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Abstract

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

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

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

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

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

Olsen's method of P extraction is recommended for analysing soils of areas identified for habitat creation; values of less than 10 mg kg^{-1} will give the greatest potential for the restoration of species-rich mesotrophic grassland.

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

Introduction

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

Two potential problems are encountered when translating this information for use in habitat restoration. Firstly, all of the existing methods were originally developed and calibrated against the growth of crop species, which are generally more productive than the species found in semi-natural habitats and less reliant on mycorrhizal fungi for access to soil P. The methods may therefore be ineffective in discriminating between the low levels of P found in semi-natural habitats (Olff 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 mg P kg^{-1}, EDTA + ammonium acetate extraction and 15 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

Materials and methods

Preparation of soil treatments

A uniform textured soil of low P content was prepared by mixing 1 part soil (pH 6.6, 18% sand, 31% silt, 51% clay) with 3 parts sand (16/30 yellow. Bardon Aggregates, Leighton Buzzard). Seventy five 2.5 litre plastic plant pots were each filled with 3.3 kg of the prepared soil. pH was reduced in 15 pots by adding 600 ml of 0.013 M H_2SO_4. pH was increased in 15 pots by mixing 9 g of CaOH into the dry soil then wetting up with 600 ml deionised water. Mycorrhizal activity was increased in 15 pots by adding 600 ml of deionised water then inoculating with 0.43 g of VAMINOX granules (Microbio, Rothamsted). Mycorrhizal activity was reduced in 15 pots by sterilising the soil in an autoclave then adding 600 ml of deionised water. 15 pots received only 600 ml deionised water without any change to their pH or mycorrhizal complement.

Potassium orthophosphate, K_3PO_4, solution was added to 5 pots from each treatment at the rate of 240 mg per pot and to a further 5 pots from each treatment at the rate of 800 mg per pot. The remaining 5 pots from each treatment received no K_3PO_4. KCl was added to the soils treated with zero and 240 mg potassium K_3PO_4 such that all treatments received an equal quantity of potassium.

420 mg of KNO_3 and 16 mg of NH_4Cl were added to each pot to ensure an adequate supply of nitrogen (after Hoagland and Arnon, 1950). The treatments were then allowed to equilibrate for a period of two weeks before planting. Soil pH was measured annually to monitor the effects of the treatments (British Standards Institute, 1990).

Plant growth

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

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

The above-ground vegetation (over 3 cm height) was harvested monthly during the growing season for two years and recorded as dry weight (dried at 30^{o}C) (MAFF 1986). Following each monthly harvest, dilute solutions of H_2SO_4 (100 ml of 0.005 *M*) and CaOH (100 ml of 0.03 *M*) were added to the acidified and calcareous treatments respectively, to maintain the soil conditions. 420 mg of KNO_3 and 16 mg of NH_4Cl were also added to each pot, to maintain adequate nitrogen and potassium concentrations. The positions of the pots were re-randomised after each harvest.

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

Soil analysis

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

Verifying mycorrhizal establishment

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

Field sampling

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

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

Data transformation

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

Vegetation survey.

At each soil sampling location a 1 m^2 quadrat was used to record the presence and percentage cover (visual estimates) of all species of vascular plants and prominent bryophytes. The field survey data comprised 199 quadrats with a total species record of 148 species. The data were subject to indirect gradient analysis using Detrended Correspondence analysis, DCA, using PC-ORD software (McCune and Mefford, 1999); axes were rescaled and species with less than five occurrences were removed from the data prior to analysis resulting in a reduced species record of 93 species. Correlations were then sought between DCA axis score and soil phosphorus. The vegetation of each quadrat was assigned to a community of the National Vegetation Classification (NVC; Rodwell, 1992) using Czekanowski co-efficients via the computer programme MATCH (Malloch, 1995). Only the quadrat locations where the programme classified the vegetation as mesotrophic grassland were used in the subsequent analysis. Communities with less than five representations were excluded from the subsequent analysis, giving 176 sample locations. The communities recorded at each site are shown in Table 3. Descriptions and relationships to European alliances (Rodwell et al., 2007) are given in Table 4.

Gaussian bivariate ellipses for each grassland NVC community are plotted onto the DCA diagram. These ellipses are centred on the mean axis scores for each NVC community. The axes of the ellipses are based on the sample standard deviation and their orientation is based on the covariance between the scores for the two axes (Systat v8.0)

Results

pH in the treated soil

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

Correlation between P extracted from soil and biomass harvested

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

Correlation between P extracted from soil and P uptake in vegetation

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

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

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

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

Inspection of the roots at the end of the second year showed a large number of vesicles to be present in the VAM inoculated treatment, indicating a high level of mycorrhizal activity. Vesicles were also observed in the roots of the control treatment, but at a lower density. Very few vesicles were observed in the sterilised treatment, indicating that mycorrhizal development had largely been prevented over the period of the experiment.

All extraction methods were significantly correlated ($p<0.01$) with P uptake in the sterilised treatment, whereas only the Olsen, Bray, membrane and resin extractions were significantly 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,

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

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

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

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

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

Discussion

Possible reasons for variation in correlation coefficient between extraction methods

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

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

The acidic extractants of EDTA-ammonium acetate, Truog and acetic acid exchange anions with phosphate ions and dissolve P held as Ca-P, Al-P and to some extent Fe-P. The different pH and concentrations of the three solutions cause them to extract different amounts of P from soil. In general, these acid extractants have performed the least well of all the methods tested. Kamprath and Watson (1980) suggested that this could be because Ca-P compounds are 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

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

Ideally, the extraction methods should be easy to use so that a wide range of laboratories can carry out the measurements within defined limits of precision. The resin extraction method does not fulfil this criterion since the retrieval of the resin presents a problem. In this work, polyester sachets were used to hold the resin, but cleaning the soil completely from the sachets before elution of the P is a subjective process. The other six extraction methods were found to be easy to undertake and therefore likely to be repeatable in different laboratories.
To enable comparison of results between researchers, it would be helpful if a single method were used for the analysis of soils from semi-natural grasslands and the assessment of soils prior to the commencement of the restoration of this habitat. Olsen P has been chosen here to measure available P in semi-natural grassland soils as it correlated well with plant P uptake and is the most frequently used test in the UK for measuring available soil P.

Olsen P and plant community

The results presented in this paper show that there are differences between the available P concentrations found in the soil supporting different plant communities. In general, species-rich communities (e.g. MG3, 4, 5 and 8) occurred on soils with lower available P concentrations whereas the species-poor communities, often of the same alliance and with similar hydrological profiles, occurred on soils with higher available P concentrations. Vegetation which is classified as MG6, 7, 9 and 10 may occur in species-rich and species-poor forms. Water regime, soil P and land management are the main factors affecting species richness in European 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^{-1} was sufficient to lower the median species richness
391 from 22 to 14 species m^{-2}, 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^{-2} was restricted to soils with less
395 than 15 mg l^{-1} Olsen P. Janssens et al. (1998) found that soil with over 50 mg kg^{-1} EDTA P
396 supported fewer than 20 species per 100 m^2, although inspection of Figure 2 shows that a value
397 of 50 mg kg^{-1} 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^{-1} and 26% of soils had concentrations which were much higher (Gilbert et al.,

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

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

The species-poor vegetation (<10 spp m^{-2}) in this study occurred over a wide range of concentrations from 8 to 18 mg kg^{-1}. 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^{-1}.

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466

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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: 0.03*M* ammonium fluoride and 0.1*M* hydrochloric acid	Page et al. (1982)
EDTA	EDTA – ammonium acetate: 0.02 *M* ethylene diaminotetraacetic acid, 0.5 *M* ammonium acetate and 0.5 *M* acetic acid (pH 4.65)	Lanaken and Ervio (1971)
Truog	0.001*M* sulphuric acid and 0.02*M* 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 0.5*M* sodium bicarbonate before use	Qian et al. (1992)
Resin	Ion exchange resin in polyester sachets Amberlite IRN-150 resin (Johnson Matthey) saturated in 0.5*M* 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	*Anthoxanthum odoratum-Geranium sylvaticum* grassland Northern hay meadow	Triseto-Polygonion
MG4	*Alopecurus pratensis – Sanguisorba officinalis* grassland Flood meadow	Alopecurion
MG5	*Cynosurus cristatus – Centaurea nigra* grassland Old hay meadow	Centaureo-Cynosuretum
MG6	*Lolium perenne – Cynosurus cristatus* grassland Ordinary pasture	Lolio-Cynosuretum
MG7	*Lolium perenne* leys and related grasslands Intensively managed grassland	Lolio-Plantaginion
MG8	*Cynosurus cristatus – Caltha palustris* grassland Water meadow	Calthion
MG9	*Holcus lanatus – Deschampsia cespitosa* grassland Tussocky neutral grassland	Calthion
MG10	*Holcus lanatus – Juncus effusus* grassland Rushy grassland	Calthion
'14' Ag-Cx	*Agrostis stolonifera-Carex nigra-Senecio aquaticus* community	Calthion/Elymo-Rumicion *
MG13	*Agrostis stolonifera – Alopecurus geniculatus* 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^{-1})		
	No P added to soil	*240 mg P added to soil*	*800 mg P added to soil*
Control	1.44	1.59	2.32
	(0.03)	(0.03)	(0.07)
Acidified	1.63	1.81	2.21
	(0.10)	(0.03)	(0.04)
Calcareous	1.51	1.93	2.02
	(0.07)	(0.03)	(0.07)
VAM inoculated	1.78	1.63	2.36
	(0.38)	(0.02)	(0.08)
Sterilised	1.64	1.96	2.38
	(0.03)	(0.01)	(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[#]
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 [#] 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

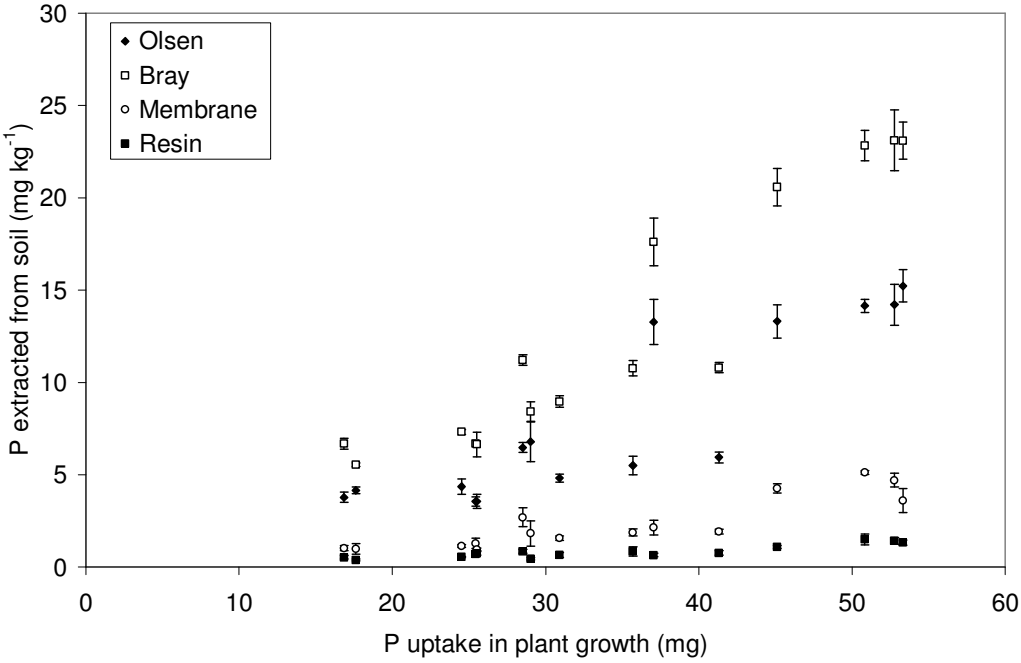
605 Figure 6 Relationship between median values of species richness and Olsen P for NVC
606 communities (Error bars show interquartile range)
607 (n=5, 23, 8,14, 12, 53, 5, 10, 30, 16 for MG3, 4, 5,6, 7, 8, 9, 10, 13 and Agrostis-Carex
608 grassland respectively). Number labels represent NVC community.

609

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

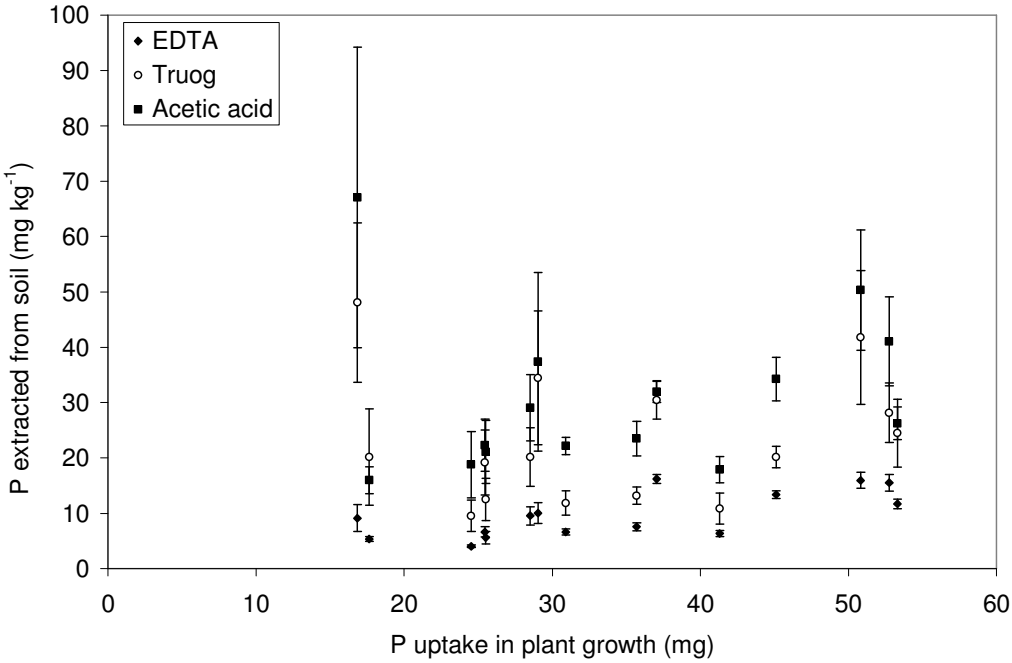
612

613



614
615 Figure 1

616



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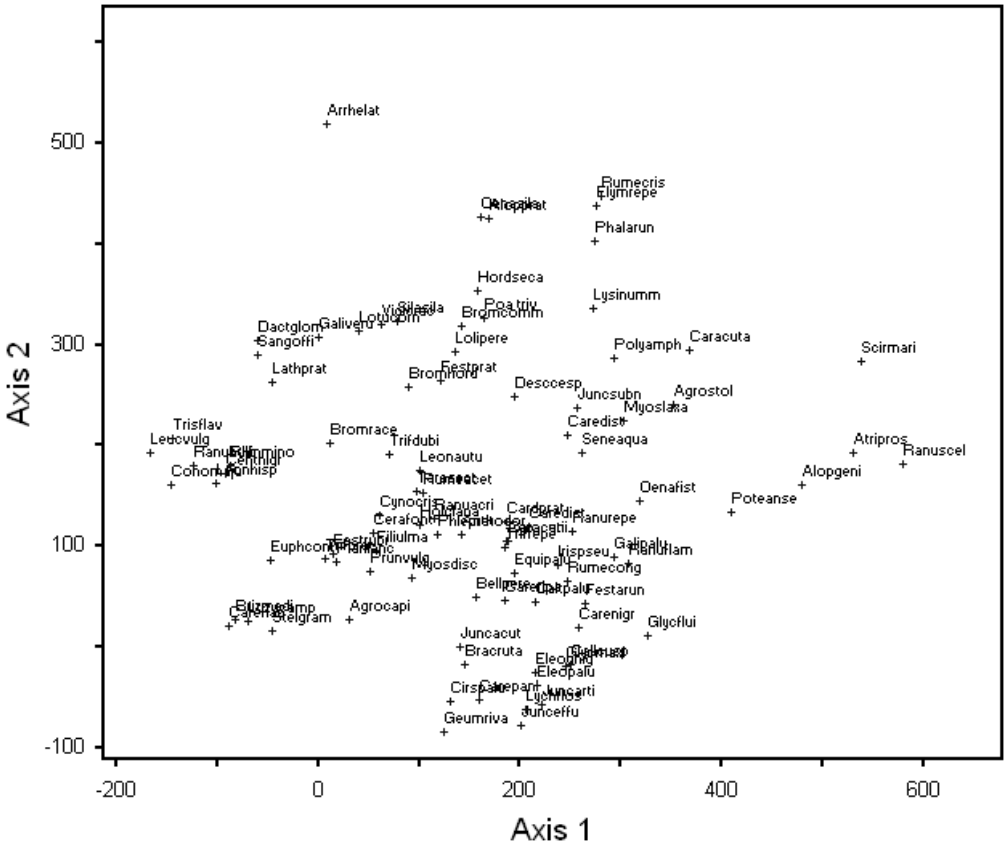
618 Figure 2



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621 Figure 3

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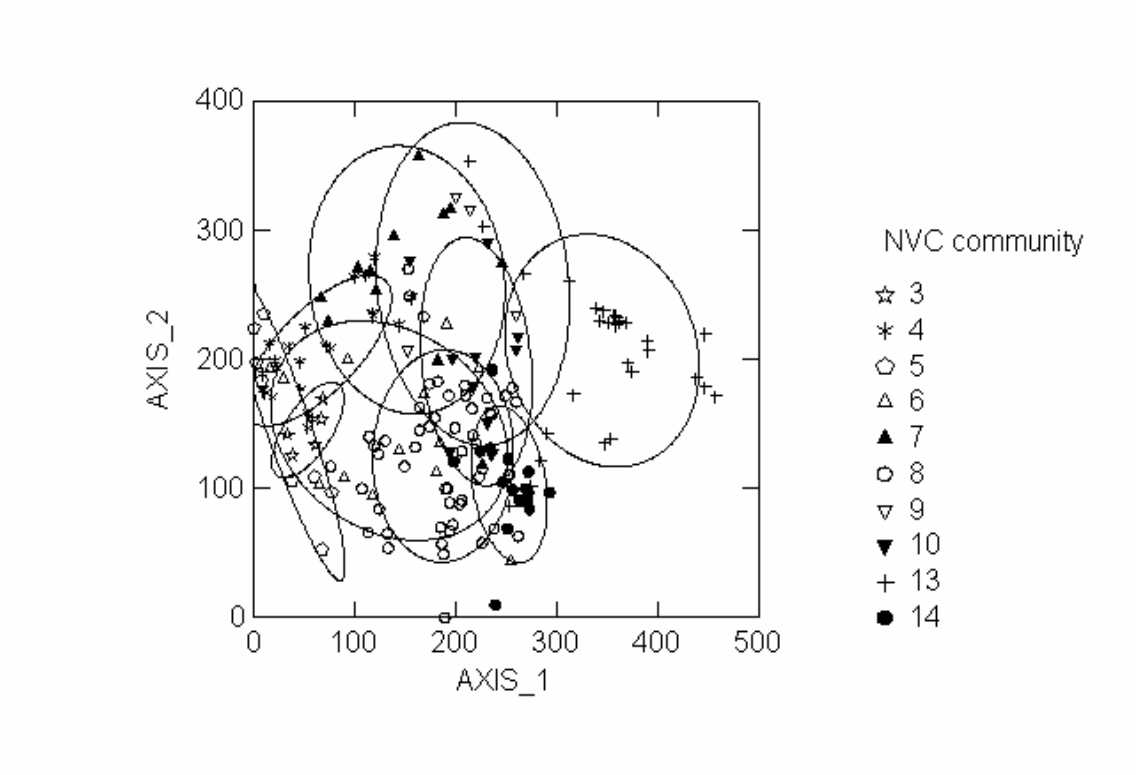
623 Figure 4



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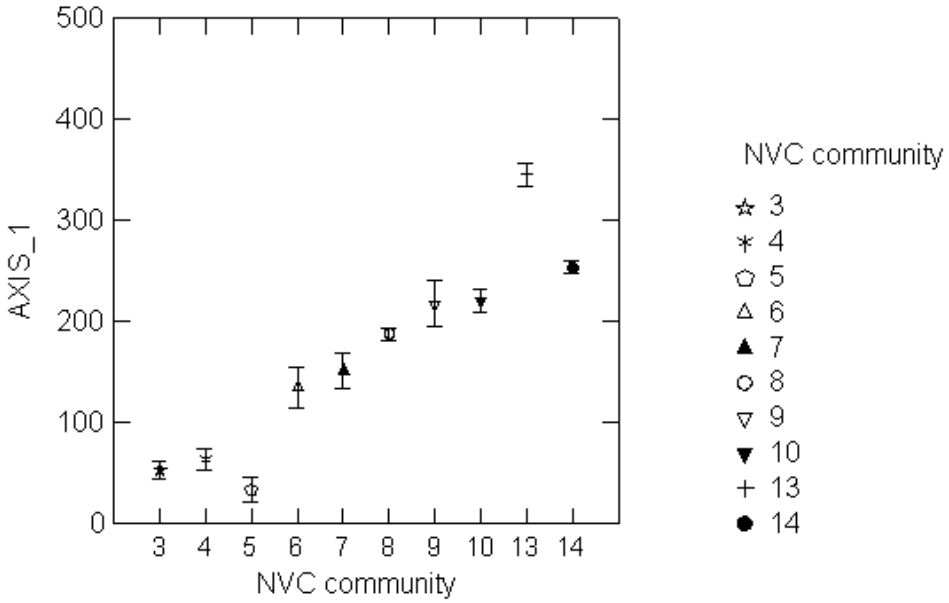

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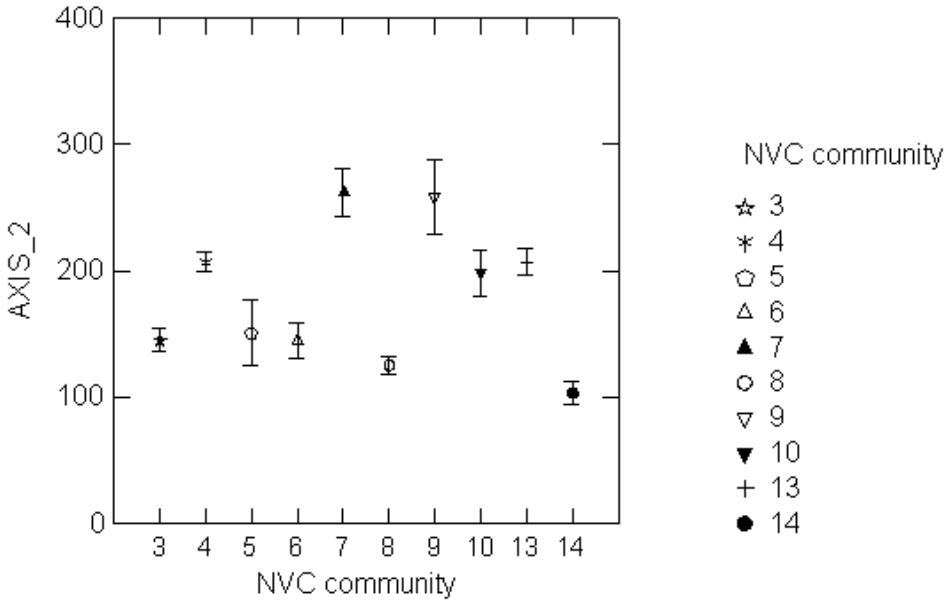
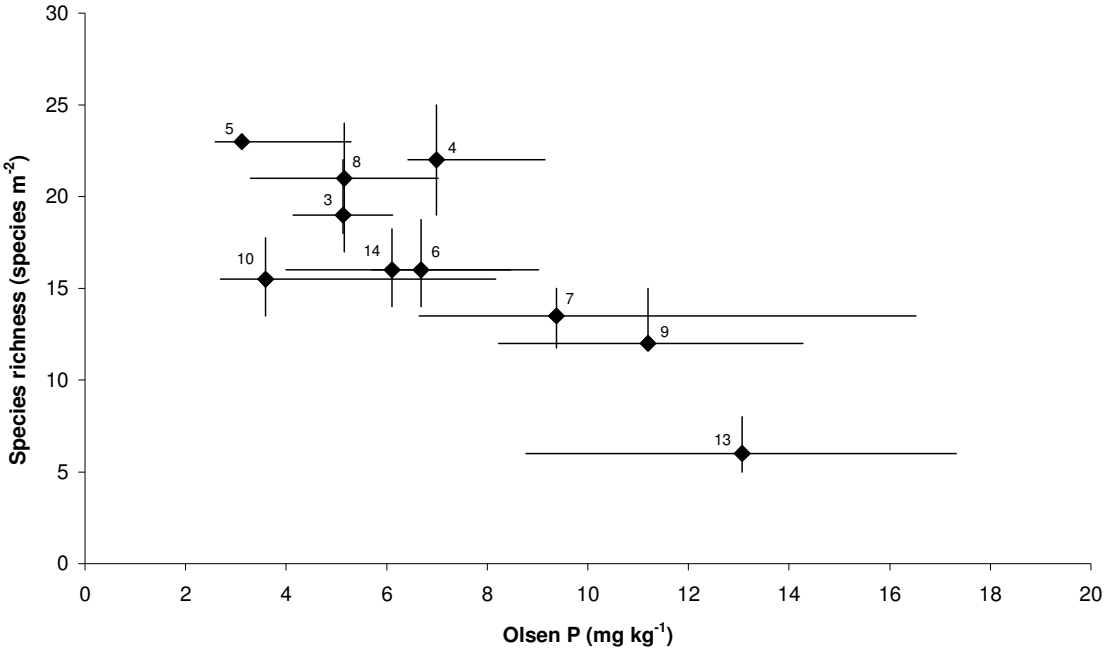


630

631 Figure 5.

632



633
634 Figure 6

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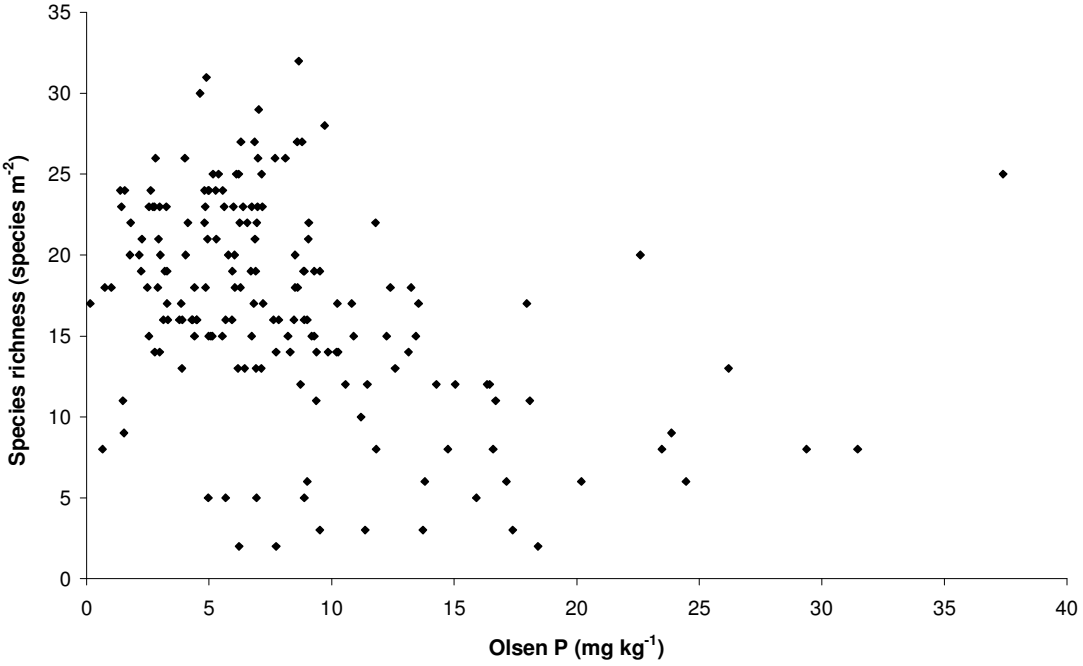
636



637
638 Figure 7

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