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Phoenix, G.K., Johnson, D.A., Muddimer, S.P. et al. (2020) Niche differentiation and plasticity in soil phosphorus acquisition among co-occurring plants. Nature Plants, 6 (4). pp. 349-354. ISSN: 2055-026X

https://doi.org/10.1038/s41477-020-0624-4

Niche differentiation and plasticity in soil phosphorus acquisition among co-occurring plants

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How species co-exist despite competing for the same resources that are in limited supply is central to our understanding of the controls on biodiversity[1,2]. Resource partitioning may facilitate co-existence as co-occurring species use different sources of the same limiting resource[3,4]. In plant communities however, direct evidence for partitioning of the commonly limiting nutrient phosphorus (P) has remained scarce due to the challenges of quantifying P acquisition from its different chemical forms present in soil[5]. To address this we used ^{33}P to directly trace P uptake from DNA, orthophosphate and calcium phosphate into monocultures and mixed communities of plants growing in grassland soil. We show that co-occurring plants acquire P from these important organic and mineral sources in different proportions, and that the differences in P source use are consistent with the species' root adaptations for P acquisition. Furthermore, the net benefit arising from niche plasticity (the gain in P uptake for a species in a mixed community compared to monoculture) correlates with species abundance in the wild, suggesting that niche plasticity for P is a driver of community structure. This evidence for P resource partitioning and niche plasticity may explain the often high levels of biodiversity in P-limited ecosystems worldwide[6,7].

The apparent incongruity between competition for a limiting resource and high levels of biodiversity is particularly striking in P-limited communities, which harbour some of the most biodiverse plant communities globally and have greater proportions of rare or endangered species than ecosystems limited by other nutrients[7-8]. If co-occurring species could share the limiting resource by preferentially acquiring P from different chemical forms in soil, this would reduce interspecific competition for P and facilitate co-existence through resource partitioning[5,9]. Indeed, it has been proposed that resource partitioning of soil P is

likely to occur given the diversity of plant adaptations for P acquisition and the range of different chemical forms of the element in soil for these to act on[9]. However, proving contrasting uptake from different chemical forms of P in soil among co-occurring species is challenging given the need to determine uptake of P from any one particular source against a background of many sources of the element in soil. To date therefore, most advances in P partitioning have been made by studies investigating plant growth and tissue P responses in plants supplied with single P sources, often in large doses or using artificial growth media[5,10-12]. These approaches, however, change natural plant-soil-microbial interactions that significantly influence, and play a substantial role in, plant P acquisition[13,14], or may alter availability and competition for P, especially where large doses are supplied. Without normal competition for P with microbes and soil exchange sites, or realistic activity of plant adaptations for P acquisition that may be lost under artificial conditions such as high rates of P additions, elucidation of partitioning as occurs in nature (i.e. differentiation of realised niches) may not be reliably determined. While an isotope dilution experiment has recently been used to infer interspecific differences in plant uptake of different P sources under realistic conditions[5], such an approach cannot directly quantify uptake of P from contrasting sources. Here, we provide evidence of P partitioning in co-occurring plant species by using ^{33}P radio-isotope labelled organic and inorganic P compounds to directly trace P uptake from these contrasting P chemical forms in grassland soil.

We use limestone grasslands as our model study system since these are important and widespread P-limited ecosystems with high floristic diversity[15]. The co-occurring species have contrasting adaptations for P acquisition that are likely to differentially gain access to different chemical forms of P. Most forbs and grasses form arbuscular mycorrhizal

symbioses[16], which enhance plant access to organic and particularly inorganic P sources[18,19], while sedges are often non-mycorrhizal and may form dauciform roots that secrete low molecular weight organic acids and chelators in spatially localized 'temporal bursts'[20] to mobilise P and compete effectively with soil microorganisms. Some forbs are non-mycorrhizal but compensate with long root hairs and by secreting large amounts of organic acid exudates that mobilise P from minerals such as calcium phosphate[21,22]. These adaptations for P acquisition can act upon the large diversity of chemical forms of P in soil[9] including orthophosphate (PO_4^{3-}) which is taken up directly by plants, and organic and precipitated mineral P from which the P must be liberated by the action of enzymes, organic acids and protons[13]. Phosphodiesters such as DNA, followed by phosphomonesters are the principle organic sources entering the soil from plant and microbial litter, and in calcium-rich mineral soils most inorganic P is immobilized into microbial biomass or precipitated as calcium phosphates[23,24]. We predicted, that when supplied with these different forms of P (orthophosphate, DNA and calcium phosphate), plant species with different strategies for acquisition of P would show different uptake from these, consistent with P partitioning. Further, we predicted that differences in P form use between species would be more apparent under conditions of interspecific competition in mixed communities (realised niche), than when each species was grown in monoculture (fundamental niche).

We established model turfs comprising monocultures and mixed communities of seven co-occurring species in pots of fresh soil collected from a limestone grassland for which P is known to limit plant growth[25]. The species included the main plant functional groups of grassland with different P acquisition strategies: mycorrhizal grasses (*Agrostis capillaris* and *Festuca ovina*); a mycorrhizal forb and legume (*Plantago lanceolata* and *Lotus corniculatus*);

a non-mycorrhizal forb (*Rumex acetosa*); and non-mycorrhizal sedges that form dauciform roots (*Carex flacca*, *C. caryophyllea*). We determined each species use of each of three contrasting and naturally occurring soil P sources by quantifying uptake over six days from the addition of ^{33}P labelled compounds of orthophosphate, DNA (applied in solution) and microcrystalline calcium phosphate (applied as a suspension). We found that in monoculture, all plants used calcium phosphate and orthophosphate as a P source more than DNA (Fig 1a; P-source main effect, $F_{2,122} = 54.31$, $P < 0.001$). Importantly, the species differed in their relative use of these P sources (Fig 1a; P-source *x* species interaction, $F_{12,122} = 2.59$, $P < 0.01$) ranging from the legume (*L. corniculatus*) that obtained P in very similar proportions from orthophosphate and calcium phosphate (47% and 41% respectively of the ^{33}P taken up per g shoot dry weight from all three P forms), through a sequence of sedges (*C. flacca*, *C. caryophyllea*) and then grasses (*A. capillaris* and *F. ovina*) showing increasing use of calcium phosphate relative to orthophosphate. The non-legume forbs (*P. lanceolata* and *R. acetosa*) showed greatest relative use of calcium phosphate, with the non-mycorrhizal *R. acetosa* acquiring a considerable 88% of the ^{33}P taken up from all three P forms from this source. Although it was the least utilised form, all plants obtained P from DNA (Fig 1a), ranging from 12% of uptake from all three P forms in *L. corniculatus* and *C. flacca*, to just 2% in *R. acetosa*. Greater use overall of calcium phosphate as a P source may seem surprising given its low solubility. However, in the limestone soil orthophosphate will be readily acquired by soil microbes or chemically bound, while microcrystalline calcium phosphate offers a greater opportunity for plant root adaptations for P acquisition (such as exudation of organic acids or symbioses with mycorrhizal fungi) to out-compete free-living microbes[20-22].

The differences in relative use of contrasting P sources between species demonstrates differentiation in the fundamental niches of plants for soil P. While there is niche overlap (as to be expected in the broadly overlapping resource use in plant communities[26]), these findings show that plants in species rich grassland utilise P from soluble inorganic, calcium bound precipitates and organic forms to different extents, which if also apparent under conditions of interspecific competition, should facilitate co-existence.

When grown in competition (mixed communities), few species showed significant changes in shoot ^{33}P concentrations compared to when grown in monoculture, indicating relatively limited niche shifts overall (Fig. 2,a,b,c). None-the-less, concentrations of ^{33}P derived from calcium phosphate in shoots of the non-mycorrhizal forb *R. acetosa* was halved in mixed communities ($t_{12} = 4.59$, $P < 0.01$) contrasting with its five-fold greater concentration of ^{33}P from this source compared to other species when in monoculture (Fig. 2b). Conversely, the grass *F. ovina*, significantly increased its concentration of ^{33}P obtained from calcium phosphate by 2.5 fold (Fig. 2b) ($t_{12} = -5.89$, $P < 0.001$). Possibly, the mycorrhizal grass with its finely branching root system benefited from the phosphate liberated by the high rates of acid secretion of the non-mycorrhizal *R. acetosa*. This would be consistent with a type of P-nutrition facilitation suggested for other interacting plant species where the action of one species may liberate P for uptake by others[27,28]. In contrast, the grass *A. capillaris* had 66 and 67% lower concentrations of ^{33}P derived from orthophosphate and DNA in mixed communities compared to when in monoculture (Fig. 2a,c) ($t_{11} = 4.20$, $P < 0.01$ and $t_{12} = 2.24$, $P < 0.05$) suggesting relatively weak competitive ability for these sources. In the latter case of DNA, this was possibly of benefit to the sedge *C. flacca* which showed a considerable (though statistically not significant) 167% greater ^{33}P concentration derived from DNA in

mixed communities compared to monocultures (Fig. 2c). This gain is consistent with the sedges adaptation of dauciform roots that may increase the sedge's competitive ability to capture organic P by releasing organic acids and enzymes (including phosphodiesterase and phosphomonoesterase that act on DNA) in a exudate burst that overwhelms competition from soil microbes[20,29,30].

Broadly, the differences in ^{33}P shoot concentrations in monoculture and mixed communities appear unrelated to differences in growth (e.g. reductions in ^{33}P tissue concentrations were not simply due to greater growth diluting the ^{33}P; Extended Data Fig. 1). A few individual cases can be noted among the 21 comparisons of mixed versus monoculture communities (7 species *x* 3 P sources). In calcium phosphate pots, *R. acetosa* grew relatively better in mixed communities compared to monoculture (Extended Data Fig. 1), which could cause its lower ^{33}P tissue concentration in mixed communities receiving calcium phosphate (Fig. 2b). Similarly, in DNA pots, *C. flacca* grew relatively better in monoculture which could explain its lower ^{33}P concentration in monoculture compared to mixed communities. Many species did not show such changes, and furthermore, if growth dilution was important for a species, it might be expected to be seen across all P sources, which it was not. We therefore maintain that differences in ^{33}P uptake between mixed communities and monocultures (Fig. 2) are due to characteristics of the species P acquisition and changes in competition for that P, rather than changes in growth.

Given the evidence for complementarity in P source use, we would expect a greater total amount of ^{33}P to be taken up, and greater biomass, in the mixed communities compared to

monocultures. However, both biomass per pot and total ^{33}P were only marginally, and non-significantly, greater in mixed communities compared to monocultures (biomass: 1.55g, s.e. 0.040g *vs* 1.53g, s.e. 0.058g; ^{33}P: 0.0111 MBq s.e. 0.002 MBq *vs* 0.0101 MBq, s.e. 0.001 MBq). Without significant differences, we must conclude that any P partitioning did little to alleviate P limitation, and hence is unlikely to be the sole driver facilitating species co-existence in our experiment. We note though that our experiment was not designed with detection of biodiversity-function relationships as a priority, in particular with our use of only two biodiversity levels (monocultures and mixtures of 7 species) in small pot. This contrasts with many studies on biodiversity-function relationships that have used multiple levels of species richness and large mesocosms or field plots with areas many orders of magnitude greater than our pots. These latter approaches are better suited to detecting biodiversity-productivity relationships of plant communities with small scale heterogeneity. Furthermore, since our study uses contrasting plant species (selected for their different P uptake adaptations), we cannot rule out that some niche differentiation may also be provided by complementarity in other traits not associated with P acquisition, but instead associated with, for instance, water uptake and light capture (though arguably these will be less important than P in our well-watered communities with relatively short swards).

While there were some changes in uptake from the three P sources between species when in monoculture compared to when in mixed communities, relatively similar P uptake patterns among species were still found for the mixed communities (Fig. 1b) compared to monocultures (Fig. 1a). In mixed communities, as with monocultures, there was overall relatively greater use of P from calcium phosphate and orthophosphate compared to DNA (P-source main effect, $F_{2,115} = 155.21$, $P < 0.001$), and interspecific differences in the

proportions in which the three sources were used, consistent with P partitioning (P-source *x* species interaction, $F_{12,115} = 4.05$, $P < 0.001$). Broadly, interspecific differences were similar between mixed communities and monocultures, with those species that showed the greatest relative use of either calcium phosphate or orthophosphate in monoculture (Fig 1a), also tending to do so in mixed communities (Fig 1b). While this suggests relatively limited niche plasticity, we found that those species with the greatest increase in ^{33}P uptake from all three forms in mixed communities compared to monocultures (i.e. showing the greatest net benefit of niche plasticity) also have the greatest abundance in the limestone grassland from where the soil was taken (Fig. 3). This suggests that even the relatively limited niche plasticity could contribute to the abundance of species in the field, and highlights the potential of competition for P to control plant community structure in a P-limited ecosystem. Previous studies have demonstrated that greater use of the most abundant P sources (or N sources in N-limited ecosystems) may drive greater plant abundance[3,5]. Instead, our suggestion of a role of niche plasticity in community structure has greater parallels with resource partitioning of N seen in alpine plants where competitively superior species showed the greatest capacity to alter N form "preference" in response to inter-specific competition[26]. Further work on the relative importance of P form use versus plasticity in driving community structure is warranted.

Since our study did not assess uptake from all soil P chemical forms, it may be that poor performance of some species in terms of their gain from niche plasticity is due to their preferred P chemical form not being represented in the ^{33}P labelled sources added. However, given the P forms used are examples of a broader suite of compounds the benefit of niche plasticity are likely to be reflected by the three P sources used. For example, DNA is a

phosphate diester which includes the nucleic acids, phospholipids and some cyclic phosphates, and these require the same action of organic acids and enzymes to liberate orthophosphate for plant uptake[9]. Once injected into the soil, the ^{33}P sources will be subjected to microbial turnover and chemical fixation so uptake by the plant will not always be directly from the P source injected. Therefore, some P form "preference" could arise from differences in competition by microbes for particular P forms, and plant species having differences in, for instance, their effect on soil microbes and microbial P transformations. That is a natural process that our methodology of using fresh grassland soil deliberately allowed to take place, and is essential for determining the realised niches of species. However, daily monitoring of sward radioactivity shows that the relative differences in uptake between P forms determined on the day-six harvest were also seen on the previous five days (Extended Data Fig. 2). This suggests that the P source use observed was not greatly influenced by P transformations over the time course of our study.

Looking to future work, we note that our study did not consider additional partitioning in space (soil depth) and time, which are factors that have been investigated in N partitioning[3,31]. Such contributors to P partitioning need further work, and it may be that the extent of niche differentiation revealed in our study is a conservative estimate of the total niche differentiation that could occur from the many different P forms, over time and different soil depths.

The necessary use of radio-labelled P sources to directly quantify P uptake raises additional challenges when studying resource partitioning, and this, combined with the limited range of soil-relevant ^{33}P labelled compounds available commercially has significantly limited the

investigation of P partitioning to date. The radiochemical synthesis approach used here provides an important tool to advance this field of research. Our synthesised calcium phosphate, however, had much lower specific activity than the other P sources, so the calcium phosphate supplied much more unlabelled P than the DNA or pure ^{33}P orthophosphate, though the total amount of P added remained small. Further tests (see Methods) investigating the influence of increasing the specific activity of the calcium phosphate showed no significant change in P uptake for nearly all species (Extended Data Fig. 3), and made little difference when used to adjust the original data on P form "preference" among species (compare Fig. 1 with Extended Data Fig 4.). Evidence for P partitioning concluded from the main study remained strong. Similar adjustments to the original data on the relationship between species abundance in the wild and the benefit of niche plasticity still revealed a highly significant relationship, though the strength of that relationship was less than in the original data (Extended Data Fig. 5)

Overall, our evidence of contrasting use among co-occurring species of different P chemical forms in natural soils supports the theory of niche differentiation in P source use as a driver of plant diversity[5,9,10,11], and that plasticity in niche differentiation may drive species abundance[26]. Given that P limitation is prevalent globally and the adaptations for P acquisition of the plant species in this study are ubiquitous, these mechanisms may drive community structure and high levels of biodiversity in some of the many P-limited plant communities worldwide.

Methods

Model grassland turfs established on limestone grassland soil

Seven plant species chosen for the model communities to cover the range of P acquisition strategies and functional groups typically found[20,32,33]. These were *Agrostis capillaris* and *Festuca ovina* (both mycorrhizal grasses), *Carex flacca* and *Carex caryophyllea* (both non-mycorrhizal sedges possessing dauciform roots), *Lotus corniculatus* (mycorrhizal legume), *Plantago lanceolata* (mycorrhizal forb) and *Rumex acetosa* (non-mycorrhizal forb). All species are widespread species that commonly co-occur in many types of grasslands, including limestone grasslands.

The soil was taken from an ancient limestone grassland (Wardlow Hay Cop) in the Derbyshire Dales National Nature Reserve, UK, and sieved fresh (2 mm mesh). Nutrient addition studies have demonstrated P-limitation of plant productivity in this grassland[25]. The soil, which forms a thin layer (10cm max depth) directly over limestone, has a sandy loam texture with an organic matter content of 33% dry weight loss on ignition and a pH of 6.3. It was a humic rendzina of the Lulsgate Complex but as a result of acid deposition is transitioning to a ranker of the Wetton series (Umbric Leptosol)[34]. Total P content is 0.8 g P kg^{-1} soil with an $NaHCO_3$ (Olsen's) extractable phosphate concentration of 3.5 mg P kg^{-1} soil, DNA of 50 mg P kg^{-1} (by ^{31}P NMR) and acid extractable (1M HCl) calcium phosphate P of 65 mg P kg^{-1} soil[35-37].

Plant communities were grown in 1 L pots (Emorsgate, Kings Lynn, UK) either as monocultures or mixed plant communities containing all seven species. For the mixed communities the percentage seed-germination was determined in trials for each species (*A.*

capillaris 70%, *F. ovina* 15%, *L. corniculatus* 50%, *P. lanceolata* 85%, and *R. acetosa* 35%) and then seeds were added to pots in appropriate quantities to produce approximately similar numbers of plants. Due to very low germination rates, the sedge species were not seeded into the pots, but instead small individuals were collected as plants from the limestone grassland where the soil was taken, with 10 individuals per pot in the sedge monocultures, and 2 individuals per pot in the mixed communities. Communities were allowed to establish over 30 weeks in a climate controlled greenhouse (day/night temperatures of 18^{o}C/15^{o}C).

To study P-partitioning, three ^{33}P-labelled phosphorus sources were chosen as examples of the main types of P available to plants in soil: inorganic P (orthophosphate), organic P (DNA) and mineral P (calcium phosphate). ^{33}P labelled orthophosphate was purchased commercially (Perkin Elmer, UK).

Production of ^{33}P labelled DNA

^{33}P labelled DNA was synthesised from random primed DNA labelling kits (Roche Applied Science, West Sussex, UK) following the method developed by Feinberg and Vogelstein[38,39]. In a micro-centrifuge tube 2 µL of control DNA (λDNA 12.5 µg/mL) was first denatured by heating the DNA in a water bath at 95 ^{o}C for 10 minutes and then chilling quickly in an ice bath. After brief centrifugation, 2 µL of the supplied reaction mixture (hexanucleotide mixture in 10× concentrated reaction buffer) was added to the tube on ice. Then 5 µL of [α-^{33}P]dATP (3000 Ci/mmol), 5 µL of [α-^{33}P]dCTP (3000 Ci/mmol) and 6 µL of [α-^{33}P]dTTP (3000 Ci/mmol) (Perkin Elmer, UK) were added to the tube along with 1 µL of unlabelled dGTP. Finally, 1 µL of Klenow enzyme (2 U/µL in glycerol, 50% v/v) was added to the tube before it was centrifuged briefly and then incubated for 30 minutes at 37 ^{o}C in a water bath.

The reaction was stopped with 5 µL 0.2M EDTA (pH 8) and unincorporated nucleotides were removed using Sephadex G-50 Quick Spin Columns (Roche Applied Science, West Sussex, UK). Each run with the DNA kit produced 31 µl of ^{33}P DNA solution (3 MBq), 23 runs were performed in total. To determine ^{33}P incorporation, spin columns were oven-dried at 80 °C for 24 hours, weighed and digested in 1 mL of concentrated sulphuric acid and heated to 350 °C in a heating block (Grant Instruments, Shepreth, Cambridgeshire, UK) for 10 minutes. To achieve oxidation to a colourless solution 100 µL of 30% hydrogen peroxide was added. Digests were made up to 10 mL with distilled water, and a 1 mL aliquot was mixed with 10 mL of emulsifying scintillant (Ecosafe, Fisher Biosciences, UK) for liquid scintillation counting of ^{33}P (Packard Tri-carb 3100TR; Isotech).

Production of ^{33}P labelled calcium phosphate, and testing its solubility at soil pH

The calcium phosphate source was microcrystalline ^{33}P-hydroxyapatite. This was synthesised by adding 100 µL (3.6 MBq) of aqueous ^{33}P orthophosphate (Perkin Elmer, UK) to 1 mL 0.05M calcium nitrate and 0.8 mL 0.05M ammonium hydrogen orthophosphate in a 10 mL centrifuge tube. To this, 0.6 mL of 32% ammonia solution was added to raise the pH and speed up the reaction. After leaving to stand for one hour, and centrifuging at 805 ×*g* for 5 minutes, the supernatant containing unprecipitated reactants was removed with a 1 mL pipette. Remaining unprecipitated reactants were removed through repeated cycles of resuspension of the pellet in 5 ml distilled water, followed by centrifugation at 358 ×*g* for 5 minutes, and removal of the supernatant. Hand monitoring with a Geiger counter (Series 900 mini-monitor, Mini-Instruments Ltd, Burnham-on-Crouch, UK) of the pellet and the removed supernatant indicated when all the unprecipitated reactants had been removed. ^{33}P

incorporation was determined by liquid scintillation counting of the unprecipitated reactants and aliquots of calcium phosphate samples.

We investigated whether the synthesised ^{33}P-labelled calcium phosphate was largely insoluble at pH 6.3 of the limestone grassland soil (as would be expected of natural calcium phosphate). The P source was suspended in 6 mL distilled water to which HCl was added (10-70 uL) to provide a pH range (2.4-8.4) that extended above and below the soil pH. The suspension was centrifuged to form a pellet and an aliquot of supernatant was removed and phosphate measured by the Murphy-Riley method[40]. Between pH 8.4 and 4.7, less than 2% of the P was solubilised, at pH 3.8 less than 4% was solubilised. Under the most extreme acidity of pH 2.4, more than 25% was solubilised.

Application of ^{33}P-labelled compounds to established model plant communities

After 30 weeks of plant growth, 7 replicate turfs of each monoculture and mixed community were each supplied with 1 MBq ^{33}P (less than 4 ng ^{33}P) in the form of either orthophosphate, DNA or calcium phosphate. The P sources were injected in a total of 10 mL distilled water at 10 points across the soil surface to a depth of 5 cm using a syringe with a two-sideport needle which was slowly withdrawn during application to distribute the P through the soil. As the calcium phosphate was supplied as a hydroxyapatite suspension it was repeatedly whirlimixed between production and injection to prevent it from settling. This included whirlimixing when dividing stock into aliquots for addition to pots (with replicate aliquots scintillation counted to check radioactivity), and prior to loading into the syringe immediately before injection into the soil.

Monitoring of ^{33}P uptake into shoots over 6 days and its measurement in biomass.

After applying the different P-forms, ^{33}P uptake was monitored immediately by measuring the radioactive counts per second (CPS) with a hand-monitor Geiger counter and on successive days until harvesting.

Aboveground biomass was harvested six days following ^{33}P injection. On harvesting, plant biomass was sorted into species for the mixed communities. This biomass was freeze-dried, ground, homogenised and then *c*.50 mg was digested in 1 mL of concentrated sulphuric acid at 350^{o}C in a heating block for 10 minutes. The solution was cooled and cleared colourless with 100 µL of 30% hydrogen peroxide and made up to 10 mL with distilled water. A 1 mL aliquot was added to 10 mL of emulsifying scintillant and the ^{33}P content of the aboveground biomass was then determined by liquid scintillation counting.

Statistics and calculations

P uptake was expressed per gram dry weight of plant shoot. In Fig 1, to determine differences in species "preference" for P sources (i.e. relative uptake of a source compared to the other sources), relative uptake was expressed as ^{33}P uptake from that P source into one gram plant tissue as a % of each species summed ^{33}P uptake from all three sources into one gram plant tissue (equation (1)).

Equation (1):

$$\%P,i,c = \frac{P,i,c}{(\bar{U}\,ortho,c + \bar{U}\,DNA,c + \bar{U}\,CaP,c)} x100$$

%P,i,c is the relative uptake of ^{33}P from a source in replicate pot *i* of a species monoculture or mixed community type *c* from the ^{33}P source added to that replicate pot *i*. (*%P,i,c* are the individual data points shown on Fig.1). *P,i,c* is the ^{33}P uptake per g of plant tissue from the P source in replicate pot *i* (Bq in the plant shoot per g). $\bar{U}ortho,c$ is the mean uptake of ^{33}P per g plant tissue from all replicate pots supplied with ^{33}P-orthophosphate of species monoculture or mixed community type *c* for which *%P,i,c* is being calculated. $\bar{U}DNA,c$ $\bar{U}CaP,c$ are the equivalent means for ^{33}P uptake from all replicated pots receiving either DNA or calcium phosphate sources and containing species monoculture or mixed community type *c*. Data were subsequently arcsine square root transformed and analysed by General Linear Model, testing for P form, species and interaction effects. Because % uptake (data in Fig. 1) was expressed as a % of the P uptake from all three sources averaged across all replicates of a species, uptake from a single P source in a high ^{33}P uptake replicate can have a relative uptake of greater than 100%.

Changes in ^{33}P concentrations in shoots (Bq per g plant shoot) between mixed communities and monocultures were determined with *t*-tests (two-tailed, $\log_{10}+1$ transformed data).

The relationship between the net benefit of niche plasticity and species abundance in the wild was determined through linear regression of % cover abundance data taken from 30 field survey plots against the % gain in P uptake in monoculture verses mixed communities for each species (equation (2)).

Equation (2):

$$\%Plasticity,s = \left(\frac{\left((\bar{U}\ ortho,s,x\ \bar{U}\ DNA,s,x + \bar{U}\ CaP,s,x) - (\bar{U}\ ortho,sm\ \bar{U}\ DNA,s,m + \bar{U}\ CaP,s,m)\right)}{(\bar{U}\ ortho,s,m\ \bar{U}\ DNA,s,m + \bar{U}\ CaP,s,m)}\right) x100$$

%Plasticity,s is the P gain from niche plasticity for species *s* (i.e. the *x*-axis data on Fig. 3), which is the % gain in P uptake per g plant tissue from all three ^{33}P sources in mixed communities compared to when grown in monoculture. $\bar{U}$*ortho,s,m* is the mean uptake of ^{33}P per g plant tissue (Bq per g) from all replicate pots of species *s* monocultures receiving orthophosphate. $\bar{U}$*DNA,s,m* and $\bar{U}$*CaP,s,m* are the equivalent mean ^{33}P uptakes per g plant tissue (Bq per g) from all replicate monoculture pots of species *s* receiving either DNA or calcium phosphate as the P source. $\bar{U}$*ortho,s,x* $\bar{U}$*DNA,s,x*, $\bar{U}$*CaP,s,x* are the mean uptake of ^{33}P per g plant tissue (Bq per g) from all replicate pots of species *s* in mixed communities receiving orthophosphate, DNA or calcium phosphate respectively. % cover data were arcsine square root transformed prior to analysis. Plant survey data was collected from thirty 50 x 50 cm quadrats taken on the limestone grassland at Wardlow Hay Cop (Peak District National Park, UK) where the experiment's soil was taken from. Since replicate monoculture and mixed communities were not paired there are not replicate niche plasticity values, hence P gain from niche plasticity (*x*-axis data on Fig. 3) was expressed for each species as a single value. All data were analysed using IBM SPSS Statistics v24 (IBM, Portsmouth, UK).

Comparison of ^{33}P uptake from high versus low specific activity calcium phosphate by plants in monocultures and mixture communities.

In the main experiment, the calcium phosphate synthesised had a much lower specific activity (i.e. amount of radio-isotope ^{33}P compared to total P) than the orthophosphate and DNA. This is because commercial orthophosphate can be purchased "carrier free" (i.e. all P

is ^{33}P), and DNA synthesis using commercial kits ensures high rates of incorporation of ^{33}P. We were unable to synthesise solid calcium phosphate to the same high specific activity in a form that could be handled as suspension. Therefore, while the same amount of ^{33}P was added to the mesocosms from each P source, the amount of total P added (i.e. ^{33}P and ^{31}P) with the calcium phosphate was approaching 5 orders of magnitude greater than added in orthophosphate or DNA. This raises the question of whether adding greater amounts of total P in calcium phosphate might influence plant uptake responses (though we highlight that the solutions of orthophosphate and DNA are already very different supplies of P than the solid, crystalline, calcium phosphate suspension). In follow-up work the efficiency of ^{33}P incorporation during calcium phosphate synthesis was increased such that its specific activity was increased 17-fold compared to the calcium phosphate of the original study. This allowed work to compare plant uptake of ^{33}P from calcium phosphate of the original specific activity, with uptake from the new higher specific activity calcium phosphate.

To produce ^{33}P-labelled calcium phosphate of greater specific activity, the same synthesis methodology was used as for the main experiment but with a reduction in the quantities of reactants supplied while maintaining the amount of ^{33}P supplied (Supplementary Table 1). To compare the ^{33}P uptake from the low (original) and high (new) specific activity calcium phosphate sources (Extended Data Fig. 3), we established monoculture and mixed plant communities in plant pots in the same way as described for the main study. Each of these then had either the low or high specific activity calcium phosphate suspension injected into the soil. Subsequent harvesting and processing all followed the same procedures as in the main study. The only difference between this and the original study was that we used 0.75L

pots rather than 1L pots, and hence applied 0.75MBq of calcium phosphate to each, rather than the 1MBq used in the original study.

In addition to directly comparing the uptake of ^{33}P from high and low specific activity calcium phosphate, the ratio of uptake between high and low specific activity calcium phosphate for each species was used to adjust the original data on relative P source use (recalculation of Fig. 1, presented in Extended Data Fig. 4) and benefit of niche plasticity (recalculation of Fig. 3, presented in Extended Data Fig. 5). This was done for all plants in mixed communities and monocultures assuming that any differences in ^{33}P uptake between high and low specific activity calcium phosphate were true, whether statistically significant or not (i.e. a worst-case-scenario).

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Figure legends

Figure 1 | Relative use of different P sources. a, b, ^{33}P uptake from orthophosphate, calcium phosphate and DNA by plants grown in monoculture **(a)**, and in mixed communities **(b)**. Uptake from each P source is expressed as a % (its relative use or "preference") calculated as the ^{33}P per g plant tissue from that source as a % of the summed ^{33}P per gram plant tissue from all three sources. Error bars are one standard error of the mean (n = 6-7). Both monocultures (a) and mixed communities (b) show significant differences in relative P source use overall (P-source main effect) and in the patterns of P source use between species (P-source *x* species interactions).

Figure 2 | Niche shifts in P source use between monocultures and mixed communities. a – c, shoot concentrations of ^{33}P (KBq per g plant shoot) acquired from orthophosphate **(a)**, calcium phosphate **(b)**, and DNA **(c)**. Dotted line indicates equal shoot concentrations in monoculture and mixed communities. Species to the top left are more competitive for the P source in mixed communities, species to the bottom right are more competitive for the P source in monocultures. Error bars are one standard error (n = 6-7). *Significant differences in ^{33}P shoot concentration for that plant species in monoculture compared to mixed communities (*t*-test, two-tailed, $P < 0.05$).

Figure 3 | Relationship between species abundance and the benefit of niche plasticity for P uptake. Regression of species abundance in the wild against means of net benefit in P uptake from niche plasticity (i.e. % change in ^{33}P uptake from all three sources per g shoot biomass between monocultures and mixed communities). Dotted line indicates linear regression ($R^2 = 52.3\%$; $P < 0.001$). Error bars are one standard error. % cover data taken from 30 surveyed quadrats at the limestone grassland where the soil for the experiment was sourced.

Acknowledgements

This research was supported by the NERC, UK (grant NE/H01179X/1 awarded to GKP, DDC and JRL). We thank Irene Johnson for technical assistance.

Author Contributions

GKP, DDC and JRL designed the study. GKP analysed the data and wrote the manuscript. DAJ and SPM undertook the experimental work with the assistance of GKP, JRL and DDC. DDC, JRL, DAJ and SPM discussed the results and commented on the manuscript with GKP.

Competing Interest Statement

The authors declare no competing interests.

Data Availability

The ^{33}P uptake data and plant abundance data that support the findings of this study are available at the NERC's Environmental Information Data Centre (EIDC) with the identifier https://doi.org/10.5285/87cdc267-a8c7-4f59-83b4-1bceaae837ad

Figures:

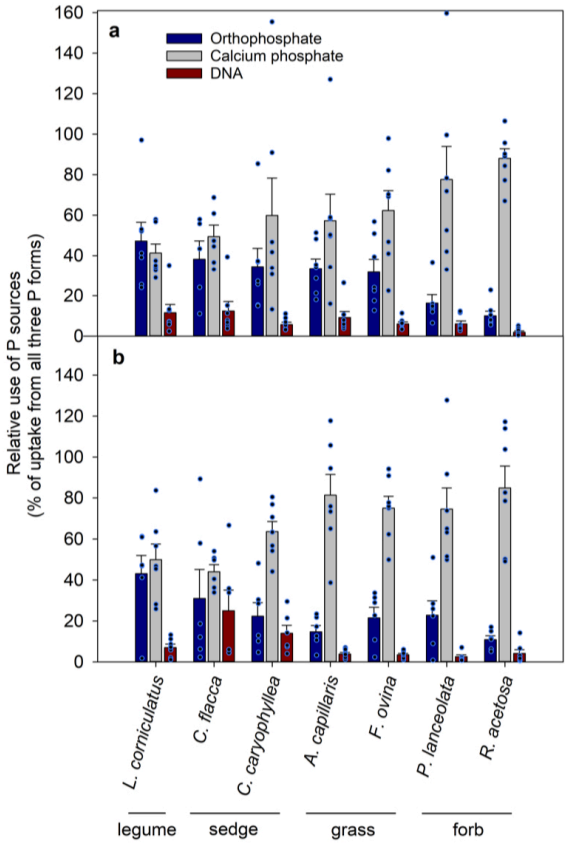

Fig. 1

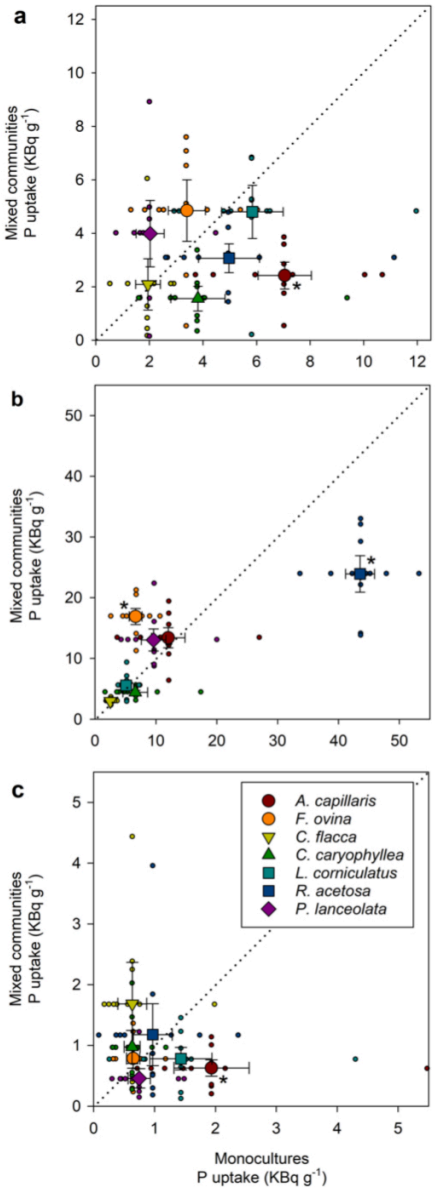
a
b
c
Mixed communities
P uptake (KBq g-1)
Monocultures
P uptake (KBq g-1)
A. capillaris
F. ovina
C. flacca
C. caryophyllea
L. corniculatus
R. acetosa
P. lanceolata

Fig. 2

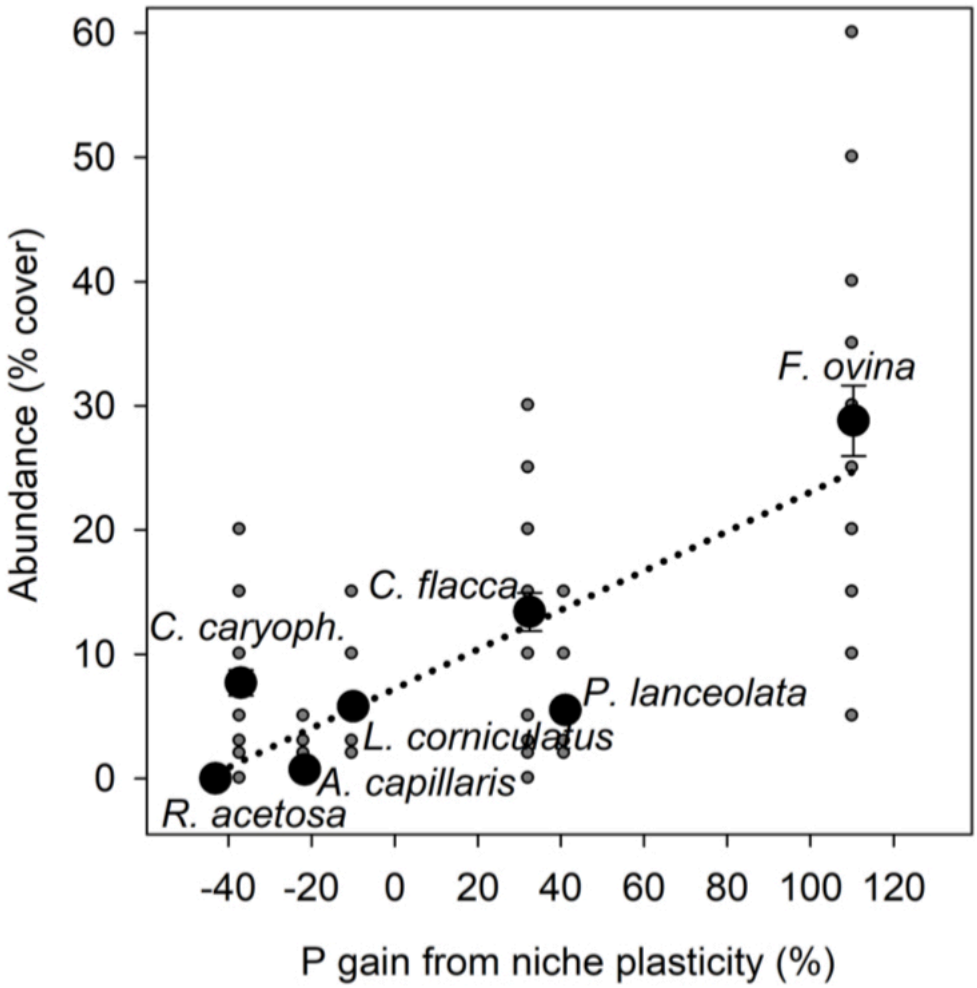
Abundance (% cover)
60
50
40
30
20
10
0
F. ovina
C. flacca
C. caryoph.
P. lanceolata
L. corniculatus
A. capillaris
R. acetosa
-40
-20
0
20
40
60
80
100
120
P gain from niche plasticity (%)

Fig. 3