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RESEARCH ARTICLE

Green hay transfer for grassland restoration: species capture and establishment

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Green hay transfer from species-rich donor sites is now commonly used in Europe to restore species-rich semi-natural grassland, both on ex-arable land and on former intensive grassland. However, species transfer rates are usually well below 100%, and due to lack of further colonization by additional target species after initial restoration, continued progress toward the target plant community is often very slow. We used data from a restoration experiment aiming to reestablish species-rich grazed meadows of the MG5 grassland type according to the British National Vegetation Classification to investigate relationships between species abundance at a donor site, species capture by green hay and its seed content, and success of species establishment on experimental plots in formerly intensively managed species-poor grassland undergoing restoration. Our results show that species with higher abundance at the donor site were more likely captured as seed in green hay, and were more likely to establish after hay application at the recipient site. Species with low abundance at the donor site that also possessed specific germination requirements that might prevent immediate establishment after green hay transfer were particularly unlikely to get established after transfer. These findings can provide guidance for additional measures aimed at ensuring establishment of a wider range of target species. Such measures could include targeted sowing of species in addition to green hay application, and management of restored grassland swards to extend or reopen an initial window of opportunity for the establishment of green hay species that might not be germinable immediately after hay transfer.

Key words: effective sowing rate, greenhouse emergence, hay seed content, MG5 grassland, seed dormancy, supplementary sowing

Implications for Practice

- Failure to capture species as seeds in green hay for meadow restoration and to establish them at a recipient site is more likely for species less abundant at the donor site, and establishment is also less likely for species with germination requirements preventing efficient germination after green hay transfer.
- These obstacles can be addressed, for example, by supplementing green hay transfer with targeted additional sowing of species, or by managing the recipient site to extend the initial window of opportunity for target species establishment, or reopen it in the spring after hay transfer.
- Greenhouse emergence trials to determine seed content of green hay must be carefully designed and interpreted, as species differ from each other in their requirement for cold stratification

Introduction

Species-rich European and U.K. lowland semi-natural grassland has strongly declined in extent over the last 70 years (Isselstein et al. 2005; Bullock et al. 2011). The principal driver of this decline was agricultural intensification, mainly in the form of conversion to intensively managed grassland (Walker et al. 2004). For example, of grazed meadows of the MG5 type according to the British National Vegetation Classification (NVC; see Rodwell 1992), once the most widespread type of lowland hay meadow in Britain (Rodwell et al. 2007), less than 10,000 ha are now left across England and Wales (Maddock 2008). To restore a coherent and resilient network of this and other types of semi-natural grassland, both the diversification of extant species-poor grassland and the creation of additional sites of high botanical quality are required (Lawton

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et al. 2010). However, unassisted recovery of species-rich semi-natural grassland often requires many decades, due to key constraints in the form of seed limitation and microsite limitation (Bakker & Berendse 1999; Walker et al. 2004). Seed limitation occurs due to the slow and limited dispersal of target species propagules (Bakker & Berendse 1999; Walker et al. 2004). Spontaneous colonization of sites undergoing reversion to grassland by restoration target species is very limited even when species-rich grassland that could act as a source of propagules is present nearby (Stampfli & Zeiter 1999; Coulson et al. 2001; Bischoff 2002). Microsite limitation, on the other hand, occurs due to site-specific constraints preventing the establishment of desired target species after their propagules have reached the site (Bakker & Berendse 1999; Walker et al. 2004).

To overcome seed limitation, target species are actively introduced during restoration (Bakker & Berendse 1999; Walker et al. 2004). The two main methods for meadow creation on ex-arable land and diversification of existing species-poor grassland are species-rich seed mixtures and transfer of green hay from high-quality local grassland (Hedberg & Kotowski 2010; Kiehl et al. 2010). The use of seed mixtures is generally straightforward as species composition can be adjusted as required. With seed mixtures, the seeds of many species will have had an opportunity to afterripen during dry storage, thus being capable of fast germination. Accordingly, on former arable land, establishment of 75–100% of sown species is usually achieved (Kiehl et al. 2010).

Several operational stages are involved during green hay application (Trueman & Millett 2003), and there is less control over species composition of seeds transferred with the hay, which depends on choice of donor site and timing of the hay cut. After the hay cut, transport of harvested material and its spreading at the recipient site must take place within 24 hours. In line with the fact that the set of species found as seeds in the green hay is usually only a subset of the set of species found at the donor site, two types of species transfer rate can be calculated. Calculation of the absolute transfer rate is based on the full complement of species found at the donor site, calculation of the relative transfer rate is based on the set of species that have actually been captured by the hay cut (Kiehl et al. 2010). When restoring species-rich noncalcareous mesic to wet meadows and floodplain grassland on former arable land, absolute transfer rates using green hay are usually around 36–53%, with relative transfer rates usually around 57–66% (Kiehl et al. 2010). Thus, a significant proportion of species from green hay donor sites regularly fail to establish at recipient sites, and for a sizable number of these species this is the case in spite of their seeds having been transferred with the hay. Both kinds of transfer rate are even lower when green hay is used to diversify species-poor existing grassland (Kiehl et al. 2010). This is the case even though in this situation, additional bare ground creation via cultivation is usually carried out prior to hay spreading, to temporarily produce a competition-free environment for establishment of target species transferred with the hay (Trueman & Millett 2003; Bischoff et al. 2018).

Irrespective of whether the recipient site is former arable land or extant species-poor grassland, if absolute transfer rates are low, this means that in the short term, only a core set of species from the target community can be established. Furthermore, given the already mentioned slow colonization, further progress to a more complete target community is often slow or absent (e.g. Sullivan et al. 2019), as those target species not already established during initial restoration also fail to colonize subsequently. Thus, it might be better to combine green hay application with seeding during initial restoration, to maximize establishment of as wide a range of species as possible. This could be done either via additional sowing of hand-collected seed of species underrepresented or absent in the green hay (Poschlod & Biewer 2005), or via additional sowing of commercially supplied seed (Baasch et al. 2016). However, the cost of purchased seed can be high, and about two thirds of European grassland species are commercially unavailable (Ladouceur et al. 2018). Hand collection on the other hand is very labor-intensive (Stevenson et al. 1997). It would thus be advantageous to have better knowledge of which species can be reliably transferred via green hay, and which species cannot. Such knowledge requires both an understanding of the factors that determine which species are captured as seed via green hay and which of these captured species then successfully establish after hay transfer. However, due to the rapid transfer requirement when applying green hay, seed species composition of the applied green hay remains unknown at the time of hay transfer. Species capture can only subsequently be determined, either via greenhouse emergence (Poschlod & Biewer 2005; Kiehl et al. 2006; Kirmer & Tischew 2014) or via manual seed extraction (Scotton et al. 2009; Albert et al. 2019). The results of such efforts show that, as a rule, representation of different donor-site species in green hay strongly varies, with seeds of a few species present in large quantities, and those of many other species only present at very low densities (Poschlod & Biewer 2005; Scotton 2016, 2018). However, among those studies relying on greenhouse emergence, treatment of samples varies, with some authors advocating a cold-moist stratification pretreatment of samples, others allowing for such stratification halfway during emergence trials (Kirmer & Tischew 2014), and yet others not making any mention of cold stratification being applied. Hence, when using greenhouse emergence to determine species capture, it appears plausible that results might depend on the exact protocol used.

Species capture with green hay may also have consequences for species establishment at the recipient site. One might expect that species better represented in the hay are more likely to establish than species whose seeds are only present at low densities. Other factors including plant traits such as seed weight or the presence of primary seed dormancy in many forb and sedge species of grassland (Grime et al. 1981), which has also been confirmed for freshly harvested seed in green hay for restoration (Rasran et al. 2006), might also affect whether species from green hay can successfully establish.

In this study, we investigated the factors determining seed capture by green hay and the effects of green hay sample pretreatment on determination of seed content using greenhouse emergence. In addition, we explored the factors determining species establishment from green hay at the recipient site. In particular, we were interested in whether establishment success at the recipient site could be predicted by a combination of species composition and flowering phenology in the donor grassland, and plant traits that might affect seed capture with green hay and/or plant establishment at the recipient site. To this end, we investigated four questions:

(1) Does greenhouse emergence from green hay depend in a species-specific manner on length of prior cold-moist stratification?

(2) Are species more likely to be captured as seed in green hay if they have higher abundance at the donor site?

(3) Is species establishment at the recipient site influenced by abundance at the donor site and by effective sowing rate via green hay transfer?

(4) Can successful species establishment be predicted from a combination of abundance and/or phenology at the donor site, and species traits that might affect either seed capture with green hay and/or plant establishment after hay transfer?

Methods

Field Site and Experimental Design

A 4-year meadow restoration experiment was set up in July 2013 in three species-poor grassland fields at the Hillesden Estate, a c. 1,000 ha arable farm in Buckinghamshire, England (51°57′58″N 0°58′15″W), along the western bank of the small river Padbury Brook, a tributary to the River Twins. These fields were arable land until 2007, and were then sown with a mixture of perennial ryegrass *Lolium perenne* and white clover *Trifolium repens*. Subsequently, until the start of the experiment, they were species-poor agriculturally improved grassland, corresponding to the *L. perenne*–*T. repens* subcommunity (MG7a) of *L. perenne* grassland in the U.K. NVC (Rodwell 1992). The soil in all three fields is alluvial clay and clay loam, with a pH of about 6.5. Mean annual temperature at Hillesden is 9.7°C and annual rainfall is 648 mm, of which 381 mm falls during the months of April to October (based on data from 1981 to 2010; Met Office 2019).

The experimental design was a randomized complete block design with four replicate blocks, two of which were located in the largest of the three fields. Within each replicate block, three restoration treatments were applied to experimental plots of between 0.95 and 2.7 ha in size, depending on the overall size and shape of the field in which the block was located. These treatments were: (1) a green hay treatment to which target species were introduced by application of freshly harvested hay from a species-rich donor meadow; (2) a diverse seeding treatment to which target species were introduced by sowing of a specifically tailored diverse seed mixture containing a large number of grasses and forbs; and (3) a control treatment as provided by the species-poor extant grassland. Treatments (1) and (2) were designed to restore *Cynosurus cristatus*–*Centaurea nigra* grassland, that is, MG5 grassland according to the NVC (Rodwell 1992), which had been determined as a suitable restoration target for the experimental area based on its location, soil, hydrology, and proposed management as a grazed hay meadow. A comparative analysis of vegetation development across all treatments over a 4-year period is presented in a separate article (Wagner et al. 2020). Here, we focus exclusively on the green hay treatment, to investigate the factors that determine species transfer by green hay application.

To prepare experimental plots for green hay application, a silage cut took place in the w/c 17 June 2013. This was followed by marking out experimental plots, and spraying with glyphosate followed by cultivation, to create a suitable shallow tilth and bare ground for facilitating seedling establishment of restoration target species. Glyphosate spraying was carried out in the w/c 24 June 2013 using a self-propelled sprayer, and cultivation was carried out in the w/c 8 July 2013. Cultivation consisted of a series of operations including plowing, power harrowing, and ring rolling, carried out in that order. Thus, we followed recommendations by Natural England (2010) to combine creation of a short sward with creation of bare ground prior to green hay application, while exceeding their recommendation of single-step cultivation by power harrowing. At the same time, as suggested by Trueman and Millett (2003), our chosen site preparation regime combined herbicide spraying and cultivation, thus resulting in more lasting bare ground creation, and a widening of the temporal window of opportunity for target species establishment. The site preparation regime used in our experiment combining all these elements resulted in close to 100% bare ground.

The green hay donor site was species-rich MG5 grassland, 3.68 ha in size and located within Rushbeds Wood SSSI, a nature reserve c. 15 km south of the Hillesden experimental site. The hay was cut on 24 July 2013 using a disk mower, and a forage harvester was used to load a farm tractor trailer for transport of the freshly cut hay to the experimental site. On the same day, using a muck spreader, the hay was evenly spread onto the green hay treatment plots. After the green hay was spread, fields were once more ring rolled. The total area of the four replicate green hay restoration plots was 8.15 ha, resulting in a ratio of donor to recipient area of 1:2.2, and green hay application at a higher rate than with an area ratio for green hay application of 1:3 as recommended by Natural England (2010). Post-establishment management from 2014 onwards was similar to traditional management of MG5 grassland, involving a single cut in the summer, followed by aftermath grazing and winter grazing (Rodwell 1992).

Vegetation Monitoring

Vegetation recording in the green hay donor meadow at Rushbeds Wood SSSI was carried out on 3 July 2013, 3 weeks before the green hay cut. Twenty-four quadrats of 1 m^2 were randomly placed within the meadow, avoiding a margin of 2 m width around the edge, and percentage cover was visually estimated for all vascular plants, following the nomenclature of Stace (2010). For each quadrat, we also recorded which species flowered or were setting seed. The assessment also involved a site-level assessment using the DAFOR scale (Kershaw & Looney 1985), to capture any additional species not picked up during the quadrat-based assessment.

Vegetation sampling in the green hay experimental plots and in the other two treatments was carried out annually in July between 2014 and 2017, when within each replicate plot, 14 randomly placed quadrats of 1 m^2 were recorded. Again, care was taken to avoid a margin of 2 m width around the edge of each plot. For the analyses presented here on transfer and establishment of donor-site species in the green hay treatment plots, vegetation data from the 56 quadrats recorded in the control treatment plots in July 2014 were used to determine the extant species pool at the recipient site. We took this approach as no vegetation recording had been carried out at the recipient site prior to the initiation of the experiment in 2013. We considered this an adequate approximation of the extant species pool prior to the onset of the experiment, as the experimental control plots were very large, and as vegetation recording in 2014 took place prior to the shift in management toward a regime more typically associated with the restoration target vegetation.

Green Hay Seed Content and Species Composition

Green hay samples were collected during application at the recipient sites to determine rate of application and seed content. In each plot, prior to the hay being spread, 10 polythene bags of 0.45 m × 0.60 m size were placed on the ground and weighed down with stones at equal distances along a transect crossing the length of the plot from near the river channel to furthest away from it. After the hay was spread, the hay on top of each bag was bagged and its fresh weight determined in the field. Samples were then transferred into the laboratory where they were stored with bags opened to allow air-drying, and turned daily for 2 weeks to allow thorough drying and shedding of the seeds. Once all seeds had been shed, each dried sample was again weighed to determine dry weight, and then processed through a 5-mm sieve several times until no further seeds appeared. The resultant seed-containing chaff was homogenized, again weighed, and then by weight split into two halves. Each half was bagged separately, thus effectively creating two sets of samples. Prior to spreading in trays, each resulting sample was again halved, and each quarter-sample was spread evenly in a plastic tray of 15.5 cm × 21 cm, and pressed firmly onto a 3.5-cm layer of a 3:1 mixture of peat-free multipurpose compost and sharp sand. Then, all trays were moistened, placed into grip-seal bags, and put into cold dark storage at 4°C in a refrigerator. One set of half-samples, with each half-sample consisting of two trays, was retrieved for further processing after 5 weeks of cold-moist stratification on 10 September 2013, and the other set was retrieved after 5.5 months of cold-moist stratification on 20 January 2014. The underlying rationale for this was that, as some species might require a longer period of cold stratification, by doing so, we would be more likely to detect species that might otherwise have gone undetected if their requirements for germination would have been insufficiently met. Also, this enabled us to investigate any potential differences in seedling emergence that might arise from using different pre-treatment protocols with respect to length of stratification. Furthermore, assuming that such differences indeed existed, this would allow us to use the higher of the two estimates of green hay seed content thus obtained as an estimate for the effective sowing rate of individual species with green hay application.

Upon removal from the refrigerator, each set of trays was placed on growing benches in a heated glasshouse to allow germination. In each of the two trials we included five control trays containing only the compost-sand mixture, to monitor for seed contamination of the substrate and for airborne contaminants. Trays were watered regularly from underneath via capillary matting to avoid seed loss. Starting 3 weeks after samples had been spread into trays, seedlings were identified weekly, counted, and carefully removed. Unidentifiable seedlings were potted on for later identification. The soil in all trays was scarified once, after the first flush of seedling emergence, to stimulate further emergence. Each of the two trials was terminated after no more additional seedlings had emerged for at least 4 weeks.

Data Analysis

Green Hay Species Composition and Effective Sowing Rates. For each species captured as seed with the green hay, two estimates, one per stratification pre-treatment, were derived of its effective sowing rate per m^2, based on the area of ground covered by the polythene bags used for sampling. To find out whether length of stratification affected seedling emergence of species during greenhouse emergence, we carried out two-tailed Wilcoxon signed rank tests (at $p < 0.05$) for all species with a total cumulative emergence of >20 seedlings across both trials, comparing seedling emergence from paired half-samples ($n = 40$). As a much larger number of species than expected by chance were identified as being affected by length of stratification (see “Results” section), the higher of two estimates was subsequently assumed to represent the more realistic estimate of the effective sowing rate for each species.

Species Capture With Green Hay and Establishment After Hay Transfer. To test whether species captured as seed in the green hay, as evidenced by greenhouse seedling emergence, were characterized by higher abundance in the donor site vegetation than species not detected in the greenhouse emergence trials, we carried out a one-tailed Mann–Whitney test (at $p < 0.05$). In addition, the range in donor site abundance for both species groups was visualized using a box–whisker plot.

With respect to analyzing species establishment after hay transfer, additional consideration had to be given to the fact that the experiment also included a control treatment of extant grassland and another restoration treatment that involved the sowing of a diverse seed mixture. For example, some of the species transferred with green hay were also introduced with the seed mixture used in adjacent plots assigned to the seeding treatment, and even though plot sizes were very large compared to those used in similar experiments, there was a possibility of cross-colonization of green hay plots by these species from seeded plots. In addition, some generalist grassland species that might have been transferred with green hay were already present in the extant species-poor grassland that served as control treatment. To avoid “false positives” for green hay transfer as a result of seed dispersal from outside the green hay plots, for example from adjacent diverse seeding plots that had received a seed mixture containing a number of species also present in the sward of the green hay donor site (Tables S1 & S2; Fig. S1), or from adjacent nonexperimental areas, any species detected in only one or two quadrats in a single year of the experiment was assumed to not have established via green hay, but to have migrated into the green hay plots during the experiment. The only exception was if a species was recorded at such low frequency 1 year after green hay application and was then not subsequently recorded, as in this case the species would not yet have had the opportunity to set seed after establishment in the diverse seeding treatment, and to disperse into the green hay treatment. Furthermore, we considered any species found in ≥3 of the 56 quadrats recorded within the control treatment in 2014, that is, 1 year after green hay application, to be extant species that had already been present at the experimental site. Such species were completely excluded from analyses of species establishment. With these adjustments made, we then carried out one-tailed Mann–Whitney tests to test whether species successfully established at the recipient site had higher abundance in the vegetation at the donor site and/or higher abundance as seed in the transferred green hay, compared to species that failed to establish. In the first analysis we included all species recorded at the donor site but not initially present at the experimental site, and in the second analysis we included all species whose seeds were captured in the green hay but that were initially absent at the experimental site. In addition, potential differences between the groups of species successfully captured or established and the groups of species to which this did not apply were visualized, again using box–whisker plots.

To explore in more detail whether a priori predictions can be made as to which species from donor sites establish after green hay transfer, and which species do not, and hence might need supplementing, for example via additional seed sowing, we constructed a classification tree model. We specified success of species establishment from hay as the dependent binary factor. Species abundance and phenological status 3 weeks prior to the hay cut at the donor site, and several readily available plant and seed traits with potential to affect either seed capture at the hay donor site and/or initial establishment from seed at the recipient site, served as candidate predictors (Table 1). Seed densities of species in the green hay were not included as predictor, as the specific intention of this analysis was to facilitate decision making at the time of hay transfer, that is, at a time when such knowledge is not available.

Species abundance at the donor site in the year of the hay cut entered the analysis in the form of five-level DAFOR scale values, recoded numerically (rare = 1; occasional = 2; frequent = 3; abundant = 4; dominant = 5) to reflect this scale's ordinal nature. This abundance measure was used instead of quadrat frequencies as several species found in the vegetation at the donor site and as seed in the green hay had gone unrecorded in the 24 individual vegetation quadrats. Furthermore, a site-level assessment of donor site vegetation in the form of a DAFOR assessment or similar may be more within the means of restoration practitioners than a detailed quadrat-based survey.

We also included two categorical variables based on the vegetation survey to characterize whether species were present only as vegetative plants or only as plants that were seeding but no longer flowering. We did this to account for the possibility that seeds of certain donor site species might not have been present in the vegetation when the hay cut took place. Plant height as specified in Hill et al. (2004) was included as potential explanatory variable to account for the possibility that some species at the donor site may have been too small for their seeds to be captured by green hay. Capacity of fresh seeds for immediate germination, as listed in Grime et al. (2007), was included as potential predictor. Many grassland species display primary physiological seed dormancy (Grime et al. 1981), as also demonstrated in at least one experiment of green hay sampling (Rasran et al. 2006). In addition, grassland species from some plant families display other types of seed dormancy, such as, for example, physical dormancy in the case of Fabaceae, and morphophysiological dormancy in the case of Apiaceae (Baskin & Baskin 2014). Such seed dormancy could put species at a disadvantage during restoration, given that bare ground creation during grassland restoration only provides target species with a relatively short-lived window of opportunity for initial establishment (Wagner et al. 2011, 2016). Seed weight as listed in the Seed Information Database (Royal Botanic Gardens Kew 2008) was included as potential explanatory variable as it is possible that large-seeded species are better able to establish. On the other hand, seed size may also be negatively correlated with seed number in green hay, given that it might negatively correlate with total seed output per plant at the species level.

The classification tree was built using the "rpart" R package vs. 4.1–9 (Therneau et al. 2015). The approach implemented in this package starts with a full tree, which is then pruned back with the aim of optimizing predictive accuracy of the final "pruned" tree (Maindonald & Braun 2010). Splits were determined using the Gini criterion. To confirm optimal tree size according to the 1-SE rule, we carried out 50 sets of 10-fold cross-validation, and averaged estimated errors for each tree size and their standard errors across all 50 sets (De'ath & Fabricius 2000). We then calculated the absolute cross-validated percent error rate (Maindonald & Braun 2010) as a measure of predictive accuracy.

Results

Species Capture by Green Hay and Effective Sowing Rates

Sixty-two herbaceous plant species were recorded at the green hay donor site (Table S1). Using greenhouse emergence, we verified the presence of 47 of these species as seeds in the green hay, with their individual contributions adding up to an overall effective sowing rate of 5,119 viable propagules per m^2 across all species (Table S3). As indicated by a Mann–Whitney test, the 47 species captured by green hay occurred on average at higher quadrat frequencies in the donor grassland than the 15 species not captured ($W = 229.5$, $p = 0.021$; $n = 62$), thus indicating that the chances of individual species

Table 1. Candidate predictors used in the construction of the classification tree model to predict whether species present at the green hay donor site will actually establish at the recipient site. Candidate predictors have either been derived from the vegetation survey at the donor site or extracted from literature databases of plant traits. Note that, as the vegetation survey took place 3 weeks prior to the hay cut, species phenology at the time of the cut was seasonally slightly more advanced.

Predictor	*Data Type*	*Description*	*Source*
DAFOR score	Ordinal	Species abundance at donor site	Vegetation survey
Vegetative only	Categorical	Species only non-flowering at donor site	Vegetation survey
Seeding only	Categorical	Species already past flowering at donor site	Vegetation survey
Plant height	Continuous	Typical height of plants in the British flora	Hill et al. (2004)
Seed weight	Continuous	Median seed weight in RBG Kew's Seed Information Database	Royal Botanic Gardens Kew (2008)
Germination requirement	Categorical	Presence of specific requirements preventing efficient germination of fresh seed	Grime et al. (2007)

being captured depended to some extent on abundance at the donor site (Fig. 1).

Estimation of effective sowing rates of species using the higher of two values for emergence following different stratification pre-treatments was justified, as Wilcoxon signed rank tests were significant for 15 of 27 species with a cumulative total emergence exceeding 20 seedlings, indicating a response to length of stratification in more than half of the tested species (Table S3). Ten species had higher emergence after extended cold-stratification, and five had higher emergence after only short stratification (Table S3).

Agrostis capillaris, by far the most abundant species in the green hay, had an estimated effective sowing rate of 3,902 seeds per m^2 (Table S3; Fig. S1). In contrast, 14 species were characterized by estimated effective sowing rates of less than one seed m^{-2} (Table S3; Fig. S1).

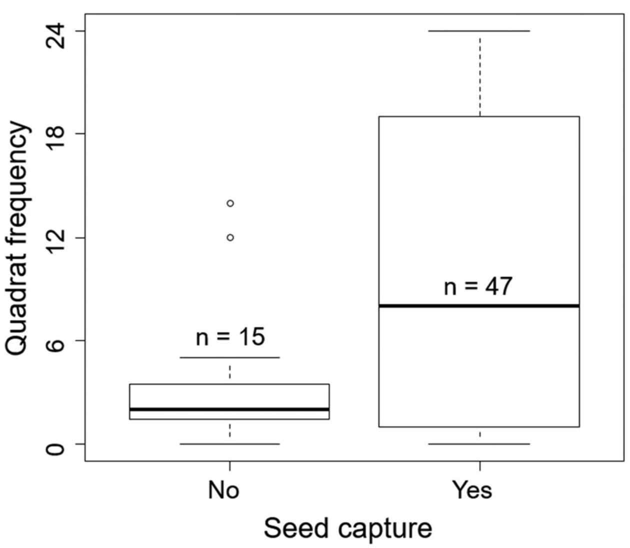

Figure 1. Species capture in green hay in relation to abundance at the donor site. Box–whisker plots illustrate the distribution of quadrat frequencies (maximum = 24) at which a species was found at the donor site, both for species captured as seed by green hay transfer and for species not captured, as evidenced by greenhouse emergence trials.

Species Establishment

For the analysis of species establishment from green hay, we a priori excluded six species that were already present at the experimental site: *Holcus lanatus*, *Lolium perenne*, *Cirsium arvense*, *Ranunculus repens*, *Taraxacum officinale* agg., and *Trifolium repens*. Of the remaining 56 species, 29 successfully established via green hay application, equivalent to an absolute transfer rate of 52%. A one-tailed Mann–Whitney test comparing median quadrat frequencies at the green hay donor site of the 29 species established after hay transfer with those of the 27 species that failed to do so was highly significant ($W = 212.5$, $p = 0.002$; $n = 56$), with frequencies of the former over four times as high as those of the latter (Fig. 2A).

Of the 29 species successfully established after green hay transfer, 21 were first recorded in 2014, with a further 6 species first recorded in 2015, and 2 species first recorded in 2016. Not included in this count were four species only recorded in a single year either 2 or 3 years after green hay application, and then in only one or two quadrats. Transfer of these species with green hay was instead considered to have failed. Three of these species—*Achillea millefolium*, *Galium verum*, and *Vicia cracca*—were only recorded in 2016 and may have cross-colonized from the diverse seeding treatment in which they were also included as seed (Table S2). The fourth species, *Senecio jacobaea*, which was recorded in a single quadrat in 2015, may have spontaneously colonized from an adjacent nonexperimental area where it had also been observed. The interpretation of these species having colonized from sources other than green hay was further underlined by the fact that in the greenhouse emergence trials, only two seedlings of *G. verum* had emerged, and none of *A. millefolium*, *S. jacobaea*, and *V. cracca* (Table S3).

The transfer of 29 of the 41 species found as seed in the green hay and not already present at the recipient site is equivalent to a relative transfer rate of 71%. Median effective sowing rate across the 29 species successfully established was 9.4 seeds m^{-2}, whereas across the 12 species that were present as seed in the green hay but failed to established after green hay application it was only 1.6 seeds m^{-2} (Fig. 2B). However, a one-tailed Mann–Whitney comparing effective sowing rates of both groups of species fell short of significance ($W = 124$, $p = 0.078$; $n = 41$).

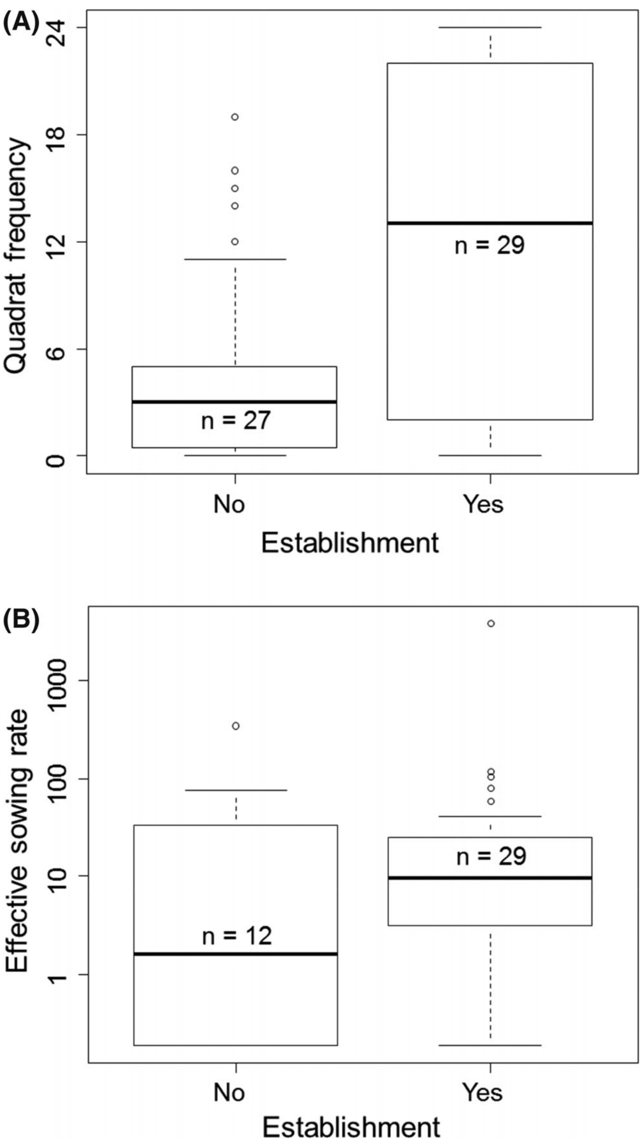

Figure 2. Species establishment after green hay transfer in relation to (A) quadrat frequencies (maximum = 24) at which a species was found at the donor site (out of 24 quadrats) for species established after hay transfer and for species that failed to establish, and (B) effective sowing rate as determined by greenhouse emergence trials.

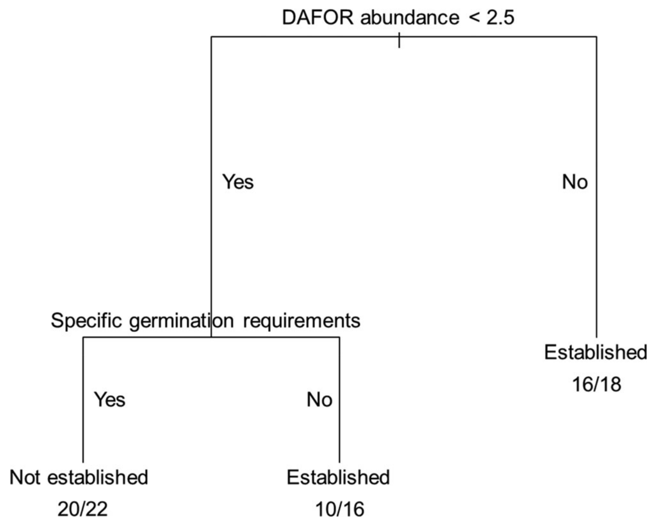

Figure 3. Classification tree model to predict which species from the green hay donor site successfully establish after green hay application. Predictors selected by the final model include species abundance at the donor site in the form of ordinal DAFOR scale values and the presence of species-specific germination requirements thought to prevent immediate germination after hay transfer. Absolute cross-validated error rate of the model is 21.9%. DAFOR abundance was coded as follows: rare = 1; occasional = 2; frequent = 3; abundant = 4; dominant = 5. Proportions supplied with each terminal node denote the proportion of species correctly classified using this model.

The optimal classification tree for predicting species establishment from donor vegetation characteristics and plant traits had two decision nodes. The final model used two predictors, DAFOR abundance at the donor site and existence of specific germination requirements (Fig. 3). The model predicts species that are at least “Frequent” at the donor site according to the DAFOR scale to establish successfully regardless of germination requirements, and species that are “Rare” or “Occasional” to fail to establish if they have specific germination requirements preventing them from quick establishment, but to establish successfully in the absence of such requirements. Absolute cross-validated error of the model was 21.9%.

Discussion

Effective Sowing Rates and Seed Capture

We found significant differences in seedling emergence between stratification pre-treatments in 15 of 27 tested species. By chance alone, only one or two species should have produced significant results. Thus, our results confirm that greenhouse emergence from green hay depends in a species-specific manner on length of prior cold-moist stratification. This means that if germination requirements of a species are not successfully met during greenhouse emergence, actual seed content of the hay for this species could be underestimated by greenhouse emergence. Moreover, 10 species behaved one way, and 5 species another way, indicating that an optimal length of cold stratification might have been somewhere between 5 weeks and 5.5 months. Our results indicate that if the seed content of green hay is determined via greenhouse emergence, numbers of seedlings emerging of a given species might be affected by the chosen stratification pre-treatment. Reassuringly, none of the species reasonably common as seed in the green hay were found in only one stratification treatment, underlining the fact that such species should be reliably recorded regardless of the exact length of cold stratification, even though numbers recorded might somewhat vary.

We were also interested in the question of whether the probability of species being captured as seed in green hay would depend on species abundance in the vegetation at the green hay donor site. As indicated by nonparametric statistical testing, the 15 species that remained undetected during greenhouse emergence were on average less abundant at the donor site than the 47 species whose emergence was recorded, thus providing an answer to our second question.

Species Establishment

We hypothesized that species successfully establishing after green hay transfer would have been more abundant in the vegetation at the donor site and would have occurred at higher seed densities in the green hay than the species failing to establish. Nonparametric statistical testing confirmed the first relationship, but fell short of significance for the second relationship. However, the control group of 27 species that failed to establish but were present in the vegetation at the donor site was larger than the group of 12 species that failed to establish in spite of having been present as seed in the green hay, resulting in a less powerful test for the second relationship. This means that the nonsignificance of the statistical test, while failing to establish such a relationship, does not preclude it either. Two studies by Scotton (2016, 2018) also provide some indication for the existence of such a relationship, with species transferred with harvested material at densities of greater than 10 seeds m^{-2} having mostly reliably established, and species transferred at densities below that having more frequently failed to establish. As indicated by our classification tree model, the capacity of species' seeds for immediate germination appears to also play a role in whether species get successfully established after transfer. We found that if a species occurs at very low abundance at the donor site, failure to be able to germinate quickly often results in failure to establish at the recipient site. Previous work led by one of us (Pywell et al. 2003) has shown that when seed mixtures are used to restore grassland, high germinability of species increases their chances for successful establishment. The results of our tree model suggest that a similar relationship might apply during green hay restoration. Green hay is spread at the recipient site almost immediately after it has been cut at the donor site, usually on the same day. As shown by Rasran et al. (2006), at this point, the fresh seeds of many species transferred with green hay still exhibit primary seed dormancy. However, in species-poor grassland undergoing restoration, the temporal window of opportunity for initial species establishment that is created by preparatory soil cultivation often closes rapidly (Wagner et al. 2016). Thus, when restoring temperate semi-natural grassland using green hay, species whose freshly produced seeds are capable of rapid germination might establish more reliably than species lacking this capacity (Wagner et al. 2011). In other situations and in ecosystems where windows of opportunity for establishment are less predictable such germination behavior can, however, be disadvantageous, as it could result in loss of the whole seed population if establishment fails (Cavers et al. 2000). To test the hypothesis of more easily germinable species doing better during green hay restoration, experiments like ours would have to be complemented by measuring the germinability of freshly produced seed at the time of hay transfer.

Delayed germination of species whose seeds are initially dormant at the time of green hay application could also help explain why during green hay restoration, often, a number of species only establish in the second or third year after hay application (see, e.g. Patzelt 1998; Mann & Tischew 2010). The same was found in our study, in which only 21 of the 29 species successfully transferred with green hay were recorded in the first year after hay application, with a further six and two species, respectively, recorded for the first time in the second and third years after hay application.

Overall, our findings suggest that species abundance at the donor site, and the presence of specific germination requirements in species have the potential to affect whether a species from a green hay donor site can successfully establish at the recipient site. The practical implications of these findings for restoration practice are discussed below. However, due to the relatively small number of 56 species present at our hay donor site that had also been initially absent from the recipient experimental plots, we were likely only able to identify the most relevant factors. This does, however, not mean that other factors might not also have affected which species were successfully transferred with green hay and which were not. One factor known to play such a role is plant species' timing of seed-set. The relevance of this factor for seed harvesting has for example been demonstrated for continental European *Arrhenatherum elatius* meadows that are traditionally cut twice during the growing season, and that contain late-flowering species that are evidently specialized to produce flowering shoots and seeds only after first hay cut in mid-June (Scotton & Ševčíková 2017). In contrast, in our study, we focused on the restoration of a type of grassland, MG5 grassland according to the British NVC, traditionally managed by a single hay cut, followed by aftermath grazing. Moreover, at our green hay donor site, Rushbeds Wood SSSI, hay usually gets cut between 15 July and the early part of August, with the exact date depending on weather conditions (M. Vallance, pers. comm.). Such a late cutting date likely limits the possibility for specialized late-flowering species to complete their life cycle, and may have thus selected against the presence of such species at our donor site. However, some typical species of MG5 grassland that were present at the site, notably *Primula veris* and *Luzula campestris*, may have not been captured by green hay due to their early phenology, with most of their seeds having already potentially been dispersed by the time of the hay cut.

Recommendations for Restoration

As indicated by our own results and those of other studies, even though green hay application is a very useful method for restoring a large number of species from target communities, nonetheless a sizeable proportion of species from green hay donor sites usually fails to establish at green hay recipient sites. Our results clearly indicate that this deficit is not just due to microsite limitation at the recipient site (as, for example, demonstrated by Pywell et al. 2003; Wagner et al. 2019; Löfgren et al. 2020), but also due to limitations inherent to green hay method. Species that are less abundant at a green hay donor site are less likely to be captured as seeds, and in grassland types with a wide spread in species phenology, using just a single cut may further limit the range of species captured by the method (Scotton & Ševčíková 2017). Moreover, even if species are represented as seed in the green hay, some may not establish because their seeds lack the capacity for quick and reliable germination while establishment microsites are available at the recipient site during the window of opportunity created by site preparation in advance of hay application.

However, our findings can also be used to provide some guidance on how to reduce the deficit in species establishment from green hay application, by highlighting which species are likely underrepresented in the green hay, based on plant composition at the donor site, and more generally by highlighting which species might be least likely to establish from green hay, also due to an inability to take advantage of the temporary window of opportunity created during initial restoration. Being able to identify such critical species, their establishment can then be promoted by targeted supplementation in the form of seed sowing alongside green hay application. These additional seeds could be either hand-collected (Poschlod & Biewer 2005), or where this is not possible, purchased seeds could be used (Baasch et al. 2016). Our results suggest that most of the species that are common at a green hay donor site, and whose seeds can be captured by a well-timed hay cut, should reliably establish after transfer. Targeted supplementation in the form of additional seed sowing should focus on target species absent from the donor site, target species whose seed production does not coincide with planned green hay cuts, and target species that are less common or even rare in the vegetation of the donor site.

Among the latter group, those species least likely to establish from green hay are those that are additionally characterized by germination requirements that may prevent them from efficient germination when spread as freshly produced seeds with green hay. If such species are sown alongside an application of green hay, the exact nature of their additional requirements for efficient germination might warrant additional seed treatment prior to sowing. For example, the seeds of many U.K. grassland forbs and sedges display primary physiological seed dormancy at the time of seed shedding, and require afterripening to become germinable (Grime et al. 1981). For a subset of these species that is characterized by nondeep physiological dormancy, dry storage prior to sowing may provide sufficient afterripening to allow germination when sown (Baskin & Baskin 2020), and germination testing prior to sowing can help confirm if this is the case. For other species, whose germination requirements might be more complex, specific methodologies for ex situ dormancy alleviation can and should be applied (Kildisheva et al. 2020) prior to sowing, to ensure efficient germination.

Another way to promote the establishment of species that might not germinate efficiently soon after sowing, that would not require additional sowing alongside the application of green hay, would be to further extend the window of opportunity, for example by heavy grazing soon after green hay transfer (Garrouj et al. 2019). Alternatively, the window could be reopened, for example by creating additional bare ground, possibly in the first spring after hay application, when transferred seed characterized by primary seed dormancy has experienced natural cold stratification.

The findings of our study provide some indication as to which species are less likely to be established via standard green hay application in the absence of additional measures. Such information can feed into decisions regarding targeted additional measures specifically for these species. Ultimately, this could help realize more ambitious grassland restoration projects that aim to establish as many species as possible from a pre-defined reference plant community early on during restoration.

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Literature Cited

Albert Á-J, Mudrák O, Jongeperiová I, Fajmon K, Frei I, Ševčíková M, Klimešová J, Doležal J (2019) Grassland restoration on ex-arable land by transfer of brush-harvested propagules and green hay. Agriculture, Ecosystems and Environment 272:74–82

Baasch A, Engst K, Schmiede R, May K, Tischew S (2016) Enhancing success in grassland restoration by adding regionally propagated target species. Ecological Engineering 94:583–591

Bakker JP, Berendse F (1999) Constraints in the restoration of ecological diversity in grassland and heathland communities. Trends in Ecology and Evolution 14:63–68

Baskin CC, Baskin JM (2014) Seeds: ecology, biogeography, and evolution of dormancy and germination. 2nd edition. Academic Press, San Diego, California

Baskin CC, Baskin JM (2020) Breaking seed dormancy during dry storage: a useful tool or major problem for successful restoration via direct seeding? Plants 9:636

Bischoff A (2002) Dispersal and establishment of floodplain grassland species as limiting factor in restoration. Biological Conservation 104:25–33

Bischoff A, Hoboy S, Winter N, Warthemann G (2018) Hay and seed transfer to re-establish rare grassland species and communities: how important are date and soil preparation? Biological Conservation 221:182–189

Bullock JM, Jefferson RG, Blackstock TH, Pakeman RJ, Emmett BA, Pywell RF, et al. (2011) Semi-natural grasslands. Pages 161–195. In: The UK National Ecosystem Assessment technical report. UNEP-WCMC, Cambridge, United Kingdom

Cavers PB, Qaderi MM, Manku R, Downs MP (2000) Intermittent germination: causes and ecological implications. Pages 363-374. In: Black M, Bradford KJ, Vázquez-Ramos J (eds) Seed biology: advances and applications. CABI, Wallingford, United Kingdom

Coulson SJ, Bullock JM, Stevenson MJ, Pywell RF (2001) Colonization of grassland by sown species: dispersal versus microsite limitation in response to management. Journal of Applied Ecology 38:204–216

De'ath G, Fabricius KE (2000) Classification and regression trees: a powerful yet simple technique for ecological data analysis. Ecology 81:3178–3192

Garrouj M, Alard D, Corcket E, Marchand L, Benot M-L (2019) The effects of management on vegetation trajectories during the early-stage restoration of previously arable land after hay transfer. Ecology and Evolution 9: 13776–13786

Grime JP, Hodgson JG, Hunt R (2007) Comparative plant ecology: a functional approach to common British species. Castlepoint Press, Dalbeattie, United Kingdom

Grime JP, Mason G, Curtis AV, Rodman J, Band SR, Mowforth MAG, Neal AM, Shaw S (1981) A comparative study of germination characteristics in a local flora. Journal of Ecology 69:1017–1059

Hedberg P, Kotowski W (2010) New nature by sowing? The current state of species introduction in grassland restoration, and the road ahead. Journal for Nature Conservation 18:304–308

Hill MO, Preston CD, Roy DB (2004) PLANTATT. Attributes of British and Irish plants: status, size, life history, geography and habitats. Centre for Ecology and Hydrology, Huntingdon, United Kingdom

Isselstein J, Jeangros B, Pavlu V (2005) Agronomic aspects of biodiversity targeted management of temperate grasslands in Europe—a review. Agronomy Research 3:139–151

Kershaw KA, Looney JHH (1985) Quantitative and dynamic plant ecology. 3rd edition. Edward Arnold, London, United Kingdom

Kiehl K, Kirmer A, Donath TW, Rasran L, Hölzel N (2010) Species introduction in restoration projects—evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. Basic and Applied Ecology 11:285–299

Kiehl K, Thormann A, Pfadenhauer J (2006) Evaluation of initial restoration measures during the restoration of calcareous grasslands on former arable fields. Restoration Ecology 14:148–156

Kildisheva OA, Dixon KW, Silveira FAO, Chapman T, Di Sacco A, Mondoni A, Turner SR, Cross AT (2020) Dormancy and germination: making every seed count in restoration. Restoration Ecology 28: S256–265

Kirmer A, Tischew S (2014) Conversion of arable land to lowland hay meadows – what influences restoration success? Pages 118–140. In: Kiehl K, Kirmer A, Shaw N, Tischew S (eds.) Guidelines for native seed production and grassland restoration. Cambridge Scholars Publishing, Newcastle upon Tyne, United Kingdom

Ladouceur E, Jiménez-Alfaro B, Marin M, De Vitis M, Abbandonato H, Iannetta PPM, et al. (2018) Native seed supply and the restoration species pool. Conservation Letters 11:e12381

Lawton JH, Brotherton PNM, Brown VK, Elphick C, Fitter AH, Forshaw J, et al. (2010) Making space for nature: a review of England's wildlife sites and ecological network. Report to Defra

Löfgren O, Hall K, Schmid BC, Prentice HC (2020) Grasslands ancient and modern: soil nutrients, habitat age and their relation to Ellenberg N. Journal of Vegetation Science 31:367–379

Maddock A (2008) UK biodiversity action plan priority habitat descriptions. Lowland meadows. http://archive.jncc.gov.uk/Docs/UKBAP_BAPHabitats-29-Lowland%20Meadows.doc (accessed 18 July 2019)

Maindonald J, Braun WJ (2010) Data analysis and graphics using R: an example-based approach. 3rd edition. Cambridge University Press, Cambridge, United Kingdom

Mann S, Tischew S (2010) Die Entwicklung von ehemaligen Ackerflächen unter extensiver Beweidung (Wulfener Bruch). Hercynia N.F. 43:119–147

Met Office (2019) UK climate summaries: Hillesden climate https://www.metoffice.gov.uk/public/weather/climate/gcpr91z6k (accessed 17 April 2019)

Natural England (2010) Sward enhancement: diversifying grassland by spreading species-rich green hay. Natural England Technical Information Note TIN063. 2nd ed. https://webarchive.nationalarchives.gov.uk/20150303045930/http://publications.naturalengland.org.uk/file/93010 (accessed 17 April 2019).

Patzelt A (1998) Pages 297. Vegetationsökologische und populationsbiologische Grundlagen für die Etablierung von Magerwiesen in Niedermooren. Dissertationes Botanicae. Cramer, Berlin, Germany

Poschlod P, Biewer H (2005) Diaspore and gap availability are limiting species richness in wet meadows. Folia Geobotanica 40:13–34

Pywell RF, Bullock JM, Roy DB, Warman L, Walker KJ, Rothery P (2003) Plant traits as predictors of performance in ecological restoration. Journal of Applied Ecology 40:65–77

Rasran L, Vogt K, Jensen K (2006) Seed content and conservation evaluation of hay material of fen grasslands. Journal for Nature Conservation 14: 34–45

Rodwell JS (1992) British plant communities. Vol 3. Grasslands and montane communities. Cambridge University Press, Cambridge, United Kingdom

Rodwell JS, Morgan V, Jefferson RG, Moss D (2007) The European context of British lowland grasslands. JNCC Report 394. Joint Nature Conservation Committee, Peterborough, United Kingdom

Royal Botanic Gardens Kew (2008) Seed Information Database (SID). Version 7.1. http://data.kew.org/sid/ (accessed 26 December 2018)

Scotton M (2016) Establishing a semi-natural grassland: effects of harvesting time and sowing density on species composition and structure of a restored *Arrhenatherum elatius* meadow. Agriculture, Ecosystems and Environment 220:35–44

Scotton M (2018) Calcareous grassland restoration at a coarse quarry waste dump in the Italian Alps. Ecological Engineering 117:174–181

Scotton M, Piccinin L, Dainese M, Sancin F (2009) Seed harvesting for ecological restoration: efficiency of haymaking and seed-stripping on different grassland types in the eastern Italian Alps. Ecological Restoration 27:66–75

Scotton M, Ševčíková M (2017) Efficiency of mechanical seed harvesting for grassland restoration. Agriculture, Ecosystems and Environment 247: 195–204

Stace C (2010) New Flora of the British Isles. 3rd edition. Cambridge University Press, Cambridge, United Kingdom

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

Stevenson MJ, Ward LK, Pywell RF (1997) Re-creating semi-natural communities: vacuum harvesting and hand collection of seed on calcareous grassland. Restoration Ecology 5:66–76

Sullivan E, Hall N, Ashton P (2019) Restoration of upland hay meadows over an 11-year chronosequence: an evaluation of the success of green hay transfer. Restoration Ecology 28:127–137

Therneau T, Atkinson B, Ripley B (2015) Rpart: recursive partitioning and regression trees. R package version 4.1-9

Trueman I, Millett P (2003) Creating wild-flower meadows by strewing green hay. British Wildlife 15:37–44

Wagner M, Bullock JM, Hulmes L, Hulmes S, Peyton J, Amy SR, Savage J, Tallowin JB, Heard MS, Pywell RF (2016) Creation of micro-topographic features: a new tool for introducing specialist species of calcareous grassland to restored sites? Applied Vegetation Science 19:89–100

Wagner M, Fagan KC, Jefferson RG, Marrs RH, Mortimer SR, Bullock JM, Pywell RF (2019) Species indicators for naturally-regenerating and old calcareous grassland in southern England. Ecological Indicators 101:804–812

Wagner M, Hulmes L, Hulmes S, Nowakowski M, Redhead JW, Pywell RF (2020) Green hay application and diverse seeding approaches to restore grazed lowland meadows: progress after 4 years and effects of a flood risk gradient. Restoration Ecology: In press. https://doi.org/10.1111/rec.13180

Wagner M, Pywell RF, Knopp T, Bullock JM, Heard MS (2011) The germination niches of grassland species targeted for restoration: effects of seed pre-treatments. Seed Science Research 21:117–131

Walker KJ, Stevens PA, Stevens DP, Mountford O, Manchester SJ, Pywell RF (2004) The restoration and re-creation of species-rich lowland grassland on land formerly managed for intensive agriculture in the UK. Biological Conservation 119:1–18

Supporting Information

The following information may be found in the online version of this article:

Figure S1 Species composition and effective sowing rates of green hay and of the diverse seed mixture that was used in an adjacent experimental treatment plots.
Table S1. Plant species composition of the green hay donor grassland at Rushbeds Wood SSSI.
Table S2. Species composition of the seed mixture used in the diverse-seeding treatment and estimated effective sowing rates for individual species.
Table S3. Seed species composition of the green hay based on greenhouse emergence from hay samples collected after transfer to recipient plots.

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