A meta-analysis of phosphatase activity in agricultural settings in response to phosphorus deficiency

Victoria Janes-Bassett [a,b,*], Martin S.A. Blackwell [c], Gordon Blair [b], Jess Davies [a], Philip M. Haygarth [a], Malika M. Mezeli [a], Gavin Stewart [d]

[a] Lancaster Environment Centre, Lancaster University, Bailrigg, LA1 4YQ, UK
[b] School of Computing and Communications, Lancaster University, Bailrigg, LA1 4YQ, UK
[c] Sustainable Agricultural Systems, Rothamsted Research, North Wyke, EX20 2SB, UK
[d] School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK

ABSTRACT

Phosphorus (P) is a key limiting factor in crop growth and essential for agriculture. As plant uptake of P is inefficient, it is commonly applied to maintain crop yields leading to a range of negative environmental issues when applied in excess. Additionally, P in mineral fertilisers is derived from mined rock phosphate, which is a finite resource that needs to be sustainably managed in order to maintain food security in the long-term.

Phosphatase activity is one of several mechanistic responses to P deficiency in the plant-soil system, enabling the mineralization of organic P to increase P availability for both plants and soil organisms. In this study we address the need to further understanding of the role of phosphatase enzyme activity in P acquisition in agricultural settings, using a systematic review of the literature and subsequent meta-analysis.

We find that monoesterase activity is inhibited by availability of inorganic P (-23%, -39.8 to -2.2%) yet is enhanced by the availability of organic P ($+74\%$, 8.4–232.1%). This indicates that phosphatase enzyme activity is important in P deficient agricultural systems, yet that the availability of organic P is more important in determining phosphatase activity than the level of P deficiency. We also investigated the role of other factors such as nitrogen addition, pH of growth substrate and changes in plant composition and physiology but, none of these factors explained significant variance in the data. We highlight need for consistent recording and reporting of additional variables in association with phosphatase enzyme assay data, which is required to enable quantification of the potential utilisation of organic P resources in agriculture, and the contribution of phosphatase activity to P acquisition in both agricultural and semi-natural ecosystems.

1. Introduction

Phosphorus (P) is essential for agricultural production as crop growth is commonly limited by the availability of either nitrogen (N) and/or P. Fertilisers containing P are applied to agricultural land to maintain and enhance crop yields, with global averages of P fertiliser use estimated around 1.2 g P m^{-2} yr^{-1} (Lu and Tian, 2017). The vast majority of this P is not taken up by plants (Sattari et al., 2012), with excess application resulting in losses to rivers and lakes, causing negative impacts on water quality and exceedance of planetary boundaries (Conley et al., 2009; Ockenden et al., 2017; Powers et al., 2016; Steffen et al., 2015). Mineral-based P fertilisers (inorganic orthophosphate) are primarily derived from phosphate rock, a non-renewable and therefore finite resource, which further questions the long-term viability of current practices and security of food production (Cordell et al., 2009).

When added to the soil, orthophosphate is either taken up by plants or transformed into other inaccessible forms; becoming 'fixed' by sorption to other soil minerals or taken up by soil organisms and converted to organic forms upon metabolisation and decomposition. The limited availability and high competition for P in soils has resulted in fertiliser additions in excess of plant requirements to maintain optimal crop production leading to an accumulation of residual P in soil (Syers et al., 2008) referred to as 'legacy P' (Haygarth et al., 2014). Whilst much research has focused on the recovery of residual inorganic P (Doydora et al., 2020) a significant portion (up to 54%) of residual P in agricultural soils is in organic form (Stutter et al., 2012). Soil organic P could

---

therefore represent an important P resource for food production, and a mechanism for reducing the environmental impacts of agriculture; Menezes-Blackburn et al. (2018) estimated that monoesters accounted for 33% of P reserves globally within agricultural soils, equating to over 100 years of sufficient P supply for agricultural production. In order to address the global P imbalance Haygarth and Rufino (2021) note the need to understand which crop varieties can best utilise existing stores of P, enabling the shift to increased usage of legacy P and reduced dependence on fertiliser additions.

Plants respond to P deficiency by using less P and facilitating greater uptake of P in several ways, including morphological changes such as favouring root growth, cluster roots, and the formation of root hairs (foraging mechanisms) and the release of extracellular anions and phosphatase enzymes (mining mechanisms) (George et al., 2011; Vance et al., 2003; Wang and Lambers, 2020). Through these mining mechanisms, plants can alter the availability of P in soil, releasing ortho-phosphate from organic P forms. Phosphatases can originate from both plants and soil microorganisms, and whilst the majority of phosphatase enzyme activity in rhizosphere soil is thought to originate from microorganisms (Nannipieri et al., 2011) the relative contribution of each and utilisation of organic P by plants remains unclear (George et al., 2011).

Several studies have linked soil phosphatase activity to low soil inorganic P content, depletion of soil organic P and mineralization of organic P (Bünemann et al., 2012; Spohn et al., 2013; Tarafdar and Jungk, 1987). Additionally, phosphatase activity in low-P soils has been observed to be correlated with plant biomass and yields, indicating the potential importance of the activity of these enzymes in organic P mineralization and plant acquisition of P (Giles et al., 2017a, 2017b; Speir and Cowling, 1991). Organic P mineralization and enzymatic hydrolysis is influenced by a range of factors including soil physiochemical properties (such as temperature, moisture, pH), the chemical form of organic P, and soil microorganisms (Bünemann, 2015; Nash et al., 2014). Current understanding of the mechanisms associated with the transformation of organic to inorganic P is limited, and this information is crucial to understand the potential contribution of organic P to food security (George et al., 2018; Nash et al., 2014).

There remains a key knowledge gap in quantifying the role of phosphatase enzymes in relation to organic P access in agricultural environments. Previous meta-analyses have analysed the response of phosphatase enzymes to P deficit and N deposition (Chen et al., 2020; Marklein and Houlton, 2012; Xiao et al., 2018) yet these studies focus solely on (semi-)natural systems. Biogeochemical modelling (integrated carbon-nitrogen-phosphorus cycling) of agricultural systems has also highlighted this knowledge gap in relation to P cycling and the potential role of organic P cycling in supporting crop production and determining ecosystem C–N response (Janes-Bassett et al., 2020).

Here, we address the need to further scientific understanding of plant organic P accessibility, particularly in agricultural settings where utilisation of this significant source of P could provide a range of benefits including resilience of future food production. Through conducting a systematic review and subsequent meta-analysis, we consider the evidence for the role of phosphatase enzymes in P acquisition by comparing enzyme activity in response to P sufficient and P deficient conditions.

2. Methods

In order to collate all published data on agricultural plant-based phosphatase activity in response to P deficiency, a systematic review of the literature was conducted using the electronic databases Web of Science, Scopus and Google Scholar. Search terms used were soil* AND (phosphatase OR "phosphatase enzyme*") AND (plant OR root) AND ("organic phosphorus" OR phytate OR SOP OR phosphate OR mono-esters OR diesters OR "phosphorus pool" OR "phosphorus stock"). Searches were conducted in June 2020. The most relevant 1000 results from Google scholar were extracted using crawling software (Publish or Perish). Efforts were made to find publications translated into English

but if no translations were found non-English publications were excluded.

Studies were included that reported phosphatase activity (measured from root or rhizosphere soil) associated with agricultural plants (arable or grassland species), included phosphatase activity measurement for a control (no addition of P) and at least one experimental comparator (with P addition). For factorial experiments we only considered comparisons between control and treatments that differed solely in P addition. Hydroponic, soil-based pot and field experiments were all included, however enzyme activity recorded from bulk soil (as opposed to rhizosphere soil) was excluded. Studies that did not report the number of replicates, means and standard errors were excluded. In order to address the specific research question of the influence of P deficiency, plants inoculated with arbuscular mycorrhizal fungi (AMF) and P additions through manure were excluded from the study as these interventions are known to influence phosphatase activity. Data were extracted on phosphatase activity, type of phosphatase activity measured (e.g. monoesterase, diesterase or phytase), plant type(s), level of P addition, type of P addition (organic/inorganic), and methodology of phosphatase activity quantification (root intact or extract assay methods, rhizosphere soil assays). Where not stated, we distinguished between monoesterase and diesterase based on enzyme assay methodology detailed within each study. Within the literature we acknowledge the interchangeable use of terms of the terms 'monoesterase' and 'phosphomonoesterase', and therefore have chosen to use the term 'monoesterase' throughout for clarity.

Studies quantifying phosphatase activity using zymography were not included as these are not necessarily comparable enzyme assay methods. Where available, pH of soil/growth solution, total plant dry weight biomass, root and shoot P content, root:shoot ratio, and N addition (if applicable) were also recorded. Where not reported in table format, data was extracted using digitising software (WebPlotDigitzer).

Nutrient additions to substrates were converted to mols P in hydroponic experiments, and to mg kg^{-1} for soil-based experiments. Where additions were reported in kg ha^{-1} soil depth and bulk density (where not provided in the experiment details) were assumed as 25 cm and 1.5 g cm^3 respectively for conversion (similarly to Mezeli et al., 2020). Phosphorus content of root/shoot was converted to a percentage (or excluded where not possible) and dry weight biomass was converted to grams. A critical appraisal was conducted to assess the rigor of included studies through 3 domains: 1) number of replicates, 2) treatment allocation (purposive/randomised), 3) risk of baseline confounding (reporting of background P in substrate). For each domain studies were awarded a score of 0–2, similarly to (Haddaway et al., 2017), with summed scores providing validity categories (see supplementary information S1 for details).

Data were paired according to control and comparable experiment (no P and P addition) and analysed using the metafor package in R (Viechtbauer, 2010). Effect sizes per group were calculated from response ratios from individual studies (an index of response magnitude, calculated as experimental mean divided by control mean) and their associated variance (as per Hedges et al., 1999). These were then used to explore the influence of P deficiency on phosphatase activity across groups using random effects models. Results were considered significant where 95% confidence intervals did not cross 0. Due to the distinct differences between hydroponic and soil-grown plants, phosphatase enzyme types (monoesterase/phytase) and enzyme assay methodologies (root intact, root extract and rhizosphere soil activity) these data were grouped separately. Enzyme assay methods for root extract data involve grinding plant roots in a buffer solution which is centrifuged and used for the enzyme assay (e.g. Sharma and Sahi, 2011) whereas root intact assays assess enzyme activity on the root surface (e.g. Johnson et al., 1999). Soil rhizosphere assay methodologies involve sampling of soil strongly adhered to roots (e.g. Deng et al., 2018). Publication bias was assessed using funnel plots and Egger's regression test.

Mixed-effects models were then used to investigate moderators

influencing the effects of P addition on phosphatase activity. Initially, models were constructed using all available pairwise moderators including the following: magnitude of P addition (and N addition in soil-based studies), arable/grassland species, and organic/inorganic P addition. The Akaike Information Criterion (AIC) was used to guide multivariate model choice and reduce overfitting using the glmulti package (Calcagno and de Mazancourt, 2010) with a cut-off of 0.8 used to differentiate between essential and non-essential moderators (Calcagno and de Mazancourt, 2010; Chen et al., 2020).

To explore the potential mechanisms of phosphatase activity on P acquisition and plant growth we checked the remaining variables for correlations with phosphatase activity (pH of growth substrate, change in root:shoot ratio, plant biomass as dry weight, P content of plant root and shoot in response to P addition). As analysis was conducted on a complete case basis and several studies did not report on these variables, where a significant correlation was observed mixed-effects model selection was repeated using a subset of studies including this data. To mitigate the potential for bias introduced by small sample sizes and high dimensionality, where mixed-effects models included statistically significant moderators analyses were repeated using only data from studies within the high validity category (from the critical appraisal).

3. Results

3.1. Data included

In total we collated 163 paired observations from 37 studies listed in Table 1, including data on both monoesterase and phytase activity from hydroponic and soil-based experiments (results of the search and selection process and a full list of studies included within the meta-analysis can be found in supplementary information S2 and S3). Summary statistics of random effects models are presented in Table 2. Mixed effects models were then performed on all subsets of data presented in Table 2, below we present only models that included significant moderators.

3.2. Monoesterase – soil-based experiments

Across all soil-based root intact, root extract and rhizosphere soil experiments, monoesterase activity in experiments with P addition was not significantly different to controls. Between-experiment variation was statistically significant across all sub-groups as indicated by the heterogeneity of effect sizes (see Q values in Table 2). Effect sizes expressed as a percent are shown in Fig. 1. Forest and funnel plots, and outcomes of Egger's regression tests for each random effects model are shown in supplementary information S5.

Analysis using mixed effects models across root intact data indicated monoesterase activity in response to P addition was best explained by organic/inorganic addition (see supplementary information S8 for model-averaged importance of moderators). Inclusion of organic/inorganic P addition as a categorical moderator within the mixed effects model accounted for 46% of total heterogeneity, and indicated a 144% increase in monoesterase activity where organic P was added, in comparison to the control. Across all rhizosphere soil data, monoesterase activity was best explained by magnitude of P addition and organic/inorganic P addition. Inclusion of these moderators in the model explained 30% of heterogeneity, and indicated that for every mg g^{-1} increase in P addition, monoesterase activity decreased by 0.8% in comparison to control, and with organic P addition monoesterase activity increased by 65%.

Monoesterase activity in root intact data in response to P addition was significantly correlated with soil pH, change in biomass between control and P addition plants, and change in shoot P content of plants (R = −0.790 p < 0.01 n = 11, R = 0.857 p < 0.05 n = 7, R = −1.000 p < 0.01 n = 6 respectively, see supplementary information S9 for correlations with additional variables across all data subsets). When repeating model selection for the subset of studies reporting these variables, pH

Table 1

Publications used for the meta-analysis. Number of response ratios indicates the number of paired data (control/P addition) calculated from each publication.

Ref no.	Author and Year	No. of response ratios	Plant type(s)
1	Fries et al. (1998)	4	Maize
2	Gilbert et al. (1999)	4	Lupin
3	Johnson et al. (1999)	6	Ribwort plantain, Common bent
4	Colvan et al. (2001)	2	Various Grassland
5	Tarafdar and Claassen (2003)	12	Wheat
6	George et al. (2004)	2	Clover
7	Goicoechea et al. (2004)	1	Barley
8	Liu et al. (2004)	4	Maize
9	Fragoso et al. (2005)	3	Soyabean
10	Nuruzzaman et al. (2006)	4	Wheat, Lupin, Pea, Faba bean
11	Raiesi and Ghollarata (2006)	1	Clover
12	Du et al. (2009)	3	Stylo
13	Priya and Sahi (2009)	8	Duo Grass
14	Zhang et al. (2010)	4	Rapeseed
15	Ding et al. (2011)	2	Maize
16	Sharma and Sahi (2011)	8	Ryegrass
17	Bünemann et al. (2012)	1	Various Grassland
18	Tang et al. (2013)	1	Lupin
19	Abdel-Fattah et al. (2014)	1	Soyabean
20	Rotaru, 2015a	8	Soyabean
21	Al-Amri et al. (2016)	1	Corriander
22	Ding et al. (2016)	1	Various Grassland
23	Lyu et al., 2016a	14	Maize, Wheat, Rapeseed, Lupin, Soyabean, Faba bean, Chickpea
24	Zebrowska et al. (2017)	8	Oat
25	Deng et al. (2018)	10	Wheat
26	Ikoyi et al. (2018)	3	Ryegrass
27	Naureen et al. (2018)	3	Cucumber
28	Shen et al. (2018)	10	Wheat
29	Zebrowska et al. (2018)	3	Oat
30	Cardinale et al. (2019)	1	Barley
31	Chen et al. (2019)	6	Sedge, Ryegrass
32	Dey et al. (2019)	4	Cowpea
33	de Medeiros et al. (2019)	2	Maize
34	Redel et al. (2019)	12	Wheat, Oat, Barley
35	Bechtaoui et al. (2020)	2	Faba bean
36	Sun et al. (2020)	2	Maize, Alfalfa
37	Wang et al. (2020)	2	Various Grassland

and change in shoot P content were not included in model selection as a moderators, yet change in biomass was (see supplementary information S10). This indicated in plants with P addition 1% increase in biomass was associated with a 2.7% decrease in monoesterase activity compared to control plants.

Across experiments adding inorganic P, monoesterase activity was not significantly different to controls across root intact and rhizosphere soil data, but was significantly lower in root extract data (−23.27%, −39.83 to −2.16%, p < 0.05). For those adding organic P, monoesterase activity was significantly higher in rhizosphere soil compared to controls (+74.42%, 8.4–232.09%, p < 0.01).

Analysis using mixed effects models across rhizosphere soil data with inorganic P addition indicated that the magnitude of P addition best

Table 2

Summary statistics of random effects models for all data subgroups; n = number of paired observations, CI = confidence intervals, Q = heterogeneity among true effect sizes, RI = Root intact, RE = Root extract, RS = Rhizosphere soil.

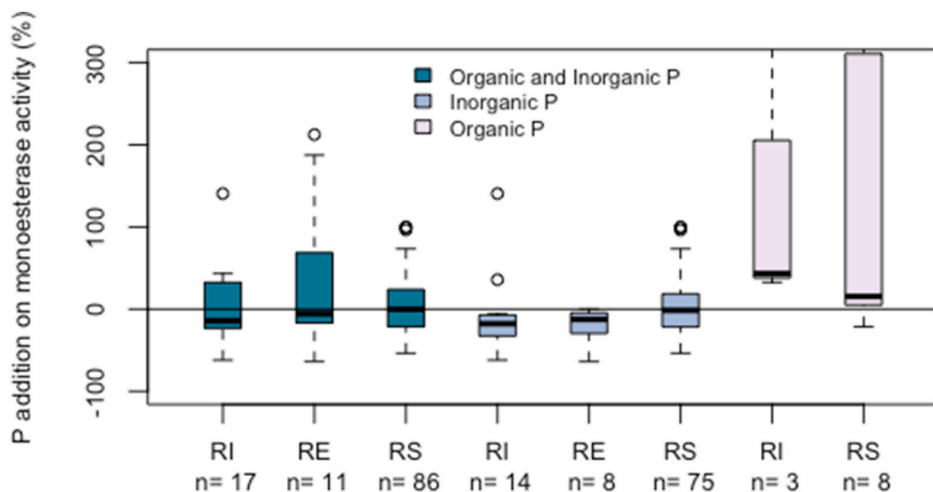| Enzyme	Soil/ Hydroponic	All/Organic/ Inorganic	Sub-group	n	No. Papers	Effect size	p-val (Effect size)	95% CI Lower	95% CI Upper	Q	p-val (Q)
Monoesterase	Soil	All	RI	17	4	−3.2	0.818	−26.3	27.3	228.1	<0.0001
			RE	11	3	9.4	0.658	−26.4	62.5	1200.1	<0.0001
			RS	86	21	−1.0	0.825	−9.5	8.3	953.9	<0.0001
		Inorganic	RI	14	4	−18.0	0.077	−34.2	2.2	110.9	<0.0001
			RE	8	3	−23.3	0.033	−39.8	−2.2	189.4	<0.0001
			RS	75	20	−6.6	0.087	−13.7	1.0	652.8	<0.0001
		Organic	RI	3	1	104.3	0.075	−6.9	348.4	33.7	<0.0001
			RE	0	0						
			RS	8	4	74.4	0.091	8.4	232.1	176.5	<0.0001
	Hydroponic	All	RI	20	8	−43.6	<0.0001	−57.1	−25.9	907.9	<0.0001
			RE	16	7	9.3	0.398	−11.1	34.4	41.2	<0.0001
		Inorganic	RI	13	8	−46.5	0.000	−61.6	−25.4	367.4	<0.0001
			RE	13	7	3.3	0.775	−17.6	29.5	466.3	<0.0001
		Organic	RI	7	4	−37.8	0.066	−62.4	3.1	366.9	<0.0001
			RE	3	2	40.1	0.145	−10.9	120.3	25.4	<0.0001
Phytase	Hydroponic	All	RI	5	4	291.8	0.005	50.4	920.4	33.7	<0.0001
			RE	8	4	12.0	0.365	−12.4	43.2	35.0	<0.0001
		Inorganic	RI	4	4	305.5	0.046	2.6	1501.7	21.6	<0.0001
			RE	5	4	1.7	0.939	−34.4	57.8	28.5	<0.0001
		Organic	RI	1	1	225.0	<0.0001	189.7	264.6	0.0	1
			RE	3	2	26.8	0.014	4.9	53.3	5.8	0.0545

Fig. 1. Response of monoesterase activity to phosphorus addition in soil-based experiments relative to control. RI = Root intact, RE = Root extract, RS = Rhizosphere soil. Colours indicate addition of organic/inorganic P/both. Boxplots show interquartile range and median, whiskers show 1.5 x interquartile range. Note: three RS experiments included addition of both inorganic and organic P, and therefore were not included in subgroups of organic/inorganic addition. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

explained monoesterase activity. Inclusion of this within the model accounted for 29% of heterogeneity, and indicated for every mg g^{-1} increase in P addition, monoesterase activity decreased by 0.7% compared to controls.

Monoesterase activity in root intact data in response to inorganic P addition was significantly correlated with change in shoot P content of plants (R = 1.000 p < 0.01 n = 6) yet when repeating model selection for the subset of studies reporting this variable, it was not included as a moderator. For the root extract subgroup, monoesterase response to inorganic P addition was significantly correlated with change in root: shoot ratio (R = 0.904 p < 0.01 n = 8) and was included as a moderator in model selection, indicating for every unit increase in root to shoot ratio in control plants, monoesterase activity increased by 420% compared with P addition plants.

These mixed effects models were repeated using only data with the highest critical appraisal scoring to assess the transitivity assumption. However, of the 17 data points of root intact monoesterase data only 1 met this criteria, and none of the 8 rhizosphere soil with organic P additions, meaning this could not be repeated on these subsets. Of the rhizosphere soil data (not specifying organic/inorganic additions) 18 of the 85 data met this criteria. Similarly, to using all data, the random

effects model did not indicate a significant difference between monoesterase activity in P addition and control plots (15.8%, −0.76 - +53.3%, p = 0.06), yet none of the moderators could explain significant variance to justify inclusion in the model. However, both magnitude of P addition and organic/inorganic P addition were still the most influential moderators (see supplementary information S11).

3.3. Monoesterase – hydroponic experiments

Monoesterase activity in hydroponic root intact experiments with P addition was significantly lower than controls (−43.6%, −57.06 to −25.92%, p < 0.0001). Root extract experiments showed no significant difference between experiment and controls. Between-experiment variation was statistically significant across all sub-groups as indicated by the heterogeneity of effect sizes (see Q values in Table 2). Effect sizes expressed as a percent are shown in Fig. 2. Forest and funnel plots, and outcomes of Egger's regression tests for each random effects model are shown in supplementary information S6. Tests indicate significant publication bias for root intact data, yet this may also be a factor of the high level of heterogeneity within the data. Mixed effects models did not indicate that organic/inorganic addition, magnitude of P addition or
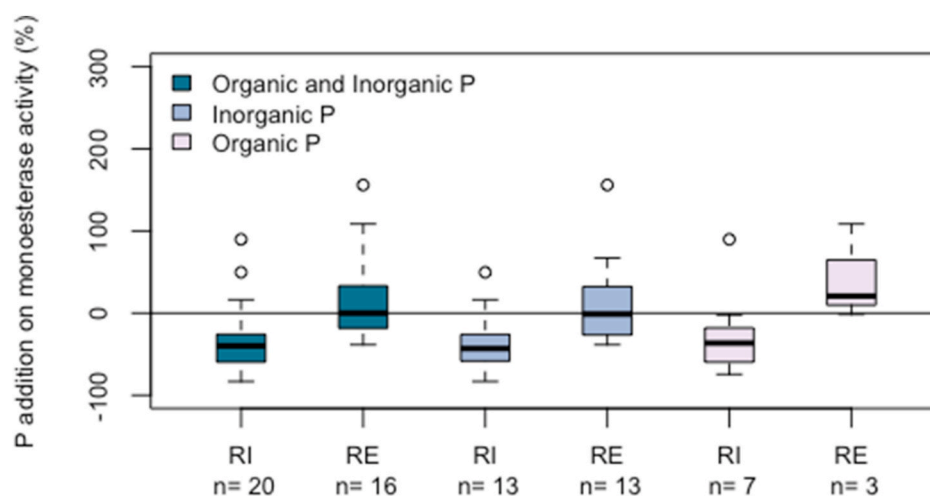
Fig. 2. Response of monoesterase activity to phosphorus addition in hydroponic experiments relative to control. RI = Root intact, RE = Root extract. Colours indicate addition of organic/inorganic P/both. Boxplots show interquartile range and median, whiskers show 1.5 x interquartile range. **(For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)**

arable/grassland species could account for significant heterogeneity of monoesterase activity in either root intact or extract hydroponic data.

Monoesterase activity across root intact experiments with inorganic P addition was significantly lower than controls (−46.46%, −61.55 to −25.45%, p < 0.001). Root extract experiments with inorganic P, and both root extract and intact experiments with organic P addition showed no significant differences in monoesterase activity compared with controls. No moderators could explain significant heterogeneity within mixed effects models across any of the hydroponic monoesterase data groups. Monoesterase activity from hydroponic data in response to P addition was not significantly correlated with any additional variables (see supplementary information S9).

3.4. Phytase – hydroponic experiments

Phytase activity in hydroponic root intact experiments with P addition was significantly higher than controls (291.8%, 50.44–920.40%, p < 0.01). Root extract experiments showed no significant difference to controls. Between-experiment variation was statistically significant across all sub-groups as indicated by the heterogeneity of effect sizes (see Q values in Table 2). Effect sizes expressed as a percent are shown in Fig. 3. Forest and funnel plots, and outcomes of Egger's regression tests

for each random effects model are shown in supplementary information S7. Mixed effects models did not indicate that organic/inorganic addition, magnitude of P addition or arable/grassland species could account for significant heterogeneity of phytase activity in either root intact or extract data.

Root intact hydroponic experiments with inorganic P additions showed significantly greater phytase activity compared with controls (305.5%, 2.65–1501.72%, p < 0.05). Root intact experiments with organic P additions also showed higher activity than controls, however only one data point was available. Root extract experiments with inorganic or organic P additions showed no significant difference in phytase activity to controls.

Mixed effects models did not indicate that inorganic P addition, magnitude of P addition or arable/grassland species could account for significant heterogeneity of phytase activity in either root intact or extract hydroponic data. Mixed effects models could not be created for phytase activity with organic P additions due to insufficient data. Phytase activity from hydroponic data in response to P addition was not significantly correlated with any additional variables (see supplementary information S9).
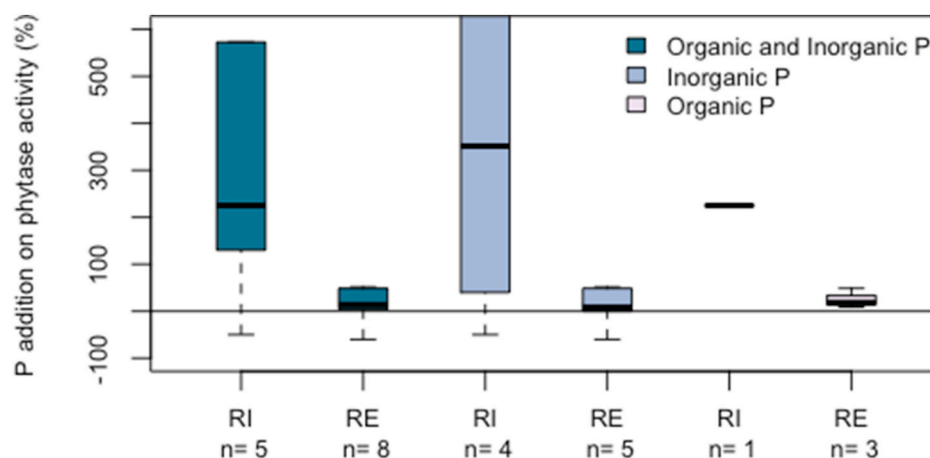

Fig. 3. Response of phytase activity to phosphorus addition in hydroponic experiments relative to control. RI = Root intact, RE = Root extract. Colours indicate addition of organic/inorganic P/both. Boxplots show interquartile range and median, whiskers show 1.5 x interquartile range. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

This meta-analysis demonstrates that monoesterase activity is inhibited in plant roots and the rhizosphere soil of agricultural ecosystems by inorganic P addition and stimulated under P deficient conditions. Whilst this result is statistically significant in root extract data only, we observe a similar trend across both root intact and rhizosphere soil data also. This consistent evidence suggesting phosphatase inhibition with addition of inorganic P is similar to comparable meta-analyses conducted in semi-natural ecosystems (Margalef et al., 2021; Marklein and Houlton, 2012; Xiao et al., 2018). Several previous studies of agricultural ecosystems (not included in this meta-analysis due to inclusion criteria) have also observed inhibition of phosphatase activity in soils due to addition of phosphate (Brandt et al., 2011; Nannipieri et al., 1978; Yadav and Tarafdar, 2001). Additionally, application of inorganic P inhibits the expression of *PHO* genes and therefore can repress the synthesis of phosphomonoesterase in soil (Oshima et al., 1996).

Conversely, we found monoesterase activity is enhanced in plant roots and the rhizosphere soil of agricultural ecosystems by organic P addition. Whilst statistically significant in rhizosphere soil data only, this trend was also observed from root intact enzyme assays and is consistent with literature evidence suggesting that phosphatase activity increases with organic P addition (Brandt et al., 2011; Yadav and Tarafdar, 2001) and is regulated by organic matter content (Sinsabaugh et al., 2008; Štursová and Baldrian, 2011). Additionally, Jarosch et al. (2019) observed mineralization of organic P by non-phytate phosphomonoesters was limited by organic P substrate rather than enzyme availability.

Our results indicate that the magnitude of monoesterase stimulation as a result of organic P addition is much greater than that of inhibition as a result of inorganic P addition (+74% with organic P addition, −23% with inorganic P addition). This suggests monoesterase activity is substrate limited and may be influenced more by substrate availability than level of P deficiency. Where organic P stores, and legacy P are sufficient in agricultural settings, this finding indicates potential enhancement of phosphatase activity, and therefore the potential of this source of P in maintaining crop yields.

Our analysis indicated level of P addition had a significant influence on phosphatase response across several of the soil-based data subsets (root intact, rhizosphere soil and rhizosphere soil under inorganic P addition), with greater inhibition of monoesterase activity in P addition experiments relative to controls as the level of P addition increases. This suggests that as the level of P deficiency increases, so does monoesterase activity (or as level of P availability increases monoesterase activity decreases) and is similar to findings from previous studies (Margalef et al., 2021; Spiers and McGill, 1979; Yadav and Tarafdar, 2001). Level of P addition could not explain significant variance in organic P addition experiments, likely due to the considerably smaller sample size of these data subsets.

Across root intact data (both organic and inorganic P additions), increased biomass in plants with P addition was linked to further inhibition of monoesterase, likely indicating that with increasing P availability, monoesterase activity decreases. Across root extract data with inorganic P addition, as root:shoot ratio of control plants increased, monoesterase activity increased relative to P addition. This follows previous studies that have observed a correlation between root length density and phosphatase activity (Schneider et al., 2001) and indicates increased root growth as a plant response to P deficiency, increasing its P scavenging potential, combined with either enhanced plant-exuded phosphatases and/or excretion of organic anions and stimulation of soil microbial phosphatases (Giles et al., 2012; Wang et al., 2016).

All mixed effects models across soil-based data sub-groups showed significant heterogeneity after inclusion of significant moderators (where applicable), indicating considerable unexplained variance in the data. Our analysis did not indicate a significant influence of factors such as N addition, arable/grassland species, pH, or plant P content. Most

surprising, perhaps, is the lack of a significant influence of N on phosphatase activity in agricultural settings; synthesis of phosphatase enzymes requires high levels of N, and as a result several previous studies and meta-analyses of semi-natural systems have observed enhancement of phosphatase activity with increasing N addition (Chen et al., 2020; Margalef et al., 2021; Marklein and Houlton, 2012; Xiao et al., 2018). Additionally, as ecosystems are often limited by either N or P, addition of N can push systems to P limitation, resulting in greater phosphatase activity (Johnson et al., 1999; Phoenix et al., 2004). Not all experiments within the meta-analysis conducted in this study included N addition as an experimental variable, and background levels of N availability between soil-based experiments will vary naturally and were not always reported which could partly explain the lack of observed relationship here. Additionally, 32 of the included 163 paired data points observed phosphatase activity in association with leguminous plants (soil-based monoesterase; RI n = 4, RE n = 1, RS n = 17, hydroponic monoesterase RI n = 3, RE n = 4, hydroponic phytase RI = 2, RE = 1) which could nullify the impacts of N addition due to N fixation by these species. However, Chen et al. (2019) showed long-term N addition decreased soil phosphatase activity due to shifts in microbial communities to those with the capacity to mineralise P and Chen et al. (2020) found N induced phosphatase activity decreased over time. As agricultural systems commonly rely heavily on inputs of both N and P, field-based experiments included within this analysis are likely to have undergone N addition for considerable periods meaning that N is in sufficient supply/excess, therefore this could also be a factor in the lack of significance observed in this study.

The magnitude of P deficiency in control vs P addition in soil-based experiments will also vary between studies, partly due to varying soil types, and is likely to result in significant heterogeneity within the data. Phosphorus deficiency will be influenced strongly by background P levels in soil (which are not always reported) and variation in susceptibility of plant species studied to P deficient conditions. Whilst studies included aimed to compare P deficient and sufficient conditions, it is not possible to quantify and directly compare the level of P deficiency across studies and this is a limitation of the meta-analysis approach used here.

Monoesterase activity in root intact hydroponic experiments was inhibited with P addition across all types of P addition and with inorganic P, similarly to soil-based experiments. No other statistically significant results were observed across hydroponic monoesterase activity, yet the data indicated these experiments broadly behaved differently to soil-based experiments, which has been observed in several previous studies (Jones and Oburger, 2011), particularly as none of the moderators could explain a significant amount of heterogeneity in the data. Root extract activity in particular showed no clear effect as a result of P deficiency, regardless of organic/inorganic P addition, which could be in part due to the small sample sizes of these sub-groups (max n = 16). Given the well-known observed differences between soil-based and hydroponically grown plants, this raises the question if experiments should be carried out hydroponically and how well understanding from this data can be translated to the field? However, due to the control over hydroponic conditions, these paired data compare truly P deprived plant growth with P sufficient growth, which could partly explain the differences observed between soil-based and hydroponic data.

Phytase activity in hydroponic experiments also behaved differently to soil-based monoesterase, with an increase in phytase activity observed in root intact experiments with inorganic P addition. The contribution of plant-derived phytase in P acquisition is questionable, as previous studies have observed low levels of root-exuded phytase suggesting most phytase comes from microbial rather than plant sources (Hunter et al., 2014; Richardson et al., 2000). Furthermore, soil phytate and phytase are rapidly adsorbed onto mineral components in soil (George et al., 2005), and Jarosch et al. (2019) found that in contrast to monoesters, phytate hydrolysis was enzyme limited. As such, only a small amount of the total soil phytate pool is likely to be plant-available.

Various other factors known to influence phosphatase enzyme

activity have not been considered within this analysis including water availability; whilst Brandt et al. (2011) observed an increase in phosphatase activity in response to drought conditions, Margalef et al. (2021) observed a decrease with recurring drought conditions. There is evidence that in some plants the root architectural responses to drought and to P deficiency in soils are similar. This is driven by the fact that both limitations can be alleviated by the exploration of greater volumes of soil by roots to find pools of water or phosphate, and by increasing the area of root in contact with the soil when resources are encountered. For example, root extension into deeper soil layers during periods of drought can provide access to sub-soil water (Wasaya et al., 2018), while in P deficient soils, the strategy of growing long, thin roots enables exploitation of recently weathered bedrock which may contain phosphate that has not been exploited by shallower rooting plants (Steingrobe, 2001; Yuan et al., 2016). Another strategy that is used to address both limitations is the development and proliferation of root hairs upon encountering either soil water or available P. In both cases, this increases the total root surface area at a relatively low carbon cost to the plant and enhances the ability to take up water (Wasaya et al., 2018) and P (Dolan, 2001). Whether or not this is reflected by phosphatase enzyme activity is unclear, but it does mean that in some cases the spatial distribution of phosphatase activity within the soil may be similar under both conditions.

Enzyme activity in field-based studies has been shown to vary throughout the year (Grierson and Adams, 2000; Schneider et al., 2001). Several studies, some of which are included within the meta-analysis, demonstrate variation in phosphatase activity amongst plant species (Lyu et al., 2016; Redel et al., 2019; Rotaru, 2015), with greater activity observed in species tolerant of P deficiency regardless of their P status (Zhang et al., 2009). Whilst plant species were recorded within data collection for this meta-analysis, due to sizes of data sub-groups only an arable/grassland categorisation was possible to include within statistical analyses and no significant difference was observed between these groups.

Other limitations of the meta-analysis considered here include the enzyme assay methodologies used, which we have attempted to minimise by sub-grouping data according to methodological choice: root intact, root extract and rhizosphere soil assay. It is important to recognise that all assays included in this meta-analysis measure potential, as opposed to real, enzymatic activity (Nannipieri et al., 2011). Additionally, Bünemann (2015) highlighted the relationship between observed organic P mineralization rates and monoesterase activity; whilst linear within studies, disparities exist between studies, highlighting optimal conditions of assay methods (such as pH and temperature) and how this varies between studies. Whilst zymography can provide a measure of actual activity these are not comparable with assay data used in this study. The systematic review found only two papers that included zymography that met the inclusion criteria (Spohn et al., 2015; Spohn and Kuzyakov, 2013), which is an insufficient sample size for statistical meta-analysis.

In order to conduct the statistical analysis here, studies were only included that compared control (no P addition) with P addition, and reported mean phosphatase activity, standard errors and sample sizes. Several studies were excluded due to omission of these comparisons/ information (see supplementary information S4). Due to the limited number of studies and small sample sizes for certain sub-groups of data, the analysis is susceptible to biasing due to researcher degrees of freedom. The influence of this was mitigated through testing of the transitivity assumption; repeating analyses based on critical appraisal scoring. The high dimensionality of the data (numerous factors influencing phosphatase activity) is further complicated by the complete case analysis resulting in further reductions in sample size. Small sample sizes of the sub-groups analysed limits confidence in the conclusions drawn and was particularly an issue when investigating the influence of additional moderators (such as pH, change in biomass, plant stoichiometries). Consistent recording and reporting of this data would be a

huge benefit for future meta-analyses. Future research should seek to quantify the contribution of phosphatase activity to P acquisition in agricultural, and semi-natural ecosystems. To do this additional data such as P mineralization rates would be required in conjunction with data from enzyme assays. Additionally, we acknowledge the potential for variations on studies returned from literature searches based on the search terms used. However, in using a range of keywords associated with phosphatase activity we feel other possible search variants would have little influence on the final data set used and therefore would not likely significantly affect the findings.

5. Conclusions

This meta-analysis evaluates current available data to establish the importance of phosphatase enzyme activity in relation to P acquisition in agricultural settings in P deficient and sufficient conditions. We find that inorganic P addition decreases monoesterase activity associated with agricultural plants in both soil and hydroponic settings, indicating that both the plant and soil microbial community play a role in responding to P deficit in this way. Statistical analysis of the data indicates the availability of organic P is an important control of monoesterase activity in rhizosphere soils, more so than the level of P deficiency. Whilst difficult to determine what phosphatase enzymes are plant/soil derived, consistent results from both hydroponic and soil-based data indicate both likely have a role to play.

Our analysis indicated N addition did not have a significant influence on phosphatase activity in agricultural settings. However, it is worth noting that not all studies included here conducted varying N addition as an experimental variable, background levels of N were not always reported, and some studies evaluated phosphatase activities in association with legumes which could nullify the impacts of N addition. Where N supply is already sufficient/in excess, which is likely to be common in agricultural settings, further additions of N are unlikely to enhance phosphatase activity, and this could partly explain the lack of significance observed here.

Unanswered questions are how much phosphatase activity contributes as a process to P acquisition. To answer this, phosphatase activity in conjunction with background P levels, P mineralization rates, and data on plant growth (biomass and plant stoichiometries) are needed, under varying and known conditions of N availability.

Combined, these findings indicate in agricultural settings phosphatase enzyme activity plays an important role in P acquisition from organic sources, and further research should seek to quantify their role to enable utilisation this P source to enhance resilience of food production.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108537.

References

Abdel-Fattah, G.M., Asrar, A.A., Al-Amri, S.M., Abdel-Salam, E.M., 2014. Influence of arbuscular mycorrhiza and phosphorus fertilization on the gas exchange, growth and phosphatase activity of soybean (Glycine max L.) plants. Photosynthetica 52, 581–588. https://doi.org/10.1007/s11099-014-0067-0.

Al-Amri, S.M., Elhindi, K.M., Sharaf El-Din, A.F., 2016. Effects of arbuscular mycorrhizal fungus Glomus mosseae and phosphorus application on plant growth rate, essential oil content and composition of coriander (Coriandrum sativum L.). Progress in Nutrition 18, 443–454.

Bechtaoui, N., Raklami, A., Benidire, L., Tahiri, A., Gottfert, M., Oufdou, K., 2020. Effects of PGPR Co-inoculation on growth, phosphorus nutrition and phosphatase/phytase activities of faba bean under different phosphorus availability conditions. Polish Journal of Environmental Studies 29, 1557–1565. https://doi.org/10.15244/pjoes/110345.

Brandt, C., Balko, C., Eichler-Loebermann, B., 2011. Interactive effects of soil water content and phytin supply on phosphorus nutrition of different crops species. Landbauforschung 61, 317–325.

Bünemann, E.K., 2015. Assessment of gross and net mineralization rates of soil organic phosphorus - a review. Soil Biology and Biochemistry 89, 82–98. https://doi.org/10.1016/j.soilbio.2015.06.026.

Bünemann, E.K., Oberson, A., Liebisch, F., Keller, F., Annaheim, K., Huguenin-Elie, O., Frossard, E., 2012. Rapid microbial phosphorus immobilization dominates gross phosphorus fluxes in a grassland soil with low inorganic phosphorus availability. Soil Biology and Biochemistry.

Calcagno, V., de Mazancourt, C., 2010. glmulti: an R package for easy automated model selection with (generalized) linear models. Journal of Statistical Software 34, 29. https://doi.org/10.18637/jss.v034.i12.

Cardinale, M., Suarez, C., Steffens, D., Ratering, S., Schnell, S., 2019. Effect of different soil phosphate sources on the active bacterial microbiota is greater in the rhizosphere than in the endorhiza of barley (Hordeum vulgare L.). Microbial Ecology 77, 689–700. https://doi.org/10.1007/s00248-018-1264-3.

Chen, J., van Groenigen, K.J., Hungate, B.A., Terrer, C., van Groenigen, J.W., Maestre, F. T., Ying, S.C., Luo, Y., Jørgensen, U., Sinsabaugh, R.L., Olesen, J.E., Elsgaard, L., 2020. Long-term nitrogen loading alleviates phosphorus limitation in terrestrial ecosystems. Global Change Biology 26, 5077–5086. https://doi.org/10.1111/gcb.15218.

Chen, Xiao, Hao, B., Jing, X., He, J.-S., Ma, W., Zhu, B., 2019. Minor responses of soil microbial biomass, community structure and enzyme activities to nitrogen and phosphorus addition in three grassland ecosystems. Plant and Soil 444, 21–37. https://doi.org/10.1007/s11104-019-04250-3.

Chen, Xiaodong, Jiang, N., Condron, L.M., Dunfield, K.E., Chen, Z., Wang, J., Chen, L., 2019. Soil alkaline phosphatase activity and bacterial phoD gene abundance and diversity under long-term nitrogen and manure inputs. Geoderma 349, 36–44. https://doi.org/10.1016/j.geoderma.2019.04.039.

Colvan, S.R., Syers, J.K., O'Donnell, A.G., 2001. Effect of long-term fertiliser use on acid and alkaline phosphomonoesterase and phosphodiesterase activities in managed grassland. Biology and Fertility of Soils 34, 258–263. https://doi.org/10.1007/s003740100411.

Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, C., Likens, G.E., 2009. Ecology - controlling eutrophication: nitrogen and phosphorus. Science 323, 1014–1015. https://doi.org/10.1126/science.1167755.

Cordell, D., Drangert, J.O., White, S., 2009. The story of phosphorus: global food security and food for thought. Global Environmental Change 19, 292–305. https://doi.org/10.1016/j.gloenvcha.2008.10.009.

de Medeiros, E.V., Silva, A.O., Duda, G.P., dos Santos, U.J., de Souza Junior, A.J., 2019. The combination of Arachis pintoi green manure and natural phosphate improves maize growth, soil microbial community structure and enzymatic activities. Plant and Soil 435, 175–185. https://doi.org/10.1007/s11104-018-3887-z.

Deng, Y., Teng, W., Tong, Y.-P., Chen, X.-P., Zou, C.-Q., 2018. Phosphorus efficiency mechanisms of two wheat cultivars as affected by a range of phosphorus levels in the field. Frontiers in Plant Science 9, 1614. https://doi.org/10.3389/fpls.2018.01614.

Dey, S.K., Chakrabarti, B., Purakayastha, T.J., Prasanna, R., Mittal, R., Singh, S.D., Pathak, H., 2019. Interplay of phosphorus doses, cyanobacterial inoculation, and elevated carbon dioxide on yield and phosphorus dynamics in cowpea. Environmental Monitoring and Assessment 191. https://doi.org/10.1007/s10661-019-7378-3.

Ding, N., Guo, H., Kupper, J.V., McNear Jr., D.H., 2016. Shoot specific fungal endophytes alter soil phosphorus (P) fractions and potential acid phosphatase activity but do not increase P uptake in tall fescue. Plant and Soil 401, 291–305. https://doi.org/10.1007/s11104-015-2757-1.

Ding, X., Fu, L., Liu, C., Chen, F., Hoffland, E., Shen, J., Zhang, F., Feng, G., 2011. Positive feedback between acidification and organic phosphate mineralization in the rhizosphere of maize (Zea mays L.). Plant and Soil 349, 13–24. https://doi.org/10.1007/s11104-011-0783-1.

Dolan, L., 2001. The role of ethylene in root hair growth in Arabidopsis. Journal of Plant Nutrition and Soil Science 164, 141–145. https://doi.org/10.1002/1522-2624(200104)164:2<141::AID-JPLN141>3.0.CO;2-Z.

Doydora, S., Gatiboni, L., Grieger, K., Hesterberg, D., Jones, J.L., McLamore, E.S., Peters, R., Sozzani, R., Van den Broeck, L., Duckworth, O.W., 2020. Accessing legacy phosphorus in soils. Soil Systems 4, 1–22. https://doi.org/10.3390/soilsystems4040074.

Du, Y.-M., Tian, J., Liao, H., Bai, C.-J., Yan, X.-L., Liu, G.-D., 2009. Aluminium tolerance and high phosphorus efficiency helps Stylosanthes better adapt to low-P acid soils. Annals of Botany 103, 1239–1247. https://doi.org/10.1093/aob/mcp074.

Fragoso, S., Martínez-Barajas, E., Vázquez-Santana, S., Acosta, J., Coello, P., 2005. Soybean (Glycine max) response to phosphate deficiency. Agrociencia 39, 303–310.

Fries, L.L.M., Pacovsky, R.S., Safir, G.R., Kaminski, J., 1998. Phosphorus effect on phosphatase activity in endomycorrhizal maize. Physiologia Plantarum 103, 162–171. https://doi.org/10.1034/j.1399-3054.1998.1030203.x.

George, T.S., Fransson, A.-M., Hammond, J.P., White, P.J., 2011. Phosphorus nutrition: rhizosphere processes, plant response and adaptations. In: Bünemann, E., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 245–271. https://doi.org/10.1007/978-3-642-15271-9_10.

George, T.S., Giles, C.D., Menezes-Blackburn, D., Condron, L.M., Gama-Rodrigues, A.C., Jaisi, D., Lang, F., Neal, A.L., Stutter, I.M., Almeida, D.S., Bol, R., Cabugao, K.G., Celi, L., Cotner, J.B., Feng, G., Goll, D.S., Hallama, M., Krueger, J., Plassard, C., Rosling, A., Darch, T., Fraser, T., Giesler, R., Richardson, A.E., Tamburini, F., Shand, C.A., Lumsdon, D.G., Zhang, H., Blackwell, M.S.A., Wearing, C., Mezeli, M. M., Almas, A.R., Audette, Y., Bertrand, I., Beyhaut, E., Boitt, G., Bradshaw, N., Brearley, C.A., Bruulsema, T.W., Ciais, P., Cozzolino, V., Duran, P.C., Mora, M.L., de Menezes, A.B., Dodd, R.J., Dunfield, K., Engl, C., Frazao, J.J., Garland, G., Jimenez, J.L.G., Graca, J., Granger, S.J., Harrison, A.F., Heuck, C., Hou, E.Q., Johnes, P.J., Kaiser, K., Kjaer, H.A., Klumpp, E., Lamb, A.L., Macintosh, K.A., Mackay, E.B., McGrath, J., McIntyre, C., McLaren, T., Meszaros, E., Missong, A., Mooshammer, M., Negron, C.P., Nelson, L.A., Pfahler, V., Poblete-Grant, P., Randall, M., Seguel, A., Seth, K., Smith, A.C., Smits, M.M., Sobarzo, J.A., Spohn, M., Tawaraya, K., Tibbett, M., Voroney, P., Wallander, H., Wang, L., Wasaki, J., Haygarth, P.M., 2018. Organic phosphorus in the terrestrial environment: a perspective on the state of the art and future priorities. Plant and Soil 427, 191–208. https://doi.org/10.1007/s11104-017-3391-x.

George, T.S., Richardson, A.E., Hadobas, P.A., Simpson, R.J., 2004. Characterization of transgenic Trifolium subterraneum L. which expresses phyA and releases extracellular phytase: growth and P nutrition in laboratory media and soil. Plant, Cell and Environment 27, 1351–1361. https://doi.org/10.1111/j.1365-3040.2004.01225.x.

George, T.S., Richardson, A.E., Simpson, R.J., 2005. Behaviour of plant-derived extracellular phytase upon addition to soil. Soil Biology and Biochemistry 37, 977–988. https://doi.org/10.1016/j.soilbio.2004.10.016.

Gilbert, G.A., Knight, J.D., Vance, C.P., Allan, D.L., 1999. Acid phosphatase activity in phosphorus-deficient white lupin roots. Plant, Cell and Environment 22, 801–810. https://doi.org/10.1046/j.1365-3040.1999.00441.x.

Giles, C.D., Brown, L.K., Adu, M.O., Mezeli, M.M., Sandral, G.A., Simpson, R.J., Wendler, R., Shand, C.A., Menezes-Blackburn, D., Darch, T., Stutter, M.I., Lumsdon, D.G., Zhang, H., Blackwell, M.S.A., Wearing, C., Cooper, P., Haygarth, P. M., George, T.S., 2017a. Response-based selection of barley cultivars and legume species for complementarity: root morphology and exudation in relation to nutrient source. Plant Science 255, 12–28. https://doi.org/10.1016/j.plantsci.2016.11.002.

Giles, C.D., George, T.S., Brown, L.K., Mezeli, M., Shand, C.A., Richardson, A.E., Mackay, R., Wendler, R., Darch, T., Menezes-Blackburn, D., Cooper, P., Stutter, M.I., Lumsdon, D.G., Blackwell, M.S.A., Wearing, C., Zhang, H., Haygarth, P.M., 2017b. Linking the depletion of rhizosphere phosphorus to the heterologous expression of a fungal phytase in Nicotiana tabacum as revealed by enzyme-labile P and solution 31P NMR spectroscopy. Rhizosphere 3, 82–91. https://doi.org/10.1016/j.rhisph.2016.11.004.

Giles, C.D., Richardson, A.E., Druschel, G.K., Hill, J.E., 2012. Organic anion-driven solubilization of precipitated and sorbed phytate improves hydrolysis by phytases and bioavailability to Nicotiana tabacum. Soil Science 177, 591–598. https://doi.org/10.1097/SS.0b013e318272f83f.

Goicoechea, N., Sánchez-Díaz, M., Sáez, R., Irañeta, J., 2004. The association of barley with AM fungi can result in similar yield and grain quality as a long term application of P or P-K fertilizers by enhancing root phosphatase activity and sugars in leaves at tillering. Biological Agriculture & Horticulture 22, 69–80. https://doi.org/10.1080/01448765.2004.9754989.

Grierson, P.F., Adams, M.A., 2000. Plant species affect acid phosphatase, ergosterol and microbial P in a Jarrah (Eucalyptus marginata Donn ex Sm.) forest in south-western Australia. Soil Biology and Biochemistry 32, 1817–1827. https://doi.org/10.1016/S0038-0717(00)00155-3.

Haddaway, N.R., Hedlund, K., Jackson, L.E., Kätterer, T., Lugato, E., Thomsen, I.K., Jørgensen, H.B., Isberg, P.E., 2017. How does tillage intensity affect soil organic carbon? A systematic review. Environmental Evidence. https://doi.org/10.1186/s13750-017-0108-9.

Haygarth, P.M., Jarvie, H.P., Powers, S.M., Sharpley, A.N., Elser, J.J., Shen, J., Peterson, H.M., Chan, N.-I., Howden, N.J.K., Burt, T., Worrall, F., Zhang, F., Liu, X., 2014. Sustainable phosphorus management and the need for a long-term perspective: the legacy hypothesis. Environmental Science & Technology 8417–8419. https://doi.org/10.1021/es502852s.

Haygarth, P.M., Rufino, M.C., 2021. Local solutions to global phosphorus imbalances. Nature Food 2, 459–460. https://doi.org/10.1038/s43016-021-00301-0.

Hedges, L., Gurevitch, J., Curtis, P., 1999. The meta-analysis of response ratios in experimental ecology. Ecological Society of America 80, 1150–1156.

Hunter, P.J., Teakle, G.R., Bending, G.D., 2014. Root traits and microbial community interactions in relation to phosphorus availability and acquisition, with particular

reference to Brassica. Frontiers of Plant Science 5. https://doi.org/10.3389/fpls.2014.00027.

Ikoyi, I., Fowler, A., Schmalenberger, A., 2018. One-time phosphate fertilizer application to grassland columns modifies the soil microbiota and limits its role in ecosystem services. The Science of the Total Environment 630, 849–858. https://doi.org/10.1016/j.scitotenv.2018.02.263.

Janes-Bassett, V., Davies, J., Rowe, E.C., Tipping, E., 2020. Simulating long-term carbon nitrogen and phosphorus biogeochemical cycling in agricultural environments. The Science of the Total Environment 714. https://doi.org/10.1016/j.scitotenv.2020.136599.

Jarosch, K.A., Kandeler, E., Frossard, E., Bunemann, E.K., 2019. Is the enzymatic hydrolysis of soil organic phosphorus compounds limited by enzyme or substrate availability? Soil Biology and Biochemistry 139. https://doi.org/10.1016/j.soilbio.2019.107628.

Johnson, D., Leake, J.R., Lee, J.A., 1999. The effects of quantity and duration of simulated pollutant nitrogen deposition on root-surface phosphatase activities in calcareous and acid grasslands: a bioassay approach. New Phytologist 141, 433–442. https://doi.org/10.1046/j.1469-8137.1999.00360.x.

Jones, D.L., Oburger, E., 2011. Solubilization of phosphorus by soil microorganisms. In: Bunemann, E.K., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. Springer, Berlin Heidelberg. https://doi.org/10.1007/978-3-642-15271-9_7.

Liu, Y., Mi, G.H., Chen, F.J., Zhang, J.H., Zhang, F.S., 2004. Rhizosphere effect and root growth of two maize (Zea mays L.) genotypes with contrasting P efficiency at low P availability. Plant Science 167, 217–223. https://doi.org/10.1016/j.plantsci.2004.02.026.

Lu, C., Tian, H., 2017. Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. Earth System Science Data 9, 181–192. https://doi.org/10.5194/essd-9-181-2017.

Lyu, Y., Tang, H., Li, H., Zhang, F., Rengel, Z., Whalley, W.R., Shen, J., 2016. Major crop species show differential balance between root morphological and physiological responses to variable phosphorus supply. Frontiers of Plant Science 7. https://doi.org/10.3389/fpls.2016.01939.

Margalef, O., Sardans, J., Maspons, J., Molowny-Horas, R., Fernández-Martínez, M., Janssens, I.A., Richter, A., Ciais, P., Obersteiner, M., Peñuelas, J., 2021. The effect of global change on soil phosphatase activity. Global Change Biology. https://doi.org/10.1111/gcb.15832.

Marklein, A.R., Houlton, B.Z., 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytologist 193, 696–704. https://doi.org/10.1111/j.1469-8137.2011.03967.x.

Menezes-Blackburn, D., Giles, C., Darch, T., George, T.S., Blackwell, M., Stutter, M., Shand, C., Lumsdon, D., Cooper, P., Wendler, R., Brown, L., Almeida, D.S., Wearing, C., Zhang, H., Haygarth, P.M., 2018. Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. Plant and Soil 427, 5–16. https://doi.org/10.1007/s11104-017-3362-2.

Mezeli, M.M., Page, S., George, T.S., Neilson, R., Mead, A., Blackwell, M.S.A., Haygarth, P.M., 2020. Using a meta-analysis approach to understand complexity in soil biodiversity and phosphorus acquisition in plants. Soil Biology and Biochemistry 142. https://doi.org/10.1016/j.soilbio.2019.107695.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of phosphatase enzymes in soil. In: Bünemann, E., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 215–243. https://doi.org/10.1007/978-3-642-15271-9_9.

Nannipieri, P., Johnson, R., Paul, E., 1978. Criteria for measurement of microbial growth and activity in soil. Soil Biology and Biochemistry 10, 223–229. https://doi.org/10.1016/0038-0717(78)90100-1.

Nash, D.M., Haygarth, P.M., Turner, B.L., Condron, L.M., McDowell, R.W., Richardson, A.E., Watkins, M., Heaven, M.W., 2014. Using organic phosphorus to sustain pasture productivity: a perspective. Geoderma 1–41. https://doi.org/10.1016/j.geoderma.2013.12.004.

Naureen, Z., Sham, A., Al Ashram, H., Gilani, S.A., Al Gheilani, S., Mabood, F., Hussain, J., Al Harrasi, A., AbuQamar, S.F., 2018. Effect of phosphate nutrition on growth, physiology and phosphate transporter expression of cucumber seedlings. Plant Physiology and Biochemistry 127, 211–222. https://doi.org/10.1016/j.plaphy.2018.03.028.

Nuruzzaman, M., Lambers, H., Bolland, M.D.A., Veneklaas, E.J., 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant and Soil 281, 109–120. https://doi.org/10.1007/s11104-005-3936-2.

Ockenden, M.C., Hollaway, M.J., Beven, K.J., Collins, A.L., Evans, R., Falloon, P.D., Forber, K.J., Hiscock, K.M., Kahana, R., MacLeod, C.J.A., Tych, W., Villamizar, M.L., Wearing, C., Withers, P.J.A., Zhou, J.G., Barker, P.A., Burke, S., Freer, J.E., Johnes, P.J., Snell, M.A., Surridge, B.W.J., Haygarth, P.M., 2017. Major agricultural changes required to mitigate phosphorus losses under climate change. Nature Communications 8. https://doi.org/10.1038/s41467-017-00232-0.

Oshima, Y., Ogawa, N., Harashima, S., 1996. Regulation of phosphatase synthesis in Saccharomyces cerevisiae - a review. Gene 179, 171–177. https://doi.org/10.1016/S0378-1119(96)00425-8.

Phoenix, G.K., Booth, R.E., Leake, J.R., Read, D.J., Grime, J.P., Lee, J.A., 2004. Simulated pollutant nitrogen deposition increases P demand and enhances root-surface phosphatase activities of three plant functional types in a calcareous grassland. New Phytologist 161, 279–290. https://doi.org/10.1046/j.1469-8137.2003.00910.x.

Powers, S.M., Bruulsema, T.W., Burt, T.P., Chan, N.I., Elser, J.J., Haygarth, P.M., Howden, N.J.K., Jarvie, H.P., Lyu, Y., Peterson, H.M., Sharpley, A.N., Shen, J., Worrall, F., Zhang, F., 2016. Long-term accumulation and transport of anthropogenic phosphorus in three river basins. Nature Geoscience 9, 353–356. https://doi.org/10.1038/ngeo2693.

Priya, P., Sahi, S.V., 2009. Influence of phosphorus nutrition on growth and metabolism of Duo grass (Duo festulolium). Plant Physiology and Biochemistry 47, 31–36. https://doi.org/10.1016/j.plaphy.2008.09.002.

Raiesi, F., Ghollarata, M., 2006. Interactions between phosphorus availability and an AM fungus (Glomus intraradices) and their effects on soil microbial respiration, biomass and enzyme activities in a calcareous soil. Pedobiologia 50, 413–425. https://doi.org/10.1016/j.pedobi.2006.08.001.

Redel, Y., Staunton, S., Durán, P., Gianfreda, L., Rumpel, C., de la Luz Mora, M., 2019. Fertilizer P uptake determined by soil P fractionation and phosphatase activity. Journal of Soil Science and Plant Nutrition 19, 166–174. https://doi.org/10.1007/s42729-019-00024-z.

Richardson, A.E., Hadobas, P.A., Hayes, J.E., 2000. Acid phosphomonoesterase and phytase activities of wheat (Triticum aestivum L.) roots and utilization of organic phosphorus substrates by seedlings grown in sterile culture. Plant, Cell and Environment 23, 397–405. https://doi.org/10.1046/j.1365-3040.2000.00557.x.

Rotaru, V., 2015. Responses of acid phosphatase activity on the root surface and rhizospheric soil of soybean plants to phosphorus fertilization and Rhizobacteria application under low water supply. Scientific Papers Series A-Agronomy 58, 295–300.

Sattari, S.Z., Bouwman, A.F., Giller, K.E., Van Ittersum, M., 2012. Residual soil phosphorus as the missing piece in the global phosphorus crisis puzzle. Proc. Natl. Acad. Sci. U.S.A. 109, 6348–6358.

Schneider, K., Turrion, M.B., Grierson, P.F., Gallardo, J.F., 2001. Phosphatase activity, microbial phosphorus, and fine root growth in forest soils in the Sierra de Gata, western central Spain. Biology and Fertility of Soils 34, 151–155. https://doi.org/10.1007/s003740100387.

Sharma, N.C., Sahi, S.V., 2011. Enhanced organic phosphorus assimilation promoting biomass and shoot P hyperaccumulations in Lolium multiflorum grown under sterile conditions. Environmental Science and Technology 45, 10531–10537. https://doi.org/10.1021/es200942v.

Shen, Q., Wen, Z., Dong, Y., Li, H., Miao, Y., Shen, J., 2018. The responses of root morphology and phosphorus-mobilizing exudations in wheat to increasing shoot phosphorus concentration. AOB PLANTS 10. https://doi.org/10.1093/aobpla/ply054.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M. P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252–1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x.

Speir, T.W., Cowling, J.C., 1991. Phosphatase activities of pasture plants and soils: relationship with plant productivity and soil P fertility indices. Biology and Fertility of Soils 12, 189–194. https://doi.org/10.1007/BF00337200.

Spiers, G.A., McGill, W.B., 1979. Effects of phosphorus addition and energy supply on acid phosphatase production and activity in soils. Soil Biology and Biochemistry 11, 3–8. https://doi.org/10.1016/0038-0717(79)90110-X.

Spohn, M., Ermak, A., Kuzyakov, Y., 2013. Microbial gross organic phosphorus mineralization can be stimulated by root exudates - a 33P isotopic dilution study. Soil Biology and Biochemistry 65, 254–263. https://doi.org/10.1016/j.soilbio.2013.05.028.

Spohn, M., Kuzyakov, Y., 2013. Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation - coupling soil zymography with 14C imaging. Soil Biology and Biochemistry 67, 106–113. https://doi.org/10.1016/j.soilbio.2013.08.015.

Spohn, M., Treichel, N.S., Cormann, M., Schloter, M., Fischer, D., 2015. Distribution of phosphatase activity and various bacterial phyla in the rhizosphere of Hordeum vulgare L. depending on P availability. Soil Biology and Biochemistry 89, 44–51. https://doi.org/10.1016/j.soilbio.2015.06.018.

Steffen, W., Richardson, K., Rockström, J., Cornell, S.E., Fetzer, I., Bennett, E.M., Biggs, R., Carpenter, S.R., de Vries, W., de Wit, C.A., Folke, C., Gerten, D., Heinke, J., Mace, G.M., Persson, L.M., Ramanathan, V., Reyers, B., Sörlin, S., 2015. Planetary boundaries: guiding human development on a changing planet. Science 347, 1259855. https://doi.org/10.1126/science.1259855.

Steingrobe, B., 2001. Root renewal of sugar beet as a mechanism of P uptake efficiency. Journal of Plant Nutrition and Soil Science 164, 533–539. https://doi.org/10.1002/1522-2624(200110)164:5<533::AID-JPLN533>3.0.CO;2-D.

Štursová, M., Baldrian, P., 2011. Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity. Plant and Soil 338, 99–110. https://doi.org/10.1007/s11104-010-0296-3.

Stutter, M.I., Shand, C.A., George, T.S., Blackwell, M.S.A., 2012. Recovering Phosphorus from Soil: a Root Solution. ACS Publications.

Sun, B., Gao, Y., Wu, X., Ma, H., Zheng, C., Wang, X., Zhang, H., Li, Z., Yang, H., 2020. The relative contributions of pH, organic anions, and phosphatase to rhizosphere soil phosphorus mobilization and crop phosphorus uptake in maize/alfalfa polyculture. Plant and Soil 447, 117–133. https://doi.org/10.1007/s11104-019-04110-0.

Syers, J.K., Johnston, A.E., Curtin, D., 2008. Efficiency of Soil and Fertilizer Phosphorus Use. FAO Fertilizer and Plant Nutrition Bulletin.

Tang, H., Li, X., Zu, C., Zhang, F., Shen, J., 2013. Spatial distribution and expression of intracellular and extracellular acid phosphatases of cluster roots at different developmental stages in white lupin. Journal of Plant Physiology 170, 1243–1250. https://doi.org/10.1016/j.jplph.2013.04.015.

Tarafdar, J.C., Claassen, N., 2003. Organic phosphorus utilization by wheat plants under sterile conditions. Biology and Fertility of Soils 39, 25–29. https://doi.org/10.1007/s00374-003-0671-9.

Tarafdar, J.C., Jungk, A., 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biology and Fertility of Soils 3, 199–204.

Vance, C.P., Uhde-Stone, C., Allan, D.L., 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytologist 157, 423–447. https://doi.org/10.1046/j.1469-8137.2003.00695.x.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor. Journal of Statistical Software 36, 1–48. https://doi.org/10.18637/jss.v036.i03.

Wang, Y., Krogstad, T., Clarke, J.L., Hallama, M., Ogaard, A.F., Eich-Greatorex, S., Kandeler, E., Clarke, N., 2016. Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in P availability. Frontiers of Plant Science 7. https://doi.org/10.3389/fpls.2016.01664.

Wang, Y., Lambers, H., 2020. Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. Plant and Soil 447, 135–156. https://doi.org/10.1007/s11104-019-03972-8.

Wang, Z., Yang, S., Wang, R., Xu, Z., Feng, K., Feng, X., Li, T., Liu, H., Ma, R., Li, H., Jiang, Y., 2020. Compositional and functional responses of soil microbial communities to long-term nitrogen and phosphorus addition in a calcareous grassland. Pedobiologia 78, 150612. https://doi.org/10.1016/j.pedobi.2019.150612.

Wasaya, A., Zhang, X., Fang, Q., Yan, Z., 2018. Root phenotyping for drought tolerance: a review. Agronomy 8, 1–19. https://doi.org/10.3390/agronomy8110241.

Xiao, W., Chen, X., Jing, X., Zhu, B., 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. Soil Biology and Biochemistry 123, 21–32. https://doi.org/10.1016/j.soilbio.2018.05.001.

Yadav, R.S., Tarafdar, J.C., 2001. Influence of organic and inorganic phosphorus supply on the maximum secretion of acid phosphatase by plants. Biology and Fertility of Soils 34, 140–143.

Yuan, H.M., Blackwell, M., McGrath, S., George, T.S., Granger, S.H., Hawkins, J.M.B., Dunham, S., Shen, J.B., 2016. Morphological responses of wheat (Triticum aestivum L.) roots to phosphorus supply in two contrasting soils. Journal of Agricultural Science 154, 98–108. https://doi.org/10.1017/S0021859615000702.

Zebrowska, E., Milewska, M., Ciereszko, I., 2017. Mechanisms of oat (Avena sativa L.) acclimation to phosphate deficiency. PeerJ 2017. https://doi.org/10.7717/peerj.3989.

Zebrowska, E., Zujko, K., Kuleszewicz, A., Ciereszko, I., 2018. The effects of diversified phosphorus nutrition on the growth of oat (Avena sativa L.) and acid phosphatase activity. Acta Societatis Botanicorum Poloniae 87, 1–13. https://doi.org/10.5586/asbp.3571.

Zhang, H., Huang, Y., Ye, X., Shi, L., Xu, F., 2009. Genotypic differences in phosphorus acquisition and the rhizosphere properties of Brassica napus in response to low phosphorus stress. Plant and Soil 320, 91–102. https://doi.org/10.1007/s11104-008-9873-0.

Zhang, H., Huang, Y., Ye, X., Xu, F., 2010. Analysis of the contribution of acid phosphatase to P efficiency in Brassica napus under low phosphorus conditions. Science China Life Sciences 53, 709–717. https://doi.org/10.1007/s11427-010-4008-2.