



Article (refereed) - postprint

Glen, Emma; Price, Elizabeth A.C.; Caporn, Simon J.M.; Carroll, Jacky A.; Jones, Laurence M.; Scott, Richard. 2017. Evaluation of topsoil inversion in U.K. habitat creation and restoration schemes. *Restoration Ecology*, 25 (1). 72-81. <u>10.1111/rec.12403</u>

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2	can be found at:
3	http://onlinelibrary.wiley.com/doi/10.1111/rec.12403/full
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5	Glen, E., Price, E.A.C., Caporn, S.J.M, Carroll, J.A., Jones, L.M., and
6	Scott, R. (2017) Evaluation of topsoil inversion in UK habitat creation
7	and restoration schemes. Restoration Ecology, 25(1), 72–81. DOI:
8	10.1111/rec.12403
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11	RESEARCH ARTICLE
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13	Evaluation of topsoil inversion in UK habitat creation
14	and restoration schemes.
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16	Running head
17	Habitat creation using topsoil inversion.
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30 Author contributions

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

34 Abstract

35 Habitat creation and restoration schemes on former agricultural soils can be constrained by high 36 residual soil fertility, a weedy seed bank, and a lack of suitable species in the seed rain. Topsoil 37 inversion has been trialled across the UK as a novel technique to address these constraints. We 38 investigated 15 topsoil inversion sites ranging in age (time since inversion) from 6 months to five 39 years. We assessed surface soil fertility compared to adjacent non-inverted soil, and vegetation 40 composition with respect to the species introduced at each site. Soil organic matter, total and 41 extractable N and P were lower in topsoil inversion surface soils, demonstrating that topsoil 42 inversion can successfully reduce surface soil fertility prior to habitat creation and restoration. 43 This reduction was maintained over the timescale of this study (five years). Cornfield annual 44 nurse crops provided instant visual appeal and gave way to grassland species over time. Sown 45 species varied widely in their establishment success, and sowings were more successful than plug 46 plantings. Grasses colonised naturally following sowing forb-only seed mixes, allowing 47 introduced forbs to establish early on with reduced competition from the seed bank. Plant 48 communities did not yet resemble semi-natural communities, but all were in the early stages of 49 community development. Results indicate that topsoil inversion can successfully lower surface 50 soil fertility and reduce competition between sown species and agricultural weeds.

51

52 Key words

- 53 Deep ploughing
- 54 Nurse crop
- 55 Seed addition
- 56 Soil fertility
- 57 Soil phosphorous
- 58 Species-rich grassland
- 59

60 Implications for Practice

- Topsoil inversion is effective in lowering surface fertility of former agricultural soil. This
 reduction is maintained over a minimum timescale of five years.
- Topsoil inversion is effective in reducing competition between sown species and
 agricultural weeds present in topsoil.
- Using a forb-only seed mix allows sown species to establish without competition from
 grasses, which colonise well naturally. Cornfield annual nurse crops provide early visual
 appeal and give way to grassland species over time.

68	•	Sown native wildflower species vary widely in their establishment success, with sowings
69		being more effective than plug planting.

- Although topsoil inversion and seed sowing aid the creation of species-rich grassland,
 there is low resemblance to semi-natural communities in the early stages.
- 72

73 Word count

- 74 Author contributions 35
- 75 Abstract 223
- 76 Implications for Practice 119
- 77 Main Text 5442 (of which Acknowledgements 24; Literature Cited 915)
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83 Introduction

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Semi-natural habitats of high plant diversity are frequently associated with infertile soils (Marrs & Gough 1989). Agricultural soils generally have significantly higher levels of nutrients, particularly extractable P, than semi-natural soils (Marrs et al. 1998), preventing establishment of species characteristic of less fertile soil (Walker et al. 2004). Thus, successful habitat restoration may be more likely when soil nutrient levels are closer to those of target habitats (Critchley et al. 2002). Natural community regeneration may also be constrained if species present in the soil seed bank do not match those required in the restored habitat (Hutchings & Booth 1996). Seed bank 92 impoverishment tends to become more pronounced with increasing time since habitat93 degradation, and with increasing agricultural intensity (Clarke 1997).

94 Traditional methods for lowering soil fertility include grazing, vegetation off-take, 95 burning and topsoil removal (Marrs 1993; Gilbert et al. 2003; Lawson et al. 2004; Jones et al. in 96 press). Topsoil removal aims to cause a rapid simultaneous reduction in surface soil fertility and 97 removal of the soil seed bank in order to achieve successful restoration (Walker et al. 2004). 98 Topsoil inversion has been suggested as a novel alternative technique to topsoil removal (Gilbert 99 et al. 1996; Marrs 2002), although the effects are much less researched. Gilbert et al. (2003) 100 suggest that deep ploughing may be an effective means of reducing P availability in the soil, 101 providing initial concentration decreases with depth. In clay soils where the P enrichment is 102 largely confined to the top 20 cm Gilbert et al. (1996) suggest that topsoil stripping to 20 cm or 103 deep ploughing to 40 cm would be most likely to be successful in reducing surface soil available 104 P. Studies indicate that soil nutrients can be reduced by ploughing more deeply than conventional 105 soil preparation. For example ploughing to 30 cm was found to reduce surface soil total N and 106 extractable K (Allison & Ausden 2004), and to reduce surface soil available P and K (Pywell et 107 al. 2002).

108 Deep ploughing to >50 cm has been used in Scandinavian forestry as a method of 109 improving plantation success. Deep ploughing keeps areas free from vegetation in the first 110 growing season (Matthesen & Damgaard 1997). Pywell et al. (2002) investigated the use of deep 111 cultivation to a depth of 30 to 40 cm in the restoration of species-rich grassland on arable land. 112 Results found that deep cultivation improved restoration success by reducing soil P and K, 113 improving the establishment of sown forbs, and reducing the number of unsown weedy grasses, 114 although the effect weakened after four years. Restored habitat on deep cultivated soil provided 115 the closest match to the target UK National Vegetation Classification (NVC) community 116 (Rodwell 1992) when sown with a species-rich seed mix on a suitable soil type. Other results are

117 less clear. For example Allison and Ausden (2004) found that reduction of soil fertility by deep 118 ploughing was less effective than topsoil removal. In this case, however, the ploughing depth was 119 less than is achievable with the Danish deep plough.

120 In 2002, Landlife set up the first trial site to investigate the use of topsoil inversion in 121 habitat creation, under the project name 'Break New Ground' (Landlife 2008), and was inspired 122 by the use of the deep plough (50-80 cm) in Danish forestry (Pywell et al. 2002; Allison & 123 Ausden 2004). Topsoil inversion results in burial of the topsoil under a layer of subsoil, so that 124 the original layers are intact but their position in the profile is changed. Despite its increasing use 125 by conservation organisations, there are limited published data on the effects of this practice on 126 soil nutrient profiles and plant community establishment. The objectives of this paper were to 127 evaluate the effect of topsoil inversion at 15 trial sites across the UK by evaluating the success 128 and duration of topsoil inversion in altering soil properties, and evaluating the success of sown 129 species and plant community composition and how this relates to soil properties. This will 130 address the requirement for a better understanding of the ecological effects of topsoil inversion in 131 habitat creation and restoration.

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133 Methods

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

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

153 At each site, we took five surface soil cores at random to a depth of 15 cm from the TI 154 areas, avoiding the outermost 5 m edge. Five soil samples were also taken from the surrounding 155 or adjacent area of soil which had not been inverted, to provide a control. We analysed soil 156 samples separately, giving five replicates each for TI and control surface soils at each site. Soil 157 samples were analysed for pH by adding fresh soil to water at a ratio of 1:2.5 by mass and 158 shaking for 15 minutes (Sykes & Lane 1996). Soil was oven dried at 105°C for 12 hours to 159 calculate gravimetric water content. Nitrate and ammonium were extracted using the KCl 160 extraction method (Allen 1989). Extractable nitrate and ammonium concentrations were then 161 measured using ion exchange chromatography (Dionex (UK) Ltd., Camberley, Surrey) and 162 converted to mg/kg of dry soil. Extractable phosphate was measured using the Olsen (bicarbonate 163 extraction) method (Rowell 1994). Dry soil was homogenised and sieved through 2 mm square 164 mesh sieve. Organic matter was measured by % loss on ignition at 375°C (Ball 1964). Dry soil 165 samples were digested in 2 ml concentrated sulphuric acid at 360°C for 4 hours with 50 mg 100:1 LiCl:Se catalyst (Carroll et al. 2003). Digests were analyzed for concentrations of total N (as 166

ammonium) using ion exchange chromatography (Dionex (UK) Ltd., Camberley, Surrey), and
total P using optical emission spectrometry (Inductively Coupled Plasma OES, Varian Inc.,
USA).

170 We analysed differences in surface soil characteristics with a paired *t*-test comparing TI 171 surface soil to adjacent surface soil. Organic matter, total N and total P were arcsin transformed 172 prior to analysis, and Olsen P was log transformed, in order to meet the assumption of normality. 173 We used Mann-Whitney tests for extractable nitrate and ammonium data since these were highly 174 right-skewed. Sown species persistence was calculated as percentage of sown species recorded 175 during the survey. We tested for relationships between measures of plant diversity and site 176 variables (age since TI and surface soil measurements) using linear regression analysis or 177 Pearson's correlations. All t-tests, Mann-Whitney tests and Pearson's correlations were 178 performed using Minitab (Minitab Statistical Software, Release 14 for Windows, State College, 179 Pennsylvania, USA). For each t-test, the number of degrees of freedom was adjusted to account 180 for unequal variances. N values refer to the number of replicates at each data point. Grime's plant 181 strategies were determined from quadrat data using MAVIS (Modular Analysis of Vegetation 182 Information System Plot Analyser v 1.00, Centre for Ecology and Hydrology, UK). Data are 183 presented untransformed.

184

185 **Results**

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187 Surface Soil Characteristics

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

195 For 14 sites, surface soil water content was higher in control soil than TI soil, and of 196 these, 11 were significantly different (Table 2). The site with the reverse trend was CH, where TI 197 surface soil was significantly wetter that control surface soil. The magnitude of the difference in 198 surface soil water content between TI and control soils varied greatly, from over 25% difference 199 at PT to just over 3% difference at OP. When all sites were analyzed, there was no significant 200 relationship between the difference in surface soil water content between TI and control, and the 201 age of the site. When the anomalous site was omitted, however, there was a significant negative relationship between age of site and the soil water content difference ($R^2 = 22.9\%$, P = 0.048), 202 203 suggesting a decline in this treatment effect with time.

For all sites, surface soil OM was lower in TI soils than controls, with 11 of these differences being significant (Table 2). The magnitude of the difference in surface soil OM between TI and control soils varied considerably, from over 9% difference at PT to 1.8% difference at LU. There was no significant relationship between the difference in surface soil OM between TI and control, and the age of the site.

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

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

222 For all 15 sites, surface soil total P was lower in TI soils than controls, with 12 sites being 223 significantly different (Table 3). Data for surface soil Olsen P levels was highly right-skewed. 224 Surface soil Olsen P levels varied from around 7 to > 80 mg/kg in control soils, and from <1 to >225 35 mg/kg in TI soils. Ten of the 15 sites had a significant difference between surface soil Olsen P 226 in control and TI soils (Table 3). Of these, two sites were significantly higher in TI than control 227 soils (BH and EP), and the remaining eight sites were significantly higher in control soils than TI 228 soils. The sites with significantly higher Olsen P in control soils varied considerably in the 229 magnitude of this difference, with the smallest difference of 5.6 mg/kg at CD, and the largest 230 difference of > 73 mg/kg at FW. All but one of the five sites with no significant difference 231 followed the general trend of having higher Olsen P in control than TI surface soils.

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Plant Community Establishment

234 Between 21 and 49 non-woody plant species were recorded at the TI sites. Grasses were present 235 as natural colonisers at all sites, from one to eight species per site. Small numbers of ferns, 236 horsetails and bryophytes were present at some sites. The numbers of species recorded as total, 237 sown, natural colonisers and grasses were not significantly related to the age of the site in months 238 since topsoil inversion. Of all the TI surface soil factors recorded at each site, only extractable nitrate showed a significant relationship with the total number of species ($R^2 = 20.9$, p = 0.049). 239 240 The number of sown species, natural colonisers and grasses showed no significant relationship 241 with any soil factor.

242 The UK NVC communities determined from the plant species present at each topsoil 243 inversion site included 10 mesotrophic grasslands, three open vegetation communities, one sand 244 dune and one calcifugous grassland community (Table 4). The number of sown species varied 245 from 12 to 28 (Table 5) with the exception of CD which was unsown. The majority of species 246 were introduced as seed; only three sites had plug planting in addition to sowing. None of the 247 species introduced by planting were recorded. Persistence of sown species varied from 20% at 248 BH to 86% at CH (Table 5). There was no relationship between the persistence of sown species 249 and the age of the site in months since topsoil inversion (Figure 1).

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

254 Sown non-cornfield annual species across all sites numbered 36 species, including 255 biennials and perennials associated with meadow and woodland edge habitats (Table S2). Of 256 these, field scabious Knautia arvensis, common knapweed Centaurea nigra, red campion Silene 257 dioica and wild carrot Daucus carota were the most frequently sown species. Sixteen species 258 were sown at only one or two sites, reflecting tailoring of the seed mix to individual sites. Of the 259 36 sown non-cornfield annual species, three (chicory Cichorium intybus, red campion and ribwort 260 plantain *Plantago lanceolata*) were found at all sites where sown (although for chicory this was 261 only one site) and 16 were not recorded at any site where they were sown. The remaining species 262 varied in establishment success from one in nine sites for meadowsweet Filipendula ulmaria 263 (11% persistence), to seven out of eight sites for viper's-bugloss Echium vulgare (88% 264 persistence).

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

271 Age of site and eight surface soil measures (pH, water, organic matter, total N, nitrate, 272 ammonium, total P, Olsen P) were tested for their relationship with nine vegetation 273 characteristics (total vegetation cover, forb cover, sown forb cover, unsown forb cover, grass 274 cover, bryophyte cover, bare ground, species richness and species diversity). Older sites had 275 significantly more grass cover and less bare ground (Figure 3). Surface soil total P was 276 significantly positively related to both grass cover and total vegetation cover (Figure 3). 277 Significantly greater bryophyte cover was also found at sites with higher surface soil nitrate 278 levels. Sites with wetter surface soils had significantly less bare ground. All three Grime's plant 279 strategies showed significant trends with age of site. Competitor (C) and stress tolerator (S) 280 scores both had a significant positive relationship with age of site, and ruderal scores (R) had a 281 significant negative relationship with age of site (Figure 4).

282

283 **Discussion**

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285 Surface Soil Characteristics

Topsoil inversion had a significant impact on soil pH, organic matter, water content, and both N and P content in the surface layer compared to control soils. Topsoil inversion raised soil pH to approximately neutral levels, ideal for mesotrophic grassland creation. Different communities of mesotrophic grassland have soil pH values that vary from pH 5.7 (MG13) to pH 7.5 (MG1) (Critchley et al. 2002). This suggests that the pH increase caused by deep ploughing could be sufficient to modify the subsequent vegetation community development, although it may not directly affect plant species diversity (Janssens et al. 1998). There was no significant negative correlation between age of site and difference in soil pH. An advantage of topsoil inversion is its potential to achieve rapid changes in surface soil fertility, rather than waiting several decades for natural processes to revert the soil to its pre-intensive agricultural condition (Marrs 1993). For social and political reasons it is usually desirable that ecological restoration produces relatively rapid results (Gilbert et al. 2003).

TI surface soils generally had lower water content than control surface soils. The exception to this was CH, due to damaged land drains. This site aside, the decrease in surface soil moisture caused by TI is likely to be a result of the reduction in water-retentive humus and the decrease in vegetation cover, which would increase evaporation of water from the soil surface. Older TI sites had closer soil water content to control soils, probably because they had developed more vegetation cover which increased soil shading (Matthesen 1997).

304 Organic matter levels were generally lower in TI surface soils than controls. This is 305 similar to results found by Allison and Ausden (2004) for deep ploughing to 30 cm. This 306 indicates burial of topsoil which is strongly associated with high soil OM. Many nutrients are 307 associated with the topsoil, and this is reflected by the results for total N and total P, both of 308 which had significantly lower levels in surface TI soil than control soils for 12 of the 15 sites. 309 Results for extractable N and P were more variable, but in general sites had lower levels in TI 310 surface soils, which were significantly lower for at least five sites. The extreme right skew in the 311 data for nitrate and ammonium made analyses problematic, and some extremely high values 312 amongst mainly low values made the relationship between total and available N unclear. The rate of N mineralization, which was not measured, would give a clearer picture of N availability to 313 314 plants (Gilbert & Anderson 1998). The data for Olsen P were less right-skewed, and total P 315 appears to have been a good predictor of Olsen P levels, which supports findings by others that 316 total P and Olsen P are well correlated (Pilgrim et al. 2007).

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

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Plant Community Establishment

326 Semi-natural grasslands in the UK are those with high species richness and managed at low 327 intensity by traditional techniques such as horse grazing. Although it has been shown that UK 328 semi-natural grassland diversity is generally associated with lower levels of soil nutrients 329 (Critchley et al. 2002), it does not seem to be the case for the early stages of habitat creation at 330 these trial sites. Higher levels of soil nutrients appear to favour an increase in plant cover due to 331 their enhancement of plant growth, and have no initial effect on plant diversity, suggesting that 332 species input from the seed rain has a more significant effect on species diversity. Seed rain is 333 likely to vary between sites according to their proximity to seed sources in the wider landscape, 334 and dispersal abilities of each species (Bakker et al. 1996). Species characteristic of semi-natural 335 grassland and associated with low soil fertility may not have been able to colonise from natural 336 sources at the survey sites. It is likely that all sites exhibited a much lower plant species diversity 337 than would have occurred if the ground had been conventionally prepared rather than deep 338 ploughed due to the reduction of the surface soil seed bank caused by topsoil burial. However it is 339 precisely this reduction in ground cover that is likely to favour successful restoration. 340 Establishment of specialist species by seeding has been shown to be more successful when bare

ground conditions are created prior to sowing, with more severe soil disturbances favouring fastergrowth and reproduction of introduced specialist plants (Wagner et al. 2015).

343 The relationship between the number of sown species and the vegetation diversity at each 344 trial site is not straightforward. Introducing more species by sowing and planting did not result in 345 a more species-rich plant community, during the timescale of this study. The role of the seed mix 346 used in habitat creation and restoration schemes is much debated (Brand-Hardy 1996). It is 347 possible to tailor seed mixes to match a target NVC community, although this was not done in 348 this study. However, it appears from these results that the initial appearance and subsequent 349 survival of sown species is a stochastic process. At no sites were all introduced species present, 350 despite the young ages of all sites. The maximum introduced species persistence was 80% and for 351 nine sites was 50% or less. Individual species performance varied from 100% appearance to 352 complete absence. Wildflower seeds have not been subjected to artificial selection for good 353 viability, and wildflower seed quality is limited by the UK climate, which is not optimum for 354 seed ripening and harvesting. The seeds used at each site were not always of local provenance. 355 Using locally sourced seeds is thought to improve performance due to the genotypes being better 356 adapted to the local soil and weather conditions (Gilbert & Anderson 1998). There is also concern that non-local strains could hybridise with local populations and erode their genetic 357 358 distinctiveness (Krauss & Kock 2004).

Poor-performing species may have specific germination requirements which were not met at the site. Unless a target community is specified it is recommended that species chosen for a seed mix are those which germinate easily over a wide range of conditions, using a mix including mid- to late-successional species (Brand-Hardy 1996). Plug planting is sometimes recommended for species that establish slowly from seed (Holden et al. 2003). However we found no evidence of any of the species introduced by plug-planting in this study. This may be due to competition from sown species, or to weather conditions such as drought that compromised their survival. 366 Cornfield annuals were sown to give the sites instant visual appeal and to act as a nurse 367 crop. Nurse crops are used in restoration to improve the establishment of target species by 368 providing shelter for seedlings during the early stages, and by suppressing weed growth (Walker 369 et al. 2004). However the effectiveness of cover crops in improving performance of other sown 370 species is unclear (Pywell et al. 2002). Although in this survey the nurse crop showed a 371 significant decline in persistence over time, it is not clear whether it improved the establishment 372 of other sown species or colonisation by specialist species, as there was no comparable control 373 without a nurse crop. However, the visual element did serve to popularise the sites and involve 374 local communities (Landlife 2008).

375 One unusual feature of the seed mixes used was the lack of grass species. At the fourteen 376 sites that were sown, only one of these (LU) included grasses in the seed mix, and even here the 377 species used (red fescue Festuca rubra and sheep's fescue Festuca ovina) were chosen as 378 standard forestry mixes. It is usually recommended that grasses form 70 to 80% of the seed mix 379 (by seed numbers) for a meadow creation scheme (Holden et al. 2003). The low grass cover at the 380 survey sites is likely to be a result of their non-inclusion in the seed mix, although grasses had 381 colonised relatively easily from the seed rain. Sowing a mixture of forbs may therefore improve 382 the overall species diversity of the new habitat by allowing forbs to establish early on in the 383 absence of grasses. Fagan et al. (2008) recommend that seed mixes used to restore calcareous 384 grassland should aim to prevent assembly of low-value communities dominated by grasses. It is 385 usually the presence of many forb species that is used to judge the relative species richness of 386 grassland habitats, rather than the number of grass species (Rodwell 2006). Reduction or 387 elimination of grass seeds in restoration seed mixes may therefore be a positive step towards 388 improving restoration success.

Results from the NVC analysis showed that the survey sites were closest to a range of semi-natural habitats, including disturbed ground, mesotrophic grasslands, upland acidic

391 grassland and sand dune communities. The fit of the NVC to the vegetation data was generally 392 poor, and it was not possible to assign NVC communities manually using tables due to the 393 dissimilarity of the observed species compositions with those listed. Many of the constant species 394 were absent. The survey sites are relatively young artificially created habitats, none of which had 395 any specific NVC restoration goal, and they do not closely resemble any semi-natural habitat.

396 Many attempts at restoring or creating species-rich semi-natural grassland highlight the importance of selecting sites with low soil fertility, in particular P (Öster et al. 2009). The results 397 398 from this survey show that where this is not possible, soil fertility can be reduced by the use of 399 topsoil inversion to bury topsoil under the lower-fertility subsoil. It is notable that none of the 400 topsoil inversion sites had a management plan such as a mowing or grazing regime, which would 401 have the potential to influence plant community development. However, it is clear from this 402 evaluation of 15 sites that topsoil inversion can significantly aid the establishment of a new, more 403 species-rich community by creating a lower-fertility substrate to begin habitat creation or 404 restoration. We will next focus on data from a replicated field experiment that investigates 405 detailed changes to soil profile chemistry before and after topsoil inversion in comparison to 406 conventional ploughing, and follows the development of plant communities during three growing 407 seasons post-restoration.

408

409 Acknowledgements

410 Funding was provided by the Dalton Research Institute at Manchester Metropolitan University,

411 Landlife (Knowsley, Liverpool), and The Centre for Ecology and Hydrology (Bangor).

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

	Site Code	Date of topsoil	Time since	Number of species sown	Number of species	Cornfield annual
		inversion	topsoil inversion		plug planted	nurse crop?
-	BH	03/03	52	25	0	Yes
	BW	08/05	23	12	0	Yes
	CD	01/04	39	0	0	No
	СН	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
	РМ	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

Table 2. Comparisons between control and topsoil inversion (TI) surface soil at 15 topsoil inversion sites for surface soil pH, soil moisture and soil organic matter (OM) content, using ttests. *, significant at the 5% level; **, significant at the 1% level; ***, significant at the 0.1% level; N = 5; df adjusted to account for unequal variances.

Site	Mean soil	рН	t-value	Mean soil	water (%)	t-value	Mean soil	OM (% LOI)	t-value
	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***
СН	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*
РМ	5.37	6.64	-8.94	24.8	23.0	1.43	6.96	3.59	9.34***
РТ	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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Table 3. Comparisons between control and topsoil inversion (TI) surface soil at 15 topsoil inversion sites for total N, nitrate, ammonium, total P and Olsen (extractable) P content, using ttests (total N and P; Olsen P) and Mann-Whitney tests (nitrate and ammonium). Asterisks (*, **, and ***) indicate significance at the 5, 1, and 0.1% level, respectively; N = 5 and df adjusted to account for unequal variances.

Site	Mean tota	ıl N	t-value	Median ni	itrate	W	Median a	nmonium	W	Mean tota	ul P (mg/g)	t-value	Mean Ols	en P	t-value
	(mg/g)			(mg/kg)			(mg/kg)						(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***
СН	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

590 Table 4. Age of site (months since topsoil inversion) and plant species and community outcomes 591 on inverted soil at each of the 15 topsoil inversion survey sites. Number of non-woody species 592 recorded shown separated into functional groups (grasses includes sedges and rushes; ferns 593 includes horsetails). Numbers in parentheses show of which were introduced (sown or planted) 594 species. No species were introduced at CD. NVC communities derived from all species recorded 595 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
СН	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
РТ	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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Table 5. Total number of species sown and planted at each of the 15 topsoil inversion survey sites, and number of these recorded during the survey. Numbers in parentheses show of which were cornfield annual species. Creative conservation seed mixes and plug plants were used which comprised of 100% forbs.

Site	Age	Number o	f spp.	Number recorded spp.		Persistence (%)	
	(months)	Sown	Planted	Sown	Planted	_	
BH	52	25 (2)	0	5 (0)	-	20	
BW	23	12 (5)	0	4 (0)	-	33	
CD	39	0	0	-	-	-	
СН	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	
РТ	23	12 (5)	0	5 (2)	-	42	
TW	6	18 (6)	0	9 (5)	-	50	
WC	46	28 (5)	0	16 (4)	-	57	







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

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

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

657 survey sites. A: R²=53.8, P=0.001; B: R²=52.7, P=0.001; C: r=0.585, P=0.022; D: r=0.614,

658 P=0.015.



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

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668 Supplementary Material

669 **Table S1.** Topsoil inversion survey site codes, names, GPS locations (latitude and longitude) date of deep ploughing (month/year), partner

670 organisation(s) and the objective of using deep ploughing at each site in terms of habitat creation or other general aim.

Site code	Site name	GPS location	Date deep	Partner	Objective
			ploughed	organisation(s)	
ВН	Billa Barra Hill	52.699630, -1.312536	3/03	National Forest	Creation of wildflower meadow adjacent to woodland on former arable land
BW	Burrough's Wood	52.651050, -1.265638	8/05	Woodland Trust	Creation of woodland edge / meadow habitat
					adjacent to a new wood on former arable land
CD	Carloggas Down	50.374101, -4.791875	1/04	Cornish Heathland	Trial the success of deep ploughing China clay
				Project / English	waste overlaid with imported topsoil as a means of
				Nature	creating heathland habitat on China clay waste spoil
					heaps
СН	Cowick Hall	53.683002, -1.006788	2/06	Woodland Trust	Creation of wildflower meadow on former arable
					land

Site code	Site name	GPS location	Date deep ploughed	Partner organisation(s)	Objective
CR	Centenary Royal Tigers Wood	52.657109, -1.329191	8/05	Woodland Trust	Creation of woodland edge / meadow habitat adjacent to a new wood on former arable land
CW	Coton Wood	52.729180, -1.637238	8/05	Woodland Trust	Creation of woodland edge / meadow habitat adjacent to a new wood on former arable land
ЕР	Eden Project	50.359898, -4.740167	11/02	Eden Project	Loosen compacted topsoil heaps which were removed from building areas and deposited onto former arable land, to create meadow habitat
FW	Foxley Wood	52.747622, -1.588158	8/05	Woodland Trust	Creation of woodland edge / meadow habitat adjacent to a new wood on former arable land
НН	Hedley Hall Wood	54.900945, -1.662938	12/05	Woodland Trust	Creation of woodland edge / meadow habitat adjacent to a new wood on former arable land
LU	Lunt	53.506511, -2.989687	9/02	Forest Enterprise	Creation of woodland / woodland edge / meadow habitat on former arable land

Site code	Site name	GPS location	Date deep	Partner	Objective
			ploughed	organisation(s)	
OP	Old Pale Farm, Delamere	53.223993, -2.684681	11/02	Forest Enterprise	Creation of wildflower meadow on former arable
	Forest				land
РМ	Paddington Meadow	53.391046, -2.553761	12/03		Creation of wildflower meadow on former arable
					land on flood plain adjacent to river
РТ	Pear Tree Wood	52.655066,1258530	8/05	Woodland Trust	Creation of woodland edge / meadow habitat
					adjacent to a new wood on former arable land
TW	Thorn Wood	53.252795, -2.562195	1/07	Woodland Trust	Creation of woodland edge / meadow habitat
					adjacent to a new wood on former arable land
WC	Wheeldon Copse	53.255148, -2.745293	9/03	Woodland Trust	Creation of woodland / woodland edge / meadow
					habitat on former arable land

Table S2. Summary of plant species introduced at the 15 TI survey sites (separated into sown cornfield annuals, sown other species, and plug planted), showing number of sites introduced at, number of sites recorded at during the survey, and percentage persistent introductions (recorded species as a percentage of introduced). This does not include species recorded at sites where they were not introduced. Some species were both sown and planted; these species are included twice and tallied separately. Names according to Stace (2010).

682	Species		Number of si	ites	% persistent introductions
683			Introduced	Recorded	
684	Sown:				
685	Cornfield	Annuals			
686		Agrostemma githago Corncockle	13	9	69
687		Anthemis arvensis Corn chamomile	12	5	42
688		Centaurea cyanus Cornflower	13	8	62
689		Chrysanthemum segetum Corn marigold	13	7	54
690		Papaver rhoeas Common poppy	13	5	38
691		Spergula arvensis Corn spurrey	2	1	50
692					
693	Sown:				
694	Other	Achillea millefolium Yarrow	3	2	67
695		Agrimonia eupatoria Common agrimony	2	0	0
696		Alliaria petiolata Garlic mustard	3	0	0
697		Anthriscus sylvestris Cow parsley	2	1	50
698		Anthyllis vulneraria Kidney vetch	5	3	60
699		Campanula trachelium Nettle-leaved bellflower	3	0	0
700		Centaurea nigra Common knapweed	12	10	83
701		Cichorium intybus Chicory	1	1	100
702		Daucus carota Wild carrot	13	10	77
703		Digitalis purpurea Foxglove	4	1	25
704		Dipsacus fullonum Wild teasel	2	0	0
705		Echium vulgare Viper's-bugloss	8	7	88
706		Filipendula ulmaria Meadowsweet	9	1	11

707		Galium mollugo Hedge bedstraw	2	0	0
708		Galium verum Lady's bedstraw	7	3	43
709		Geranium pratense Meadow cranesbill	2	0	0
710		Geum rivale Water avens	1	0	0
711		Geum urbanum Wood avens	2	0	0
712		Hyacinthoides non-scripta Bluebell	3	0	0
713		Hypericum perforatum Perforate St John's-wort	1	0	0
714		Iris pseudacorus Yellow iris	1	0	0
715		Knautia arvensis Field scabious	14	5	36
716		Leucanthemum vulgare Oxeye daisy	9	7	78
717		Malva moschata Musk mallow	2	1	50
718		Oenothera biennis Evening-primrose	3	1	33
719		Plantago lanceolata Ribwort plantain	5	5	100
720		Primula veris Cowslip	5	0	0
721		Prunella vulgaris Self-heal	4	3	75
722		Ranunculus acris Meadow buttercup	9	6	67
723		Reseda luteola Weld	1	0	0
724		Rhinanthus minor Yellow-rattle	2	1	50
725		Sanguisorba minor Salad burnet	1	0	0
726		Silene dioica Red campion	10	10	100
727		Silene latifolia White campion	2	1	50
728		Stachys officianalis Betony	1	0	0
729		Succisa pratensis Devil's-bit scabious	8	0	0
730					
731	Planted	Campanula trachelium Nettle-leaved bellflower	1	0	0
732		Centaurea nigra Common knapweed	1	0	0
733		Glechoma hederacea Ground-ivy	2	0	0
734		Iris pseudacorus Yellow iris	1	0	0
735		Primula veris Cowslip	1	0	0
736		Primula vulgaris Primrose	2	0	0
737					



Figure S1. Approximate locations of the fifteen topsoil inversion survey sites. Sites are
represented by unique codes, used throughout this work. Five sites (BW, CRT, CW, FW, PT)
are in close proximity, and these have been shown as one point. For site names, see Table S1.