Biological Conservation 142 (2009) 1074–1083

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Available soil phosphorus in semi-natural grasslands: Assessment methods and community tolerances

Joanne Gilbert [a,*], David Gowing [b], Hilary Wallace [b]

[a] Cranfield University, Silsoe, Bedfordshire MK45 4DT, UK
[b] Department of Life Sciences, Open University, Walton Hall, Milton Keynes MK7 6AA, UK

ARTICLE INFO

Article history:
Received 17 February 2008
Received in revised form 18 January 2009
Accepted 25 January 2009
Available online 5 March 2009

Keywords:
Chemical extraction
Species-rich
Grassland
Meadows

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.

1. 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 growth of single species in glasshouse or field experiments, whereas semi-natural

* Corresponding author. Present address: Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, UK. Tel.: +44 1767 680551; fax: +44 1767 689836.
E-mail addresses: jo.gilbert@rspb.org.uk (J. Gilbert), d.j.gowing@open.ac.uk (D. Gowing), mikehilary@ecosurvey.demon.co.uk (H. Wallace).

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doi:10.1016/j.biocon.2009.01.018

Table 1
Summary of extraction techniques previously used for soils supporting semi-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

grasslands contain a mixture of species growing together, which may enable a greater utilisation of soil P.

Secondly, the quantity of 'available P' in soil is a variable rather than a constant, since P exists in a range of different forms which vary in space and time. The methods used to measure 'available P' rely on a chemical equilibrium between the soil and the extractant. Hence, the concentration of 'available P' measured in a soil depends on the method used (Fixen and Grove, 1990). The results from two different extraction methods, therefore, cannot be compared directly. Previous research comparing available P in semi-natural habitats with those of restoration sites has used a variety of methods (Table 1).

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

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

2. Materials and methods

2.1. 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 l 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 five pots from each treatment at the rate of 240 mg per pot and to a further five pots from each treatment at the rate of 800 mg per pot. The remaining five 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.

Four hundred twenty milligram 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).

2.2. 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 °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.

2.3. 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).

2.4. 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).

2.5. Field sampling

Soil samples (199) were collected from eleven lowland grassland sites in England. At each sampling location, four cores were