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1 **Available soil phosphorus in semi-natural grasslands: assessment**  
2 **methods and community tolerances**

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11 **Available soil phosphorus in semi-natural grasslands: assessment**  
12 **methods and community tolerances**

13 **Abstract**

14 Restoration of diverse semi-natural grasslands is potentially limited by high availability of soil  
15 phosphorus (P). Successful targeting of restoration effort requires a knowledge of plant  
16 community tolerances to soil P availability. Many extraction methods for P availability have  
17 been developed but most are calibrated against the growth and P uptake of crop species grown  
18 in monoculture.

19

20 To test which methods are most suitable for measuring available P in soils of mesotrophic  
21 grasslands, a bioassay experiment was undertaken to compare seven extraction methods with  
22 the growth and P uptake of grassland species. Five species were grown together on a soil treated  
23 to create a range of conditions of pH, mycorrhizal infection and P availability.

24

25 Olsen P and Bray P were found to be significantly correlated with P uptake in plant growth  
26 across the range of soil treatments whilst ion exchange membrane P and resin P were  
27 significantly correlated with P uptake in plant growth in all but the calcareous soils. The acid  
28 extractions of Truog, acetic acid and EDTA-ammonium acetate were found to be less correlated  
29 with P uptake in plant growth. All extraction methods correlated more strongly with P uptake in  
30 the sterilised treatments than in those inoculated with mycorrhizal spores.

31

32 The method of Olsen was therefore selected to analyse P availability in soils supporting a range  
33 of mesotrophic grassland communities from eleven sites across England. At each sampling  
34 location, the species composition of the vegetation was assessed and classified using the British  
35 National Vegetation Classification (NVC). Species-rich hay meadows across a range of  
36 alliances were found to occur on soils with low phosphorus availability. Species-poor

37 communities, such as inundation grassland, were found to occur on soils with higher  
38 phosphorus availability. Pasture communities, of intermediate species richness, tended to occur  
39 on soils of intermediate phosphorus availability.

40

41 Olsen's method of P extraction is recommended for analysing soils of areas identified for  
42 habitat creation; values of less than 10 mg kg<sup>-1</sup> will give the greatest potential for the restoration  
43 of species-rich mesotrophic grassland.

44 *Keywords:* chemical extraction, species-rich, grassland, meadows

45

## 46 Introduction

47 High phosphorus (P) availability in soils has been identified as a limitation in the restoration of  
48 semi-natural vegetation (Pywell et al., 2007; Critchley et al., 2002a; Wassen et al., 2005;  
49 Janssens et al., 1998; Marrs and Gough, 1989). Many methods of measuring P availability have  
50 been proposed over the last 100 years and a vast literature has been produced comparing  
51 methods over a range of situations (Bates, 1990; Hislop and Cooke, 1968; Kamprath and  
52 Watson, 1980; Sibbesen, 1983). The majority of methods are based on a chemical extraction  
53 intended to simulate the conditions provided by root exudate and therefore measure the  
54 proportion of P in the soil available for use by plants. Most of these methods were designed to  
55 assess the fertiliser requirements of soil to prevent P limitation in crops.

56

57 Two potential problems are encountered when translating this information for use in habitat  
58 restoration. Firstly, all of the existing methods were originally developed and calibrated against  
59 the growth of crop species, which are generally more productive than the species found in semi-  
60 natural habitats and less reliant on mycorrhizal fungi for access to soil P. The methods may  
61 therefore be ineffective in discriminating between the low levels of P found in semi-natural  
62 habitats (Olf and Pegtel, 1994). Further, calibrations have almost always been against the

63 growth of single species in glasshouse or field experiments, whereas semi-natural grasslands  
64 contain a mixture of species growing together, which may enable a greater utilisation of soil P.  
65 Secondly, the quantity of 'available P' in soil is a variable rather than a constant, since P exists  
66 in a range of different forms which vary in space and time. The methods used to measure  
67 'available P' rely on a chemical equilibrium between the soil and the extractant. Hence, the  
68 concentration of 'available P' measured in a soil depends on the method used (Fixen and Grove,  
69 1990). The results from two different extraction methods, therefore, cannot be compared  
70 directly. Previous research comparing available P in semi-natural habitats with those of  
71 restoration sites has used a variety of methods (Table 1).

72

73 Janssens et al. (1998) and Critchley et al. (2002b) have provided threshold values for P  
74 availability in soil ( $50 \text{ mg P kg}^{-1}$ , EDTA + ammonium acetate extraction and  $15 \text{ mg P l}^{-1}$  Olsen  
75 extraction respectively) suggesting that beyond these values, species-rich grassland cannot be  
76 maintained. To enable information to be used widely in the field of habitat restoration, it would  
77 be helpful to select a single method of measuring P availability that is reliable across a range of  
78 conditions.

79

80 This paper compares seven P-extraction methods in terms of the P uptake of a mixed species  
81 sward across soils varying in pH, mycorrhizal infection and available P, to identify which  
82 technique is most suitable for use in natural grassland communities. The resulting 'best'  
83 technique is then used in a field survey to measure available P across a range of English  
84 mesotrophic grasslands of differing species-richness

85

86

87

## 88 Materials and methods

### 89 *Preparation of soil treatments*

90 A uniform textured soil of low P content was prepared by mixing 1 part soil (pH 6.6, 18% sand,  
91 31% silt, 51% clay) with 3 parts sand (16/30 yellow. Bardon Aggregates, Leighton Buzzard).  
92 Seventy five 2.5 litre plastic plant pots were each filled with 3.3 kg of the prepared soil. pH was  
93 reduced in 15 pots by adding 600 ml of 0.013 M H<sub>2</sub>SO<sub>4</sub>. pH was increased in 15 pots by mixing  
94 9 g of CaOH into the dry soil then wetting up with 600 ml deionised water. Mycorrhizal  
95 activity was increased in 15 pots by adding 600 ml of deionised water then inoculating with  
96 0.43 g of VAMINOX granules (Microbio, Rothamsted). Mycorrhizal activity was reduced in 15  
97 pots by sterilising the soil in an autoclave then adding 600 ml of deionised water. 15 pots  
98 received only 600 ml deionised water without any change to their pH or mycorrhizal  
99 complement.

100

101 Potassium orthophosphate, K<sub>3</sub>PO<sub>4</sub>, solution was added to 5 pots from each treatment at the rate  
102 of 240 mg per pot and to a further 5 pots from each treatment at the rate of 800 mg per pot. The  
103 remaining 5 pots from each treatment received no K<sub>3</sub>PO<sub>4</sub>. KCl was added to the soils treated  
104 with zero and 240 mg potassium K<sub>3</sub>PO<sub>4</sub> such that all treatments received an equal quantity of  
105 potassium.

106

107 420 mg of KNO<sub>3</sub> and 16 mg of NH<sub>4</sub>Cl were added to each pot to ensure an adequate supply of  
108 nitrogen (after Hoagland and Arnon, 1950). The treatments were then allowed to equilibrate for  
109 a period of two weeks before planting. Soil pH was measured annually to monitor the effects of  
110 the treatments (British Standards Institute, 1990).

111

112 *Plant growth*

113 Three bare-root, eight week old, seedlings of each of *Holcus lanatus* L., *Festuca rubra* ssp.  
114 *rubra* L., *Cynosurus cristatus* L., *Anthoxanthum odoratum* L. and *Trifolium repens* L.,  
115 (nomenclature for vascular plants follows Tutin et al., 1964) all of which occur in typical  
116 British mesotrophic grassland swards, were transplanted into each pot. Seeds were obtained  
117 from Emorsgate Seeds (King's Lynn, England) and raised in trays of proprietary compost.  
118 The roots of the plants introduced to the sterilised treatments were dipped in Benomyl (methyl  
119 1-(butylcarbamoyl)-2-benzimidazolecarbamate) prior to planting to reduce the likelihood of  
120 fungal hyphae infection.

121

122 The 75 pots were placed on a bench outside, in a randomised order, where they were able to  
123 receive rainwater and drain freely. 100 ml of distilled water were added to the pots on a daily  
124 basis during the summer months when evapotranspiration exceeded rainfall. Any excess water  
125 was allowed to drain from the pots.

126

127 The above-ground vegetation (over 3 cm height) was harvested monthly during the growing  
128 season for two years and recorded as dry weight (dried at 30°C) (MAFF 1986). Following each  
129 monthly harvest, dilute solutions of H<sub>2</sub>SO<sub>4</sub> (100 ml of 0.005 M) and CaOH (100 ml of 0.03 M)  
130 were added to the acidified and calcareous treatments respectively, to maintain the soil  
131 conditions. 420 mg of KNO<sub>3</sub> and 16 mg of NH<sub>4</sub>Cl were also added to each pot, to maintain  
132 adequate nitrogen and potassium concentrations. The positions of the pots were re-randomised  
133 after each harvest.

134

135 The concentration of P in the biomass harvested in July of year 1 was measured using the  
136 method of dry combustion followed by dissolution in HCl and ammonium molybdate-  
137 ammonium metavanadate reagent (MAFF, 1986). P uptake has been calculated by multiplying  
138 the P concentration in the vegetation by the biomass produced.

139 *Soil analysis*

140 Soil samples were collected from three locations in each pot using a 0.01 m diameter auger  
141 extending over the full depth of soil. The soils were sampled on two occasions, September year  
142 1 and July year 2. The samples were air-dried, ground to pass through a 2 mm sieve and  
143 analysed for available P using the extraction methods outlined in Table 2. Resin P could only  
144 be assessed in year 2 as a larger quantity of soil had to be extracted for the test.  
145 P concentration in all extracts was determined using the molybdenum blue method described in  
146 MAFF (1986).

147

148 *Verifying mycorrhizal establishment*

149 Root samples were collected from each pot in July year 2. The mass of roots could not be  
150 separated into its constituent species, but was analysed as a mixed sample. The roots were  
151 washed, stained with trypan blue and inspected on a gridded Petri dish using a binocular  
152 microscope, as described in Brundrett et al. (1996).

153

154 *Field sampling*

155 199 soil samples were collected from eleven lowland grassland sites in England. At each  
156 sampling location, four cores were taken from the top 0.15 m of the soil profile using a Dutch  
157 auger. These were mixed together to provide a bulked sample.

158

159 All of the soil samples were air-dried, ground to pass through a 2-mm sieve and then analysed  
160 for available P using Olsen's extraction (MAFF, 1986). Olsen's extraction was used as the  
161 results later in this paper indicated it to correlate well with P uptake in plant growth of a mixed  
162 species grassland.

163

164 *Data transformation*

165 The resulting data set of P extracted from the soil was transformed using the square root  
166 function to reduce the skew in the variance. The data were then analysed using Pearson  
167 correlations.

168

169 *Vegetation survey.*

170 At each soil sampling location a 1 m<sup>2</sup> quadrat was used to record the presence and percentage  
171 cover (visual estimates) of all species of vascular plants and prominent bryophytes.

172 The field survey data comprised 199 quadrats with a total species record of 148 species.

173 The data were subject to indirect gradient analysis using Detrended Correspondence analysis,  
174 DCA, using PC-ORD software (McCune and Mefford, 1999); axes were rescaled and species

175 with less than five occurrences were removed from the data prior to analysis resulting in a

176 reduced species record of 93 species. Correlations were then sought between DCA axis score

177 and soil phosphorus. The vegetation of each quadrat was assigned to a community of the

178 National Vegetation Classification (NVC; Rodwell, 1992) using Czekanowski co-efficients via

179 the computer programme MATCH (Malloch, 1995). Only the quadrat locations where the

180 programme classified the vegetation as mesotrophic grassland were used in the subsequent

181 analysis. Communities with less than five representations were excluded from the subsequent

182 analysis, giving 176 sample locations. The communities recorded at each site are shown in

183 Table 3. Descriptions and relationships to European alliances (Rodwell et al., 2007) are given

184 in Table 4.

185

186 Gaussian bivariate ellipses for each grassland NVC community are plotted onto the DCA

187 diagram. These ellipses are centred on the mean axis scores for each NVC community. The

188 axes of the ellipses are based on the sample standard deviation and their orientation is based on

189 the covariance between the scores for the two axes (Systat v8.0)

## 190 Results

### 191 *pH in the treated soil*

192 pH was reduced from 5.2 to 5.0 in the acidified treatment in year 1 and to 4.6 in year 2. Between  
193 year 1 and year 2 the pH of the control soil also decreased from 5.2 to 5.0. pH was increased to  
194 7.0 in year 1 and 6.6 in year 2 in the calcareous treatment.

195

### 196 *Correlation between P extracted from soil and biomass harvested*

197 Correlation matrices were calculated from the transformed data (Tables 5 and 6). In the first  
198 year, P extracted from the soil using Olsen and Bray was significantly correlated ( $p < 0.05$ ) with  
199 plant biomass on all soil treatments apart from VAM inoculated. EDTA extracted P and  
200 membrane P were significantly correlated ( $p < 0.05$ ) with three out of five soil treatments. Truog  
201 P and acetic acid P showed no correlation with biomass except for the sterilised soil treatment.  
202 Data collected in the second year showed the strength of the correlations with Olsen P and Bray  
203 P had weakened and were only significant ( $p < 0.05$ ) for two out of five soil treatments. The  
204 correlation with EDTA P remained weak, with only one out of five soil treatments showing a  
205 significant correlation ( $p < 0.05$ ). Truog P and acetic acid P showed no correlation with biomass  
206 in any treatment. Membrane P strengthened in its correlation with biomass showing a  
207 significant correlation with four out of five treatments ( $p < 0.05$ ). The resin P extraction showed  
208 a significant correlation with biomass in three out of five soil treatments ( $p < 0.05$ ).  
209 Biomass from soils inoculated with VAM did not correlate significantly with any of the P  
210 extractions, whereas biomass from the sterilised soils correlated with all extraction methods in  
211 year 1 and four out of seven extraction methods in year 2.

212

### 213 *Correlation between P extracted from soil and P uptake in vegetation*

214 P uptake in vegetation is likely to be a better assessment of P availability than simply measuring  
215 biomass, since plants may adjust the ratio of N, P and K uptake depending on the relative

216 availability of each nutrient (Koerselman and Meuleman, 1996). Table 7 shows the  
217 concentration of P measured in the vegetation harvested from each soil treatment. In all soil  
218 treatments, the P concentration in the vegetation increased with the concentration of P in the  
219 soil.

220

221 The correlation coefficients were found to be much higher between P extracted from the soil  
222 and P uptake (Table 8) than between P extracted from the soil and biomass. The strength of the  
223 correlation between soil extracted P and P uptake varied between treatments and extraction  
224 method. Olsen P gave the strongest correlation (significant at  $p < 0.01$  in all soil treatments) with  
225 Bray P also strongly correlated with P uptake showing a significant correlation ( $p < 0.01$ ) in four  
226 out of five soil treatments with the fifth significant at the  $p < 0.05$  confidence level. Membrane P  
227 and resin P were significantly correlated with P uptake in four out of five soil treatments  
228 ( $p < 0.05$ ) and EDTA P was correlated with P uptake in three out of five soil treatments ( $p < 0.01$ ).  
229 Truog P and acetic acid P were only correlated with P uptake in the sterilised soil treatment. The  
230 relationship between the concentrations of soil P extracted by each of the seven methods and P  
231 uptake in plant growth are shown in Figures 1 and 2.

232

233 *Effect of mycorrhizal treatments on correlation coefficient between P uptake and P extracted*

234 Inspection of the roots at the end of the second year showed a large number of vesicles to be  
235 present in the VAM inoculated treatment, indicating a high level of mycorrhizal activity.

236 Vesicles were also observed in the roots of the control treatment, but at a lower density. Very  
237 few vesicles were observed in the sterilised treatment, indicating that mycorrhizal development  
238 had largely been prevented over the period of the experiment.

239

240 All extraction methods were significantly correlated ( $p < 0.01$ ) with P uptake in the sterilised  
241 treatment, whereas only the Olsen, Bray, membrane and resin extractions were significantly  
242 correlated ( $p < 0.05$ ) with P uptake in the VAM inoculated treatment. In every case, the

243 correlation coefficient was higher in the sterilised treatment than in the VAM inoculated  
244 treatment.

245

246 *The relationship between Olsen P concentrations and plant community*

247 The coefficients of determination for the DCA ordination axes indicate approximately 30%  
248 explanation of floristic variation in the first three axes (Table 9).

249

250 The species ordination plot suggests that axis 1 relates to soil moisture and axis 2 to soil fertility  
251 (Figure 3). Species with low tolerance to soil wetness (*Leucanthemum vulgare*, *Trisetum*  
252 *flavescens*, *Conopodium majus*, *Briza media*) are clustered at low axis 1 scores, whilst those  
253 tolerant of moderate waterlogging have high axis 1 scores (*Scirpus maritimus*, *Ranunculus*  
254 *sceleratus*, *Alopecurus geniculatus*). Species typical of low fertility soils (*Danthonia*  
255 *decumbens*, *Briza media*, *Juncus articulatus*) have low scores on axis 2 whilst those typical of  
256 fertile soils (*Arrhenatherum elatius*, *Cirsium arvense*, *Phalaris arundinacea*, *Elymus repens*)  
257 occur with high axis 2 scores.

258

259 The DCA diagram of the 176 grassland quadrats (Figure 4) shows a clear segregation of  
260 communities along axes 1 and 2. The meadows MG3, 4 and 5 occur at the dry end of axis 1 ,  
261 semi-improved grasslands MG6 and MG7 together with communities of the *Calthion* alliance  
262 occur in the mid-range and MG13, inundation grassland, occurs at the wetter end of the scale.  
263 Axis 2, which probably represents soil fertility, is broadly aligned with the Olsen P  
264 measurements (Pearson correlation coefficient 0.46). The dispersal of communities on axis 2  
265 indicates that it is not hydrology alone that is driving variation in floristic composition of these  
266 grassland communities; this is seen clearly when mean axis scores are plotted for each  
267 community (Figure 5). At the dry end of the gradient MG3 and MG5 occupy less fertile  
268 situations than MG4; however, too few stands of more fertile, dry vegetation (e.g.MG1) were  
269 available for comparison. In the central range of axis 1 the fertile analogue to MG6 is MG7,

270 whilst, within the *Calthion* MG8 and *Agrostis-Carex* occur at lower axis 2 scores with MG9  
271 and MG10 appearing to represent the more fertile analogue. The inundation community  
272 (MG13), at the wettest end of the hydrological gradient, only occurs on sites with relatively high  
273 Axis 2 scores, it appears to be absent from sites with low fertility. The relationship between  
274 species richness of the mesotrophic grassland communities and their Olsen P measurements can  
275 be seen in Figure 6. It is again evident that the communities form loose groupings on a gradient  
276 of available P concentration. At the lowest Olsen P measurements are the species-rich  
277 communities of MG5, MG3 and MG8 (medians of 3.1, 5.1 and 5.2 mg kg<sup>-1</sup> Olsen P  
278 respectively). At a slightly higher Olsen P value is the flood meadow community of MG4 (7.0  
279 mg kg<sup>-1</sup> Olsen P). Historically, flood meadows would have been enriched with silt during flood  
280 events which may explain the slightly higher value (Rodwell, 1992). Slightly overlapping, but  
281 generally at higher Olsen P values are the grassland communities with lower species richness.  
282 MG6 and MG7 are agriculturally improved communities and would typically occur in areas  
283 which have received fertiliser. MG9 and MG10 (typically pastures) are often derived from poor  
284 management of these semi-improved grasslands. MG13 (also a pasture) occurs at sites with the  
285 highest Olsen P concentration but also shows a wide range of measured P values. The inter-  
286 quartile range of the MG3, MG4, MG5, MG6, MG8 and *Agrostis-Carex* communities all fell  
287 below a threshold of 10 mg kg<sup>-1</sup>.

288

289 None of the communities had an upper quartile value exceeding 20 mg kg<sup>-1</sup> available P, but a  
290 few individual samples did show values much higher than this. It is possible that the data  
291 collected were biased towards the low end of their potential range of Olsen P as none of the  
292 fields in the study had been fertilised in the last ten years.

293

294 The relationship between species richness and Olsen P is less clear when individual quadrat data  
295 are considered (Figure 7). The data form a cloud where the outer limit of the cloud represents  
296 the maximum species richness recorded at each concentration of Olsen P (after Janssens et al.,  
297 1998 and Critchley et al., 2002b). The hump-back shape of the data cloud is similar to that

298 found by both Janssens et al. (1998) and Critchley et al. (2002b) and shows that low species  
299 richness was found at very low values of Olsen P, as well as declining with increasing Olsen P  
300 above a concentration of approximately 5 mg kg<sup>-1</sup>.

301

302 Occasional outliers may be due to other factors, such as water regime or limitation by a different  
303 nutrient, allowing high species richness to occur on soils of high Olsen P.

304

## 305 Discussion

### 306 *Possible reasons for variation in correlation coefficient between extraction methods*

307 There are four mechanisms by which ‘available P’ is extracted from soil; anion exchange, acid  
308 dissolution, cation hydrolysis and cation complexation (Fixen and Grove, 1990). The extraction  
309 methods tested here utilise different combinations of these mechanisms.

310

311 Olsen’s reagent is buffered at pH 8.5 and is able to extract P by exchange of bicarbonate ions  
312 with phosphate ions, hydrolysis of Fe-P and Al-P with hydroxide ions and by precipitation of  
313 soluble Ca as calcium carbonate causing the release of Ca-bound P. The acidic extractant of  
314 Bray is able to extract P by dissolution of Ca-P, Al-P and Fe-P and by complexation of Al-P  
315 compounds with fluoride anions (Fixen and Grove, 1990).

316

317 The acidic extractants of EDTA-ammonium acetate, Truog and acetic acid exchange anions  
318 with phosphate ions and dissolve P held as Ca-P, Al-P and to some extent Fe-P. The different  
319 pH and concentrations of the three solutions cause them to extract different amounts of P from  
320 soil. In general, these acid extractants have performed the least well of all the methods tested.  
321 Kamprath and Watson (1980) suggested that this could be because Ca-P compounds are  
322 dissolved by the acid to a much greater extent than is possible by plant roots during P uptake.

323 This leads to a larger proportion of P being extracted than would be considered 'plant-  
324 available'.

325

326 The ion exchange membranes and resin were saturated with sodium and bicarbonate ions before  
327 use, hence the mechanisms for P extraction were similar to those of the Olsen extraction. Only  
328 water is added to the soil prior to contact with the membranes/resin, thus there is no change in  
329 the ionic background of the soil solution as occurs with all of the other extraction methods. This  
330 is the reason why ion exchange methods were advocated by Abrams and Jarrell (1992) for  
331 measuring available P in a non-agricultural context, although the results presented here and by  
332 Cooke and Hislop (1963) suggest that the presence of carbonate ions may limit the effectiveness  
333 of the technique. It is possible that the membrane exchange sites are blocked by the  
334 precipitation of calcium carbonate, caused by the release of bicarbonate ions from the  
335 membrane surface. This may have prevented phosphate ions from accessing the exchange sites  
336 on the membrane and thus caused the poor correlation between the membrane and resin  
337 extractions and P uptake in the calcareous soil treatment.

338

### 339 *Mycorrhizal activity and P uptake*

340 Soils with a high degree of mycorrhizal activity had a weaker correlation between soil P  
341 extracted and P uptake, compared with sterilised soil. This is likely to be caused by mycorrhizal  
342 fungi increasing the availability of P to plant roots at low concentrations of soil P. This is  
343 supported by the data presented in Table 7 which show the P uptake from the soil with no added  
344 P was highest for the VAM inoculated soil treatment.

345

### 346 *Selecting an extraction method for measuring P availability in semi-natural grassland*

347 Selecting the most appropriate extraction method for measuring P availability in soils of semi-  
348 natural vegetation requires consideration of several factors. The method chosen must correlate

349 with P uptake in plants. Olsen and Bray extraction methods have been shown here to correlate  
350 well with P uptake across a range of conditions of pH and degree of mycorrhizal activity. This  
351 reflects the conclusion of Kamprath and Watson (1980), who reviewed soil P tests for  
352 agricultural purposes and said that Olsen and Bray appear to be the most suitable extraction  
353 methods where it is necessary to test soils with a wide range of chemical properties.

354

355 Ideally, the extraction methods should be easy to use so that a wide range of laboratories can  
356 carry out the measurements within defined limits of precision. The resin extraction method  
357 does not fulfil this criterion since the retrieval of the resin presents a problem. In this work,  
358 polyester sachets were used to hold the resin, but cleaning the soil completely from the sachets  
359 before elution of the P is a subjective process. The other six extraction methods were found to  
360 be easy to undertake and therefore likely to be repeatable in different laboratories.

361 To enable comparison of results between researchers, it would be helpful if a single method  
362 were used for the analysis of soils from semi-natural grasslands and the assessment of soils prior  
363 to the commencement of the restoration of this habitat. Olsen P has been chosen here to  
364 measure available P in semi-natural grassland soils as it correlated well with plant P uptake and  
365 is the most frequently used test in the UK for measuring available soil P.

366

### 367 *Olsen P and plant community*

368 The results presented in this paper show that there are differences between the available P  
369 concentrations found in the soil supporting different plant communities. In general, species-rich  
370 communities (e.g. MG3, 4, 5 and 8) occurred on soils with lower available P concentrations  
371 whereas the species-poor communities, often of the same alliance and with similar hydrological  
372 profiles, occurred on soils with higher available P concentrations. Vegetation which is  
373 classified as MG6, 7, 9 and 10 may occur in species-rich and species-poor forms. Water  
374 regime, soil P and land management are the main factors affecting species richness in European  
375 wet grasslands (Hardtle et al., 2006, Hajek et al. 2008). In English floodplains, species-rich

376 stands of MG4 and MG8 move towards species-poor stands of MG9, MG10 and MG13  
377 following frequent inundation and less frequency haying (Gowing et al. 2002). MG13 has the  
378 lowest species richness and highest soil P, it appears to be absent from the least fertile  
379 situations. MG13 is a community of more frequently inundated situations which are usually  
380 grazed; high P and grazing may be combining to produce the very low species richness values  
381 recorded. Work of Gowing et al. (2002) demonstrated a strong correlation between water  
382 regime and phosphorus availability on many floodplain grasslands and the separation of the  
383 relative effect of high soil moisture and high fertility remains a problem for conservation  
384 management. All of the data presented in this paper were collected from vegetation which had  
385 not received fertiliser for at least ten years, hence it is possible that P values for communities  
386 such as MG6 and MG7, which are usually managed intensively with regular fertiliser  
387 application, could be biased towards the lower end of the range of P availability at which they  
388 occur.

389

390 A doubling of Olsen P from 5 to 10 mg kg<sup>-1</sup> was sufficient to lower the median species richness  
391 from 22 to 14 species m<sup>-2</sup>, equivalent to changing a botanically interesting community into one  
392 of limited conservation value. Other researchers have also shown a reduction in maximal  
393 species-richness in grasslands at increased concentrations of soil available P. Critchley et al.  
394 (2002b) showed that vegetation with more than 30 species m<sup>-2</sup> was restricted to soils with less  
395 than 15 mg l<sup>-1</sup> Olsen P. Janssens et al. (1998) found that soil with over 50 mg kg<sup>-1</sup> EDTA P  
396 supported fewer than 20 species per 100 m<sup>2</sup>, although inspection of Figure 2 shows that a value  
397 of 50 mg kg<sup>-1</sup> is much higher than the values measured here.

398

### 399 *Implications for habitat restoration*

400 The range of Olsen P concentrations measured by the Soil Survey and Land Research Centre in  
401 arable soils of England and Wales shows 65% of soils had Olsen P concentrations between 11  
402 and 40 mg kg<sup>-1</sup> and 26% of soils had concentrations which were much higher (Gilbert et al.,

403 2000). The results presented here show that species-rich communities tend to occur on the soils  
404 with the lowest Olsen P concentrations, typically between 3 and 10 mg kg<sup>-1</sup>, with median values  
405 around 5 mg kg<sup>-1</sup>. This is in agreement with the suggestion of Gough and Marrs (1990b), that  
406 when restoring species-rich grassland, extractable P concentration of between 5 and 10 mg kg<sup>-1</sup>  
407 Olsen P would be most appropriate. It is therefore apparent that the majority of ex-arable soils  
408 are not suitable for the restoration of species-rich grassland in the absence of measures taken to  
409 reduce P availability such as soil stripping, biomass harvesting or chemical amelioration  
410 (Gilbert et al., 2003). Attention must also be given to the hydrological status of the sites; in the  
411 absence of hydrological manipulation many arable fields may only have potential for restoration  
412 of some community types. In wetter habitats it may be that the greater availability of P in  
413 waterlogged soils will restrict the potential to re-create species-rich grasslands, here pastures of  
414 lower botanical value may be the only possible outcome.

415

416 The NVC communities MG6, 7 and 9 tend to occur on soils with Olsen P in the range of 5 to 17  
417 mg kg<sup>-1</sup> and may be more appropriate habitats for ex-arable soils, although these are often not  
418 the habitats most in need of restoration. Some variants of these communities do have nature-  
419 conservation interest and it is suggested that in circumstances where P availability is  
420 significantly above 10 mg kg<sup>-1</sup>, these habitats could be restored, at least for a transitional period.  
421 Similar suggestions were made by Ford (1997) whereby MG1 grassland (*Arrhenatherion*) was  
422 recommended as an acceptable habitat for set-aside land, since it can provide ecological interest  
423 without the necessity of lowering soil fertility. As the field sites used in this study were all  
424 regularly grazed or cut, there were insufficient records of the MG1 community to estimate the  
425 available P content of soils supporting it. More recently, Walker et al. (2004) have suggested  
426 that a phased establishment of grassland is more likely to be successful, introducing species  
427 associated with more diverse NVC communities after several years.

428

429 The species-poor vegetation (<10 spp m<sup>-2</sup>) in this study occurred over a wide range of  
430 concentrations from 8 to 18 mg kg<sup>-1</sup>. In general, ecological restoration of these habitats is aimed

431 at providing suitable conditions for fauna (e.g. birds or invertebrates) with little importance  
432 placed on the botanical diversity of the resulting sward.

### 433 Conclusion

434 Each of the NVC communities investigated occurred on soils with definable ranges of P  
435 availability. The most species-rich communities tended to occur on the soils with the lowest  
436 available P concentrations. To increase the chance of successful habitat restoration on soils  
437 formerly used for intensive agricultural production, it is necessary to take account of the  
438 residual available P in the soil and target the restoration of vegetation appropriate for the  
439 conditions. The land manager is faced with three options. Firstly, measuring the soil conditions  
440 to select a site where the concentration of available P is low enough to enable the restoration of  
441 a species-rich community or secondly, tailoring the target habitat to suit the conditions found at  
442 the site. The third option is to reduce the concentration of available P in the soil, either by  
443 topsoil stripping, biomass harvesting or chemical amelioration.

444  
445 In the analysis of seven methods of P extraction against the P uptake in plant growth of a  
446 species mixture, it was shown that Olsen and Bray produced the highest correlation coefficients  
447 across a range of soil conditions varying in pH, degree of mycorrhizal activity and amount of P-  
448 addition. In specific circumstances, some of the other methods were found to have similar  
449 correlation coefficients. Ion-exchange methods were found to be unsuitable on calcareous soil  
450 and EDTA-ammonium acetate was found to be unsuitable in the control and VAM inoculated  
451 treatments. Truog and acetic acid extractions were found to be unsuitable in all but the  
452 sterilised treatment. Although a range of soil conditions were investigated in this work, only  
453 one soil texture was used. Extrapolation of these findings to other soils would need further  
454 investigation.

455  
456 Based on the findings presented here, the authors suggest that Olsen's method is used to assess  
457 P availability in soils where habitat creation is proposed and that species-rich mesotrophic

458 grasslands should only be expected to be sustained on soils where Olsen P is less than  
459 10 mg kg<sup>-1</sup>.

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466

#### 467 References

468 Abrams, M.M. and Jarrell, W.M., 1992. Bioavailability index for phosphorus using ions  
469 exchange resin impregnated membranes. *Soil Science Society of America Journal* 56, 1532-  
470 1537.

471 Allen, S.E. (Ed.), 1989. *Chemical analysis of ecological materials*, 2nd Edition Blackwells  
472 Scientific Publications, Oxford.

473 Bates, T.E., 1990. Predictions of phosphorus availability from 88 Ontario soils using five  
474 phosphorus soil tests. *Communications in Soil Science and Plant Analysis* 21, 1009-1023.

475 British Standards Institute, 1990. *Methods of tests for civil engineering purposes. Part 3.*  
476 *Chemical and electrochemical test. BS1377:Part3:1990*

477 Brundrett, M., Bougher, N., Dell, B., Grove, T. and Malajczuk, N., 1996. *Working with*  
478 *mycorrhizas in forestry and agriculture.* Australian Centre for International Agricultural  
479 Research, Canberra.

480 Cox, W.P. and Leach, S.J., 1995. *Agrostis stolonifera-Carex spp. grassland: a new plant*  
481 *community described from the Somerset Levels.* *Ecology in Somerset* 4, 221-226.

482 Cooke, I.J. and Hislop, J., 1963. Use of an anion-exchange resin for the assessment of available  
483 soil phosphate. *Soil Science* 96, 308-312.

- 484 Critchley, C.N.R., Chambers, B.J., Fowbert, J.A., Sanderson, R.A., Bhogal, A. and Rose, S.C.,  
485 2002a. Association between lowland grassland plant communities and soil properties.  
486 *Biological Conservation* 105, 199-215
- 487 Critchley, C.N.R., Chambers, B.J., Fowbert, J.A., Bhogal, A., Rose, S.C. and Sanderson, R.A.,  
488 2002b. Plant species-richness, functional type and soil properties in grasslands re-created under  
489 arable reversion agreements in the Countryside Stewardship and Environmentally Sensitive  
490 Area Schemes. *Aspects of Applied Biology*, 81, Delivering Arable Biodiversity, 285-292.
- 491 Fixen, P.E. and Grove, J.H., 1990. Testing soils for phosphorus. in: *Soil testing and plant*  
492 *analysis* Ed. Westerman. pp 141-180. SSSA, Madison, WI.
- 493 Ford, M., 1997 Successful habitat creation on set-aside. *Farming and Conservation* 3, 16-18.
- 494 Gilbert, J.C., Gowing, D.J.G., Higginbottom, P.R.G. and Godwin, R.J., 2000. The habitat  
495 creation model: a decision support system to assess the viability of converting arable land into  
496 semi-natural habitat. *Computers and Electronics in Agriculture* 28, 67-85.
- 497 Gilbert, J.C., Gowing, D.J.G. and Loveland, P., 2003. Chemical amelioration of high  
498 phosphorus availability in soil to aid the restoration of species-rich grassland. *Ecological*  
499 *Engineering*. 19, 297-304
- 500 Gough, M.W. and Marrs, R.H., 1990a. A comparison of soil fertility between semi-natural and  
501 agricultural plant communities: Implications for the creation of species-rich grassland on  
502 abandoned agricultural land. *Biological Conservation* 51, 83-96.
- 503 Gough, M.W. and Marrs, R.H., 1990b. Trends in soil chemistry and floristics associated with  
504 the establishment of a low-input meadow system on an arable clay soil in Essex, England.  
505 *Biological Conservation* 52, 135-146.
- 506 Gowing, D.J.G., Lawson, C.S., Youngs, E.G., Barber, K.R., Rodwell, J.S., Prosser, M.V.,  
507 Wallace, H.L., Mountford, J.O and Spoor, G., 2002. The water regime requirements and  
508 response to hydrological change of grassland plant communities. DEFRA Project BD1310,  
509 Cranfield University.

- 510 Hajek, M., Hajkova, P., Sopotlieva, D., Apostolova, I., and Velez, N., 2008. The Balkan wet  
511 grassland vegetation: a prerequisite to better understanding of European habitat diversity. *Plant*  
512 *Ecology* 195, 197-213.
- 513 Hardtle, W., Bernd, R., Assmann, T and Meyer, H., 2006. Vegetation responses to  
514 environmental conditions in floodplain grasslands: Prerequisites for preserving plant species  
515 diversity. *Basic and Applied Ecology* 7, 280-288.
- 516 Hislop, J. and Cooke, I.J., 1968. Anion exchange resin as a means of assessing soil phosphate  
517 status: A laboratory technique. *Soil Science* 105, 8-11.
- 518 Hoagland, D.R. and Arnon, D.I., 1950. The water culture method for growing plants without  
519 soil. *California Agricultural Experimental Station Circular* 347.
- 520 Janssens, F., Peeters, A., Tallowin, J.R.B., Bakker, J.P., Bekker, R.M., Fillat, F. and Oomes,  
521 M.J.M., 1998. Relationship between soil chemical factors and grassland diversity. *Plant and*  
522 *Soil* 202, 69-78.
- 523 Kamprath, E.J. and Watson, M.E., 1980. Conventional soil and tissue tests for assessing the  
524 phosphorus status of soils. in: *The role of phosphorus in agriculture* Ed. F. E. Khasawneh and E.  
525 C. Sample. pp 433-469. American Society of Agronomy, Madison.
- 526 Koerselman, W. and Meuleman, A.F.M., 1996. The vegetation N:P ratio: A new tool to detect  
527 the nature of nutrient limitation. *Journal of Applied Ecology* 33, 1441-1450.
- 528 Kruijne, A. A., de Vries, D. M. and Mooi, H., 1967. Bijdrage tot de oecologie van de  
529 Nederlandse graslandplanten. Centrum voor Landbouwpublicaties en Landbouwdocumentatie,  
530 Wageningen.
- 531 Lanaken, E. and Ervio, R., 1971. A comparison of eight extractants for the determination of  
532 plant available micronutrients on soils. *Acta Agralia Fennica* 123, 223-232.
- 533 McCune, B. and Mefford, M.J., 1999. PC-ORD. Multivariate Analysis of Ecological Data,  
534 Version 5. MjM Software Design, Gleneden Beach, Oregon, USA.
- 535 MAFF, 1986. ADAS Reference Book 427: The analysis of agricultural materials. HMSO.

- 536 Malloch, A.J.C., 1995. MATCH version 2. A computer program to aid the assignment of  
537 vegetation data to the communities and subcommunities of the National Vegetation  
538 Classification. University of Lancaster.
- 539 Marrs, R.H. and Gough, M.W., 1989. Soil fertility - a potential problem for habitat restoration.  
540 in: Biological Habitat Restoration Ed. G P Buckley. pp 29-44.
- 541 Olf, H. and Pöggendorf, D.M., 1994. Characterisation of the type and extent of nutrient limitation in  
542 grassland vegetation using a bioassay with intact sods. *Plant and Soil* 163, 217-224.
- 543 Page, A.L., Miller, R.H. and Keeney, D.R., 1982. Methods of soil analysis. Part 2. Chemical  
544 and microbiological properties. American Society of Agronomy Inc., Soil Science Society of  
545 America Inc., Madison, Wisconsin.
- 546 Pywell, R.F., Bullock, J.M., Tallowin, J.B., Walker, K.J., Warman, E.A. and Masters, G., 2007  
547 Enhancing diversity of species-poor grasslands: an experimental assessment of multiple  
548 constraints. *Journal of Applied Ecology* 44, 81-94
- 549 Qian, P., Schoenau, J.J. and Huang, W.Z., 1992. Use of ion exchange membranes in routine soil  
550 testing. *Communications in Soil Science and Plant Analysis* 23, 1791-1804.
- 551 Rodwell, J.S., 1992. British plant communities. Volume 3. Grasslands and montane  
552 communities. Cambridge University Press, Cambridge.
- 553 Rodwell, J.S., Morgan, V., Jefferson, R.G. and Moss, D., 2007. The European context of British  
554 Lowland Grasslands. JNCC, Peterborough.
- 555 Sibbesen, E., 1983. Phosphate soil tests and their suitability to assess the phosphate status of  
556 soil. *Journal of the Science of Food and Agriculture* 34, 1368-1374.
- 557 Tutin, T.G., Heywood, V.H., Burges, N.A., Moore, D.M., Valentine, D.H., Walters, S.M. &  
558 Webb, D.A., 1964. *Flora Europaea*. Cambridge.
- 559 Walker, K.J., Stevens, P.A., Stevens, D.P., Mountford, J.O., Manchester, S.J. and Pywell, R.F.,  
560 2004. The restoration and re-creation of species-rich lowland grassland on land formerly  
561 managed for intensive agriculture in the UK. *Biological Conservation* 119, 1-18
- 562 Wassen, M.J., Olde Venterink, H., Lapshina, E.D., and Tannberger, F., 2005. Endangered plants  
563 persist under phosphorus limitation. *Nature* 437, 547-550

- 564 Yang, J.E., Skogley, E.O., Geogitis, S.J., Schaff, B.E. and Ferguson, A.H., 1991.
- 565 Phytoavailability soil test: development and verification of theory. Soil Science Society of
- 566 America Journal 55, 1358-1365.

567 **Table 1. Summary of extraction techniques previously used for soils supporting semi-**  
568 **natural vegetation**

Study	Extraction method
Critchley et al., 2002a	Olsen and Resin
Janssens et al., 1998	EDTA-ammonium acetate
Gilbert et al., 2000	Olsen
Gough and Marrs, 1990a	Truog
Gough and Marrs, 1990b	Olsen
Abrams and Jarrell, 1992	ion exchange membranes
Kruijne, et al., 1967	citric acid

569 **Table 2. P extraction methods undertaken**

Name of extraction method	Composition of extractant	Reference for method
Olsen	0.5 M sodium bicarbonate buffered at pH 8.5	MAFF (1986)
Bray	Bray P2: 0.03M ammonium fluoride and 0.1M hydrochloric acid	Page et al. (1982)
EDTA	EDTA – ammonium acetate: 0.02 M ethylene diaminetetraacetic acid, 0.5 M ammonium acetate and 0.5 M acetic acid (pH 4.65)	Lanaken and Ervio (1971)
Truog	0.001M sulphuric acid and 0.02M ammonium sulphate buffered at pH 3	Allen (1989)
Acetic acid	2.5% v/v acetic acid	Allen (1989)
Membrane	Cation and anion membranes sheets (BDH) cut into small strips and saturated in 0.5M sodium bicarbonate before use	Qian et al. (1992)
Resin	Ion exchange resin in polyester sachets Amberlite IRN-150 resin (Johnson Matthey) saturated in 0.5M sodium bicarbonate before use	Yang et al. (1991)

570 **Table 3. Neutral grassland communities identified at the sampling locations**

Site	UK Ordnance Survey Grid reference	Number of samples	Neutral grassland communities identified by MATCH	Other communities identified by MATCH
Berney Marshes (Norfolk)	TG 465 055	20	MG13	OV29, SM28
Cricklade (Wilts)	SU 096 958	20	MG4, 7, 11, 13	OV28, S22
East Harnham (Wilts.)	SU 151 289	10	MG8, 10	M22
Moorlinch (Somerset)	ST 393 362	25	MG6, 7, 8, 10, 14*	M23
Portholme (Cambs.)	TL 238 708	20	MG4, 5, 6, 9, 10	OV19
Southlake (Somerset)	ST 364 301	19	MG6, 8, 9, 10, 14	
Stonygill-foot (Durham)	NY 926 263	11	MG3, 8	
Tadham (Somerset)	ST 416 455	24	MG1, 5, 6, 7, 8, 14	M23
Upton Ham (Worcs.)	SO 860 400	25	MG4, 7, 8, 9, 10, 13	OV28
Welney (Norfolk)	TL 547 945	5	MG13	
Wet Moor (Somerset)	ST 435 245	20	MG6, 8, 13, 14	OV28
Total		199		

571 \* Closest match score for SD17 relates to the newly described *Agrostis-Carex* community of

572 Cox and Leach (1995) within the Calthion alliance, labelled '14' above.

573 **Table 4. Description of NVC communities recorded in the study**

NVC code	Description	Relevant European Alliance
MG3	<i>Anthoxanthum odoratum</i> - <i>Geranium sylvaticum</i> grassland Northern hay meadow	Triseti-Polygonion
MG4	<i>Alopecurus pratensis</i> – <i>Sanguisorba officinalis</i> grassland Flood meadow	Alopecurion
MG5	<i>Cynosurus cristatus</i> – <i>Centaurea nigra</i> grassland Old hay meadow	Centaureo-Cynosuretum
MG6	<i>Lolium perenne</i> – <i>Cynosurus cristatus</i> grassland Ordinary pasture	Lolio-Cynosuretum
MG7	<i>Lolium perenne</i> leys and related grasslands Intensively managed grassland	Lolio-Plantaginion
MG8	<i>Cynosurus cristatus</i> – <i>Caltha palustris</i> grassland Water meadow	Calthion
MG9	<i>Holcus lanatus</i> – <i>Deschampsia cespitosa</i> grassland Tussocky neutral grassland	Calthion
MG10	<i>Holcus lanatus</i> – <i>Juncus effusus</i> grassland Rushy grassland	Calthion
'14' Ag-Cx	<i>Agrostis stolonifera</i> - <i>Carex nigra</i> - <i>Senecio aquaticus</i> community	Calthion/Elymo-Rumicion *
MG13	<i>Agrostis stolonifera</i> – <i>Alopecurus geniculatus</i> grassland Inundation grassland	Elymo-Rumicion

574 \* Status still unclear

575 **Table 5. Effect of treatments on correlation coefficients between biomass harvested and**  
 576 **the square-root transformed soil extraction measurements of available P in year 1**

Treatment	Olsen P	Bray P	EDTA P	Truog P	Acetic acid P	Membrane P
Control	0.56*	0.59*	0.06	-0.61	-0.57	0.62*
Acidified	0.57*	0.62*	0.53*	0.13	0.22	0.58*
Calcareous	0.57*	0.64**	0.61*	0.09	0.13	0.17
VAM inoculated	0.44	0.40	0.12	0.21	0.20	0.41
Sterilised	0.68**	0.68**	0.64**	0.64**	0.54*	0.75**
All treatments combined	0.52**	0.57**	0.24**	-0.05	-0.04	0.75**

577 n = 15 for treatments, n=75 for all treatments combined, \* Significant (p<0.05), \*\* Significant (p<0.01)

578 **Table 6. Effect of treatments on correlation coefficients between biomass harvested and**  
 579 **the square-root transformed soil extraction measurements of available P in year 2**

Treatment	Olsen P	Bray P	EDTA P	Truog P	Acetic acid P	Membrane P	Resin P
Control	0.31	0.41	0.20	-0.14	0.15	0.75**	0.52*
Acidified	0.47	0.42	0.50	-0.03	0.27	0.60*	0.63*
Calcareous	0.53*	0.57*	0.60*	0.27	0.40	0.63*	0.45
VAM inoculated	0.00	0.14	-0.23	-0.66	-0.24	0.44	0.20
Sterilised	0.58*	0.54*	0.36	-0.10	0.30	0.71**	0.72**
All treatments combined	0.38**	0.49**	0.19	-0.19	0.13	0.69**	0.54**

580 n = 15 for treatments, n=75 for all treatments combined, \* Significant (p<0.05), \*\* Significant (p<0.01)

581 **Table 7. P concentration in biomass harvested in year 1. Standard error given in**  
 582 **parentheses (n=5)**

Treatment	P concentration in biomass harvested (mg g <sup>-1</sup> )		
	<i>No P added to soil</i>	<i>240 mg P added to soil</i>	<i>800 mg P added to soil</i>
Control	1.44 (0.03)	1.59 (0.03)	2.32 (0.07)
Acidified	1.63 (0.10)	1.81 (0.03)	2.21 (0.04)
Calcareous	1.51 (0.07)	1.93 (0.03)	2.02 (0.07)
VAM inoculated	1.78 (0.38)	1.63 (0.02)	2.36 (0.08)
Sterilised	1.64 (0.03)	1.96 (0.01)	2.38 (0.02)

583 **Table 8. Effect of treatments on correlation coefficients between P uptake and the square-**  
 584 **root transformed soil extraction measurements of available P in year 1**

Treatment	Olsen P	Bray P	EDTA P	Truog P	Acetic acid P	Membrane P	Resin P <sup>#</sup>
Control	0.86**	0.88**	0.36	-0.48	-0.43	0.89**	0.68**
Acidified	0.77**	0.82**	0.73**	0.32	0.31	0.72**	0.64**
Calcareous	0.66**	0.72**	0.71**	0.14	0.24	0.27	0.46
VAM inoculated	0.64**	0.61*	0.35	0.31	0.35	0.55*	0.56*
Sterilised	0.88**	0.88**	0.82**	0.73**	0.66**	0.93**	0.76**
All treatments combined	0.73**	0.78**	0.47**	0.07	0.08	0.69**	0.64**

585 n = 15 for treatments, n=75 for all treatments combined, \* Significant (p<0.05), \*\* Significant (p<0.01)

586 <sup>#</sup> Soil extraction data from year 2

587 **Table 9. Coefficient of determination ( $R^2$ ) for the DCA and Pearson correlation**  
 588 **coefficients (r) with axis scores for phosphorus and pH.**

Axis	$R^2$	Cumulative $R^2$	Phosphorus (r)	pH ( r)
1	0.154	0.154	0.277	0.292
2	0.092	0.245	0.456	0.124
3	0.072	0.317	-0.123	0.094

589 **Figures**

590 Figure 1 Relationship between P extracted from soil by Olsen, Bray, Membrane and Resin  
591 extractions and plant uptake of P (Error bars show standard error n = 5)

592

593 Figure 2 Relationship between P extracted from soil by EDTA, Truog and Acetic acid  
594 extractions and plant uptake of P (Error bars show standard error n = 5)

595

596 Figure 3. DCA species plot. Species names use the convention of 4 letters for the genus and 4  
597 for the species. Nomenclature follows Tutin et al., 1964

598

599 Figure 4 DCA quadrat plot. Communities are identified by their NVC mesotrophic grassland  
600 number; 14 = the *Agrostis-Carex-Senecio* community. The superimposed ellipses are Gaussian  
601 bivariate ellipses for each NVC community.

602

603 Figure 5. Mean DCA axis scores for each grassland community.

604

605 Figure 6 Relationship between median values of species richness and Olsen P for NVC  
606 communities (Error bars show interquartile range)

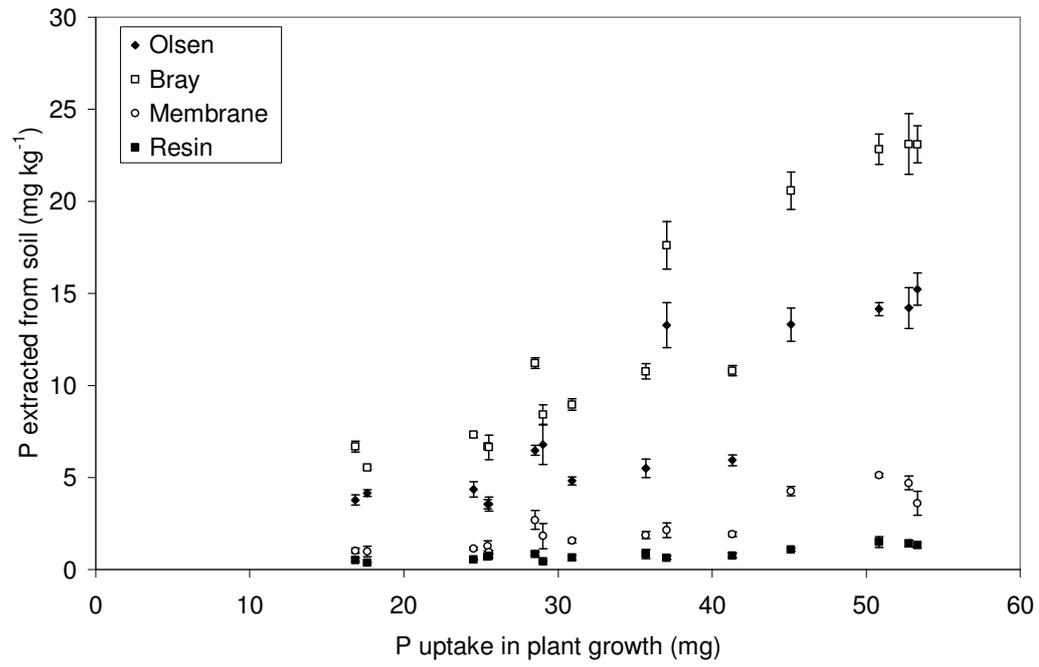
607 (n=5, 23, 8,14, 12, 53, 5, 10, 30, 16 for MG3, 4, 5,6, 7, 8, 9, 10, 13 and *Agrostis-Carex*  
608 grassland respectively). Number labels represent NVC community.

609

610 Figure 7 Relationship between species richness and Olsen P for individual quadrats of neutral  
611 grassland

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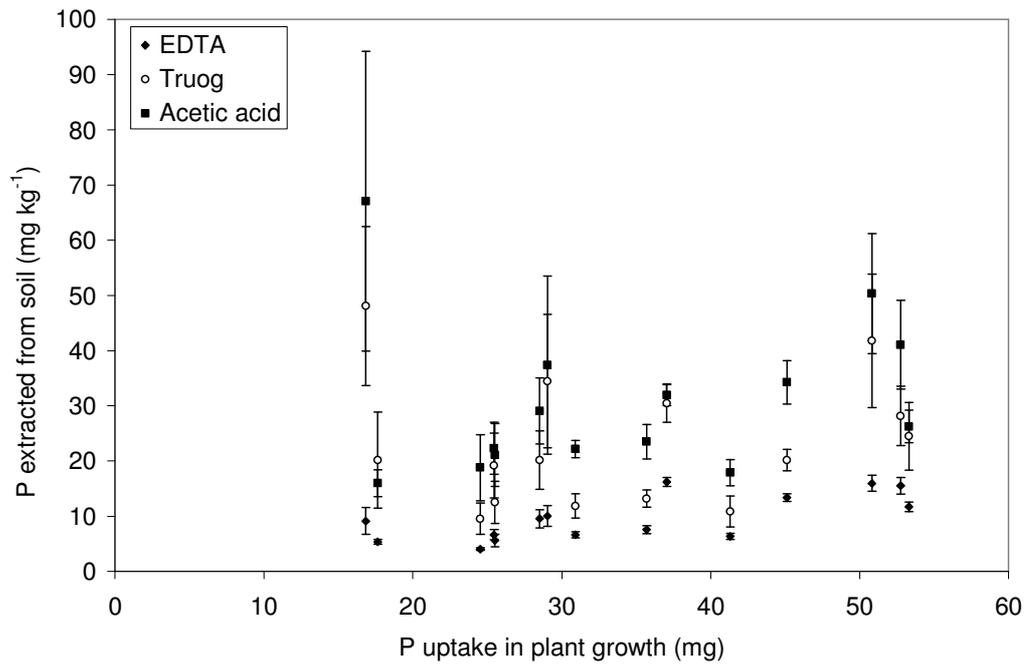


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Figure 1

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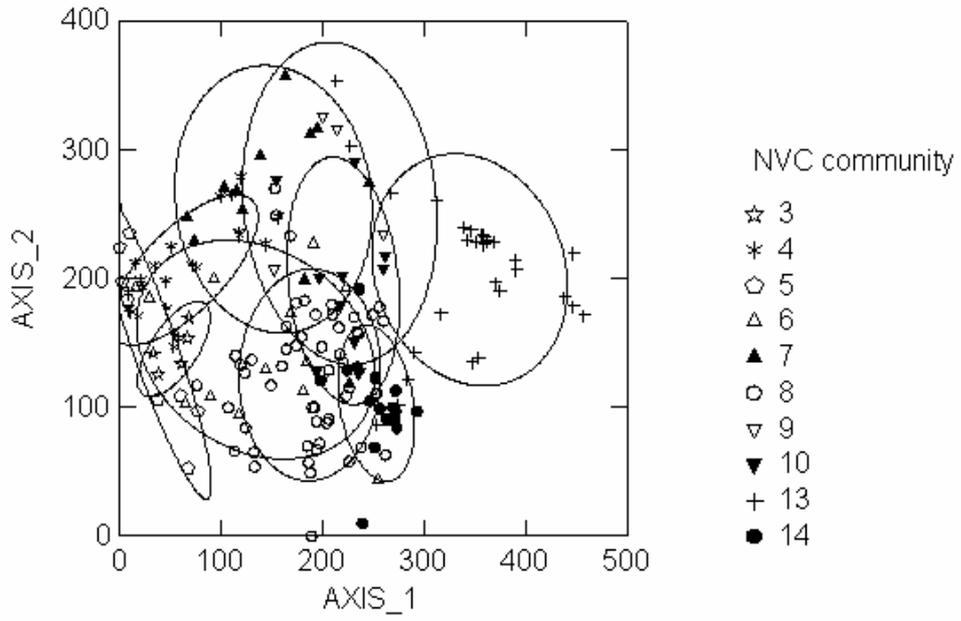
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618 Figure 2



622

623 Figure 4

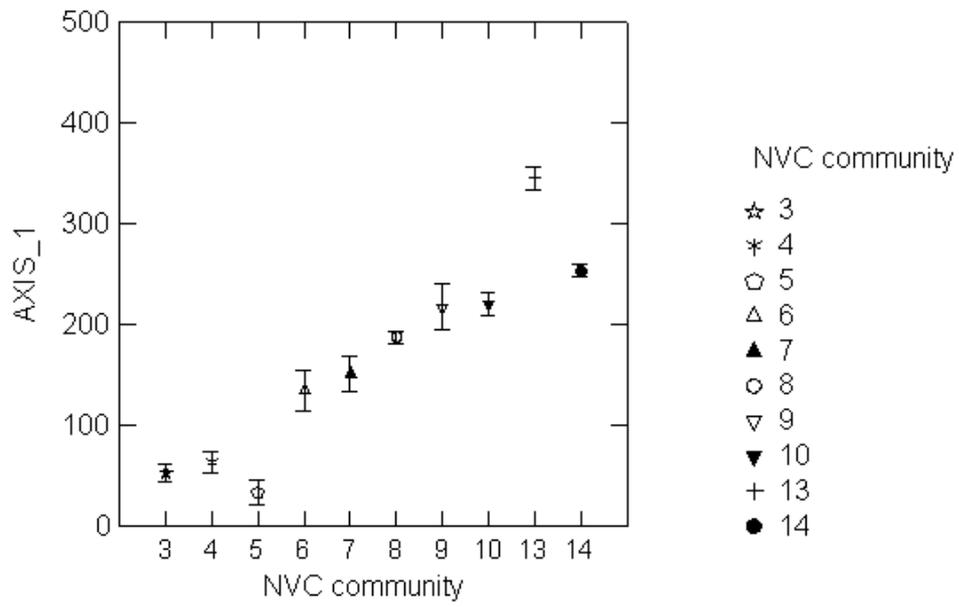


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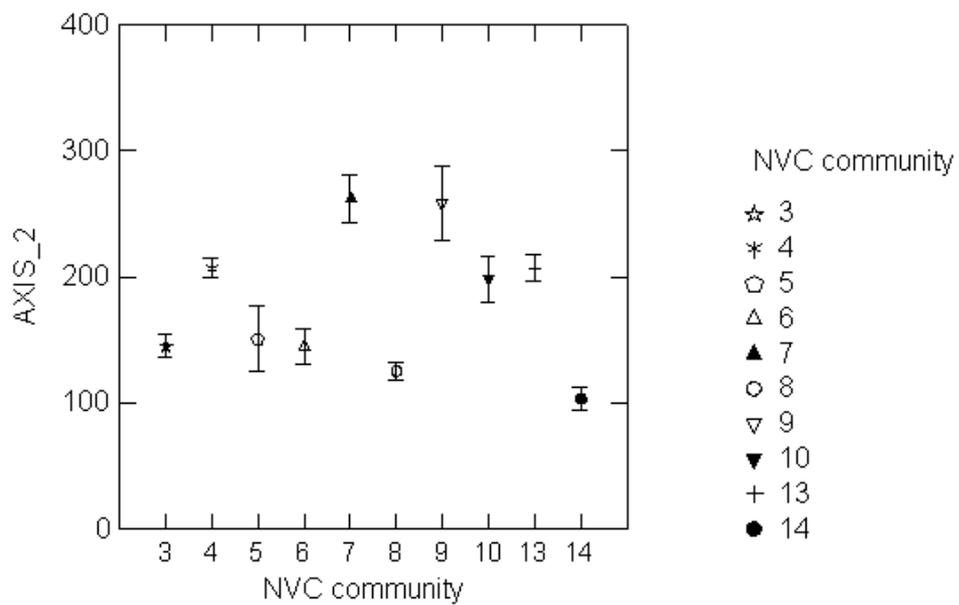
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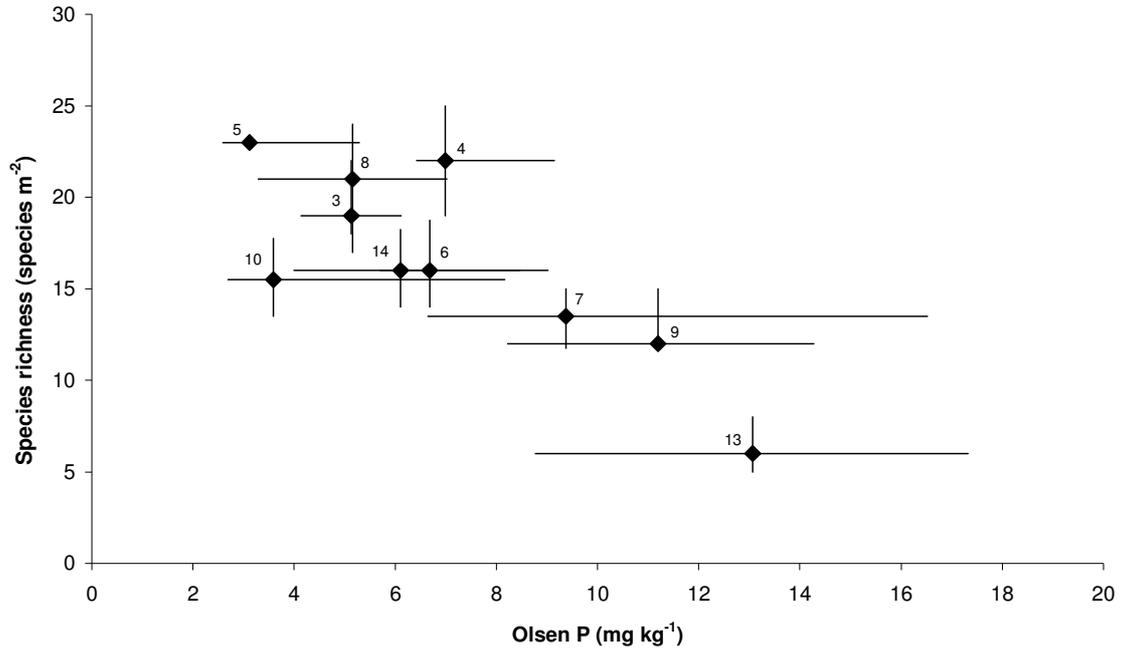
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631 Figure 5.

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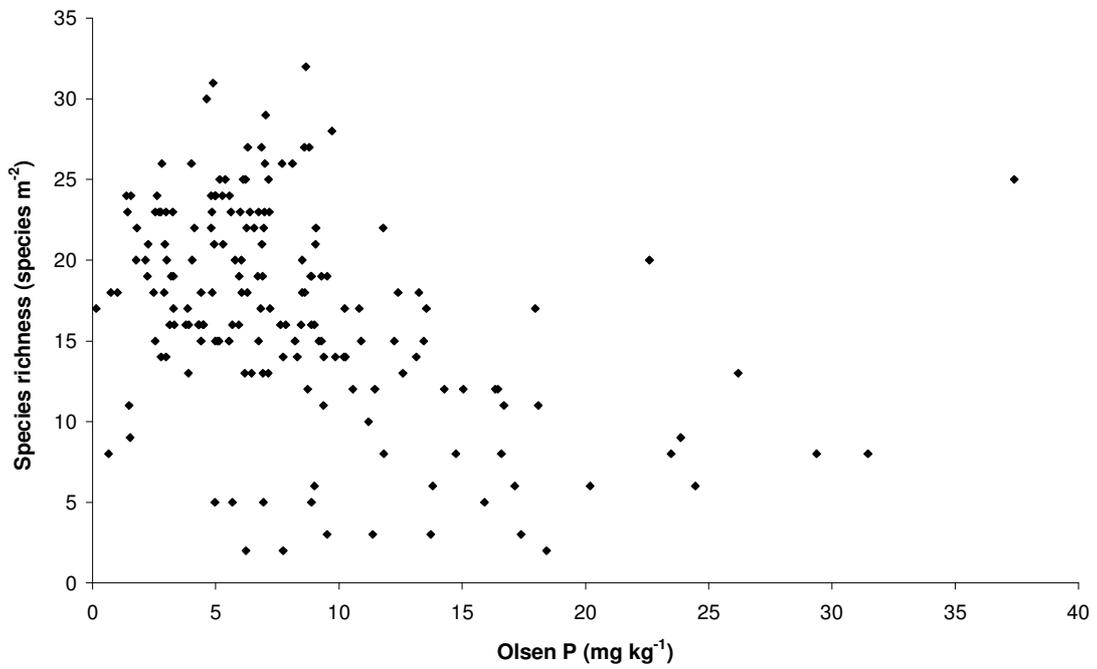


633

634 Figure 6

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636



637

638 Figure 7

639