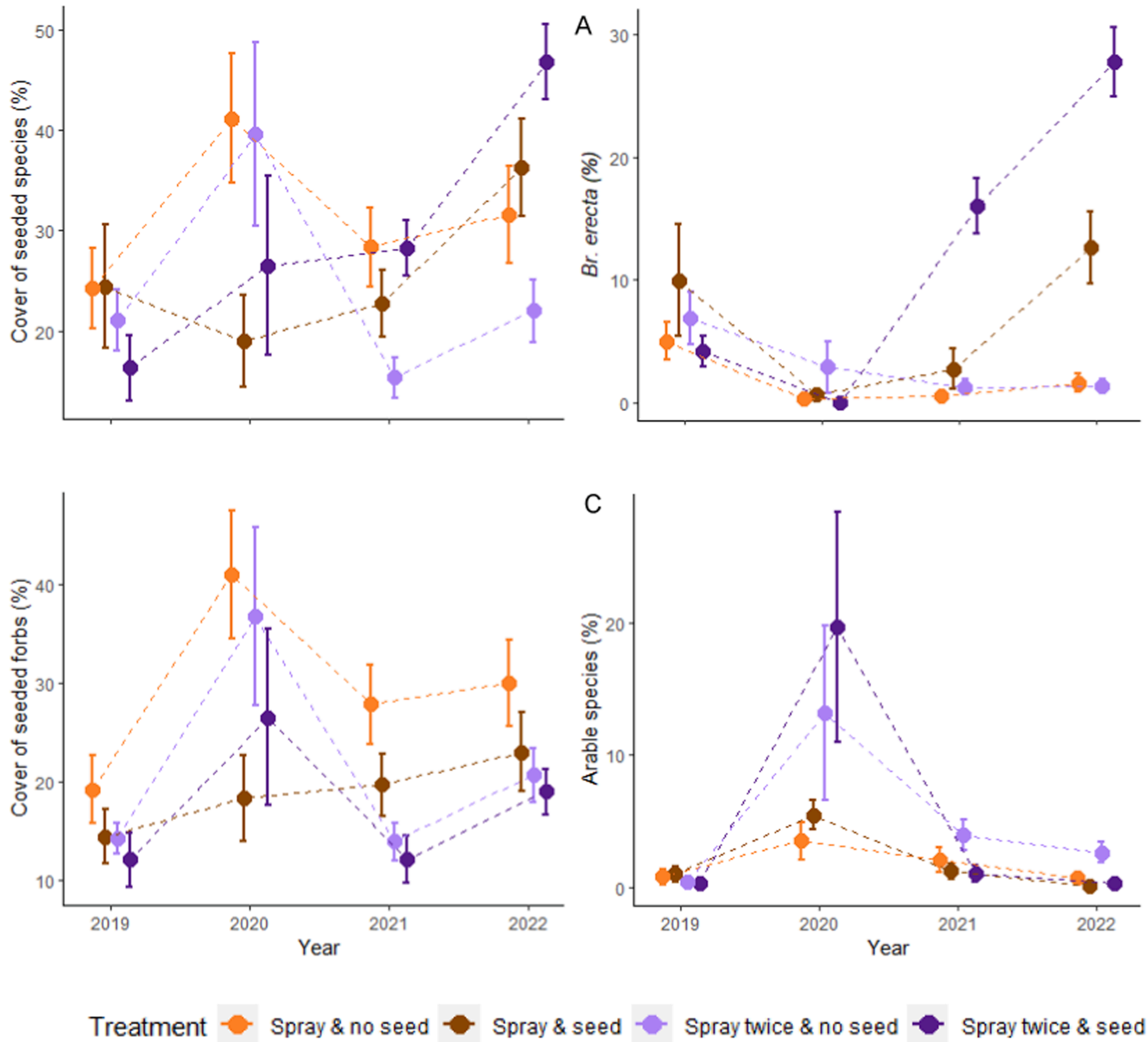

Fig. 3. Mean (±SE) cover of all seeded species (A), *Bromopsis erecta* (B), seeded forbs (C) and typical arable species (D) within each of the four treatments for the herbicide experiment at Martin Down between 2019 (baseline) and 2022 (see Figure S5 for the change from the baseline).

second spray, the cover of *B. pinnatum* is considerably lower than the equivalent of two years after the once spray treatment in 2021, suggesting that a double spray delays the recovery of *B. pinnatum*. Further observations in the years following this experiment are required to determine how long the cover of *B. pinnatum* is reduced for. Farmer and Baxter (1998) recommended that repeated applications are required long-term. Similarly, further trials are required to explore the number of applications beyond two years required to eliminate *B. pinnatum*. This will influence whether glyphosate spraying could be a viable method in the long-term.

The cover of typical arable species (e.g. *Sonchus* spp., *Myosotis arvensis*) that established after the plots were sprayed in 2020 was high compared with the baseline, as expected. Redhead et al. (2019) also found that the species which colonised after glyphosate treatment were predominately those associated with arable land or disturbance. However, in our experiment the cover of arable species decreased in 2021 and 2022. Redhead et al. (2019) used repeat glyphosate treatments, so they would not have observed this decline more than one year after treatment. In 2022, the cover of arable species was lower in the two treatments which had been seeded, however there was no evidence for this effect.

However, the results revealed that the change in the cover of seeded species over time was largely driven by the increase in *Br. erecta*. This species, like *B. pinnatum* is a dominant species of calcareous grassland, so the establishment of this species in the seeded plots may have helped reduce the cover of arable species and prevented the re-establishment of *B. pinnatum* through competition. *Br. erecta* has been found to be more tolerant of drought and disturbance than *B. pinnatum* (Corcket et al., 2003), so long-term, particularly with the threat of more droughts as a consequence of climate change (Hanlon et al., 2021), the presence of *Br. erecta* may provide competition for *B. pinnatum,* which is also expected to benefit from climate change (Buckland et al., 2001). The cover of typical calcareous grassland species was higher than the baseline across all treatments in all years. This suggests the reduction in *B. pinnatum* allowed smaller calcareous grassland forbs from the surrounding grassland and associated seedbank to establish, due to less competition for light (Jacquemyn et al., 2003), given that this pattern was seen across all plots, not just those that were seeded.

Although an additional spray of glyphosate was effective at reducing *B. pinnatum* cover, there are still concerns over the use of such herbicides (Myers et al., 2016), particularly for conservation purposes with the risk of potential drift onto non-target species (Marrs et al., 1991; Mathiassen et al., 2021; Strandberg et al., 2021). The viability of using this method depends on how many years *B. pinnatum* cover is reduced for after two sprays of glyphosate. There is also uncertainty on the long-term use of glyphosate in some countries (European Commission, 2022), however

Table 3
Results of generalised linear mixed models (species richness (exponentiated) and diversity) and mixed effects beta regression models (cover of *Brachypodium pinnatum* (logit scale) and cover of calcareous grassland species (logit scale)) for the cut-and-graze experiment at Martin Down. All models included treatment, the baseline (2019) and spatial eigenvectors as fixed factors (represented as "fitted(_.ME)"), with a random effect of year and quadrat.

Model	Explanatory variable	Coefficient	SE	Z value	p-value
Species richness	(Intercept)	13.77	0.09	28.97	<0.001
	Spring	0.94	0.06	−1.18	0.240
	Spring & Autumn	1.18	0.05	3.27	0.001
	Species richness 2019	1.01	0.01	1.74	0.082
	fitted(SR.ME)	0.39	0.35	−2.71	0.007
Diversity	(Intercept)	3.55	0.61	5.84	<0.001
	Spring	−0.42	0.66	−0.65	0.521
	Spring & Autumn	1.19	0.58	2.04	0.046
	Diversity 2019	0.22	0.08	2.85	0.006
	fitted(div.ME)	−13.19	4.07	−3.24	0.002
Cover of *B. pinnatum*	(Intercept)	−1.44	0.28	−5.11	<0.001
	Spring	0.20	0.37	0.55	0.584
	Spring & Autumn	−1.07	0.35	−3.09	0.002
	B. pinnatum 2019	0.02	0.00	4.58	<0.001
	fitted(bp.ME)	8.26	2.49	3.32	0.001
Cover of calcareous grassland species	(Intercept)	−1.22	0.31	−3.91	<0.001
	Spring	0.43	0.28	1.54	0.124
	Spring & Autumn	0.81	0.28	2.87	0.004
	CG species 2019	0.02	0.00	4.13	<0.001
	fitted(CG.ME)	−4.46	1.61	−2.76	0.006

approval may be granted for specific requirements or for other broad spectrum herbicides.

4.2. *Controlling the spread of B. pinnatum with seasonal cutting and grazing*

The aim of exploring cutting-and-grazing during different seasons was to control *B. pinnatum* and prevent further spread rather to reduce the cover as with the herbicide experiment. Because of *B. pinnatum* was much more patchy in this area of the study site compared with the location of the herbicide experiment (Figure S1). Spring grazing is useful for where the dominant species is unpalatable, such as *B. pinnatum* (Vickery et al., 2001), however, we found little evidence of an effect on *B. pinnatum* cover with only spring cut-and-graze. The spring and autumn cut-and-graze treatment, on the other hand, resulted in a reduction in *B. pinnatum* cover and an increase in species richness, diversity and the cover of calcareous grassland species over time. This suggests that undertaking cutting and grazing twice a year may be more beneficial than spring or autumn alone. This supports the findings of Bobbink and Willems (1993) who explored different cutting frequencies on a *B. pinnatum* dominated slope at Bemelerberg, Netherlands. They found that cut and removal twice a year (end of May and end of August/ early September) was adequate for controlling the dominance of *B. pinnatum* and also resulted in an increase in forb biomass and species diversity. The increase in species diversity is also consistent with our study. Redhead et al. (2019) also explored a cut-and-graze treatment (single summer cut), as part of their herbicide experiment and found that although it did not significantly reduce the cover of *B. pinnatum*, the grassland community had changed with more typical unimproved grass species present. These results are similar to the single cut-and-graze treatments in our study, where the cover of *B. pinnatum* remained fairly consistent over time with only spring or autumn cut and grazing. However, there were small increases in species richness and diversity with cutting and grazing in the autumn only. Although the analysis has accounted for the varying levels of *B. pinnatum* cover in the baseline survey and the unrandomised set-up of the experiment through the use of spatial eigenvectors, caution should still be taken with the findings from this experiment, particularly with the spring treatment. This is because infertile grassland communities usually respond slowly to changes in management (Pakeman et al., 2019), thus further monitoring is required to understand the long-term impacts of cutting and grazing in different seasons.

5. Conclusion

The control of this native invader, *B. pinnatum,* is important for the preservation of calcareous grassland which is of high conservation value across Western Europe. Our findings suggest two applications of glyphosate were more effective at reducing dense patches of *B. pinnatum*. Furthermore, seeding may restrict the cover of arable species in subsequent years and replace the dominant grass with the species expected for the target calcareous grassland community. Cutting and grazing in both spring and autumn was also found to reduce the cover of *B. pinnatum*, whilst increasing the richness and diversity of the grassland. However, continued monitoring of the grassland community and the extent of *B. pinnatum* is recommended to determine how long the treatments are effective for. Further research may explore the combination of spraying, followed up with seasonal cutting and grazing in future years once the grassland community has recovered.

CRediT authorship contribution statement

Lucy E. Ridding: Writing – review & editing, Investigation, Writing – original draft, Methdology, Conceptualization. **John W. Redhead:** Writing – review & editing, Methodology, Conceptualization. **Emily V. Upcott:** Writing – review & editing, Investigation. **Lucy Hulmes:** Writing – review & editing, Investigation. **Sarah Hulmes:** Writing – review & editing, Investigation. **Nadine Mitschunas:** Writing – review & editing, Investigation. **Katherine Howell:** Writing – review & editing, Investigation. **Jenny Christelow:** Writing – review & editing, Investigation. **Alexander R. Wickenden:** Writing – review & editing, Data curation. **Mike Fussell:** Writing – review & editing, Investigation. **James Plunkett:** Writing – review & editing, Investigation. **Richard F. Pywell:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data is published and freely available via the Environmental Information Data Centre (EIDC) https://catalogue.ceh.ac.uk/documents/f15e64c0-db65-40ec-8b6d-50573f5f6694 shared via the

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnc.2024.126566.

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