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Contact CEH NORA team at
noraceh@ceh.ac.uk

1 **Seed bank dynamics in restored grassland following the sowing of high- and low-**
2 **diversity seed mixtures**

3

4 Markus Wagner¹, Kevin J. Walker², Richard F. Pywell¹

5 ¹NERC Centre for Ecology & Hydrology, Benson Lane, Wallingford, Oxfordshire

6 OX10 8BB, UK

7 ²Botanical Society of Britain and Ireland, Room 14, Bridge House, 1-2 Station

8 Bridge, Harrogate, North Yorkshire, HG1 1SS, UK

9

10 Corresponding author:

11 M. Wagner (NERC Centre for Ecology & Hydrology, Benson Lane, Wallingford,

12 Oxfordshire OX10 8BB, UK; E-mail: mwagner@ceh.ac.uk)

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14 Running head: Seed bank dynamics in restored grassland

15

16 Author contributions:

17 RP and KW conceived and designed the research; KW carried out the experiment;

18 MW analyzed the data and led and coordinated the writing of the manuscript; RP and

19 KW edited the manuscript.

20

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26 Abstract

27 Soil seed banks on ex-arable land are dominated by undesirable ruderal species that
28 compete with ‘desirable’ target species during grassland restoration. At the same time,
29 for continued regeneration, the latter often functionally depend on gap colonization
30 from the seed bank, which serves as a buffer against local extinction. Nonetheless,
31 few studies have so far investigated the effects of restoration practices on seed bank
32 dynamics. Using a multi-site experiment investigating techniques for restoring
33 lowland mesotrophic grassland, we studied the effects of seed bed preparation
34 (shallow cultivation using harrows or discs vs deep cultivation using a plough) and of
35 seed mixtures (species-rich grass-forb mixes vs species-poor grass-only mixes vs
36 unseeded natural regeneration) on seven years of post-restoration seed bank dynamics.
37 We assessed how these practices affected density and diversity of sown and unsown
38 species in the seed bank. Seed bank dynamics were much more strongly affected by
39 seed sowing than by cultivation. Grass sowing resulted in stronger seed bank decline
40 of unsown grasses, and additional forb sowing in stronger decline of unsown forbs.
41 Higher seed densities and species richness of sown forbs colonizing from neighboring
42 plots sown with the grass-forb mix were observed under natural regeneration than in
43 the grass-only sown treatment, reflecting grass priority effects on sown forb
44 colonization in the latter. Sowing of diverse target species mixtures was associated
45 with the greatest shift in seed bank composition away from extant ruderal species
46 towards sown target species. Our results illustrate the usefulness of seed bank
47 monitoring for assessing restoration progress.

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51 Keywords: cultivation; ex-arable land; natural regeneration; priority effects; sowing

52

53 Implications for Practice:

- 54 • Use of seed mixes in grassland restoration can accelerate development of a
55 functional seed bank that is more reflective of the target community, by
56 promoting decline of unsown non-target species and accumulation of sown
57 target species in the seed bank.
- 58 • Species-rich grass-forb mixtures appear to be more effective than species-poor
59 grass-only mixtures, by enabling a faster build-up of sown forbs in the seed
60 bank, along with a faster decline of unsown non-target forbs.
- 61 • Ploughing to regular depth as opposed to shallow cultivation does not appear
62 to have any positive effects on seed bank or vegetation development.
- 63 • Complementation of vegetation monitoring during restoration by additional
64 seed bank monitoring can help obtain a more integrated picture of restoration
65 progress.

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76 **Introduction**

77 Soil seed banks play an important functional role in the continuous regeneration of
78 plant populations (Thompson 2000). In grassland, this is illustrated by the fact that re-
79 colonization of small-scale canopy openings such as those created by sward
80 management is often effected by the soil seed bank (Pakeman et al. 1998; Kalamees &
81 Zobel 2002; but see also Bullock et al. 1994; Edwards & Crawley 1999). It is also
82 known that the above-ground persistence of grassland species is positively linked to
83 their seed bank persistence (Stöcklin & Fischer 1999), underlining the fact that buried
84 seed populations act as a buffer against localised extinction during periods
85 characterized by unfavourable conditions.

86 When semi-natural grassland is restored on former arable land, the soil seed
87 bank tends to be dominated by ruderals, whereas typical species of the grassland
88 target are usually absent (Walker et al. 2004). This can have implications for success
89 of restoration of target species, which, amongst others, will be affected by the surface-
90 layer densities of the seeds both of target species and of potentially undesirable non-
91 target species (Walker et al. 2004). Accordingly, some authors have suggested soil
92 inversion as a means of reducing numbers of seeds of unwanted species, by burying
93 them deeper in the soil profile and thereby preventing them from interfering with the
94 establishment of target species (Glen et al. 2007; Czerwiński et al. 2015; Glen et al.
95 2017).

96 At newly-restored sites, the initial absence of sown target species from the seed bank
97 increases the vulnerability to environment and management factors with the potential
98 to cause of newly-established populations due to demographic events and to buffer
99 against trigger such events.

100 A rapid build-up of target species' seed populations in the soil seed bank, coupled
101 with a steady depletion of the seeds of non-target species, is thus desirable and might
102 positively affect restoration progress. However, little is known about how grassland
103 restoration techniques on ex-arable land, such as seed sowing or type of soil
104 cultivation, influence seed bank dynamics both in terms of unsown and sown species.
105 Few studies have investigated seed bank dynamics in restored grassland (Rayburn et
106 al. 2016), especially on ex-arable land (but see McDonald et al. 1996; Schmiede et al.
107 2009; Fagan et al. 2010; Török et al. 2012; Karlík & Poschlod 2014). Of the few
108 studies that have, none assessed the effects of cultivation, and only Fagan et al. (2010)
109 assessed the potential effects on seed banks of seeding with different seed mixtures.
110 However, Fagan et al. (2010) made no distinction between sown and unsown species,
111 as many of the sites included had been seeded using green hay or other plant materials
112 collected from donor sites, and species composition of these materials is usually only
113 known approximately, and often highly variable.

114 The work presented here, carried out as part of a study into the use of soil
115 cultivation and the sowing of different kinds of seed mixtures for re-creating species-
116 rich mesotrophic grassland on ex-arable land (Pywell et al. 2002), aims to help closing
117 this gap in our knowledge of seed bank dynamics during restoration. Based on
118 repeated sampling of the soil seed bank across a range of treatments in Pywell et al.'s
119 (2002) experiment, we set out to investigate the following questions:

- 120 (1) To what extent does the sowing of seed mixtures of different diversity affect
121 dynamics of unsown and sown target species in the soil, and how do these approaches
122 compare with natural regeneration?
- 123 (2) To what extent does seed bed preparation (deep cultivation versus shallow
124 cultivation) affect seed bank dynamics?

125 (3) To what extent are compositional trends in the seed bank affected by experimental
126 treatments, and how do these trends relate to compositional trends in the aboveground
127 vegetation?

128

129 **Methods**

130 *Field sites and experimental design*

131 Three experimental sites located in southern England were included in this study
132 (Table 1). At these sites, experimental restoration was carried out from September
133 1994 to determine the relative success of various cultivation methods and seed sowing
134 options when establishing species-rich grassland on ex-arable land (Pywell et al.
135 2002). Sites were selected in natural areas that had been included in the UK's
136 Environmentally Sensitive Areas (ESA) scheme, an agri-environment scheme that ran
137 from 1986 to 2005 and whose aims included both the protection of remaining diverse
138 grasslands as well as at the creation of additional grassland habitat of high
139 biodiversity value (Coates 1997). For the experiment, a specific lowland grassland
140 target community conforming to the British National Vegetation Classification
141 (Rodwell 1992) was identified for each site depending on location, soil, hydrology
142 and proposed management. For all three sites included here, targets represented
143 specific sub-communities of *Cynosurus cristatus* – *Centaurea nigra* grassland (NVC
144 category MG5; Table 1). MG5 grassland corresponds to the continental European
145 *Centaureo-Cynosuretum cristati* Br.-Bl. & Tx grassland association (Rodwell 1992),
146 and the most frequent constant species in this community are the grasses *Agrostis*
147 *capillaris*, *Cynosurus cristatus* and *Festuca rubra*, and the forbs *Lotus corniculatus*,
148 *Plantago lanceolata* and *Trifolium repens* (Rodwell 1992). *Centaurea nigra*, in spite
149 of being one of the species the community is named after, occurs somewhat less

150 frequently (Rodwell 1992). For two of our sites, the Norfolk Broads site and the
151 Upper Thames Tributaries site, we did identify the MG5a sub-community as suitable
152 target for restoration, whose preferential species include *Lathyrus pratensis* and
153 *Leucanthemum vulgare* (Rodwell 1992). For the third site, the Suffolk River valleys
154 site, we did identify the MG5c sub-community as suitable target, which amongst
155 others is characterized by preferential species including *Danthonia decumbens*,
156 *Potentilla erecta*, and *Succisa pratensis* (Rodwell 1992).

157 Specifically, Pywell et al. (2002) compared pre-sowing cultivation techniques,
158 namely no cultivation vs. shallow cultivation vs. deep cultivation, and the use of
159 different seed mixtures, namely natural regeneration without sowing vs. species-poor
160 grass-only mixture (= ‘ESA mixture’) vs. species-rich mixture including grasses and
161 forbs that are characteristic of the respective NVC target community (= ‘NVC
162 mixture’). NVC mixtures differed between the Norfolk Broads and Upper Thames
163 Tributaries sites, which had an MG5c restoration target, and the Suffolk River Valleys
164 site, which had MG5a grassland as restoration target. At the former two sites, NVC
165 mixtures included 11 grasses and 28 forbs, and at the latter eight grasses and 17 forbs,
166 reflecting the slightly smaller pool of characteristic species in MG5c grassland
167 (Rodwell 1992; for full details see Table S1, Supporting Information). The same ESA
168 mixture of six grasses was used at all three sites, with one additional species,
169 *Alopecurus pratensis*, included at the Norfolk Broads site (Table S1). The ESA
170 mixture was based on Department of Environment, Food and Rural Affairs (Defra)
171 guidelines for establishing moderately diverse grass cover on arable land. NVC
172 mixtures were made up of grasses and a sizeable number of forb species based on the
173 composition of the chosen target community. Depending on soil type, shallow
174 cultivation was achieved with harrows or discs, with a maximum cultivation depth of

175 10 cm and no soil inversion. Deep cultivation consisted of ploughing to a depth of 30-
176 40 cm, which inverted the uppermost soil layer. This is similar to conventional
177 agricultural ploughing and is not to be confused with the more recently developed
178 restoration practice of ‘deep-ploughing’ which typically involves cultivation to a
179 depth of about 80 cm (Glen et al. 2017). The experiment was set up in four replicate
180 blocks per site, and treatment plots were 6 m × 4 m with a 1 m guard row between the
181 plots (Pywell et al. 2002). Here, we focus on five of the seven treatments included in
182 Pywell et al.’s (2002) study:

183 (1) natural regeneration from cereal stubble (i.e. a control with no cultivation or
184 sowing of species);

185 (2) shallow cultivation + species-poor ESA seed mixture;

186 (3) shallow-cultivation + species-rich NVC seed mixture;

187 (4) deep cultivation + species-poor ESA seed mixture;

188 (5) deep cultivation + species-rich NVC seed mixture.

189

190 ***Vegetation monitoring***

191 Vegetation sampling was carried out annually from 1995 to 1998. In early July of
192 each year, three 40 cm × 40 cm quadrats, subdivided into 16 cells of 10 cm × 10 cm,
193 were placed at random within each plot, avoiding a 1 m buffer strip around the edge.
194 The presence of vascular plant species was recorded for each cell. *Festuca rubra* and
195 *Festuca ovina* were treated as an aggregate due to difficulties in distinguishing
196 between these species. Nomenclature follows Stace (2010).

197

198 ***Seed bank sampling and monitoring***

199 In September 1994 after cultivation, and again in October 1998 and October 2001,
200 soil was sampled to a depth of 20 cm in all experimental plots. This was done for the
201 seed bank analyses presented here as well as for nutrient analyses (for these see
202 Pywell et al. 2002), using a soil auger of 6 cm diameter, to extract ten randomly
203 placed soil cores per treatment plot. After removal of the litter layer, the soil cores
204 were divided into segments of 0-5 and 5-20 cm depth. The 10 segments for a given
205 experimental plot were pooled for each depth, and pooled plot samples thoroughly
206 mixed.

207 From each plot sample, a volume of 500 cm³ was sub-sampled for seed bank
208 analyses, from which large vegetative fragments, roots and stones were removed.
209 Sub-samples were then transferred into 20 cm × 16 cm plastic trays where they were
210 spread evenly in a layer of ca. 1.5 cm thickness over a 3 cm layer of sharp sand. In
211 1994, seed bank analysis was only carried out for the 0-5 cm surface layer, whereas in
212 1998 and 2001, it was also carried out for the 5-20 cm sub-surface layer. However,
213 here, we focus entirely on the 0-5 cm surface layer. About 5 cm is usually the
214 maximum depth from which the seedlings of grassland species can emerge (Williams
215 1983; Traba et al. 2004), and the 0-5 cm top layer of soil can thus be considered to
216 represent the functional seed bank of undisturbed grassland.

217 After processing, seed trays were transferred onto a bench in a heated glasshouse,
218 along with four randomly placed trays per year only containing a layer of sharp sand,
219 to check for potential contamination by airborne seed or by seed emerging from the
220 sand layer. However, throughout the study, no seedling emergence was observed from
221 these control trays.

222 All trays were watered initially, and subsequently kept moist using automated
223 irrigation. Seedlings were periodically identified and removed for a 12-month period,

224 during which the soil was thoroughly stirred every two months. If a seedling was not
225 identifiable, it was transplanted into a flower pot and grown until identification was
226 possible.

227

228 ***Data analysis***

229 *Seed bank density and species richness*

230 Treatment effects on seed densities and species richness in the soil seed bank were
231 analysed with SAS 9.3 (SAS Institute, Cary, NC, US). Separate analyses were carried
232 out for sown species, with sub-groupings of sown forbs and sown grasses, and for
233 unsown species, with sub-groupings of unsown forbs and unsown grasses. The
234 ‘unsown species’ grouping also contained woody species and rushes (*Juncus* spp.).
235 However, seeds of these two sub-groupings tended to occur quite unevenly across
236 sites, and in the case of woody species also very sporadically, and thus, no separate
237 analyses are presented.

238 Prior to analyses, seedling counts were summed across member taxa of each species
239 group. Analyses for unsown and sown species groups differed in one important
240 respect. The former were based on data from all three samplings, whereas the latter
241 were based on data from 1998 and 2001 only, as sown species were entirely absent
242 from the seed bank in 1994. In terms of interpreting analysis results, this means that
243 for unsown groups, for which a pre-treatment baseline was included in analyses,
244 treatment effects should primarily manifest themselves as interactions between
245 treatment and year. In contrast, for sown species groups, treatment effects may likely
246 manifest themselves also as main effects. The earliest data included in these analyses
247 was from four years after sowing, by which time some treatment effects may have
248 already occurred.

249 Because the five restoration treatments included were not fully factorial, we carried
250 out two kinds of analysis, henceforth referred to as analysis A and analysis B.
251 Analysis A focused on effects of seed sowing (natural regeneration vs. ESA seed
252 mixture vs. NVC seed mixture), including treatments (1) to (3). The shallow
253 cultivation prior to sowing in treatments (2) and (3) can be considered as the
254 minimum seed bed treatment, and is thus most comparable to natural regeneration
255 (Pywell et al. 2002). Analysis B focused on effects of seed mixture (ESA vs. NVC)
256 and cultivation depth (deep vs. shallow), including treatments (2) to (5).

257 Together with experimental factors, which were specified as fixed factors in
258 the respective analyses, site and year were also specified as fixed factors, along the
259 various interaction terms between these main factors. In both types of analysis, year
260 was specified as repeated measures factor, and blocks nested within sites were
261 included as random effects (Schabenberger & Pierce 2002).

262 Analyses were carried out using GLMMs and Poisson errors as provided by SAS Proc
263 GLIMMIX. If GLMMs failed to converge, alternative analyses were carried out using
264 computationally less demanding LMMs as provided by SAS Proc MIXED. In this
265 case, while models were specified in the same way, data was Box-Cox-transformed
266 prior to analysis to meet distributional requirements. In case of a significant main
267 effect of seed sowing for analyses A, pair-wise comparisons between the three seed
268 sowing treatments were carried out using the two-sided Tukey HSD test.

269

270 *Compositional trends*

271 To analyse compositional trends in seed bank and vegetation, we performed an
272 ordination analysis using Non-metric Multidimensional Scaling (NMDS) as provided
273 by PC-ORD, Version 6.08 (McCune & Mefford 2011). Prior to analysis, for each

274 species, quadrat cell counts from the three replicate quadrats in a given plot and year
275 were summed. Then, to make seed bank and vegetation data comparable, all data was
276 standardized to the sample total (Wagner et al. 2006).

277 As recommended by McCune and Grace (2002), NMDS was performed using
278 the Sørensen distance measure. Optimal dimensionality for the final model was
279 assessed with the ‘slow and thorough’ option of PC-ORD’s autopilot mode (McCune
280 & Grace 2002). The final run was carried out with a predefined stability criterion of
281 0.000001 and a maximum of 500 iterations. For each of the resulting NMDS axes, we
282 determined the amount of compositional variance explained by calculating R^2
283 correlation coefficients between distance in NMDS ordination space and distance in
284 raw data space (McCune & Grace 2002). Species scores were calculated on the basis
285 of weighted averages of site scores.

286 To help evaluate compositional trends in the vegetation in terms of restoration
287 progress, we calculated percentage fit of the vegetation in each treatment and site for
288 each year with the respective target community of the NVC classification (see Table
289 1), based on the species lists provided by Rodwell (1992) and using the bespoke
290 Tablefit software, Version 2.0 (Hill 2015). This was done based on quadrat
291 frequencies (Hill 2015; see also Hill 1989), based on the twelve quadrats (= three
292 replicate quadrats \times four replicate plots) per treatment in a given year at a given site.
293 Results of these goodness-of-fit calculations are presented in Fig. S1, Supporting
294 Information.

295

296 **Results**

297 A total of 5750 seedlings emerged from all soil samples across all three sample years
298 (1994: 1755 seedlings; 1998: 1751 seedlings; 2001: 2244 seedlings). Seedlings of 103

299 taxa were identified, including 10 sown grasses and 14 sown forb species. We were
300 unable to identify 368 seedlings (= 6.4% of the total), including 353 forb and 15 grass
301 seedlings. Of these 368 seedlings, 341 were from samples collected in 1994 prior to
302 sowing. As sampling in 1994 was carried out before seed mixes were sown,
303 unidentified seedlings from that year were included in the respective ‘unsown’
304 categories, as excluding these seedlings from analysis in spite of knowing that they
305 must be from unsown species would have carried a risk of biasing the results. In
306 contrast, the 27 unidentified seedlings from later years were not included in analyses
307 in the default analyses presented here. Additional analyses in which these 27 seedlings
308 were alternatively included in the respective unsown categories (results not shown)
309 indicated that this decision did not affect analytical results.

310

311 *Seed bank density and species richness*

312 Site-dependent effects, particularly those not acting in interaction with treatment
313 factors, are less relevant in terms of allowing general conclusions for restoration.
314 Therefore, here, we focus on the main and interaction effects, particularly with time,
315 of the experimental treatments. Full statistical results are presented in Tables S2 to S5,
316 Supporting Information.

317

318 *Effects of seed addition and mixture type: unsown species*

319 Analyses A and B indicated highly significant year effects on seed bank densities of
320 all unsown species groupings (Tables S1-S2), reflecting a decline in density with time
321 (Fig. 1). This decline was more pronounced for forbs than grasses (Fig. 1). Analyses
322 A indicated highly significant interactions between seed addition and year for unsown
323 species in general (GLMM; $F_{2,72} = 6.58$; $P < 0.001$) and for unsown grasses (LMM;

324 $F_{2,72} = 3.53$; $P = 0.011$). In both groupings, the decline was stronger with sown seed
325 mixtures than with natural regeneration (Fig. 1).
326 For unsown forbs, while analysis A yielded a non-significant interaction between seed
327 addition and year (GLMM; $F_{2,72} = 2.16$; $P = 0.082$), analysis B yielded a significant
328 seed mix main effect (GLMM; $F_{1,99} = 6.04$; $P = 0.016$), indicating slightly lower
329 densities on plots sown with the NVC mix than on plots sown with the ESA mix (Fig.
330 1).

331 As indicated by analyses A, species richness per sample was significantly
332 affected by seed addition for unsown species in general (GLMM; $F_{2,72} = 4.66$;
333 $P = 0.012$), and for unsown forbs (GLMM; $F_{2,72} = 3.24$; $P = 0.045$), and highly
334 significantly so for unsown grasses (LMM; $F_{2,72} = 11.61$; $P < 0.001$). For the latter
335 group, there was also a significant interaction of seed addition with year (LMM;
336 $F_{2,72} = 2.81$; $P = 0.031$). As indicated by two-sided pairwise Tukey tests, species
337 richness of unsown species in general was lower in soil samples from NVC plots than
338 in samples from natural regeneration plots (d.f. = 72; $t = 2.93$; $P = 0.012$; see Fig. 2).
339 A similar trend of samples from NVC plots being less species-rich than samples from
340 ESA plots was only marginally significant (d.f. = 72; $t = 2.39$; $P = 0.051$). Species
341 richness of unsown forbs was lower in samples from NVC plots than in those from
342 ESA plots (d.f. = 72; $t = 2.49$; $P = 0.040$; see Fig. 2). Species richness of unsown
343 grasses was lower in samples from sown plots in general than in samples from natural
344 regeneration plots (ESA mix vs. natural regeneration: d.f. = 72; $t = 3.58$; $P = 0.002$;
345 NVC mix vs. natural regeneration: d.f. = 72; $t = 4.58$; $P < 0.001$; see Fig. 2).
346 Accordingly, in analyses B, sowing of ESA vs. NVC mix affected species richness of
347 unsown forbs (GLMM; $F_{1,99} = 4.74$; $P = 0.032$) and of unsown species in general
348 (GLMM; $F_{1,99} = 6.07$; $P = 0.017$), but not of unsown grasses (LMM; $F_{1,99} = 1.95$;

349 $P = 0.166$). In the case of unsown species in general, the effect of seed mix varied
350 with year and site, as indicated by a highly significant three-way interaction (GLMM;
351 $F_{4,99} = 4.07$; $P = 0.004$).

352

353 *Effects of seed addition and mixture type: sown species*

354 Seed bank densities of sown species groupings were strongly affected by seed
355 addition, as indicated by highly significant main effects in analyses A (all $P < 0.001$;
356 all sown species: GLMM; $F_{2,45} = 20.94$; forbs: LMM; $F_{2,45} = 25.87$; grasses: LMM;
357 $F_{2,45} = 12.04$; see also Table S4). Seed densities of sown species in general were
358 highest on NVC plots and lowest on natural regeneration plots, with ESA plots
359 intermediate (Tukey tests with d.f. = 45; NVC vs natural regeneration: $t = 6.45$;
360 $P < 0.001$; ESA vs natural regeneration: $t = 4.21$; $P < 0.001$; NVC vs ESA: $t = 2.53$;
361 $P = 0.040$; see Fig. 3). Sown forb seed densities were also highest on NVC plots, but
362 were actually lower on ESA plots than on natural regeneration plots (Tukey tests with
363 d.f. = 45; NVC vs natural regeneration: $t = 4.28$; $P < 0.001$; ESA vs natural
364 regeneration: $t = -2.87$; $P = 0.017$; NVC vs ESA: $t = 7.15$; $P < 0.001$; see Fig. 3). For
365 sown forb seed densities, a significant interaction between seed addition and year
366 (LMM; $F_{2,45} = 4.61$; $P = 0.015$) reflected continued increases on NVC plots between
367 1998 and 2001 as compared to continually low levels on natural regeneration plots.
368 Seed bank densities of sown grasses were higher on seeded plots than on natural
369 regeneration plots, irrespective of mixture used (Tukey tests with d.f. = 45; NVC vs
370 natural regeneration: $t = 3.44$; $P = 0.004$; ESA vs natural regeneration: $t = 4.75$;
371 $P < 0.001$; see Fig. 3). Accordingly, in analyses B, we found significant main effects
372 of seed mix for sown species in general (GLMM; $F_{1,63} = 7.17$; $P = 0.010$) and for

373 sown forbs (GLMM; $F_{1,63} = 18.68$; $P < 0.001$), but not for sown grasses (GLMM;
374 $F_{1,63} = 1.03$; $P = 0.314$).

375 Species richness per sample was highly significantly affected by seed addition
376 in all three groupings (all sown species: GLMM; $F_{2,45} = 16.55$; $P < 0.001$; forbs:
377 LMM; $F_{2,45} = 19.36$; $P < 0.001$; grasses: $F_{2,45} = 5.47$; $P = 0.008$). Overall richness of
378 sown species was highest in NVC plots (Tukey tests with d.f. = 45; NVC vs natural
379 regeneration: $t = 4.44$; $P < 0.001$; NVC vs ESA: $t = 4.89$; $P < 0.001$; see Fig. 4), but
380 there was no difference between the ESA and natural regeneration treatments ($t = -$
381 0.57 ; $P = 0.839$). Sown forb richness was highest in samples from NVC plots and
382 lowest in those from ESA plots, with samples from natural regeneration plots
383 characterized by intermediate richness (Tukey tests with d.f. = 45; NVC vs natural
384 regeneration: $t = 3.43$; $P = 0.004$; NVC vs ESA: $t = 6.21$; $P < 0.001$; ESA vs natural
385 regeneration: $t = -2.78$; $P = 0.021$; see Fig. 4). Sown grass richness was significantly
386 higher in samples from NVC plots than from natural regeneration plots (d.f. = 45;
387 $t = 3.43$; $P = 0.004$), with sown grass richness in samples from ESA plots being
388 intermediate and not significantly different from that in the other two treatments.
389 Results of analyses B were mostly in agreement with those from analyses A, with
390 significant main effect of seed mix on overall sown species richness (GLMM;
391 $F_{1,63} = 37.64$; $P < 0.001$) and on forb richness ($F_{1,63} = 18.73$; $P < 0.001$). However,
392 with analysis B being based on data from both the shallow-cultivated plots and the
393 deep-cultivated plots, unlike in analysis A, we found a significant seed mix effect for
394 grass richness ($F_{1,63} = 8.89$; $P = 0.004$) that was indicative of higher richness in
395 samples from NVC plots than from ESA plots (Fig. 4).

396

397 *Effects of cultivation*

398 Both for unsown and sown species groupings, there were only few main or interaction
399 effects of cultivation (Tables S3 and S5). For seed density of unsown grasses, there
400 was a significant cultivation main effect (LMM; $F_{1,99} = 6.23$; $P = 0.014$), reflecting
401 slightly higher seed densities on deep-cultivated plots than on shallow-cultivated plots
402 (Fig. 1). In addition, we found one significant three-way interaction involving
403 cultivation for unsown species richness (Table S3) and one for sown species richness
404 (Table S3). Other than that, we found no evidence for choice of cultivation to affect
405 seed bank dynamics.

406

407 *Compositional trends*

408 A three-dimensional NMDS ordination proved best for representing seed bank and
409 vegetation species compositional variation. Stress of the final model was 13.4,
410 corresponding to reasonably good preservation of between-sample relationships in
411 ordination space (Clarke 1993). NMDS axes 1, 2, and 3 explained 36.1%, 16.9%, and
412 29.3% of variance, respectively. Accordingly, a combination of axis 1 with axis 3
413 provided the clearest two-dimensional representation for illustrating differences
414 between trajectories for the seed bank and vegetation of different experimental
415 treatments (Fig. 5).

416 For the sown treatments, but not for natural regeneration, seed bank and
417 vegetation were distinctly and consistently different from each other in terms of
418 species composition, as indicated by the fact that both were clearly separated in
419 NMDS ordination space, with sown vegetation of all three sites consistently being
420 located in the same part of ordination space (Fig. 5). A positive shift of vegetation
421 trajectories along NMDS axis 1 was found for all treatments at the Norfolk Broads
422 site, but only for the natural regeneration treatment at the other two sites (Fig. 5). As

423 indicated by the additional analyses of goodness-of-fit with the target community, this
424 shift along the first NMDS axis coincided with an increase in compositional similarity
425 with the respective NVC target communities (Fig. S1, Supporting Information).

426 For all three sites, trajectories differed strongly between natural regeneration
427 and seeded treatments, and differed somewhat between ESA and NVC seeded
428 treatments. Type of cultivation exerted little effect on these trajectories (Fig. 5).

429 Seed bank composition also shifted markedly towards higher NMDS axis 1
430 values (Fig. 5). For the Upper Thames Tributaries site, but not the other sites, seed
431 bank composition also shifted towards higher values along NMDS axis 3 (Fig. 5).

432 The corresponding species plot (Fig. 6) summarizes the underlying patterns
433 and trends at the species level. The positive shift in seed bank composition along
434 NMDS axis 1 was partly affected by declining seed bank densities of unsown ruderal
435 species positioned on the left-hand side, including *Capsella bursa-pastoris*, *Lamium*
436 *purpureum*, *Poa annua*, and *Polygonum aviculare* (Fig. 6). At the same time, it was
437 also partly affected by increasing seed bank densities of several sown target species
438 located on the right-hand side of the plot (Fig. 6). Of these, *Agrostis capillaris*,
439 *Cynosurus cristatus*, *Festuca ovina/rubra* agg., *Hypochaeris radicata*, *Leucanthemum*
440 *vulgare* and *Plantago lanceolata* all had accumulated substantial seed bank densities
441 of up to several hundred seeds per m² by 1998, whereas several others, including
442 *Anthoxanthum odoratum*, *Prunella vulgaris* and *Trifolium dubium* appeared to have
443 accumulated more gradually (Table S6, Supporting Information).

444 Seed bank compositional shifts along NMDS axis 3 for the Upper Thames Tributaries
445 site were driven by the rapid decline in seed bank densities of a number of ruderal
446 species largely limited to this site and located in the lower half of the plot, including
447 *Helminthotheca echioides*, *Juncus articulatus*, and *Persicaria maculosa* (Fig. 6). The

448 generally higher NMDS axis 3 scores for the vegetation than for the seed bank at the
449 three sites were affected by several sown species located at the top of the plot,
450 including *Alopecurus pratensis*, *Phleum pratense*, *Poa pratensis*, and *Schedonorus*
451 *pratensis* – that had successfully established in the vegetation of one or more sites, but
452 hardly accumulated any seeds in the soil (Table S6).

453

454 **Discussion**

455

456 *Seed mixtures*

457 The sowing of seed mixtures was associated with a decline of unsown species in the
458 seed bank, both in terms of seed densities and average species richness per sample.
459 Compared to natural regeneration, sowing of both the grass-only ESA mixtures and of
460 the diverse grass-forb NVC mixtures led to a decline in seed densities of unsown
461 grasses. At the same time, decline in unsown forb densities may have been somewhat
462 slower with ESA mixtures than with NVC mixtures, but our findings are somewhat
463 inconclusive, with analyses A and B yielding seemingly contradictory results.

464 Overall, our findings on the effects of seed mixtures on seed density dynamics
465 of unsown species groupings are in agreement with other studies showing that the
466 presence of particular functional groups of species may increase resistance to further
467 colonization by other members of the same functional group, as e.g. shown for
468 graminoids (Helsen et al. 2016) and for legumes (Turnbull et al. 2005). For our
469 results, this means that lower seed bank densities of unsown forbs and grasses in
470 seeded restoration treatments may have been the result of competitive exclusion
471 which would have prevented the continued replenishment of existing seed reserves of
472 unsown species, resulting in faster net depletion.

473 Interestingly, the seeds of sown forbs accumulated at a lower rate in the soil on
474 treatment plots sown with grass-only ESA mixtures than on naturally regenerating
475 plots. This may have been a consequence of negative priority effects exerted by sown
476 grasses on sown-forb colonization from adjacent NVC plots. In another grassland
477 restoration experiment on ex-arable land, carried out by Werner et al. (2016), grasses
478 exerted very strong negative priority effects on forbs. In fact, negative priority effects
479 from sowing grass-dominated seed mixtures can effectively prevent partially restored
480 grassland from further progressing towards forb-rich stages that would more strongly
481 resemble the target vegetation (Fagan et al 2008). Our own findings may reflect such
482 processes, albeit at a smaller spatial and temporal scale.

483 A potentially higher species richness of sown grasses in seed bank samples
484 from NVC-sown treatments, compared with those from ESA-sown treatments
485 indicated by our results most likely simply represents an artefact due to NVC mixtures
486 having included more grasses than ESA mixtures.

487

488 *Cultivation*

489 With the exception of a weak effect on seed densities of unsown grasses, depth of
490 cultivation (deep ploughing versus shallow cultivation using discs or harrows)
491 appeared to have little effect on seed bank densities of sown or unsown groups, nor
492 did it affect compositional trajectories. This may be due to the fact that deep
493 cultivation by ploughing was carried out to a depth of no more than 30-40 cm, and
494 may thus have hardly extended below the regular depth of cultivation in the years
495 prior to the experiment when sites were still managed as arable land. This means that
496 the vertical distribution of seeds across the actively managed soil profile may have
497 been fairly homogeneous at the start of the experiment, and therefore the two

498 cultivation treatments may have had little effect in terms of vertical re-distribution of
499 seeds in the soil profile. Accordingly, Glen (2008) found that, compared to deep-
500 ploughing to greater depths, conventional ploughing has very limited effects on the
501 seed bank in ex-arable restoration.

502

503 *Compositional trends*

504 Species composition of the vegetation of sown treatments was rather static throughout
505 the four years in which vegetation was monitored, with the exception of that at the
506 Norfolk Broads site, where compositional similarity with the target community
507 continued to increase. Nonetheless, in these sown treatments, any observed
508 compositional convergence between the soil seed bank and the vegetation was rather
509 limited. This is not surprising as compositional similarity between these two
510 compartments usually remains quite low even in old grassland (Hopfensperger 2007;
511 Kiss et al. 2017). This is due to i) the fact that only a limited number of specialist
512 grassland species tend to form persistent seed banks (Bekker et al. 1998), and ii) the
513 fact that the seeds of early-successional ruderal species often persist in the soil for
514 decades after conversion from arable to grassland has taken place (Chancellor 1986).
515 For the seed bank, compositional shifts during restoration involved both an increase in
516 seed densities of sown species as well as a decline in seed densities of unsown
517 species. Both trends were more pronounced in sown treatments than under natural
518 regeneration, reflecting both a suppression of unsown species and a boost to seed
519 bank formation of late-successional sown species from sowing. Without sowing, such
520 late-successional target species tend to build up only slowly in the seed bank during
521 restoration, reflecting the slowness of unassisted colonization (Helsen et al. 2015).

522 With respect to dynamics of different groups of unsown species relative to each other,
523 unsown forbs tended to decline faster than unsown grasses. This may have been due
524 to unsown forbs in our study having mainly been ruderal species confined to the seed
525 bank, whereas unsown grasses also contained generalist grassland species such as
526 *Holcus lanatus* that established rather well in the vegetation, enabling them to
527 replenish their buried seed reserves. Observed declines in seed bank densities of
528 ruderal species were broadly in line with declines observed by other authors (Akinola
529 et al. 1998). From the limited information available on seed bank composition of
530 mature MG5 grassland (Kirkham & Kent 1997), it appears that with the exception of
531 rushes (*Juncus* spp.), ruderal species tend to be poorly represented in the seed bank of
532 MG5 reference sites. On the other hand, a number of component species of MG5
533 communities species which were sown in our study, such as *Agrostis capillaris*,
534 *Cynosurus cristatus* and *Plantago lanceolata*, and which readily accumulated seeds in
535 the soil once successfully established, are also prevalent in the seed bank of mature
536 MG5 grassland (Kirkham & Kent 1997). This suggests that over the seven years
537 covered by our study, the seed bank at our experimental sites, particularly on plots
538 seeded with the NVC mixtures, has made some compositional progress towards the
539 desired reference condition.

540

541 ***Seed bank monitoring in the post-restoration phase***

542 As suggested by Rayburn et al. (2016), seed bank analysis can complement more
543 traditional methods of vegetation monitoring. Vegetation monitoring tends to focus on
544 species presence in the vegetation, but usually fails to take into account seed
545 production and recruitment aspects, which are important when assessing restoration
546 progress under functional aspects (Godefroid et al. 2011). Time required by sown

547 target species to become self-sustaining, i.e. capable of regeneration e.g. by self-
548 seeding does not just depend on initial establishment and survival, but also on how
549 well species requirements are met during restoration (Wagner et al. 2016). Seed bank
550 monitoring, by indicating whether species have succeeded in building up a functional
551 soil seed bank, can complement the information provided by traditional vegetation
552 monitoring, thus allowing for a more integrated assessment of restoration progress
553 also in functional terms. It can help differentiate between those populations that are
554 fully functionally restored and those that may only just manage to persist under sub-
555 optimal conditions. While inclusion of seed bank monitoring does involve additional
556 time and resources, it is worth doing so where such resources are available.

557

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562 identification. We thank two reviewers for valuable comments and suggestions.

563

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692 **Supporting Information**

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

694

695 Figure S1. Goodness-of-fit to NVC target communities for the vegetation in the
696 various restoration treatments at each site.

697 Table S1. Composition of species-poor ESA mixtures and of the species-rich NVC
698 mixtures used in sown treatments at the three experimental sites.

699 Table S2. Results of mixed model analyses A of the effects of seed addition on seed
700 bank density and species richness of unsown species groupings.

701 Table S3. Results of mixed model analyses B of the effects of seed mixture type and
702 cultivation depth on seed bank density and species richness of unsown species
703 groupings.

704 Table S4. Results of mixed model analyses A of the effects of seed addition on seed
705 bank density and species richness of sown species groupings.

706 Table S5. Results of mixed model analyses B of the effects of seed mixture type and
707 cultivation depth on seed bank density and species richness of sown species groups.

708 Table S6. Seed bank densities and quadrat cell counts in the vegetation of all taxa
709 recorded in the experiment.

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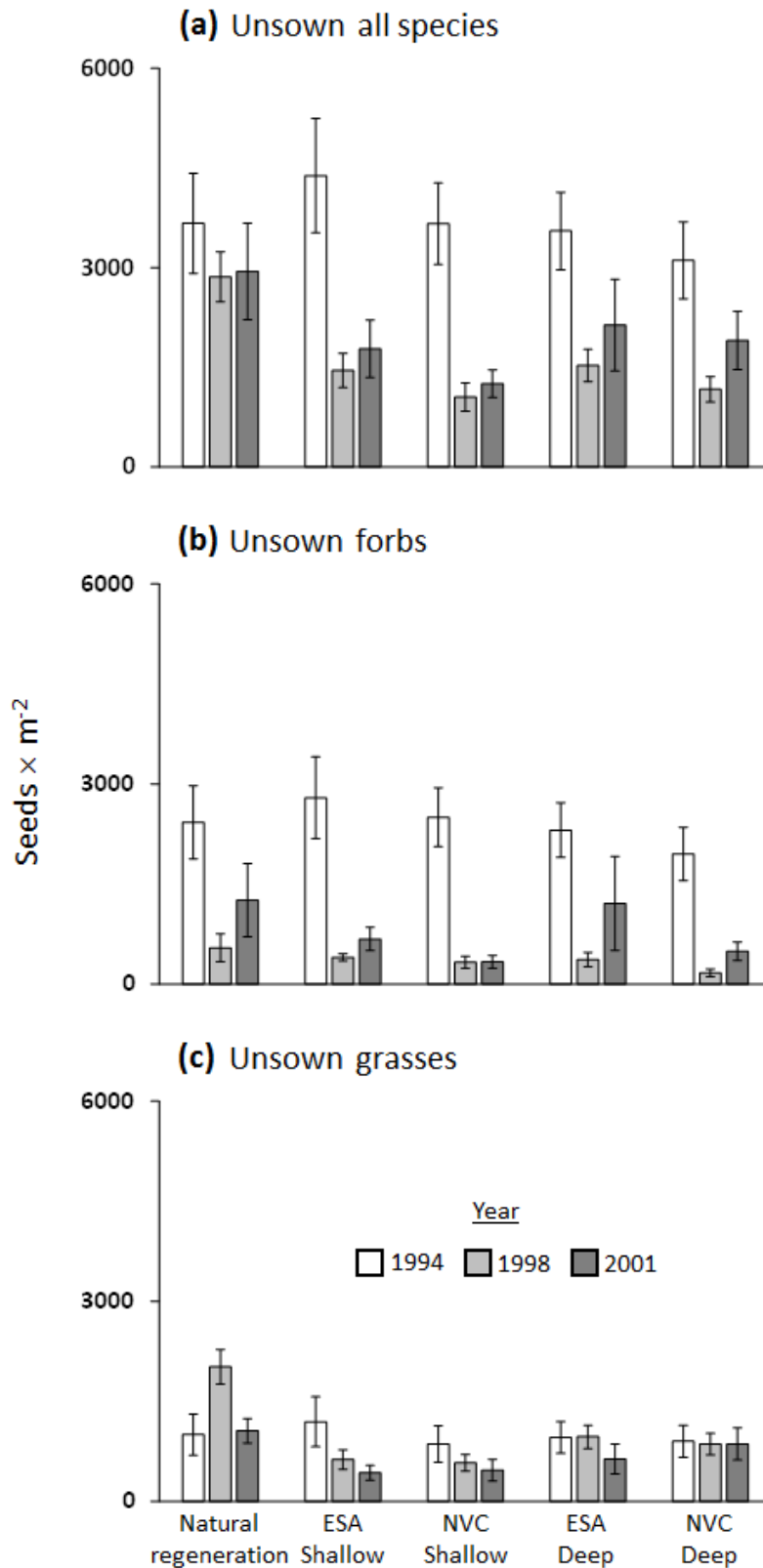
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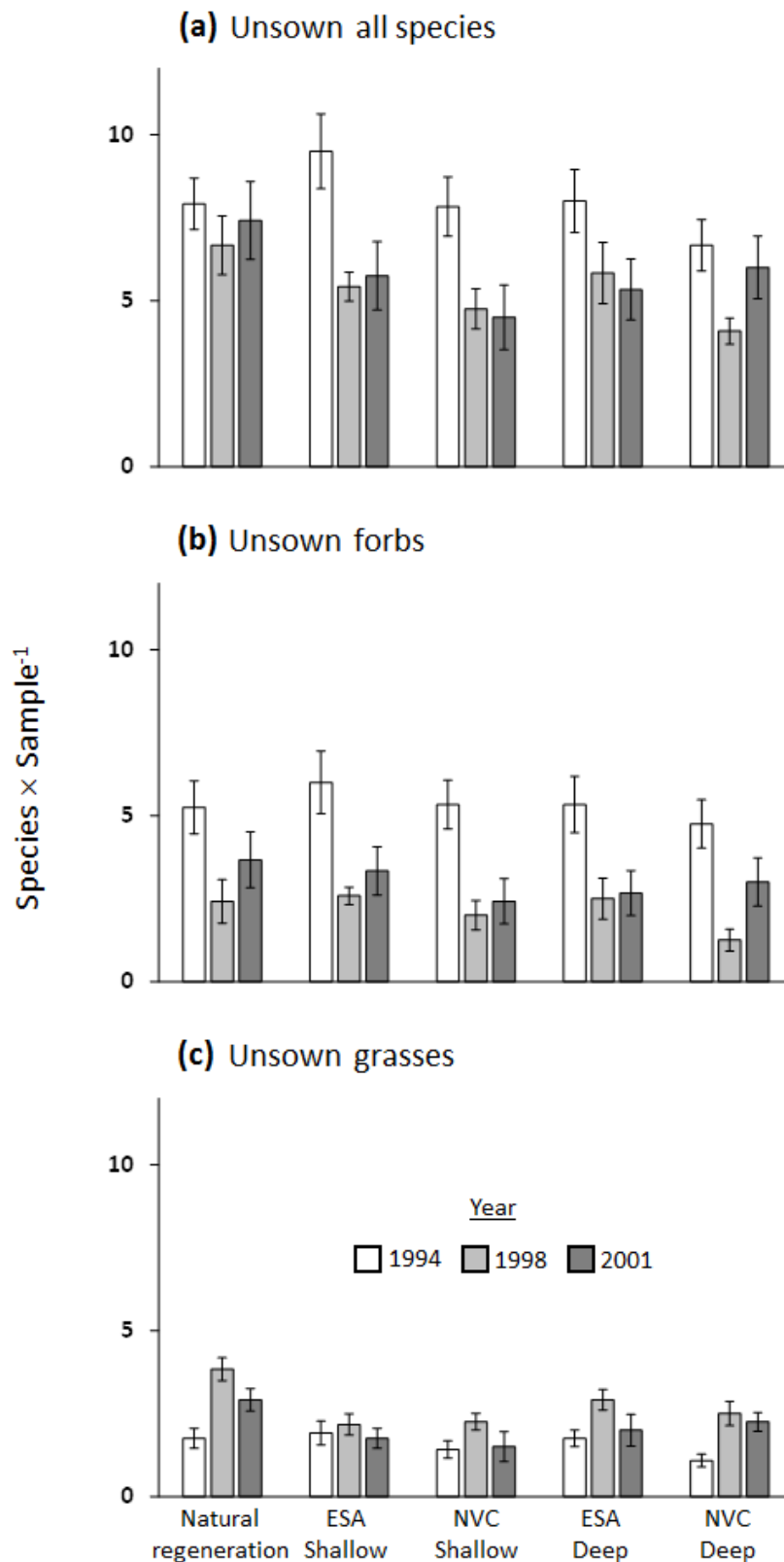
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717 Figure 1. Buried seed densities in the various restoration treatments for (a) all unsown
 718 species, (b) unsown forbs, and (c) unsown grasses. Error bars indicate \pm SE (n = 12).
 719 Seed densities (a) also include *Juncus* spp. and woody species.



742 Figure 2. Sample species richness in the various restoration treatments for (a) all
 743 unsown species, (b) unsown forbs, and (c) unsown grasses. Error bars indicate
 744 \pm SE (n = 12). Species counts for (a) also include *Juncus* spp. and woody
 745 species.



767 Figure 3. Buried seed densities in the various restoration treatments for (a) all sown
 768 species, (b) sown forbs, and (c) sown grasses. Error bars indicate \pm SE (n = 12).

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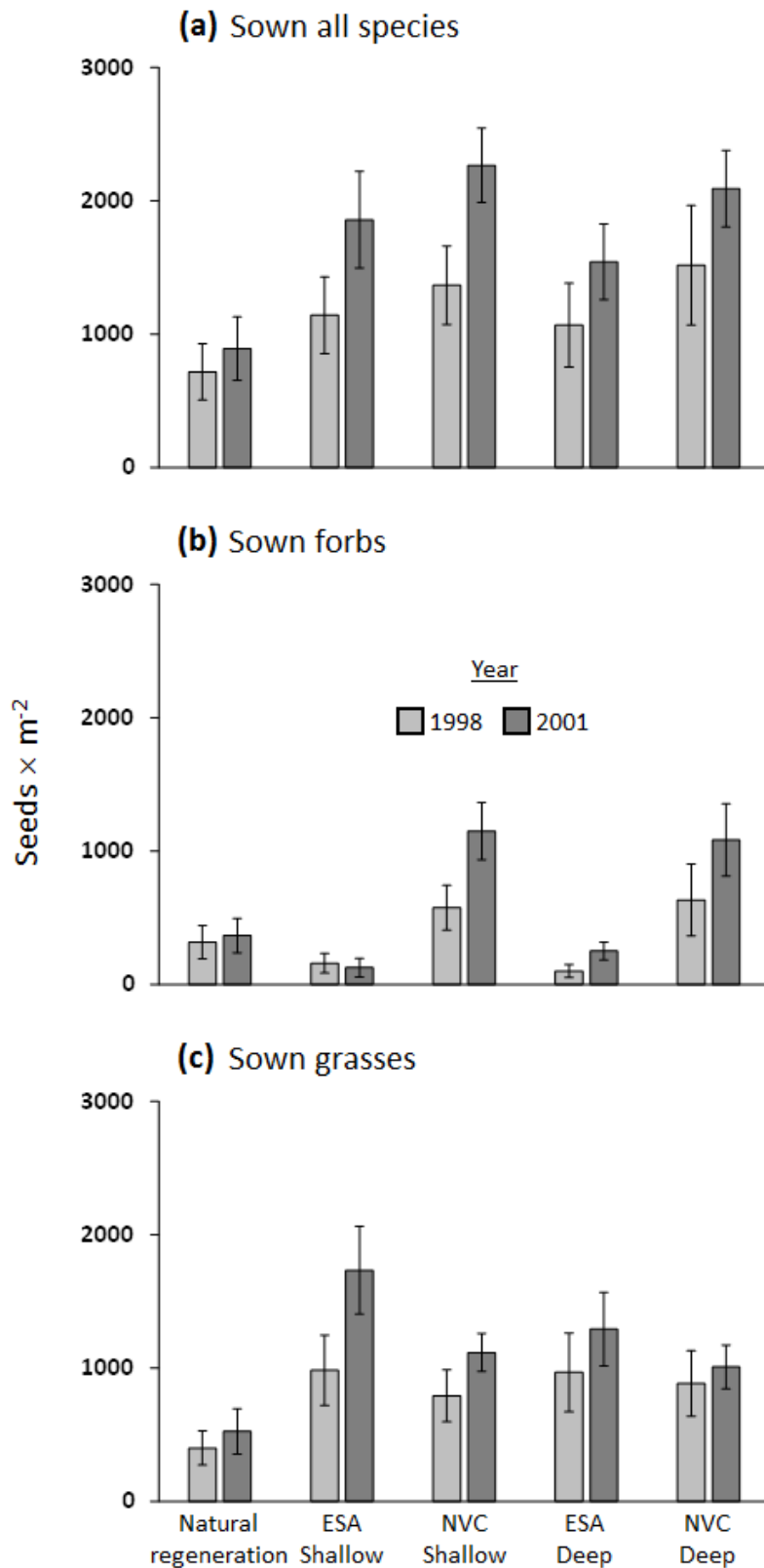
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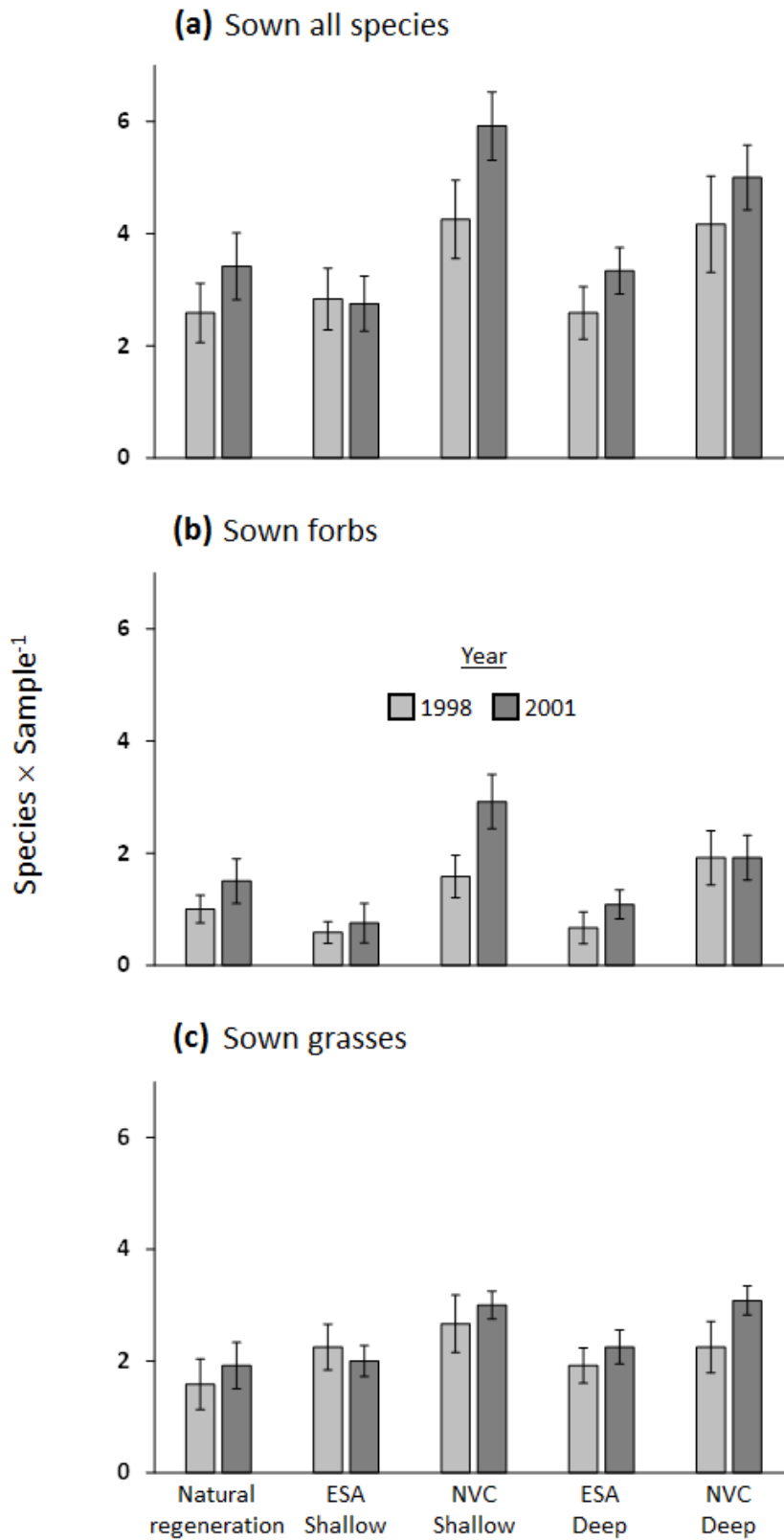
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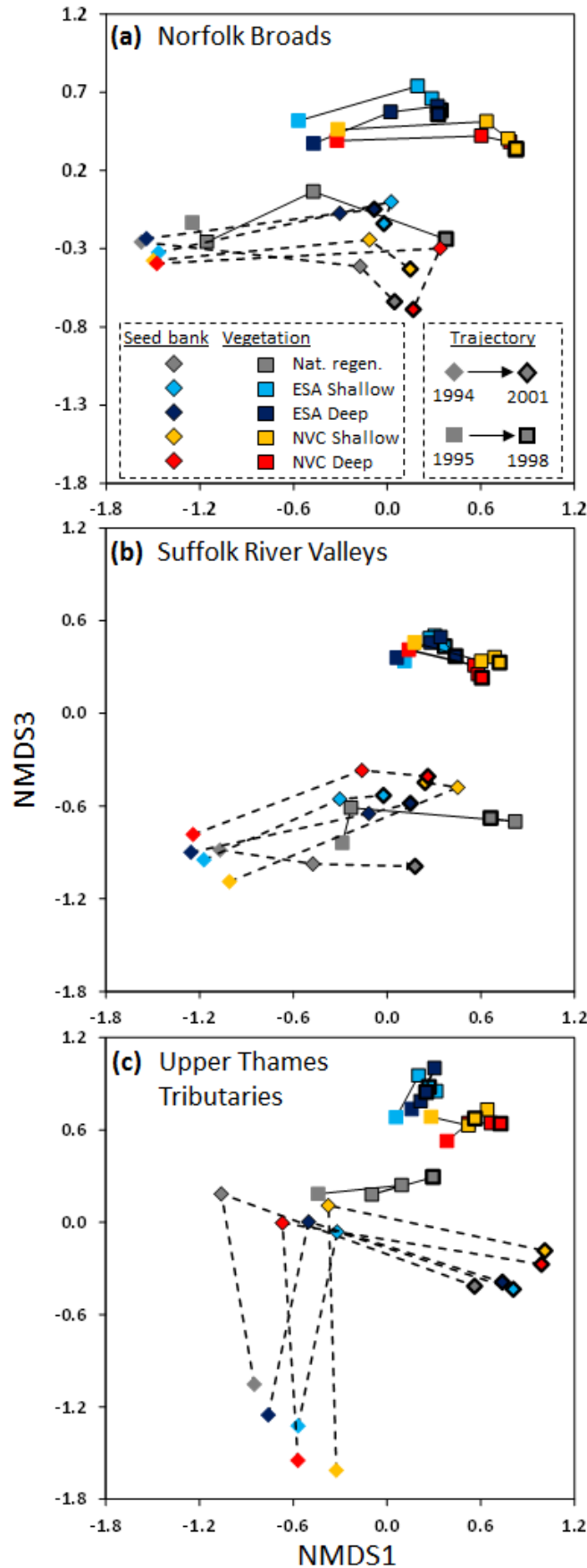
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792 Figure 4. Sample species richness in the various restoration treatments for (a) all sown
 793 species, (b) sown forbs, and (c) sown grasses. Error bars indicate \pm SE (n = 12).



816 Figure 5. NMDS sample plots of a joint analysis of seed bank and vegetation at the
 817 three experimental sites. For clarity, trajectories are depicted separately for each site
 818 in panels (a) to (c). Axes 1 and 3 explain 36.1% and 29.3% of species composition,
 819 respectively.



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840 Figure 6. NMDS species plots of a joint analysis of seed bank and vegetation at the
 841 three experimental sites. To avoid overcrowding in this plot, only those species are
 842 plotted that did occur in at least ten vegetation and/or seed bank samples. Letter codes
 843 indicate species identity (for full names see Table S6, Supporting Information).

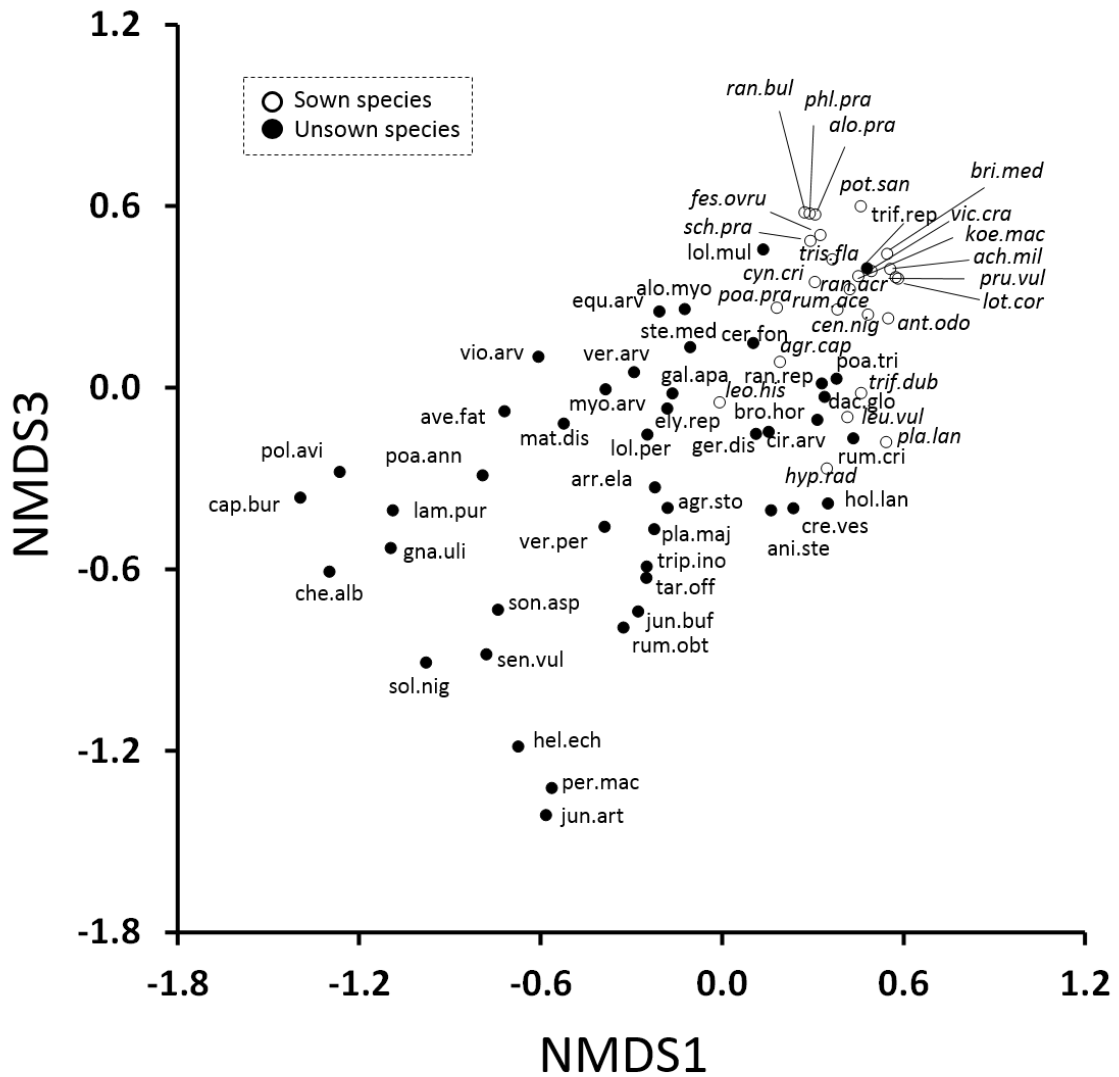


Table 1. Description of the three experimental sites.

Site	Location	Elevation (m a.s.l.)	Annual rain (mm)	Soil type	NVC target community (Rodwell 1992)
Norfolk Broads	52°44'N 1°36'E	< 5	500-600	Humic alluvial gley	MG5a <i>Cynosurus cristatus</i> – <i>Centaurea nigra</i> grassland: <i>Lathyrus pratensis</i> subcommunity
Suffolk River Valleys	52°01'N 1°20'E	<10	500-600	Brown sand	MG5c <i>Cynosurus cristatus</i> – <i>Centaurea nigra</i> grassland: <i>Danthonia decumbens</i> subcommunity
Upper Thames Tributaries	51°52'N 1°03'W	70	600-700	Alluvial gley	MG5a <i>Cynosurus cristatus</i> – <i>Centaurea nigra</i> grassland: <i>Lathyrus pratensis</i> subcommunity