

1 RESEARCH ARTICLE

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3 **Evaluation of topsoil inversion in UK habitat creation**
4 **and restoration schemes.**

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6 **Running head**

7 Habitat creation using topsoil inversion.

8

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20 **Author contributions**

21 EG, EP, SC, JC, LJ, RS conceived and designed the research; RS set up trial sites and contributed
22 materials; EG performed the experiments and analysed the data; EG, EP wrote and edited the
23 manuscript.

24 **Abstract**

25 Habitat creation and restoration schemes on former agricultural soils can be constrained by high
26 residual soil fertility, a weedy seed bank, and a lack of suitable species in the seed rain. Topsoil
27 inversion has been trialled across the UK as a novel technique to address these constraints. We
28 investigated 15 topsoil inversion sites ranging in age (time since inversion) from 6 months to five
29 years. We assessed surface soil fertility compared to adjacent non-inverted soil, and vegetation
30 composition with respect to the species introduced at each site. Soil organic matter, total and
31 extractable N and P were lower in topsoil inversion surface soils, demonstrating that topsoil
32 inversion can successfully reduce surface soil fertility prior to habitat creation and restoration.
33 This reduction was maintained over the timescale of this study (five years). Cornfield annual
34 nurse crops provided instant visual appeal and gave way to grassland species over time. Sown
35 species varied widely in their establishment success, and sowings were more successful than plug
36 plantings. Grasses colonised naturally following sowing forb-only seed mixes, allowing
37 introduced forbs to establish early on with reduced competition from the seed bank. Plant
38 communities did not yet resemble semi-natural communities, but all were in the early stages of
39 community development. Results indicate that topsoil inversion can successfully lower surface
40 soil fertility and reduce competition between sown species and agricultural weeds.

41

42 **Key words**

43 Deep ploughing

44 Nurse crop

45 Seed addition

46 Soil fertility

47 Soil phosphorous

48 Species-rich grassland

49

50 **Implications for Practice**

- 51 • Topsoil inversion is effective in lowering surface fertility of former agricultural soil. This
52 reduction is maintained over a minimum timescale of five years.
- 53 • Topsoil inversion is effective in reducing competition between sown species and
54 agricultural weeds present in topsoil.
- 55 • Using a forb-only seed mix allows sown species to establish without competition from
56 grasses, which colonise well naturally. Cornfield annual nurse crops provide early visual
57 appeal and give way to grassland species over time.
- 58 • Sown native wildflower species vary widely in their establishment success, with sowings
59 being more effective than plug planting.
- 60 • Although topsoil inversion and seed sowing aid the creation of species-rich grassland,
61 there is low resemblance to semi-natural communities in the early stages.

62

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72

73 **Introduction**

74

75 Semi-natural habitats of high plant diversity are frequently associated with infertile soils (Marrs
76 & Gough 1989). Agricultural soils generally have significantly higher levels of nutrients,
77 particularly extractable P, than semi-natural soils (Marrs et al. 1998), preventing establishment of
78 species characteristic of less fertile soil (Walker et al. 2004). Thus, successful habitat restoration
79 may be more likely when soil nutrient levels are closer to those of target habitats (Critchley et al.
80 2002). Natural community regeneration may also be constrained if species present in the soil seed
81 bank do not match those required in the restored habitat (Hutchings & Booth 1996). Seed bank
82 impoverishment tends to become more pronounced with increasing time since habitat
83 degradation, and with increasing agricultural intensity (Clarke 1997).

84 Traditional methods for lowering soil fertility include grazing, vegetation off-take,
85 burning and topsoil removal (Marrs 1993; Gilbert et al. 2003; Lawson et al. 2004; Jones et al. in
86 press). Topsoil removal aims to cause a rapid simultaneous reduction in surface soil fertility and
87 removal of the soil seed bank in order to achieve successful restoration (Walker et al. 2004).
88 Topsoil inversion has been suggested as a novel alternative technique to topsoil removal (Gilbert
89 et al. 1996; Marrs 2002), although the effects are much less researched. Gilbert et al. (2003)
90 suggest that deep ploughing may be an effective means of reducing P availability in the soil,
91 providing initial concentration decreases with depth. In clay soils where the P enrichment is
92 largely confined to the top 20 cm Gilbert et al. (1996) suggest that topsoil stripping to 20 cm or
93 deep ploughing to 40 cm would be most likely to be successful in reducing surface soil available
94 P. Studies indicate that soil nutrients can be reduced by ploughing more deeply than conventional
95 soil preparation. For example ploughing to 30 cm was found to reduce surface soil total N and
96 extractable K (Allison & Ausden 2004), and to reduce surface soil available P and K (Pywell et
97 al. 2002).

98 Deep ploughing to >50 cm has been used in Scandinavian forestry as a method of
99 improving plantation success. Deep ploughing keeps areas free from vegetation in the first
100 growing season (Matthesen & Damgaard 1997). Pywell et al. (2002) investigated the use of deep
101 cultivation to a depth of 30 to 40 cm in the restoration of species-rich grassland on arable land.
102 Results found that deep cultivation improved restoration success by reducing soil P and K,
103 improving the establishment of sown forbs, and reducing the number of unsown weedy grasses,
104 although the effect weakened after four years. Restored habitat on deep cultivated soil provided
105 the closest match to the target UK National Vegetation Classification (NVC) community
106 (Rodwell 1992) when sown with a species-rich seed mix on a suitable soil type. Other results are
107 less clear. For example Allison and Ausden (2004) found that reduction of soil fertility by deep
108 ploughing was less effective than topsoil removal. In this case, however, the ploughing depth was
109 less than is achievable with the Danish deep plough.

110 In 2002, Landlife set up the first trial site to investigate the use of topsoil inversion in
111 habitat creation, under the project name 'Break New Ground' (Landlife 2008), and was inspired
112 by the use of the deep plough (50-80 cm) in Danish forestry (Pywell et al. 2002; Allison &
113 Ausden 2004). Topsoil inversion results in burial of the topsoil under a layer of subsoil, so that
114 the original layers are intact but their position in the profile is changed. Despite its increasing use
115 by conservation organisations, there are limited published data on the effects of this practice on
116 soil nutrient profiles and plant community establishment. The objectives of this paper were to
117 evaluate the effect of topsoil inversion at 15 trial sites across the UK by evaluating the success
118 and duration of topsoil inversion in altering soil properties, and evaluating the success of sown
119 species and plant community composition and how this relates to soil properties. This will
120 address the requirement for a better understanding of the ecological effects of topsoil inversion in
121 habitat creation and restoration.

122

123 **Methods**

124

125 We selected fifteen topsoil inversion survey sites. Sites selected included a range of ages (time
126 since topsoil inversion) and geographic locations, with a variety of partner organisations and
127 habitat objectives (further details including full names and GPS locations are provided in Table
128 S1). Topsoil inversion had been undertaken using a double-bladed deep plough (Bovlund 64D,
129 Bovlund, Denmark) pulled by a 230 horsepower tractor, detailed in Glen (2009) and Jones et al.
130 (2010). Topsoil inversion results in burial of the surface soil under a layer of subsoil. Depths vary
131 according to soil type but typically will result in approximately 40 cm of topsoil buried under 40
132 cm of subsoil. We use the term ‘topsoil inversion’ (TI) to denote sites where this treatment was
133 applied. All but one site was sown with creative conservation seed mixes comprised entirely of
134 forbs. A creative conservation seed mix is one that introduces a mixture of common species as a
135 starting point for community restoration, rather than aiming for a specific target or NVC
136 (National Vegetation Classification) community (Landlife 2008). Additionally, plants at three
137 sites were introduced as plugs. All fourteen sown sites included cornfield annual species to
138 provide a visually appealing nurse crop. Surveys took place in July 2007.

139 We recorded all plant species present on TI soil, and carried out a quantitative vegetation
140 survey at each site. We placed five x 1 m² quadrats at random within the inverted area and
141 estimated percentage cover for each species, and percentage bare ground. Total percentage covers
142 were calculated by summing the mean percentage covers for each species.

143 At each site, we took five surface soil cores at random to a depth of 15 cm from the TI
144 areas, avoiding the outermost 5 m edge. Five soil samples were also taken from the surrounding
145 or adjacent area of soil which had not been inverted, to provide a control. We analysed soil
146 samples separately, giving five replicates each for TI and control surface soils at each site. Soil
147 samples were analysed for pH by adding fresh soil to water at a ratio of 1:2.5 by mass and

148 shaking for 15 minutes (Sykes & Lane 1996). Soil was oven dried at 105°C for 12 hours to
149 calculate gravimetric water content. Nitrate and ammonium were extracted using the KCl
150 extraction method (Allen 1989). Extractable nitrate and ammonium concentrations were then
151 measured using ion exchange chromatography (Dionex (UK) Ltd., Camberley, Surrey) and
152 converted to mg/kg of dry soil. Extractable phosphate was measured using the Olsen (bicarbonate
153 extraction) method (Rowell 1994). Dry soil was homogenised and sieved through 2 mm square
154 mesh sieve. Organic matter was measured by % loss on ignition at 375°C (Ball 1964). Dry soil
155 samples were digested in 2 ml concentrated sulphuric acid at 360°C for 4 hours with 50 mg 100:1
156 LiCl:Se catalyst (Carroll et al. 2003). Digests were analyzed for concentrations of total N (as
157 ammonium) using ion exchange chromatography (Dionex (UK) Ltd., Camberley, Surrey), and
158 total P using optical emission spectrometry (Inductively Coupled Plasma OES, Varian Inc.,
159 USA).

160 We analysed differences in surface soil characteristics with a paired *t*-test comparing TI
161 surface soil to adjacent surface soil. Organic matter, total N and total P were arcsin transformed
162 prior to analysis, and Olsen P was log transformed, in order to meet the assumption of normality.
163 We used Mann-Whitney tests for extractable nitrate and ammonium data since these were highly
164 right-skewed. Sown species persistence was calculated as percentage of sown species recorded
165 during the survey. We tested for relationships between measures of plant diversity and site
166 variables (age since TI and surface soil measurements) using linear regression analysis or
167 Pearson's correlations. All *t*-tests, Mann-Whitney tests and Pearson's correlations were
168 performed using Minitab (Minitab Statistical Software, Release 14 for Windows, State College,
169 Pennsylvania, USA). For each *t*-test, the number of degrees of freedom was adjusted to account
170 for unequal variances. N values refer to the number of replicates at each data point. Grime's plant
171 strategies were determined from quadrat data using MAVIS (Modular Analysis of Vegetation

172 Information System Plot Analyser v 1.00, Centre for Ecology and Hydrology, UK). Data are
173 presented untransformed.

174

175 **Results**

176

177 **Surface Soil Characteristics**

178 Soil pH was higher in TI than control surface soil for all sites except LU, which had almost
179 identical surface soil pH in TI and control soils. For the remaining sites, eight of these differences
180 were significant (Table 2). This trend occurred regardless of soil pH levels, which varied from pH
181 5.4 to 7.3 in control soils. Although the oldest site (LU) had no pH difference between TI and
182 control soils, and some of the smallest pH differences between TI and control soils were from the
183 oldest sites. There was no significant correlation between the magnitude of the pH difference (TI
184 minus control) and age of site.

185 For 14 sites, surface soil water content was higher in control soil than TI soil, and of
186 these, 11 were significantly different (Table 2). The site with the reverse trend was CH, where TI
187 surface soil was significantly wetter than control surface soil. The magnitude of the difference in
188 surface soil water content between TI and control soils varied greatly, from over 25% difference
189 at PT to just over 3% difference at OP. When all sites were analyzed, there was no significant
190 relationship between the difference in surface soil water content between TI and control, and the
191 age of the site. When the anomalous site was omitted, however, there was a significant negative
192 relationship between age of site and the soil water content difference ($R^2 = 22.9\%$, $P = 0.048$),
193 suggesting a decline in this treatment effect with time.

194 For all sites, surface soil OM was lower in TI soils than controls, with 11 of these
195 differences being significant (Table 2). The magnitude of the difference in surface soil OM
196 between TI and control soils varied considerably, from over 9% difference at PT to 1.8%

197 difference at LU. There was no significant relationship between the difference in surface soil OM
198 between TI and control, and the age of the site.

199 For 14 of the 15 sites, surface soil total N was lower in TI soils than controls, with 12 of
200 these differences being significant (Table 3). The magnitude of the difference in surface soil total
201 N between TI and control soils varied considerably, from around 5 mg/kg difference at PT to 1
202 mg/kg difference at LU. There was no significant relationship between surface soil total N
203 difference between TI and control, and the age of the site.

204 The frequency distribution of all surface soil extractable nitrate levels was highly right-
205 skewed. Surface soil nitrate levels varied between 0.5 mg/kg to over 150 mg/kg in control soils,
206 and between zero to around 19 mg/kg in TI soils. Despite this variation, all 15 sites had higher
207 surface soil nitrate levels in control soils than TI soils (Table 3); this difference was significant
208 for five sites. Surface soil ammonium levels varied between 0.2 and 14.5 mg/kg in control soils,
209 and 0 to 2.5 mg/kg in TI soils. The results for surface soil extractable ammonium levels were
210 similar to that of nitrate, with 13 sites having higher surface soil ammonium levels in control soils
211 than TI soils, five of which were significantly higher.

212 For all 15 sites, surface soil total P was lower in TI soils than controls, with 12 sites being
213 significantly different (Table 3). Data for surface soil Olsen P levels was highly right-skewed.
214 Surface soil Olsen P levels varied from around 7 to > 80 mg/kg in control soils, and from <1 to >
215 35 mg/kg in TI soils. Ten of the 15 sites had a significant difference between surface soil Olsen P
216 in control and TI soils (Table 3). Of these, two sites were significantly higher in TI than control
217 soils (BH and EP), and the remaining eight sites were significantly higher in control soils than TI
218 soils. The sites with significantly higher Olsen P in control soils varied considerably in the
219 magnitude of this difference, with the smallest difference of 5.6 mg/kg at CD, and the largest
220 difference of > 73 mg/kg at FW. All but one of the five sites with no significant difference
221 followed the general trend of having higher Olsen P in control than TI surface soils.

222

223 **Plant Community Establishment**

224 Between 21 and 49 non-woody plant species were recorded at the TI sites. Grasses were present
225 as natural colonisers at all sites, from one to eight species per site. Small numbers of ferns,
226 horsetails and bryophytes were present at some sites. The numbers of species recorded as total,
227 sown, natural colonisers and grasses were not significantly related to the age of the site in months
228 since topsoil inversion. Of all the TI surface soil factors recorded at each site, only extractable
229 nitrate showed a significant relationship with the total number of species ($R^2 = 20.9$, $p = 0.049$).
230 The number of sown species, natural colonisers and grasses showed no significant relationship
231 with any soil factor.

232 The UK NVC communities determined from the plant species present at each topsoil
233 inversion site included 10 mesotrophic grasslands, three open vegetation communities, one sand
234 dune and one calcifugous grassland community (Table 4). The number of sown species varied
235 from 12 to 28 (Table 5) with the exception of CD which was unsown. The majority of species
236 were introduced as seed; only three sites had plug planting in addition to sowing. None of the
237 species introduced by planting were recorded. Persistence of sown species varied from 20% at
238 BH to 86% at CH (Table 5). There was no relationship between the persistence of sown species
239 and the age of the site in months since topsoil inversion (Figure 1).

240 Twelve of the 15 sites had a nurse crop of five or six species of cornfield annuals (Table
241 S2). The number of cornfield annual species recorded during the survey varied from none to all
242 five or six (Table 5). There was a significant negative relationship between the persistence of
243 sown cornfield annual species and the age of the site in months since topsoil inversion (Figure 1).

244 Sown non-cornfield annual species across all sites numbered 36 species, including
245 biennials and perennials associated with meadow and woodland edge habitats (Table S2). Of
246 these, field scabious *Knautia arvensis*, common knapweed *Centaurea nigra*, red campion *Silene*

247 *dioica* and wild carrot *Daucus carota* were the most frequently sown species. Sixteen species
248 were sown at only one or two sites, reflecting tailoring of the seed mix to individual sites. Of the
249 36 sown non-cornfield annual species, three (chicory *Cichorium intybus*, red campion and ribwort
250 plantain *Plantago lanceolata*) were found at all sites where sown (although for chicory this was
251 only one site) and 16 were not recorded at any site where they were sown. The remaining species
252 varied in establishment success from one in nine sites for meadowsweet *Filipendula ulmaria*
253 (11% persistence), to seven out of eight sites for viper's-bugloss *Echium vulgare* (88%
254 persistence).

255 Total percent cover varied from 67% at BW to almost 220% at WC (Figure 2). For most
256 sites, the functional group that contributed most to the vegetation cover was forbs, with the
257 exception of LU and OP, which both had more grasses than forbs (Figure 2). Bryophyte cover
258 was variable between sites, with around half the sites surveyed having little or no bryophyte
259 cover and some having considerable amounts, in particular WC and OP, with 8% and 13%
260 respectively. Percent bare ground varied from zero at WC to almost 70% at TW.

261 Age of site and eight surface soil measures (pH, water, organic matter, total N, nitrate,
262 ammonium, total P, Olsen P) were tested for their relationship with nine vegetation
263 characteristics (total vegetation cover, forb cover, sown forb cover, unsown forb cover, grass
264 cover, bryophyte cover, bare ground, species richness and species diversity). Older sites had
265 significantly more grass cover and less bare ground (Figure 3). Surface soil total P was
266 significantly positively related to both grass cover and total vegetation cover (Figure 3).
267 Significantly greater bryophyte cover was also found at sites with higher surface soil nitrate
268 levels. Sites with wetter surface soils had significantly less bare ground. All three Grime's plant
269 strategies showed significant trends with age of site. Competitor (C) and stress tolerator (S)
270 scores both had a significant positive relationship with age of site, and ruderal scores (R) had a
271 significant negative relationship with age of site (Figure 4).

272

273 **Discussion**

274

275 **Surface Soil Characteristics**

276 **Topsoil inversion had a significant impact on soil pH, organic matter, water content, and**

277 **both N and P content in the surface layer compared to control soils.** Topsoil inversion raised soil

278 pH to approximately neutral levels, ideal for mesotrophic grassland creation. Different

279 communities of mesotrophic grassland have soil pH values that vary from pH 5.7 (MG13) to pH

280 7.5 (MG1) (Critchley et al. 2002). This suggests that the pH increase caused by deep ploughing

281 could be sufficient to modify the subsequent vegetation community development, although it may

282 not directly affect plant species diversity (Janssens et al. 1998). There was no significant negative

283 correlation between age of site and difference in soil pH. An advantage of topsoil inversion is its

284 potential to achieve rapid changes in surface soil fertility, rather than waiting several decades for

285 natural processes to revert the soil to its pre-intensive agricultural condition (Marrs 1993). For

286 social and political reasons it is usually desirable that ecological restoration produces relatively

287 rapid results (Gilbert et al. 2003).

288 TI surface soils generally had lower water content than control surface soils. The

289 exception to this was CH, due to damaged land drains. This site aside, the decrease in surface soil

290 moisture caused by TI is likely to be a result of the reduction in water-retentive humus and the

291 decrease in vegetation cover, which would increase evaporation of water from the soil surface.

292 Older TI sites had closer soil water content to control soils, probably because they had developed

293 more vegetation cover which increased soil shading (Matthesen 1997).

294 Organic matter levels were generally lower in TI surface soils than controls. This is

295 similar to results found by Allison and Ausden (2004) for deep ploughing to 30 cm. This

296 indicates burial of topsoil which is strongly associated with high soil OM. Many nutrients are

297 associated with the topsoil, and this is reflected by the results for total N and total P, both of
298 which had significantly lower levels in surface TI soil than control soils for 12 of the 15 sites.
299 Results for extractable N and P were more variable, but in general sites had lower levels in TI
300 surface soils, which were significantly lower for at least five sites. The extreme right skew in the
301 data for nitrate and ammonium made analyses problematic, and some extremely high values
302 amongst mainly low values made the relationship between total and available N unclear. The rate
303 of N mineralization, which was not measured, would give a clearer picture of N availability to
304 plants (Gilbert & Anderson 1998). The data for Olsen P were less right-skewed, and total P
305 appears to have been a good predictor of Olsen P levels, which supports findings by others that
306 total P and Olsen P are well correlated (Pilgrim et al. 2007).

307 The results show that for many sites it is possible to lower surface soil N and P (total and
308 extractable) by topsoil inversion, which is often a central objective in restoration (Gilbert et al.
309 2003) and that this reduction can persist for at least 58 months. A survey of 40 UK restoration
310 sites found that high phosphorous concentration was detrimental to restoration (Fagan et al.
311 2008). A reduction in surface soil nutrients, particularly P, is essential to favour the growth of
312 less competitive plant species and enable the development of a diverse sward (Critchley et al.
313 2002).

314

315 **Plant Community Establishment**

316 Semi-natural grasslands in the UK are those with high species richness and managed at low
317 intensity by traditional techniques such as horse grazing. Although it has been shown that UK
318 semi-natural grassland diversity is generally associated with lower levels of soil nutrients
319 (Critchley et al. 2002), it does not seem to be the case for the early stages of habitat creation at
320 these trial sites. Higher levels of soil nutrients appear to favour an increase in plant cover due to
321 their enhancement of plant growth, and have no initial effect on plant diversity, suggesting that

322 species input from the seed rain has a more significant effect on species diversity. Seed rain is
323 likely to vary between sites according to their proximity to seed sources in the wider landscape,
324 and dispersal abilities of each species (Bakker et al. 1996). Species characteristic of semi-natural
325 grassland and associated with low soil fertility may not have been able to colonise from natural
326 sources at the survey sites. It is likely that all sites exhibited a much lower plant species diversity
327 than would have occurred if the ground had been conventionally prepared rather than deep
328 ploughed due to the reduction of the surface soil seed bank caused by topsoil burial. However it is
329 precisely this reduction in ground cover that is likely to favour successful restoration.
330 Establishment of specialist species by seeding has been shown to be more successful when bare
331 ground conditions are created prior to sowing, with more severe soil disturbances favouring faster
332 growth and reproduction of introduced specialist plants (Wagner et al. 2015).

333 The relationship between the number of sown species and the vegetation diversity at each
334 trial site is not straightforward. Introducing more species by sowing and planting did not result in
335 a more species-rich plant community, during the timescale of this study. The role of the seed mix
336 used in habitat creation and restoration schemes is much debated (Brand-Hardy 1996). It is
337 possible to tailor seed mixes to match a target NVC community, although this was not done in
338 this study. However, it appears from these results that the initial appearance and subsequent
339 survival of sown species is a stochastic process. At no sites were all introduced species present,
340 despite the young ages of all sites. The maximum introduced species persistence was 80% and for
341 nine sites was 50% or less. Individual species performance varied from 100% appearance to
342 complete absence. Wildflower seeds have not been subjected to artificial selection for good
343 viability, and wildflower seed quality is limited by the UK climate, which is not optimum for
344 seed ripening and harvesting. The seeds used at each site were not always of local provenance.
345 Using locally sourced seeds is thought to improve performance due to the genotypes being better
346 adapted to the local soil and weather conditions (Gilbert & Anderson 1998). There is also concern

347 that non-local strains could hybridise with local populations and erode their genetic
348 distinctiveness (Krauss & Kock 2004).

349 Poor-performing species may have specific germination requirements which were not met
350 at the site. Unless a target community is specified it is recommended that species chosen for a
351 seed mix are those which germinate easily over a wide range of conditions, using a mix including
352 mid- to late-successional species (Brand-Hardy 1996). Plug planting is sometimes recommended
353 for species that establish slowly from seed (Holden et al. 2003). However we found no evidence
354 of any of the species introduced by plug-planting in this study. This may be due to competition
355 from sown species, or to weather conditions such as drought that compromised their survival.

356 Cornfield annuals were sown to give the sites instant visual appeal and to act as a nurse
357 crop. Nurse crops are used in restoration to improve the establishment of target species by
358 providing shelter for seedlings during the early stages, and by suppressing weed growth (Walker
359 et al. 2004). However the effectiveness of cover crops in improving performance of other sown
360 species is unclear (Pywell et al. 2002). Although in this survey the nurse crop showed a
361 significant decline in persistence over time, it is not clear whether it improved the establishment
362 of other sown species or colonisation by specialist species, as there was no comparable control
363 without a nurse crop. However, the visual element did serve to popularise the sites and involve
364 local communities (Landlife 2008).

365 One unusual feature of the seed mixes used was the lack of grass species. At the fourteen
366 sites that were sown, only one of these (LU) included grasses in the seed mix, and even here the
367 species used (red fescue *Festuca rubra* and sheep's fescue *Festuca ovina*) were chosen as
368 standard forestry mixes. It is usually recommended that grasses form 70 to 80% of the seed mix
369 (by seed numbers) for a meadow creation scheme (Holden et al. 2003). The low grass cover at the
370 survey sites is likely to be a result of their non-inclusion in the seed mix, although grasses had
371 colonised relatively easily from the seed rain. Sowing a mixture of forbs may therefore improve

372 the overall species diversity of the new habitat by allowing forbs to establish early on in the
373 absence of grasses. Fagan et al. (2008) recommend that seed mixes used to restore calcareous
374 grassland should aim to prevent assembly of low-value communities dominated by grasses. It is
375 usually the presence of many forb species that is used to judge the relative species richness of
376 grassland habitats, rather than the number of grass species (Rodwell 2006). Reduction or
377 elimination of grass seeds in restoration seed mixes may therefore be a positive step towards
378 improving restoration success.

379 Results from the NVC analysis showed that the survey sites were closest to a range of
380 semi-natural habitats, including disturbed ground, mesotrophic grasslands, upland acidic
381 grassland and sand dune communities. The fit of the NVC to the vegetation data was generally
382 poor, and it was not possible to assign NVC communities manually using tables due to the
383 dissimilarity of the observed species compositions with those listed. Many of the constant species
384 were absent. The survey sites are relatively young artificially created habitats, none of which had
385 any specific NVC restoration goal, and they do not closely resemble any semi-natural habitat.

386 Many attempts at restoring or creating species-rich semi-natural grassland highlight the
387 importance of selecting sites with low soil fertility, in particular P (Öster et al. 2009). The results
388 from this survey show that where this is not possible, soil fertility can be reduced by the use of
389 topsoil inversion to bury topsoil under the lower-fertility subsoil. It is notable that none of the
390 topsoil inversion sites had a management plan such as a mowing or grazing regime, which would
391 have the potential to influence plant community development. However, it is clear from this
392 evaluation of 15 sites that topsoil inversion can significantly aid the establishment of a new, more
393 species-rich community by creating a lower-fertility substrate to begin habitat creation or
394 restoration. We will next focus on data from a replicated field experiment that investigates
395 detailed changes to soil profile chemistry before and after topsoil inversion in comparison to

396 conventional ploughing, and follows the development of plant communities during three growing
397 seasons post-restoration.

398

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544 **Table 1.** Summary of survey sites showing date of topsoil inversion, age of site at the time of the
 545 survey (time since topsoil inversion), numbers of plant species sown and plug planted, and use of
 546 cornfield annual nurse crops.

Site Code	Date of topsoil inversion	Time since topsoil inversion	Number of species sown	Number of species plug planted	Cornfield annual nurse crop?
BH	03/03	52	25	0	Yes
BW	08/05	23	12	0	Yes
CD	01/04	39	0	0	No
CH	02/06	17	14	0	Yes
CR	08/05	23	10	2	Yes
CW	08/05	23	13	0	Yes
EP	11/02	56	15	0	Yes
FW	08/05	23	12	4	Yes
HH	12/05	19	18	1	Yes
LU	09/02	58	15	0	Yes
OP	11/02	57	16	0	Yes
PM	12/03	43	14	0	Yes
PT	08/05	23	12	0	Yes
TW	01/07	6	18	0	Yes
WC	09/03	46	28	0	Yes

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552 **Table 2.** Comparisons between control and topsoil inversion (TI) surface soil at 15 topsoil
 553 inversion sites for surface soil pH, soil moisture and soil organic matter (OM) content, using t-
 554 tests. *, significant at the 5% level; **, significant at the 1% level; ***, significant at the 0.1%
 555 level; N = 5; df adjusted to account for unequal variances.
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Site	Mean soil pH		<i>t-value</i>	Mean soil water (%)		<i>t-value</i>	Mean soil OM (% LOI)		<i>t-value</i>
	Control	TI		Control	TI		Control	TI	
BH	6.06	6.31	-2.32	30.9	22.0	3.27*	12.03	5.20	4.92***
BW	6.28	7.82	-9.75***	29.1	12.4	5.61**	10.11	1.84	28.24***
CD	5.61	5.91	-2.96*	27.0	6.4	11.85***	7.35	0.32	11.38***
CH	7.23	7.46	-2.38	15.8	20.2	-2.86*	1.86	1.45	0.87
CR	6.72	7.27	-5.99***	35.3	16.7	3.64***	10.34	1.62	6.43***
CW	6.43	7.20	-6.31***	24.5	15.9	5.20***	5.27	1.66	9.85***
EP	6.38	6.72	-2.42*	30.7	22.7	3.32*	8.00	5.72	2.56
FW	6.22	7.12	-3.60*	24.1	14.6	5.53***	4.98	1.43	8.53***
HH	6.58	6.80	-1.23	20.2	10.3	6.29***	6.22	4.05	2.33
LU	6.04	6.04	0.04	17.3	17.1	0.11	2.84	1.05	6.42***
OP	6.34	6.87	-1.84	22.5	19.2	4.22***	4.16	2.58	3.19*
PM	5.37	6.64	-8.94	24.8	23.0	1.43	6.96	3.59	9.34***
PT	6.75	7.51	-3.51**	35.5	9.6	10.33***	11.62	2.15	10.52***
TW	5.93	6.80	-7.09***	19.7	8.0	6.36***	4.88	1.15	8.19***
WC	6.85	7.15	-1.99	17.0	14.2	1.09	2.84	1.97	1.69

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560 **Table 3.** Comparisons between control and topsoil inversion (TI) surface soil at 15 topsoil
 561 inversion sites for total N, nitrate, ammonium, total P and Olsen (extractable) P content, using t-
 562 tests (total N and P; Olsen P) and Mann-Whitney tests (nitrate and ammonium). Asterisks (*, **,
 563 and ***) indicate significance at the 5, 1, and 0.1% level, respectively; N = 5 and df adjusted to
 564 account for unequal variances.

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Site	Mean total N		<i>t-value</i>	Median nitrate		W	Median ammonium		W	Mean total P (mg/g)		<i>t-value</i>	Mean Olsen P		<i>t-value</i>
	(mg/g)			(mg/kg)			(mg/kg)			(mg/g)			(mg/kg)		
	Control	TI		Control	TI		Control	TI		Control	TI		Control	TI	
BH	5.65	2.23	6.79***	154.22	1.32	40.0*	1.84	2.54	27.0	0.97	0.62	5.03**	7.09	14.58	-3.56*
BW	3.94	0.59	13.97***	3.80	0.48	40.0*	2.50	0.65	40.0*	0.73	0.16	19.59***	16.69	0.57	5.60***
CD	3.68	0.25	13.76***	0.54	0.44	29.0	14.52	0	-	0.67	0.10	9.03***	9.24	3.68	8.00***
CH	1.44	1.54	-0.36	0.52	0.42	31.0	0.90	1.00	25.0	0.36	0.16	2.32	17.11	3.12	3.02*
CR	4.03	1.06	5.26***	1.16	0.29	40.0*	4.97	0.72	40.0*	0.67	0.33	4.33***	22.45	3.22	21.48***
CW	2.43	0.83	8.99***	7.33	0	-	0.62	0.20	30.0	0.55	0.36	2.45*	33.22	5.81	5.66***
EP	5.44	3.48	3.24*	21.20	7.06	35.0	2.07	0.57	38.0*	1.03	0.90	1.36	6.81	11.62	-3.69**
FW	2.72	0.85	6.63***	12.56	0.25	37.0	0.15	0.00	32.0	0.72	0.41	3.10*	81.60	8.11	6.90***
HH	2.96	1.26	3.44*	0.61	0.00	33.0	0.57	0.47	34.0	0.72	0.19	10.31***	27.74	4.89	9.34***
LU	1.60	0.59	16.53***	0.78	0.32	37.0	1.20	0.34	30.0	0.41	0.16	5.59***	16.86	12.40	1.98
OP	1.67	1.09	2.49	35.78	11.36	35.0	3.50	1.26	38.0*	0.62	0.52	0.93	45.43	35.99	1.36
PM	3.79	2.40	5.22***	91.54	0.99	40.0*	2.31	0.00	33.5	0.81	0.41	3.64*	21.10	4.28	2.15
PT	6.15	1.04	9.81***	16.93	1.59	37.0	2.45	0.50	35.0	0.92	0.21	6.63**	15.84	2.66	4.32***
TW	3.28	0.98	10.59***	30.40	0.69	40.0*	1.42	0.72	38.0*	0.87	0.36	6.69*	21.79	27.06	-0.89
WC	1.24	0.70	2.28	14.40	18.59	29.0	1.30	0.57	37.0	0.46	0.28	3.54*	27.44	22.94	1.18

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580 **Table 4.** Age of site (months since topsoil inversion) and plant species and community outcomes
 581 on inverted soil at each of the 15 topsoil inversion survey sites. Number of non-woody species
 582 recorded shown separated into functional groups (grasses includes sedges and rushes; ferns
 583 includes horsetails). Numbers in parentheses show of which were introduced (sown or planted)
 584 species. No species were introduced at CD. NVC communities derived from all species recorded
 585 within topsoil inversion area.
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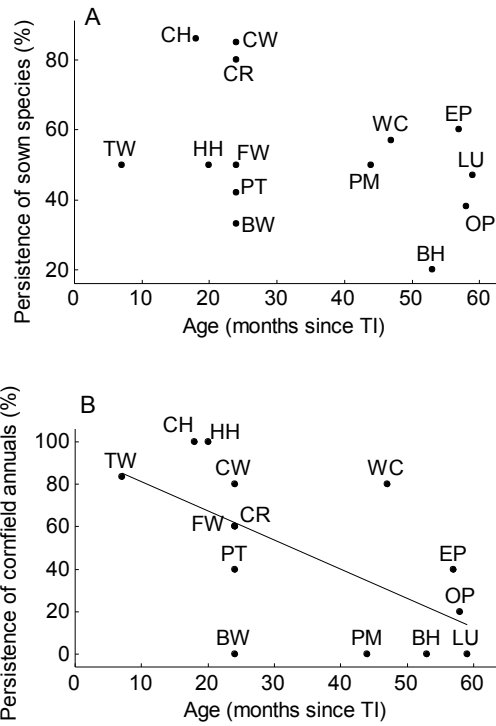
Site	Age (months)	Number of species recorded					NVC
		Forbs	Grasses	Ferns	Bryophytes	Total	
BH	52	20 (2)	4	0	0	24 (2)	MG6
BW	23	16 (4)	4	0	1	21 (4)	MG5
CD	39	25	5	0	3	33	U4
CH	17	42 (12)	6	0	1	49 (12)	MG5
CR	23	28 (8)	7	1	1	37 (8)	MG6
CW	23	27 (11)	4	0	1	32 (11)	MG1
EP	56	41 (8)	3	0	0	44 (8)	MG5
FW	23	16 (6)	6	0	1	23 (6)	MG1
HH	19	19 (9)	4	1	0	24 (9)	OV19
LU	58	19 (7)	3	0	3	25 (7)	SD8
OP	57	23 (6)	8	1	3	35 (6)	MG1
PM	43	21(7)	5	0	1	27 (7)	MG5
PT	23	20 (5)	5	0	1	26 (5)	OV23
TW	6	21 (9)	1	0	0	21 (9)	OV22
WC	46	37 (16)	6	0	1	44 (16)	MG1

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592 **Table 5.** Total number of species sown and planted at each of the 15 topsoil inversion survey
 593 sites, and number of these recorded during the survey. Numbers in parentheses show of which
 594 were cornfield annual species. Creative conservation seed mixes and plug plants were used which
 595 comprised of 100% forbs.
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Site	Age (months)	Number of spp.		Number recorded spp.		Persistence (%)
		Sown	Planted	Sown	Planted	
BH	52	25 (2)	0	5 (0)	-	20
BW	23	12 (5)	0	4 (0)	-	33
CD	39	0	0	-	-	-
CH	17	14 (5)	0	12 (5)	-	86
CR	23	10 (5)	2	8 (3)	0	80
CW	23	13 (5)	0	11 (4)	-	85
EP	56	15 (5)	0	9 (2)	-	60
FW	23	12 (5)	4	6 (3)	0	50
HH	19	18 (6)	1	9 (6)	0	50
LU	58	15 (1)	0	7 (0)	-	47
OP	57	16 (5)	0	6 (1)	-	38
PM	43	14 (5)	0	7 (0)	-	50
PT	23	12 (5)	0	5 (2)	-	42
TW	6	18 (6)	0	9 (5)	-	50
WC	46	28 (5)	0	16 (4)	-	57

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607 **Figure 1.** Persistence of A) all sown species and B) sown cornfield annual species (percentage of
 608 introductions recorded) as related to age of site in months since topsoil inversion (TI). The
 609 relationship was only significant for cornfield annuals: $R^2 = 34.7\%$, $P = 0.016$.

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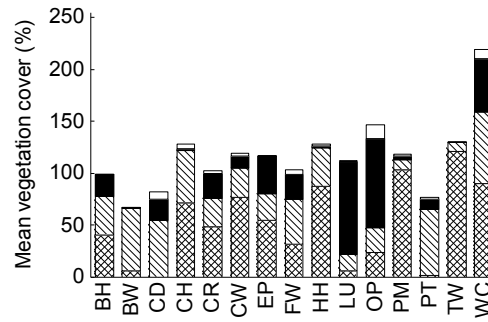
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621 **Figure 2.** Mean percent cover of vegetation in deep ploughed soil for the 15 topsoil inversion
 622 survey sites (N=5/site). Sites are identified by their unique codes. Cross hatched = sown forbs;
 623 hatched = naturally colonised forbs; black = grasses, sedges and rushes; white = ferns, horsetails
 624 and bryophytes.

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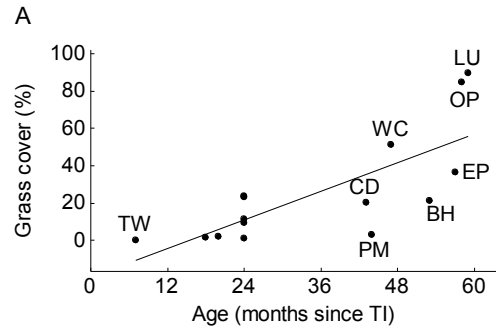
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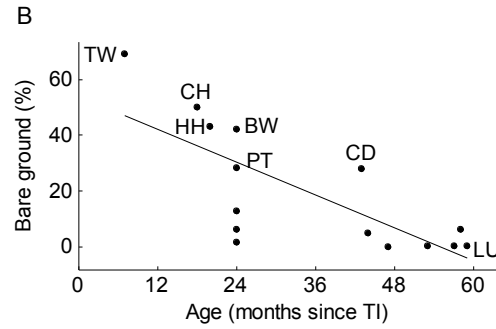
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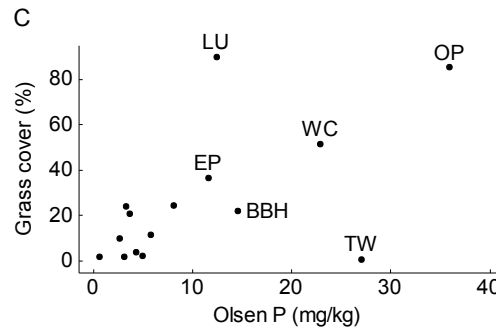
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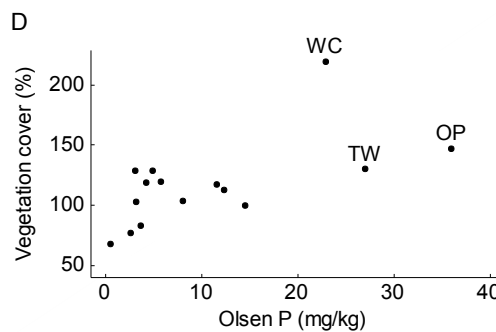
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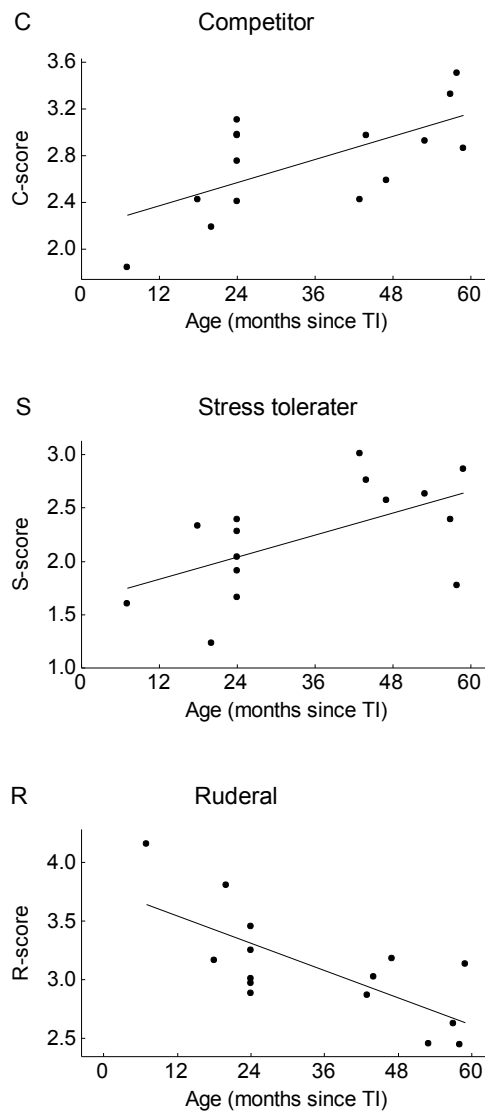
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644 **Figure 3.** Significant regressions and correlations between A) age [months since topsoil
 645 inversion] and grass cover, B) age [months since topsoil inversion] and percent bare ground, C)
 646 Olsen P and grass cover and D) Olsen P and total vegetation cover from the 15 topsoil inversion

647 survey sites. A: $R^2=53.8$, $P=0.001$; B: $R^2=52.7$, $P=0.001$; C: $r=0.585$, $P=0.022$; D: $r=0.614$,
648 $P=0.015$.



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652 **Figure 4.** Significant regressions between age of site (months since topsoil inversion) and
653 Grime's plant strategies scores (C=Competitor; S=Stress tolerater; R=Ruderal) from the 15
654 topsoil inversion survey sites. Competitor: $R^2=36.4\%$, $P=0.010$; Stress tolerater: $R^2=27.9\%$,
655 $P=0.025$; Ruderal: $R^2=48.5\%$, $P=0.002$.

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