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1 **Can species-rich grasslands be established on former intensively managed arable soils?**

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3

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8

9 **Abstract**

10 Land use change from intensive arable production to extensively managed grasslands is
11 encouraged through subsidy payments to farmers under the European Union's Common
12 Agricultural Policy. Created grasslands are sown with a species-rich seed mix and receive
13 limited or no fertiliser application with the aim of increasing the provision of non-production
14 ecosystem services. In the UK these agri-environment schemes are funded for periods of 5, 7
15 or 10 years. This study compared the plant diversity and soil properties of paired intensively
16 managed (IM) arable and recently created (3, 5, 8 and 9 years) extensively managed species-
17 rich grasslands (SRG) at 4 sites in the Scottish Borders. Botanical surveys of the newly
18 created grassland plots showed limited establishment of the species-rich seed mixes and the
19 dominance of grasses that favour more nutrient-rich environments. Soil properties at 0-10 and
20 30-40 cm depths were measured over 2 consecutive years. Total and available soil nitrogen,
21 phosphorus and soil organic carbon were not significantly different between paired plots.
22 This study indicates that in order to create edaphic conditions for species-rich grassland
23 communities to develop within a 10 year timespan on former intensively managed arable
24 land, radical changes in soil properties are required, which current de-intensification
25 managements are not achieving.

26

27 **Keywords**

28 Agri-environment; ecosystem services; land use; soil organic carbon; soil nutrients;

29 biodiversity

30

31 **1. Introduction**

32 A growing awareness of the value of non-production ecosystem services (ES) provision to
33 human health and wellbeing has encouraged the funding of agri-environment schemes in the
34 UK, through which farmers receive funding to alter management practices to increase the
35 provision of certain ES. In general, management to maximise production causes the decline
36 of other ES (MA, 2005) including the regulation of water quality and nutrient cycling and
37 maintenance of biodiversity, with mixed effects reported on climate regulation (Pilgrim et al.
38 2010).

39 In the European Union (EU) direct support and subsidies are provided to farmers through the
40 Common Agricultural Policy (CAP). Funding for environmental initiatives is provided under
41 the second pillar of the CAP through the European Agricultural Fund for Rural Development
42 (EAFRD) and includes agri-environment schemes that aim to enhance the environmental
43 value of land, such as the extensification of agricultural management through the creation of
44 semi-natural grassland (EC, 2009). Under these schemes farmers are required to carry out an
45 extensification of management practices by reducing or ceasing fertiliser application, grazing
46 and cultivation, or removing the existing crop or sward and sowing a specified seed mix of
47 desired grassland species. In England by the end of 2012 there were over 80,000 ha of created
48 or restored grassland (Wilson et al., 2013), and £3 million was spent on the creation of
49 species rich grassland and arable reversion to grassland in Scotland from 2008 to 2012
50 (Scottish Executive, 2012).

51 Across the UK SRG creation schemes are funded for periods of between 5 and 10 years. Thus
52 within 10 years of adoption the benefits of agri-environment aimed at enhancing the
53 provision of non-production ES should justify both the loss of production and the cost of the
54 financial subsidy awarded to farmers (Horrocks et al., 2014). Despite the commitment of
55 substantial sums of money and land to extensification schemes, there has been little research
56 into (i) the extent to which they enhance provision of multiple ES and (ii) the
57 potential for the legacy of intensive agriculture to continue to limit ES provision during the
58 funding period of the agri-environment scheme. The creation of SRG in Scotland is listed as
59 a land management option under the ‘biodiversity and landscape’ and ‘water quality’ regional
60 priorities (Scottish Executive 2009), so the provision of increased biodiversity and improved
61 water quality are key targets for SRG creation schemes.

62

63 The UK is a signatory of the Convention on Biodiversity (CBD) and is obliged to take
64 targeted action to restore biodiversity where intensive agriculture has led to its loss (CBD,
65 2012). The maintenance of biodiversity enhances the provision of other ES, particularly those
66 mediated by the soil, e.g. the storage, internal cycling and processing of nutrients (Haygarth
67 and Ritz, 2009) and carbon (Goldstein et al., 2012). However, intensive agricultural practices,
68 including the use of fertilisers, pesticides, tillage are incompatible with high biodiversity
69 maintenance (Pilgrim et al., 2010). Changes to soil properties, which include decreased total
70 soil nitrogen (N) increased N availability, decreased and soil organic carbon (SOC), and
71 increased total and (Knops and Tilman, 2000), decreased soil organic carbon (McLauchlan,
72 2006) and increased total and available phosphorus (P) concentration (Gough and Marrs,
73 1990; McLauchlan, 2006) decrease botanical diversity.

74

75 The most diverse grasslands with plant species of the highest conservation value tend to
76 occur on soils with low nutrient status, as large concentrations of nutrients favours dominance
77 by a small number of species capable of rapid resource utilisation (Critchley et al., 2002;
78 Janssens et al., 1998). Thus, substantial concentrations of legacy soil N and P can limit the
79 biodiversity value of created or restored grasslands (Walker et al., 2004). Legacy soil N and P
80 can also have significant implications for water quality since, increased concentrations in
81 water bodies can result in eutrophication (Søndergaard and Jeppesen, 2007; Dungait et al.,
82 2012). Nutrients leached in forms that are readily available for biotic uptake, such as NO_3^-
83 (nitrate), may have a particularly large, immediate effect on the aquatic system.

84

85 Legacy effects of past management on soil properties can still be observed after many
86 decades (Kopecký and Vojta, 2009) and in some cases thousands of years (Dupouey et al.,
87 2002) following the cessation of intensive agriculture. Yet there are very few published
88 reports of the co-dynamics of the major macronutrient (N and P) and C cycles in soils
89 following the cessation of agricultural management (Table S1).

90

91 The aim of this study was to establish the extent of the legacy effect of former intensive
92 arable management on ES provision including SOC and macronutrient cycling and
93 biodiversity in recently created (<10 years) species-rich grasslands (SRG) on working farms.
94 We focus in particular on direct measurement of botanical biodiversity provision, and soil
95 chemistry, including N and P, which are key factors regulating both biodiversity and potential
96 nutrient loss to water bodies, key targets of SRG creation.

97 We tested the hypotheses that:

98 1. Soil chemical properties (SOC, N, P and pH) will not change within the first 10 years
99 following cessation of intensive management.

100 2. Legacy macro-nutrients will create soil conditions to which prescribed species-rich seed
101 mixes are not well adapted.

102

103 **2. Materials and Methods**

104 *2.1 Field Sites*

105 Paired IM-SRG field plots (11 m x 11 m) were established in 2010 in fields on 4 farms in SE
106 Scotland. SRG seed mixes (Table 1) had been sown 3, 5, 8 and 9 years previously in a
107 portion of an IM field at each farm. Plot pairs were matched for soil type (silty loam, brown
108 earth, Lauder series; Soil Survey of Scotland, 1981) using soil particle size analysis, slope
109 and aspect. All of the IM plots continued to receive fertiliser throughout the study, in contrast
110 to the SRG plots that had received no fertiliser or biocides since conversion (full details in
111 Horrocks et al., 2015). Hereafter, each site is identified by the letter S followed by a number,
112 which refers to the age in years since establishment of the SRG. Before conversion to SRG,
113 sites S3, S8 and S9 had been under arable rotation for at least 20 years, and site S5 had been
114 under intensive arable management until 2 years prior to establishment of the SRG.

115

116 *2.2 Soil properties*

117 *2.2.1 Sampling and preparation*

118 Sites S3, S8 and S9 were sampled in spring (late March) and summer (early July) in 2010 and
119 2011. The site at S5 was not sampled in 2011 having withdrawn from the agri-environment
120 scheme at the end of 2010. Soil cores (5 cm diameter x 10 cm length, n=5) were sampled in a
121 cross diagonal pattern from each plot (Been and Schomaker, 2013). Surface soil cores (0-10
122 cm depth) were taken in spring and summer. In spring, samples from 30-40 cm depth were
123 also taken using a soil corer from a pit dug beneath the surface sample to 30 cm.

124 Fresh soil samples were sieved (2 mm) prior to analysis. The bulk density (BD) of the surface
125 soil was determined for spring soil samples in 2010 using steel cores (56 mm diameter and 40
126 mm depth; Eijelkamp, Giesbeek, the Netherlands) according to Hopkins et al. (2009) and was
127 used to calculate nutrient concentration per ha.

128 *2.2.2 Total N and SOC*

129 Total N and SOC (% mass) were determined for spring soil samples using elemental analysis
130 (Carlo Erba NA 1500 analyser; CE Instruments Ltd, Wigan UK). Approximately 15 mg
131 ground (pestle and mortar) oven dried soil was weighed into a foil capsule.

132 *2.2.3 Total available N*

133 Total available soil N and P concentrations were determined in both spring and summer soil
134 samples to determine the level of intra-annual variation in more labile nutrient forms (Hatch
135 et al., 2002 , Blake et al., 2003).

136 Total available soil N, defined as the sum of ammoniacal N ($\text{NH}_4^+\text{-N}$), nitrate N ($\text{NO}_3^-\text{-N}$)
137 and nitrite N ($\text{NO}_2^-\text{-N}$) concentrations, was measured in 5 g of fresh soil extracted with 100
138 ml of 6% potassium chloride (KCl) on an orbital shaker for 1 hour at 150 revolutions min^{-1} .

139 The suspension was allowed to settle for 10 mins before 20 ml was filtered through Whatman
140 No. 42 filter paper (Whatman plc., Maidstone, UK) and analysed for NH_4^+ and $\text{NO}_3^-/\text{NO}_2^-$
141 using a Bran & Luebbe Auto Analyser III (SPX Flow Technology, Brixworth, UK). Two
142 blanks were prepared for each run and processed in an identical manner (Pansu and
143 Gautheyrou, 2006). It was assumed that all oxidised N was present as NO_3^- since NO_2^-
144 concentrations are usually negligible relative to NO_3^- (Shen et al., 2003).

145 *2.2.4 Available P*

146 Available soil P (defined as acetic acid extractable soil P) concentration was determined
147 using the same method of extraction as for available N with 100 ml of 2.5% acetic acid in

148 place of the KCl solution (Edwards and Hollis, 1982). The concentration of phosphate (PO_4^{3-} -
149 P) in the extracts was measured using a Bran & Luebbe Auto Analyser III.

150 *2.2.5 Total P*

151 Total soil P concentrations were determined for spring soil samples using the Kjeldahl
152 method (Taylor, 2000). Twenty ml of 95% sulphuric acid (H_2SO_4) and 6 Kjeldahl copper
153 sulphate (CuSO_4) catalyst tablets (Fisher Scientific, Loughborough, UK) were added to 0.5 g
154 of oven dried and ground soil and heated in a Buchi K-437 digestion system (Buchi UK Ltd.,
155 Oldham, UK) for 30 mins at 250°C, followed by 90 mins at 350°C. Once cool, digests were
156 filtered through Whatman No. 42 filter paper, made up to 250 ml with deionised water,
157 shaken by hand and then left for 10 hours to reach equilibrium. A 60 ml aliquot was analysed
158 using a Bran & Luebbe Autoanalyser III using the same method as for available P.

159 *2.2.6 Calculating soil nutrient concentrations*

160 Gravimetric soil moisture content was determined for each homogenised batch of fresh soil
161 prior to analysis for N and P, by drying a 20 g subsample at 105°C until constant weight was
162 attained. The value was used to calculate soil N and P concentration per mass of dry soil (mg
163 kg^{-1} dry soil) and converted to nutrient content (kg ha^{-1}) using bulk density values measured
164 for each field plot.

165

166 *2.3 Botanical survey*

167 The percentage cover of plant species identified using Rose (2006) and Hubbard (1992) was
168 recorded in July 2010 and 2011 using a 1 m x 1 m quadrat subdivided into 0.1 m x 0.1 m
169 sections at 5 randomly located points within each SRG plot. The value for percentage cover
170 was converted to a Domin score using the Joint Nature Conservation Committee Standard
171 conversion table (Rodwell, 2006). Values for key traits, indicating their ecological niche,
172 were collated from references for all species identified at the sites and included in the seed

173 mixes. Traits used were i) Ellenberg indicator (EI) values (Ellenberg, 1979) for light and N
174 (after Hill et al., 1999); ii) categorisation within the Competitive (C)-Stress tolerant (S)-
175 Ruderal (R) system of plant functional types (Grime, 1974; Grime et al. 1996), with scores
176 ranging from -2 to 2 on each axis (C,S and R) allocated according to Hodgson et al. (1999);
177 and iii) canopy height taken as the maximum height according to the LEDA European plant
178 trait database (Kleyer et al., 2008).

179

180 *2.4 Data analysis*

181 The Shannon diversity index (H') was calculated for plant diversity in each plot (Equation 1),
182 using the mean % cover to determine the abundance of the i^{th} species as a proportion of total
183 total cover (P_i) for each species:

$$184 \quad H' = \sum_{i=1}^s -(p_i \times \ln p_i) \quad (1)$$

185 Where, P_i = abundance of the i^{th} species as a proportion of total cover

186 All soil analyses were conducted in duplicate and the mean of the replicate values was used
187 for the data analysis using GENSTAT14. Where a normality plot indicated non-normal data
188 distribution for a given variable, data were normalised by taking the natural logarithm
189 (constant e). Data from paired plots at each site were compared for every sampling occasion
190 using a two sample t-test, following a check for equality of variance the mean and standard
191 deviation of the measurements and indication of significance of the t-test are reported for all
192 plots and sampling occasions in tables. Subsequently a randomised block design ANOVA
193 was applied to combined data from 2010 / 2011 to identify any significant consistent effects
194 of management over the two year sampling period, management (IM / SRG) was modelled as
195 a fixed effect, across the 4 sites, with the data blocked according to the site pair, considered
196 as a random block (S3, S5, S8 and S9). Where both spring and summer analyses were
197 performed (available soil N and P), separate models were written for the spring and summer

198 data to enable the spring data to be analysed using a 2-way split plot design, with soil depth
199 (0-10 cm / 30-40 cm) and management both taken as fixed effects $p < 0.05$ was considered
200 significant. The results of the ANOVAs are reported in the text.

201

202 **3. Results**

203 *3.1 Soil organic carbon*

204 The total SOC content did not vary significantly, as a function of management ($p=0.28$) or
205 depth ($p=0.46$). The smallest SOC contents tended to occur at site S3, ranging from 11.1 (± 2)
206 to 24.2 (± 9.3) t ha^{-1} , and the greatest at site S9, ranging from 24.1 (± 11.7) to 38.7 (± 3.5) t ha^{-1}
207 in both sampling years. This pattern was observed in both the IM and SRG plots (Table 1).

208

209 *3.2 Total and available nitrogen*

210 The mean total soil N content did not vary significantly between IM and SRG plots, as a
211 function of sample depth ($p=0.55$) or year ($p=0.11$). There was a trend for the smallest total
212 soil N content to occur at site S3, ranging from 1.08 (± 0.19) to 1.85 (± 1.44) t ha^{-1} , and the
213 greatest at site S9, ranging from 0.81 (± 0.07) to 3.69 (± 0.32) t ha^{-1} for both depths (Table 1).

214 The greatest soil available N contents were at site S9, where there were peaks in total
215 available soil N ($> 70 \text{ kg ha}^{-1}$) measured in both the IM and SRG plots in spring 2011 and in
216 the IM plots in summer 2010 (Table 2); on both occasions the content in the IM plots were
217 significantly greater. The total soil available N in S3, S5 and S8 tended to be less than those
218 observed at site S9 and showed no consistent relationship with management. There
219 were significant differences between paired plots for individual sampling occasions but these
220 showed no consistent effect of management (ANOVA, spring $p=0.30$, summer $p=0.06$).

221

222 *3.3 Total and available phosphorus*

223 The mean total soil P (Table 1) did not vary significantly between IM and SRG plots, as a
224 function of sample depth ($p=0.33$) or year ($p=0.36$). Likewise soil available P content (Table
225 2) did not vary significantly with management in spring ($p=0.24$) or summer ($p=0.97$). There
226 was a trend for the smallest total soil P content to be recorded at site S3, ranging from 0.11
227 (± 0.04) to 0.40 (± 0.1) $t\ ha^{-1}$ across both depth ranges, and the greatest at site S9, ranging from
228 0.43 (± 0.09) to 0.81 (± 0.07) $t\ ha^{-1}$. There were significant differences between paired plots
229 for individual sampling occasions but these showed no consistent effect of management. The
230 SRG plot at site S5 had a significantly higher total soil P content compared to the paired IM
231 IM plot in the 0-10 cm depth. In Spring 2010 the IM plot at site S8 had significantly ($p<0.05$)
232 greater mean soil total P content in the 30-40 cm depth range compared to the paired SRG
233 plot.

234

235 *3.4 Soil nutrient ratios*

236 The soil C:N ratio was ~ 10 across all sites and did not vary significantly with management
237 ($p=0.12$) or depth ($p=34$). The N:P ratios were much more variable (3.3-12.1; Figure 1), but
238 as with the C:N ratio did not vary significantly with management ($p=0.29$) or depth ($p=0.50$).
239

240 *3.5 Botanical survey*

241 The SRG at plot S9 had the greatest diversity (as determined by the Shannon diversity index)
242 and species richness in 2010 and 2011 (Table 4). In 2011 the species richness (total number
243 of species recorded) in the S9 SRG plot was about double that for the S3 and S8 SRG plots.
244 In 2010 the lowest diversity was recorded in the SRG plot at site S3, whilst in 2011 the
245 lowest diversity occurred at site S8. The only plot at which an increase in diversity was
246 observed between 2010 and 2011 was S3 SRG, where H' increased by 0.62. In the SRG plots
247 at S8 and S9 H' decreased by 0.13 and 0.26 respectively between 2010 and 2011. In 2011 all

248 three of the SRG plots sampled showed an increase in species richness from the previous
249 year.

250

251 At all sites grass species provided over 50% of the total cover with forbs much less dominant
252 (Figure 2). The most dominant grass species (mean % cover >10; Domin score ≥ 5) tended to
253 be those not present in the seed mix, including *Agrostis stolonifera* and *Holcus lanatus* at site
254 S3, *Phleum pratense* at site S5, *Arrhenatherum elatius*, *H. lanatus*, and *A. stolonifera* at site
255 S8 and *A. stolonifera* at site S9 (Table 4). Grass species present in the seed mixes that
256 achieved >10% cover included *Dactylis glomerata* at site S3, *Cynosurus cristatus* at site S5,
257 and *Poa pratensis* at sites S8 and S9, whilst species present in the seed mix which failed to
258 establish included *Festuca pratensis* at site S3, *Alopecurus pratensis*, *F. rubra*, *P. pratensis*
259 and *Agrostis capillaris* at site S5, *A. capillaris*, *C. cristatus* and *F. ovina* at site S8 and *A.*
260 *capillaris* and *F. ovina* at site S9. The only forb species not present in the seed mix that
261 provided a mean cover of >10% (Domin ≥ 5) was *Trifolium repens* at sites S3, S5 and S9.
262 Other forb species that established despite not being present in the seed mix included *Rumex*
263 *obtusifolius* at site S5, *Cirsium vulgare*, *Ranunculus bulbosus*, *T. repens* and *R. obtusifolius* at
264 site S8 and *Bellis perennis*, *Cerastium fontanum*, *C. vulgare*, *Plantago lanceolata*, *R. bulbosus*,
265 *Silene alba*, and *Taraxacum spp.* at site S9. Forb species present in the seed mix and
266 providing >10% cover (Domin ≥ 5) included *Rhinanthus minor* at site S5 and *Lotus*
267 *corniculatus* at sites S8 and S9. At site S8, 5 out of 8 sown forb species were not recorded in
268 any quadrat in either year, whilst from the same seed mix sown at site S9, only 1 of the 8
269 species failed to establish. The percentage cover from legumes at the four sites ranged from
270 10.2% at site S8 in 2011 and 23.2% at site S9 in 2010.

271

272 *3.6 Plant traits*

273 The most dominant grass species (Domin value ≥ 5) had either a generalised strategy
274 according to C-S-R theory (Grime, 1974), scoring 0 across the three axes according to
275 Hodgson et al. (1999) or a more competitive / disturbance tolerance strategy, scoring higher
276 on the C and R axes compared to the stress tolerance (S) axis (Table 5). The EI-N scores of
277 the most dominant grasses (range 5-7; mode 6) were indicative of species found in soils of
278 intermediate to high fertility, with the exception of *C. cristatus* at site S5, which had an EI-N
279 of 4. The modal EI-light value of the dominant grasses was 7 with all species being typical of
280 well-lit environments (Hill et al., 1999). The requirement for high light environments was
281 also a characteristic of the forb species which established, as well as of those which failed to
282 establish from the seed mixes. The established forb species typically have a generalist or
283 ruderal / competitive strategy according to the CSR theory, with the exception of *Centaurea*
284 *nigra*, a stress tolerator not present in the seed mix, which established at site S9 (Domin value
285 = 4) and *Lotus corniculatus* var. *sativus* a cultivated variety of a stress tolerator present in the
286 seed mix at sites S8 and S9. Typically the forb species identified and present in the seed mix
287 had a lower EI-N compared to the grass species (range 2-9; mode 4). The non-sown species
288 that had the greatest dominance included *T. repens*, *R. obtusifolius* and *Cersatium fontanum*
289 which have EI-N values of 6, 9 and 4, respectively.

290

291 **4. Discussion**

292 The effectiveness of agri-environment schemes has been a subject of recent debate. The
293 schemes have been criticised for providing limited benefit and can also have unforeseen
294 costs, for example, by increasing production pressure and environmental damage elsewhere
295 to compensate for production losses in agri-environment schemes (Ekroos et al., 2014). The
296 current study provides valuable insight into the value of extensive grassland creation
297 schemes. Whilst the findings are primarily applicable to the specific soil type studied (brown

298 earths), the results highlight the potential for legacy effects of intensive management on soil
299 chemical properties to limit the value of agri-environment schemes for enhancing ecosystem
300 service (ES) provision.

301

302 *4.1 Enduring effects of intensive management on soil nutrients*

303 The cycling and changes in C, N and P content in soils are regulated by physical, chemical
304 and biological processes. In intensively managed systems N, P and C cycles become
305 decoupled as plants can obtain their required nutrients directly from the soil solution
306 following fertiliser application (Dungait et al., 2012; Soussana and Lemaire, 2014).

307 A transition towards more ‘natural’ soil processes would tend to reduce total P in soils and
308 increase SOC and total N (as components of organic matter), thus altering the stoichiometry
309 of the soil nutrients. In this study we focus in particular on direct measurement of botanical
310 biodiversity provision, and soil chemistry, including N and P, which are key factors
311 regulating both biodiversity and potential nutrient loss to water bodies. We hypothesised,
312 however, that in the newly created SRG sites (<10 years) in this study, legacy effects of
313 former intensive management would limit succession towards a more ‘natural’ system with
314 soil macronutrient content showing no detectable change compared to the IM sites, thus
315 limiting improvements in key ES provision. The data from four working farms in Scotland
316 largely support our hypothesis. The percentage total N in our study plots (0.1-0.3%) was
317 closer to those measured by other authors in IM soils, as opposed to semi-natural grassland
318 habitats. For example a study of UK grasslands reported a mean soil total N content of 0.5%
319 at long established semi-natural grasslands, compared to a mean value of 0.3% at adjacent
320 intensive agricultural sites (Gough and Marrs, 1990). Another study of permanent, species-
321 rich grassland in Western Europe found soil total N ranging from 0.3 to 0.9% (Janssens et al.,
322 1998). These comparisons with other IM sites and established SRGs highlight the extent of

323 the legacy effect of former intensive management on the soils in this study, as there is no
324 significant increase in total soil N, which would be expected when comparing IM sites with
325 long established SRG.

326

327 Highly managed systems can become ‘leaky’ and maintain relatively high concentrations of
328 available soil N (Wardle et al., 2004). In more ‘natural’ systems rates of N release from
329 organic matter mineralisation may be regulated through plant-soil feedbacks hence these
330 systems tend to be characterised by improved N use efficiency and retention (Chapman et al.,
331 2005). The IM and SRG plots in this study maintained similar, high contents of available soil
332 N, with no significant management effect on total available N content, supporting the theory
333 that mineralisation rates were rapid.

334

335 The availability of soil P, which remained high in the SRG sites in this study may also
336 encourage N mineralisation, by supporting elevated rates of microbial activity and
337 encouraging plant growth and the production of high quality, readily mineralised plant matter
338 (Janssens et al., 1998; section 4.2). Rates of P cycling are an order of magnitude less than N
339 (Dungait et al., 2012), thus, fertiliser applications during intensive management tend to lead
340 to soil P accumulation, which may take many decades to decline following cessation of
341 fertiliser application (Dodd et al., 2012; Falkengren-Grerup et al., 2006). Desorption or
342 dissolution of the total P pool can maintain soil available P (Koopmans et al., 2004; Vu et al.,
343 2010). The persistence of accumulated soil P following cessation of intensive management
344 was observed in this study, as was the maintenance of a consistent pool of soil available P;
345 neither total nor available soil P content differed significantly between the IM and SRG sites.

346

347 In agro-ecosystems at steady state, net loss or gain of SOC is not observed, i.e. the amount of
348 C lost through decomposition processes and harvesting is the same as the net ecosystem
349 production (Jones and Donnelly, 2004; Smith et al., 2010). There are well-recognised benefits
350 associated with increasing SOC in agricultural soils, i.e. to mitigate climate change and
351 improve soil quality. Management changes, including conversion from arable cropping to
352 permanent grassland have been found to increase SOC (Conant et al., 2001; Guo and Gifford,
353 2002), however there was no measureable difference in SOC between the paired SRG and IM
354 plots in this study. We assume that high rates of organic matter mineralisation at our sites
355 balanced SOC and N inputs from the SRG plants, thus preventing the hypothesised increases
356 in SOC and total N.

357

358 *4.2 Legacy soil nutrients limit biodiversity provision*

359 The relatively abundant soil available N and total P contents recorded at the SRG sites in this
360 study are likely to impact on the nature of the plant community established, favouring
361 dominance by a few plant species typical of more nutrient rich environments and thus
362 limiting the biodiversity, species richness and conservation value of the created SRG. The
363 seed mixes sown in the SRG plots in this study met the requirements of the Scotland Rural
364 Development programme for low productivity mixes and contained plants typical of species
365 rich grasslands that develop in relatively nutrient poor soils (Scottish Executive, 2011).

366

367 The dominance of non-sown species, particularly grasses and the limited establishment of
368 sown species, demonstrated that success in establishing the desired sward at the SRG sites
369 was limited. Analysis of the traits of the most dominant grass species found them to be
370 characteristic of generalist species able to compete effectively in environments with low
371 nutrient stress (scoring lower on the S axes relative to C and R and high EI-N) or species able

372 to take advantage of disturbance due to high fecundity and rapid growth (scoring relatively
373 high on the R axes relative to C and S score). Other authors have reported similar
374 observations, and found that high soil P content in particular can limit biodiversity and
375 prevent establishment of species typical of low nutrient environments (Pywell et al., 2003).
376 The conservation of biodiversity is a central goal of agri-environment schemes. Maintaining
377 biodiversity has been shown to support the provision of other ecosystem services, such as
378 efficient nutrient cycling and to increase ecosystem stability through functional diversity
379 (Cardinale et al., 2012), thus biodiversity is a key measure of the ability of a landscape to
380 provide multiple ES. The plant communities in the newly created SRGs in this study were
381 less diverse and differed substantially from those found in well-established species rich hay
382 meadows, which are a threatened European habitat (Garcia, 1992). Traditional hay meadows
383 in Sweden have been found to have H' 's of 2.56-3.71 and mean EI-Ns ranging from 2.3 to 4.5
384 (Linusson et al., 1998). Similarly Shannon diversity indexes ranging from 0.5 to 5 were
385 measured in old, permanent grasslands, with the diversity at the majority of low fertility sites
386 being greater than 2.5 (Janssens et al., 1998). Most of the plots in this study fail to achieve
387 such high diversity.

388

389 Grassland diversity has been shown to negatively correlate with the grass:forb ratio (Willems
390 and Nieuwstadt 2009), indicating that the dominance by a few grass species is driving the
391 relatively low diversity in the SRG plots in this study. Many of the forbs which did establish
392 generally had low abundance (Domin value of 1). All the forb species had a requirement for
393 high light environments (EI-light 7 or 8), hence reduction in light availability in the sward
394 caused by dominant tall growing grasses is likely to have been a significant factor in limiting
395 forb establishment and overall biodiversity at the sites (Hautier et al., 2009). Amongst the
396 most dominant forb species were the legumes *T. repens* and *L. corniculatus*. The former is

397 similar to other dominant species at the sites as it has low stress tolerance (low S score) and
398 typically grows in relatively nutrient rich soils (EI-N =6), however the latter is stress tolerant
399 and typically grows in relatively infertile soils (EI-N =2).

400

401 The large soil available P content in the SRG soils could explain the relative dominance by
402 legumes as P availability has been found to correlate positively with legume abundance
403 (Bobbink, 1991). Rates of N fixation from legumes in UK grasslands have been estimated to
404 be between 74-280 kg N ha⁻¹ yr⁻¹ (Cowling, 1982). The abundance of legumes in the SRG
405 plots should have a positive feedback on soil fertility through N fixation, providing a supply
406 of easily decomposable (low C:N ratio) litter, which is readily mineralisable. Another
407 potential source of N input to the SRG plots is atmospheric N deposition which is estimated
408 at 15.12 kg N ha⁻¹ yr⁻¹ in the area of the field sites (APIS; CEH 2014). The combination of N
409 fixation and deposition could explain the relatively high available and low total soil N
410 observed in the SRG plots. The soil in the SRG plots showed no significant difference in N
411 content to the IM soils, which received fertiliser applications in line with recommendations in
412 the RB209 fertiliser manual for wheat and winter Barley (DEFRA, 2010), consisting of an
413 initial fertiliser application of approximately 40 kg N ha⁻¹ in February each year followed by
414 additional applications in May / April of up to 150 kg N ha⁻¹. These fertiliser applications
415 were similar to the potential N fixation by legumes in the SRG sites.

416 Fertiliser applications to IM plots were made to coincide with crop establishment and the
417 period of maximum stem extension. During this time, N uptake rates by cereal crops are
418 likely to have been greater than those of the grassland species in the SRG, which further
419 explains the similarities in measured soil N between IM and SRG plots, despite cessation of
420 fertiliser application to the SRG (Horrocks et al., 2014).

421

422 The observation that some stress tolerant species typical of more nutrient poor soils, such as
423 *L. corniculatus* and *C. fontanum*, did establish at the sites could be due to the spatial
424 variability in soil nutrient availability observed at the sites, allowing species with lower
425 competitive ability and greater stress tolerance to establish in patches of lower nutrient
426 availability. In the case of *L. corniculatus* var. *sativus* it is possible that the particular non
427 wild type cultivar identified at the site had a superior competitive advantage, which could
428 explain why it was able to develop such dominance at sites S8 and S9 (Schröder and Rudiger,
429 2012).

430

431 As well as taking advantage of spatial variability in soil nutrient availability, some species
432 will be able to benefit from temporal environmental changes. For example, at site S3 in 2011,
433 the increased cover from forbs and increased diversity could have been in response to the 6%
434 cover from bare ground at the site in 2010, providing niches for light-loving disturbance
435 tolerant forb species such as *Taraxacum* agg. and *T. repens* that would otherwise have been
436 shaded out by dominant grass species. Such shifts could be short lived as more competitive
437 species dominate again in future years. Another factor that can affect establishment of sown
438 species is the size, composition and longevity of the weed seedbank present at a site. There
439 are not data for the weed seedbank at the study sites, but assessment of the effect of the weed
440 seedbank could be a valuable addition to future studies.

441

442 The botanical survey results support the hypothesis that high legacy soil nutrient content, in
443 particular soil P, limits biodiversity provision at the recently created SRG sites by allowing
444 the dominance of a limited number of low conservation value grasses. The success of
445 disturbance tolerant species (high R score) could also be expected as the SRG sites were all

446 ploughed prior to sowing so species able to rapidly colonise disturbed soils would have been
447 advantaged (Pywell et al., 2003).

448

449 The dominance of non-sown species and relatively poor performance of forbs suggest the
450 composition of seed mixtures selected for the sites were not appropriate to the soil conditions
451 as a limited number of competitive grass species were able to dominate. The results highlight
452 the need for management actions that decrease soil fertility prior to attempting to establish
453 species-rich semi natural swards (Pywell et al., 2003; Smith et al., 2003). Whilst these have
454 been recommended previously in the literature it is apparent from this study that wider
455 implementation is required in the field. Soil testing to identify sites suitable for SRG
456 establishment should be encouraged (Hautier et al., 2009).

457

458 **5. Conclusions**

459 Through comparisons of repeated measurements of multiple soil properties at paired IM and
460 SRG sites this study has provided a much greater insight into soil properties before and after
461 entry into agri-environment schemes. The data provide strong evidence for a substantial
462 legacy effect on soil properties which could limit the benefit of newly created SRGs in
463 supporting enhanced ES provision, including plant biodiversity provision. Despite successful
464 establishment of some target seed mix species in the newly created grassland sward, overall
465 the diversity, richness and composition of the plant communities were low when compared to
466 long established species-rich grasslands, managed extensively for many decades. Overall the
467 study draws into question the value of funding agri-environment schemes that encourage the
468 short term creation of ‘semi-natural’ grasslands as the benefits they provide in terms of ES
469 provision are limited. Instead resources (money and land) may be better prioritised to
470 maintaining existing and long established semi-natural grasslands, or sowing moderately

471 diverse mixtures containing more competitive forbs (Woodcock et al., 2014), which have
472 been demonstrated to provide significant ES benefits.

473

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482

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650 Table 1. Mean (n=5) total soil organic carbon (SOC), nitrogen (N) and phosphorus (P) content in soil samples collected from paired
 651 intensively managed (IM) and 'species-rich' grassland (SRG) plots at 4 sites in the Scottish Borders. Values in brackets show 1 standard
 652 deviation, where a t-test indicated that values were significantly greater ($p < 0.05$) than at the paired plot that figure is in bold.

Site	Season	Depth (cm)	Total SOC (tonne ha ⁻¹)				Total N (tonne ha ⁻¹)				Total P (tonne ha ⁻¹)			
			2010		2011		2010		2011		2010		2011	
			IM	SRG	IM	SRG	IM	SRG	IM	SRG	IM	SRG	IM	SRG
S3	Spring	0-10	14.4 (2.0)	16.2 (0.9)	20.1 (14.9)	24.2 (9.3)	1.40 (0.20)	1.63 (0.07)	1.85 (1.44)	1.70 (1.20)	0.12 (0.11)	0.19 (0.12)	0.29 (0.05)	0.40 (0.10)
	Spring	30-40	11.7 (1.7)	14.0 (1.1)	17.4 (12.3)	11.1 (2.0)	1.21 (0.19)	1.38 (0.14)	1.26 (1.27)	1.08 (0.19)	0.11 (0.04)	0.12 (0.04)	0.25 (0.02)	0.31 (0.04)
S5	Spring	0-10	24.8 (4.3)	28.3 (4.0)	-	-	2.46 (0.47)	2.58 (1.24)	-	-	0.29 (0.05)	0.46 (0.14)	-	-
	Spring	30-40	24.0 (6.1)	24.2 (7.5)	-	-	2.38 (0.56)	2.32 (0.63)	-	-	0.24 (0.06)	0.43 (0.19)	-	-
S8	Spring	0-10	28.2 (5.7)	23.5 (1.9)	24.5 (5.1)	19.7 (4.1)	2.67 (0.49)	2.22 (0.20)	2.31 (0.51)	1.22 (1.02)	0.46 (0.09)	0.35 (0.05)	0.48 (0.12)	0.32 (0.03)
	Spring	30-40	22.4 (2.2)	21.7 (2.4)	33.1 (12.0)	32.1 (11.6)	2.20 (0.18)	2.13 (0.24)	3.06 (1.04)	2.58 (0.65)	0.40 (0.07)	0.31 (0.04)	0.34 (0.13)	0.41 (0.06)
S9	Spring	0-10	38.7 (3.5)	31.2 (1.1)	29.6 (12.4)	24.1 (11.7)	3.69 (0.32)	2.92 (0.16)	2.72 (1.33)	1.73 (1.28)	0.81 (0.07)	0.60 (0.03)	0.64 (0.16)	0.52 (0.03)
	Spring	30-40	37.9 (2.7)	28.1 (1.6)	25.5 (16.7)	25.2 (9.3)	3.64 (0.26)	2.67 (0.22)	2.33 (1.49)	2.28 (0.83)	0.77 (0.04)	0.54 (0.05)	0.70 (0.05)	0.43 (0.09)

653

654

655

656 Table 2. Mean (n=5) available nitrogen (N) and phosphorus (P) content in soil samples collected from paired intensively managed (IM) and
 657 'species-rich' grassland (SRG) plots at 4 sites in the Scottish Borders. Values in brackets show 1 standard deviation, where a t-test indicated that
 658 values were significantly greater (p<0.05) than at the paired plot that figure is in bold.

Site	Season	Depth (cm)	Available N (kg ha ⁻¹)				Available P (kg ha ⁻¹)			
			2010		2011		2010		2011	
			IM	SRG	IM	SRG	IM	SRG	IM	SRG
S3	Spring	0-10	8.14 (2.9)	8.3 (4.7)	18.8 (0.27)	21.6 (16.4)	34.5 (19.9)	32.4 (7.5)	31.6 (5.8)	30.4 (11.4)
	Spring	30-40	8.6 (3.1)	4.3 (1.3)	12.8 (3.6)	12.8 (2.8)	33.5 (21.1)	33.0 (8.8)	32.9 (8.5)	16.4 (11.5)
	Summer	0-10	15.7 (0.6)	7.7 (0.9)	17.1 (13.6)	7.9 (4.2)	2.5 (0.24)	17.1 (3.4)	44.5 (6.1)	42.4 (5.4)
S5	Spring	0-10	4.1 (0.8)	7.2 (2.2)	NA	NA	11.0 (10.4)	36.5 (53.5)	NA	NA
	Spring	30-40	6.2 (2.9)	6.8 (3.2)	NA	NA	14.0 (13.9)	42.2 (61.9)	NA	NA
	Summer	0-10	8.9 (1.4)	14.4 (2.8)	NA	NA	5.6 (0.3)	3.3 (0.1)	NA	NA
S8	Spring	0-10	31.2 (4.1)	25.7 (3.7)	48.8 (8.1)	31.0 (9.9)	23.3 (11.9)	14.0 (6.5)	37.8 (3.8)	27.0 (7.5)
	Spring	30-40	12.8 (2.5)	13.3 (2.9)	40.0 (8.2)	15.1 (1.3)	14.6 (3.0)	11.9 (3.2)	35.5 (7.1)	27.2 (7.7)
	Summer	0-10	10.4 (0.1)	11.7 (3.2)	10.6 (5.7)	8.4 (2.4)	20.9 (1.1)	7.3 (2.5)	29.7 (5.2)	34.0 (5.2)
S9	Spring	0-10	13.5 (5.4)	29.5 (3.8)	112 (20.2)	70.7 (21.9)	22.7 (11.6)	13.1 (6.1)	36.8 (3.7)	25.3 (7.0)
	Spring	30-40	8.4 (2.6)	13.8 (2.1)	72.1 (9.4)	10.7 (2.0)	38.0 (5.4)	21.3 (11.4)	65.6 (4.2)	21.6 (4.3)
	Summer	0-10	66.8 (35.1)	10.0 (1.6)	14.4 (7.0)	14.2 (3.2)	20.3 (1.1)	6.9 (2.3)	28.9 (5.1)	31.9 (6.5)

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Table 3. Summarising the diversity, percentage of sown species established and species richness in each of 4 species rich grassland (SRG) plots in July 2010 and 2011.

Plot	Shannon diversity index		% of seed mix species which have established		Total species richness	
	2010	2011	2010	2011	2010	2011
S3 SRG	1.33	1.95	50.0	66.7	8	11
S5 SRG	1.66	NA	38.9	NA	11	NA
S8 SRG	1.82	1.69	23.1	30.8	9	11
S9 SRG	2.33	2.07	61.5	61.5	15	21

Table 4. Plant species identified at each of 4 species rich grassland plots (S3, S5 S8 and S9). Domin scores allocated according to the Joint Nature Conservation Committee Standard (Rodwell, 2006) based on mean (n=5) percentage recorded in 1 m x 1 m quadrats in July of 2010 and 2011. Ellenberg indicator values for light and nitrogen (N) were obtained from Hill et al. (1999), the C-S-R category to which each species is assigned was obtained from Grime et al. (1996) and scores from -2 to 2 for each axis allocated according to Hodgson et al. (1999). Canopy height is the maximum canopy height (m) taken from the LEDA plant trait database (Kleyer et al., 2008). Domin values are entered for all species present in the seed mix for each site; values in bold and underlined indicate species established at sites that were not present in the seed mix.

Species	Type	Domin score				C-S-R scores			Ellenberg indicators		Canopy height	
		S3	S5	S8	S9	C	S	R	Light	N		
<i>Alopecurus pratensis</i>	grass		0	<u>1</u>		1	-1	-1	7	7	0.7	
<i>Anthoxanthum odoratum</i>	grass				<u>3</u>	-1	0	0	7	3	0.25	
<i>Festuca pratensis</i>	grass	0			<u>3</u>	0	0	0	7	6	0.8	
<i>Dactylis glomerata</i>	grass	5			<u>4</u>	1	-1	-1	7	6	1.1	
<i>Arrhenatherum elatius</i>	grass			<u>6</u>		1	-1	-1	7	7	1.8	
<i>Holcus lanatus</i>	grass	<u>5</u>		<u>5</u>	<u>4</u>	0	0	0	7	5	0.5	
<i>Phleum pratense</i>	grass	4	<u>8</u>		<u>3</u>	0	0	0	8	6	0.9	
<i>Agrostis castellana</i>	grass	4			<u>3</u>	0	0	0	6	4	0.2	
<i>Festuca rubra</i>	grass	4	0		<u>4</u>	0	0	0	8	5	0.9	
<i>Agrostis stolonifera</i>	grass	<u>5</u>		<u>7</u>	<u>5</u>	0	-2	0	7	6	1.3	
<i>Agrostis capillaris</i>	grass		0	0	0	0	0	0	6	4	0.4	
<i>Cynosurus cristatus</i>	grass		7	0	1	0	0	0	7	4	0.75	
<i>Poa pratensis</i>	grass	3	0	6	5	0	0	0	7	5	0.5	
<i>Festuca ovina</i>	grass			0	0	-2	2	-2	7	2	0.35	
<i>Lolium perenne</i>	grass		<u>4</u>			0	-1	0	8	6	0.2	
<i>Achillea millefolium</i>	forb		0	1	1	0	-1	0	7	4	0.8	
<i>Bellis perennis</i>	forb				<u>1</u>	-1	-1	1	8	4	1	
<i>Centaurea nigra</i>	forb		0	0	4	-1	1	-1	7	6	0.65	
<i>Cerastium fontanum</i>	forb	<u>3</u>			<u>3</u>	-1	-1	1	7	4	0.25	
<i>Cirsium vulgare</i>	forb		0	<u>1</u>	<u>1</u>	0	-2	0	7	6	1.2	
<i>Galium verum</i>	forb		0			0	0	-1	7	2	1	
<i>Hypochaeris radicata</i>	forb		1			0	0	0	8	3	0.1	
<i>Papaver rhoeas</i>	forb					-2	-2	2	7	6	0.9	
<i>Plantago lanceolata</i>	forb		3		<u>1</u>	0	0	0	7	4	0.4	
<i>Prunella vulgaris</i>	forb		1	0	1	0	0	0	7	4	0.3	
<i>Ranunculus acris</i>	forb		3	0	1	0	0	0	7	4	0.1	
<i>Ranunculus bulbosus</i>	forb			<u>1</u>	<u>1</u>	-1	0	0	7	4	0.25	
<i>Rhinanthus minor</i>	forb		7	0	0	-2	-1	1	7	4	0.6	
<i>Rumex acetosa</i>	forb		0	3	1	0	0	0	7	4	1	
<i>Rumex obtusifolius</i>	forb		<u>3</u>	<u>3</u>		0	-2	0	7	9	1.2	
<i>Silene alba</i>	forb				<u>1</u>	-1	-2	1	7	6	1	
<i>Taraxacum agg.</i>	forb	<u>1</u>			<u>1</u>	-1	-1	1	7	6	0	
<i>Trifolium repens</i>	forb	<u>5</u>	<u>7</u>	<u>1</u>	<u>5</u>	0	-1	0	7	6	0.5	
<i>Vicia cracca</i>	forb		0			1	-1	-1	7	5	2	
<i>Lotus corniculatus var sativus</i>	forb			5	5	-1	1	-1	7	2	1.7	
<i>Lathyrus pratensis</i>	forb		0			0	0	0	7	5	1.2	
<i>Leontodon autumnalis</i>	forb		0			-1	-1	1	8	4	0.15	
<i>Leucanthemum vulgare</i>	forb		1	0	1	0	-1	0		8	4	0.6

Figure 1. Mean (n=5) soil organic carbon (SOC): total nitrogen (N) plotted against total N:total phosphorus (P) ratio at 4 sets of paired intensively managed (IM) and species rich grassland (SRG) field plots, measured in July of 2010 and 2011.

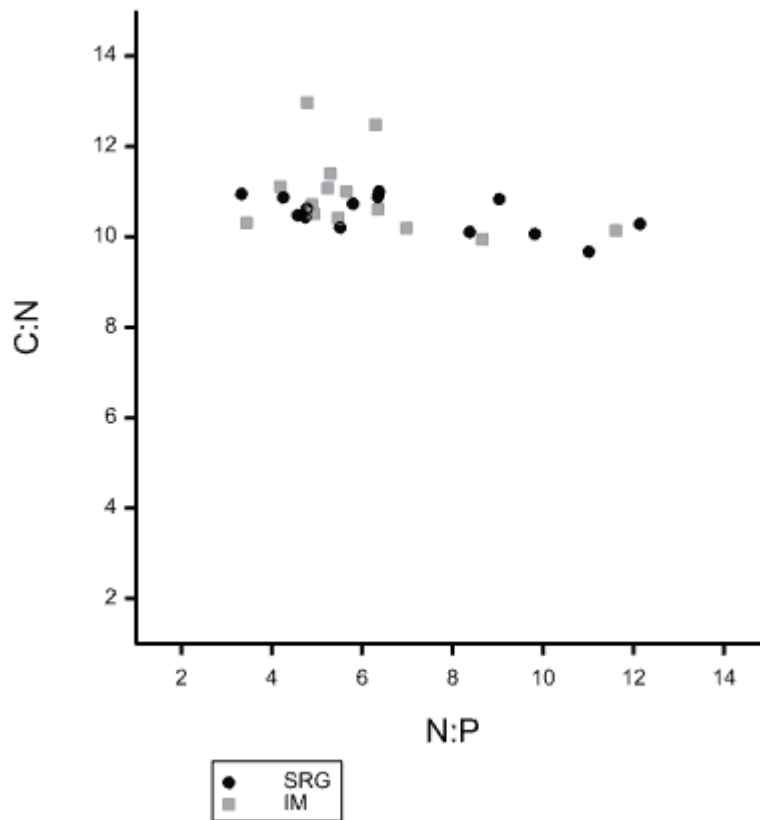


Figure 2. Mean (n=5) % cover within a 1 m x 1 m quadrat provided by grasses that were either present in the seed mix (sown) or not (non-sown) and leguminous (L) and non-leguminous (NL) forbs that were either sown or non-sown at 4 species rich grassland plots (S3, S5, S8 and S9) in July of a) 2010 and b) 2011. S5 was not surveyed in 2011 as the field had been withdrawn from the scheme 2011.

