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Exploring efficient techniques to decrease phosphorus levels in previously farmed land to promote the revival of indigenous grassland

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ABSTRACT

Restoration of native grasslands is challenging due to high soil phosphorus levels. Cultivation of plants with high phosphorus (P) absorption is an optimal solution to remove and decrease P from the soil. It has been demonstrated that native grassland taxa (species) of the genus Ptilotus have significant P-uptake. In a glasshouse study, *Ptilotus macrocephalus* and *Ptilotus polystachyus* were tested for their ability to reduce the amount of soil phosphorus that was readily available. *Lupinus albus*, a third species with a reputation for high phosphorus uptake, served as a comparison species, and a further treatment included Phoslock®, a soil additive that could bind soil phosphorus into insoluble forms.

The findings revealed that phosphorus in the soil was absorbed at a high level via *Ptilotus macrocephalus* and *Ptilotus polystachyus* showed a maximum reduction of P (-2.58 and -2.55 ppm). It is argued that several years of planting and harvesting these plants will offer a workable method for lowering soil phosphorus levels. However, this only happened at high concentrations of 1500 g/m^2 and when soil phosphorus concentrations were very high. Despite, the Phoslock®'s effectiveness in lowering soil-accessible phosphorus. At concentrations often observed in former agriculture paddocks, it proved less effective. The study's findings have improved our existing comprehension of reclaiming abandoned grassland.

Keywords: Land management, Phosphorus, Phoslock, Ptilotus, Binding, Taxa.

1. Introduction

Intense agricultural activity on previous grassland sites, including the addition of considerable amounts of inorganic fertilizer, has significantly affected the original soil's chemical structure[1]. Superphosphate, for example, has been a common agricultural fertilizer, and has been used to increase both phosphorus and nitrogen levels in the soil to stimulate the production of exotic crops. However, attempts to restore native grasslands are unsuccessful since the high nutrient levels in such soils encourage the reproduction of alien plants over native species. The regeneration of semi-natural vegetation is seriously hampered by the increased phosphorus availability in post-agricultural soil[2].

It has been observed that, in general, Australian native species prefer low nutrient levels, and thus the substantial and regular historical application of phosphorus fertilizers to cropland soil in temperate Australia will have had significant negative effects on the germination and growth of native plant species[3;4]. There are a number of innovative studies that have investigated the use of plants to reduce soil phosphorus concentration[5;6;7;8;9]. The biology of legume species, which can tolerate a wide range of phosphorus levels and may produce cluster (proteoid) roots in reaction to phosphate deficiency inside the plant, served as a foundation for several of these research[10].

As a result, the plant can concentrate phosphorus inside its structure. Accordingly, much study has been done on *Lupinus albus* (Fabaceae) with respect to cluster-root development and carboxylate release [11;12;13], and it has demonstrated a significant capacity to take phosphorus from the soil. Many Australian native species possess a range of adaptations to maintain adequate phosphorus nutrition when growing in soil with low availability of labile inorganic phosphorus[14]. These root system alterations, which also promote mycorrhizal fungi's association with plants, boost phosphorus absorption and have a several benefits[15].

Previous research has examined the uptake of phosphorus by various plant species, including *Ptilotus exaltatus, P. microcephalus, P. aerovoides* (Amaranthaceae), *Dysphania kalpari* (Chenopodiaceae), and *Abutilon oxycarpum*

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(Malvaceae), in subtropical and semi-arid Australian grasslands with low levels of phosphorus. *Ptilotus* species were found to have shoots with the highest amount of phosphorus, up to 1.75 mg per plant[16]. The growth response of *P. polystachyus* and *Cichorium intybus* (Asteraceae) in sandy soil with difecient bicarbonate-extractable phosphorus and mineral nitrogen to P and N addition was focused in another study[17]. It was formulated in an attempt to reduce the amount of Filterable Reactive Phosphorus (FRP) present in the water column and in the sediment pore water of a water body. FRP was seen to be an important growth factor for blue green algae and other algae[18]. Additionally, it was believed that Phoslock® may be expected to be particularly successful when added to flooded soils because it can create an active layer on top of the soil, reducing the mobilization of phosphate into the mobile water layer[19].

This present study aimed to reduce the amount of available phosphorus in the soil to provide native plants has a greater probability of sprouting and out-competing exotic flora. Two null hypotheses were tested: the first being that adding Phoslock® to P-rich soils would not significantly reduce the amount of available phosphorus, and the second being that planting seeds and harvesting plants in phosphorus-rich areas would not significantly decrease the amount of available phosphorus in the soil, which would hinder the growth of native plants.

2. Methods and Materials

2.1 Glasshouse experimental strategy

A total of 200 kg of soil from the top 10 cm of abandoned cropland was collected randomly at two different locations. Half of the soil was obtained from a field experiment site located northwest of Werribee, Victoria, while the other half was collected from a nearby fence line. Plastic bags were used for packing the soil samples immediately and stored in a glasshouse at a temperature of 27^{O}C upon arrival at Federation University Australia.

Four sub-samples from each site were chosen randomly from the collection. These sub-samples were sent to a NATA-approved laboratory for chemical analysis. Additionally, samples were taken from each location and sanitized through autoclaving before being placed in labeled plastic bags. The experiment was carried out in a temperature-controlled glasshouse range of 16-22^{O}C at night and 22-30^{O}C during the day.

Before the experiment, the soil subsamples were examined, and it was found that the soil at the experimental site had 21 ppm of phosphorus, while the soil at the fence line had 8 ppm of phosphorus[20]. Three different levels of phosphorus were required during the experiment, so the soil at the fence line had a "low P level," while the soil at the experiment site was considered to have a "mid P level." To create a "high-level soil P" with a concentration of 40 ppm, phosphorus was added to the experiment site soil in the form of finely powdered Ca $(H_2PO_4)_2.H_2O$[5].

A randomized complete block design (RCBD) included six treatments and a control, which was regarded as the experimental design in this present study. *Lupinus albus*, *Ptilotus polystachyus*, and *Ptilotus macrocephalu*s were the three first treatments, while (100, 500, and 1500 g/m^2), were soil with different amounts of Phoslock®, respectively, which were the other three treatments. Each treatment was applied at each of three levels of phosphorus-containing soil (low, medium and high), and there were six replicates of each treatment at each P level, resulting in a total of 126 pots. The experiment was done in the glasshouse at Federation University Australia on December 18, 2015. Pots with a diameter of 13.5 cm and a height of 16 cm were used. Pots with 16 cm height and 13.5 cm diameter were used. These pots were lined with plastic bags to prevent nutrient leaching, and filled with 5 cm of river sand soil 550-650 g and completed with 10 cm of soil which was about 1350 g of air-dried soil. To create three different concentrations of Phoslock® treatments (14.50, 71.50, and 214.50 g/pot), Phoslock was mixed into the topsoil of each treatment. The imitation pots were randomly arranged on the glasshouse benches. Each pot was planted with five seeds of each plant, but on January 4, 2016, the plants were reduced from four to two per pot. To maintain the soil moisture at field capacity, a top sprayer was used for watering automatically three minutes once a day for all pots.

2.2 Sample collection and chemical analysis

A 100-mm ruler was used to measure plant heights 45 days after the experiment started, from the soil's surface to the plants' apical meristems, and each plant's number of leaves and branches was tallied. Plants were harvested using a pair of secateurs to cut at the root collar 63 days after planting. Roots were carefully removed from the soil and washed on top of a mesh sieve to avoid the loss of fine roots. Afterward, independent measurements of the fresh weight of the shoots and roots were made.

After removing the leaves, a PATON Electronic Plano-meter was used to measure the leaf surface area. Separate shoots and roots in labeled bags dried at 70 ^{O}C for 48 hours. Then, the dried samples (shoots and roots) were ground to 450 m, and placed in labeled 5 mL plastic containers. Next, chemical analysis were done on these samples using inductively-coupled plasma weight spectrometry (ICP).

P analysis was also taken in the entire soil profile, which was from the pots, and each sample was put into a labeled plastic bag. These bags were then taken to a different glasshouse with a temperature range of 17-22^{O}C to air-dry. After crushing the dried samples by using a 2 mm filter, the phosphorus content was determined by applying the Olsen analytical method[20].

2.3 Data Analysis

MINITAB 17 statistical software was used for the data analysis[21]. Ryan-Joiner test was also applied to check the normality of the data, which is similar to the Shapiro-Wilk test at a significance level of 0.05. Additionally, the Box-Cox transformation method was used to check the non-normally of distributed data. For determination of the significant differences between the means of all factors such as; species, soil P level, and treatments, as well as their combinations, species-soil P level and treatments-soil P level, Tukey's test was used at a significance level of 0.05.

3. Results and discussion

3.1 Measurement of the plants

In the experiment, the shoot dry weight of two species of *Ptilotus* and *L. albus* were measured at changeable soil phosphorus levels. Figure 1 and Table 1 show the data in soil with medium and high P levels, *P. polystachyus* had a higher shoot dry weight than *L. albus* and *P. macrocephalus*, which is related to some ecological and physiological effects. Both *P. polystachyus* and *L. albus* had heavier shoots dry weight as soil P concentration rose. P-inhibition may have worked in *P. macrocephalus* because its shoot dry weight was lower in high soil P levels than in low and medium soil P levels. As a result of these findings, it can be concluded that the various Ptilotus species and *L. albus* reacted differentially to soil P levels, which had an impact on their shoot dry weight. At all soil P levels, as predicted, the P content of the shoot dry weights rose for all three species. *P. macrocephalus*, which grew where there was a lot of soil P, had the lowest shoot dry weight. Compared to crops and pastures, native species require substantially less N, P, and K fertilizer (Figure 1; Table 1). This in line with the discovery that a species' P uptake from the soil increases with the dry weight of the shoots it produces[17;22]. Also, it's been demonstrated that in soil with low bicarbonate-extractable phosphorus or mineral nitrogen, the *P. polystachyus* species grew significantly better and produced more biomass than chicory[17]. In the high-available phosphorus soil, the *L. albus* shoot dry weight and seed yield were much higher[23].

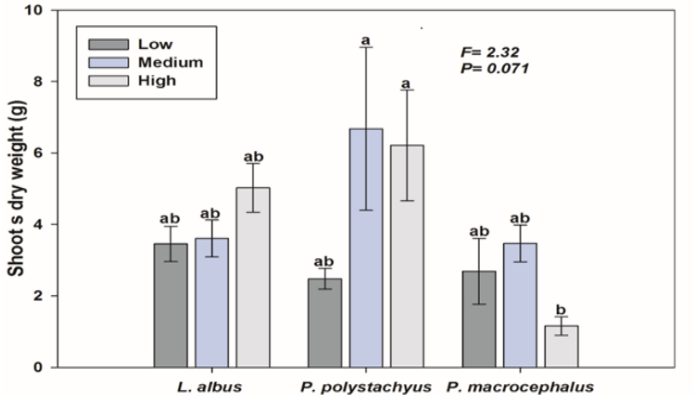

Figure 1: Mean shoot dry weght of L. albus, P. polystachyus and P. macrocephalus grown at three different P levels (low, medium and high).

In soil with high P levels, *L. albus* had a significantly higher root dry weight of 0.85 g (p= 0.013) compared to *P. macrocephalus* and *P. polystachyus*, which had root dry weights of 0.31 g and 0.35 g, respectively. At high soil P levels, the highest amount of the root dry weight was noted in *L. albus*, which was approximately three times more than both Ptilotus species. Although *P. macrocephalus* had a higher root dry weight than *L. albus and P. polystachyus* in soil with low and medium P levels, these differences were not statistically significant. (Figure 2 and Table 1.). At high soil P levels, *L. albus* had roots with a dry weight that was around three times that of the two Ptilotus species. This because of *L. albus* 's root system consists of a large tap root and irregularly spaced dense clusters of rootlets with restricted growth along the lateral roots[11]. Due to more accessible P in the soil, the increased root dry weight found in soil with higher P levels can be linked to higher P uptake. Low root dry weight was noted from Ptilotus species at the high P level. According to another study, *P. polystachyus* had heavier roots at the lowest degree of P addition than chicory[17].

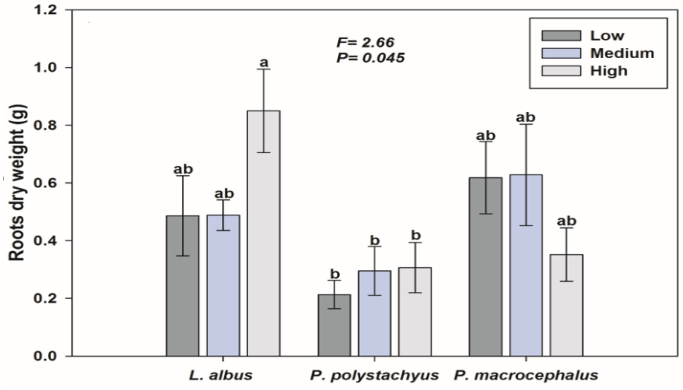

Figure 2: Mean root dry weight of L. albus, P. polystachyus and P. macrocephalus grown at three different P levels (low, medium and high).

P. polystachyus had the most significant shoot-to-root ratio ever seen (Figure 3; Table 1). While, the highest shoot-root ratio was found in the medium P level soils, which was from the *P. polystachyus* and *P. macrocephalus*. Though, the shoot-root ratio of *L. albus* increase with increasing soil P-level.

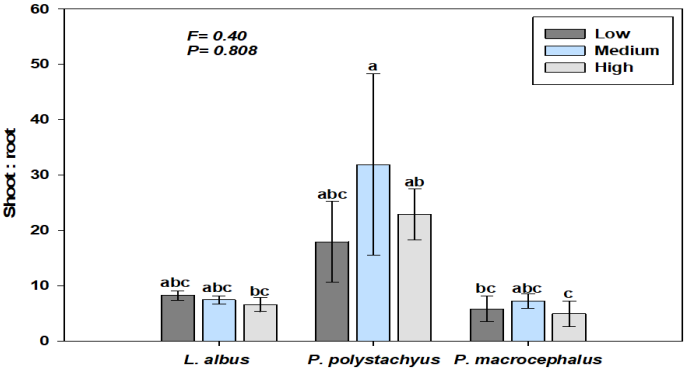

Figure 3: Mean shoot-root ratio of L. albus, P. polystachyus and P. macrocephalus grown at three different P levels (low, medium and high).

At both high and low soil P levels, *L. albus'* leaf area was noticeably more remarkable than that of *P. polystachyus* and *P. macrocephalus* (Figure 4; Table 1). However, with medium soil phosphorus, *P. polystachyus* and *P. macrocephalus* had larger leaves than *L. albus.* While *P. macrocephalus* and *P. polystachyus* species increased leaf area in response to rising phosphorus, *L. albus* species increased individual leaf size.

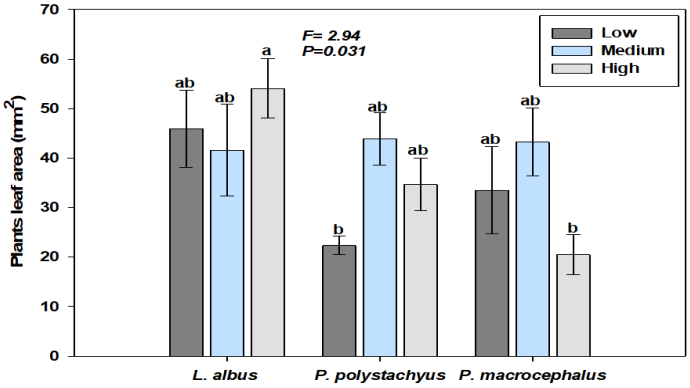

Figure 4: Mean plant leaf area of L. albus, P. polystachyus and P. macrocephalus grown at three different P levels (low, medium and high).

Comparable to shoot dry weights, plant heights followed this pattern (Figure 5; Table 1). In the high P soil, *P. polystachyus* grew taller than *L. albus* and *P. macrocephalus. P. polystachyus* and *L. albus* plants' heights were increased by raising the soil's P content. This study amply demonstrated that *L. albus* species grow more rapidly in response to high P levels than *P. macrocephalus* and *P. polystachyus* species. This might be due to the leaves smaller size and shapes in *P. macrocephalus* and *P. polystachyus* compared to *L. albus*.

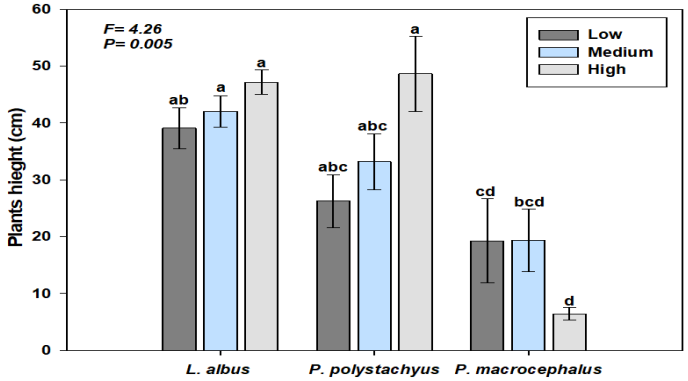

Figure 5: Mean plant height of L. albus, P. polystachyus and P. macrocephalus grown at three different P levels (low, medium and high).

Table 1: Mean ± standard error of plants growth measurements of three species L. albus, P. polystachyus and P. macrocephalus that planted at three different phosphorous level soils (low, medium and high).

	L. albus			*P. polystachyus*			*P. macrocephalus*		
Plant measurements	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
Shoots dry weight (g)	3.45 ± 0.50	3.61 ± 0.52	5.03 ± 0.68	2.48 ± 0.29	**6.68 ± 2.28**	**6.22 ± 1.55**	2.69 ± 0.92	3.47 ± 0.52	1.16 ± 0.26
Roots dry weight (g)	0.49 ± 0.14	0.49 ± 0.05	**0.85 ± 0.14**	0.21 ± 0.05	0.30 ± 0.08	0.31 ± 0.09	0.62 ± 0.13	0.63 ± 0.18	0.35 ± 0.09
Leaf area (mm^2)	**45.90 ± 7.80**	41.59 ± 9.23	**54.07 ± 5.99**	22.33 ± 1.85	43.93 ± 5.31	34.66 ± 5.34	33.51 ± 8.84	43.22 ± 6.88	20.51 ± 4.05
Plant heights (cm)	39.08 ± 3.58	42.00 ± 2.78	47.17 ± 2.19	26.25 ± 4.64	33.17 ± 4.92	**48.67 ± 6.62**	19.25 ± 7.38	19.33 ± 5.52	6.42 ± 1.13
Shoot-Root (%)	8.23 ± 0.90	7.43 ± 0.70	6.60 ± 1.28	17.95 ± 7.31	**31.91 ± 16.40**	22.92 ± 4.63	5.82 ± 2.26	7.23 ± 1.34	4.94 ± 2.29

3.2 Phosphorous concentrations in plant matter

The uptake of phosphorus by the roots and shoots of all three species was impacted by different soil phosphorus levels. *P. macrocephalus* had significantly higher phosphorus levels in the shoots than *P. polystachyus* and *L. albus* when the soil had high phosphorus levels. However, at low levels P soil, there was no significant change in shoot phosphorus concentration. The increased soil phosphorus levels had a positive effect on the shoot and root phosphorus uptake of all three species. At high soil phosphorus levels, *P. macrocephalus* had the highest shoot phosphorus concentration, significantly higher than that of *P. polystachyus* and *L. albus*. However, there was no significant difference between *P. macrocephalus* and *P. polystachyus* at medium soil phosphorus levels . The trend started to reverse when the soil phosphorus levels were lower, and no significant difference was observed in phosphorus uptake by the shoots of the three species. When the soil had high phosphorus levels, *P. macrocephalus* had the highest root phosphorus concentration, while *L. albus* had the lowest root phosphorus concentration. At low soil phosphorus levels, there was slight variation in root phosphorus concentration between the species. However, at medium and high soil phosphorus levels, root phosphorus concentration increased in all three species, reaching very high values at the highest soil phosphorus level. Some native Australian plants have evolved in phosphorus-poor environments[14], and their effective phosphorus acquisition systems appear to be poorly regulated at times of high phosphorus availability[17;10]. *Hakea prostrata*'s limited ability to down-regulate phosphorus absorption may be linked to its tendency to accumulate high amounts of phosphorus in its roots and shoots[13;24].

3.3 Phosphorus concentrations in the soil

Figure 6 shows the difference in mean soil-available phosphorus levels between the start and end of the study. The results indicate that *Ptilotus macro*cephalus and *P. polystachyus* species were most effective at reducing soil P at all three soil P levels. At low P levels, *P. macrocephalus* and *P. polystachyus* showed a maximum reduction of (-2.58 and -2.55 ppm), respectively, which was significantly different from the control and other treatments. The P reduction of *P. macrocephalus and P. polystachyus* was (-4.75 and -4.10 ppm) at medium soil P, respectively, and (-5.20 and -9.13) ppm at high soil P, showing similar patterns at both levels of P soils, respectively. At medium and high P levels, both species showed similar patterns of reduction. *L. albus* and *P. polystachyus* showed a significant response to high P levels, with *P. polystachyus* showing the highest shoot dry weight, plant height, and shoot: root ratio at medium and high P-level soils. *L. albus* had the maximum root dry weight and leaf area at high and low P-level soils.

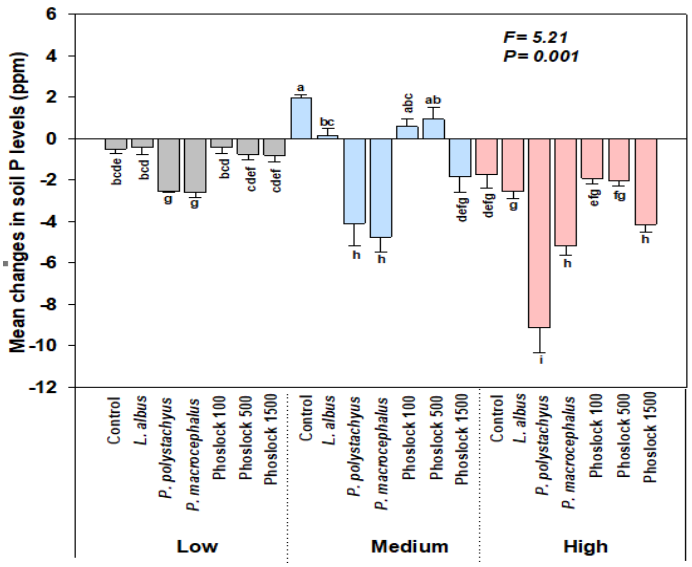

Figure 6: Mean change in soil phosphorus levels that were treated with three concentrations of Phoslock® (100, 500 and 1500g/m^2) and planted with three species (L. albus, P. polystachyus and P. macrocephalus) at three different P level (low, medium and high).

Previous studies have also shown similar findings for *P. polystachyus*, with increased shoot and root dry weight at low and high phosphorus content soils[5;7]. On soils with high phosphorus levels, studies have observed an increase in the growth response and phosphorus uptake of *L. albus* species[23]. It could be argued that *L. albus* species maintained a constant homeostasis and P concentration in the tissues rather than taking up the available phosphorus[24].

Phoslock® treatments (Phoslock 100, Phoslock 500, and Phoslock 1500) have not a significant impact on soil P reduction at low and medium soil P-levels. However, Phoslock 1500 treatment had a significant effect on P reduction in high P level soil. Phoslock® binds diverse types of phosphorus and precipitates as the stable mineral rhabdophane [19], which is distinguished by a deficient solubility product. The study suggests that the use of high levels of Phoslock® can reduce the available phosphorus content in the soil by binding it without removing it. However, the prohibitive cost makes it less than ideal as a management strategy for the reduction of soil P in the terrestrial environment [25].

The results also indicate that *L. albus* is not an effective means of managing phosphorus reduction in ex-arable cropping land in the Victorian Volcanic Plain (VVP) grasslands, while *P. macrocephalus* and *P. polystachyus* species are well-suited for phytoremediation, as they can accumulate high levels of phosphorus in their shoot and root tissues while maintaining biomass production. Therefore, these native species can be effectively used for restoration management of ex-arable cropping land in the VVP grasslands, where high levels of phosphorus pose an environmental threat to native species and require removal from the system.

Conclusions

The purpose of this study was to look at several strategies for lowering the phosphorus content of the soil. The study used soil treatments and plants to test two hypotheses in a controlled setting. The initial assumption was that encouraging the development of native plants by applying Phoslock® to soil with high phosphorus levels would not work. The second theory held that phosphorus-absorbent plants would not lessen the quantity of phosphorus in the soil when used in locations with high phosphorus concentrations. According to the study, Phoslock® is ineffective at low or medium amounts of phosphorus but can reduce accessible phosphorus in soils with high phosphorus content. In contrast, Ptilotus species were effective in reducing soil phosphorus at all levels and also helpful in absorbing heavy metals from contaminated areas such as Halabja, Iraq, which suffered a chemical gas attack in 1988. Further research will explore the potential use of Ptilotus species to reduce heavy metals in affected areas.

Author's contribution

Authors attest that the submitted manuscript is original, and they confirmed that it has not been published or is not under consideration for publication elsewhere.

Conflict of interests

The authors declare no conflict of interest.

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References

1. Dorrough, J., Ash, J., & McIntyre, S. (2004). Plant responses to livestock grazing frequency in an Australian temperate grassland. *Ecography*, *27*(6), 798-810.
2. Pywell, R. F., Bullock, J. M., Tallowin, J. B., Walker, K. J., Warman, E. A., & Masters, G. (2007). Enhancing diversity of species-poor grasslands: an experimental assessment of multiple constraints. *Journal of Applied Ecology*, *44*(1), 81-94.
3. Hobbs, R. J., & Yates, C. J. (2000). *Temperate eucalypt woodlands in Australia: Biology, conservation, management and restoration*. Surrey Beatty & Sons Pty., Ltd.,.
4. Kirkpatrick, J. B., Gilfedder, L., Bridle, K., & Zacharek, A. (2005). The positive and negative conservation impacts of sheep grazing and other disturbances on the vascular plant species and vegetation of lowland subhumid Tasmania. *Ecological Management & Restoration*, *6*(1), 51-60.
5. Brennan, R. F., Webb, M. G., & Crowhurst, A. M. (2000). Yield responses of mulla mulla (*Ptilotus exaltatus Nees.*) seedlings to additions of nitrogen, potassium and phosphorus fertiliser. *Australian journal of experimental agriculture*, *40*(6), 867-871.
6. Watt, M., & Evans, J. R. (2003). Phosphorus acquisition from soil by white lupin (*Lupinus albus L.*) and soybean (Glycine max L.), species with contrasting root development. *Plant and soil*, *248*, 271-283.

7. Bayon, R. L., Weisskopf, L., Martinoia, E., Jansa, J., Frossard, E., Keller, F., & Gobat, J. M. (2006). Soil phosphorus uptake by continuously cropped *Lupinus albus*: a new microcosm design. *Plant and Soil*, *283*, 309-321.
8. Gilbert, J., Gowing, D., & Wallace, H. (2009). Available soil phosphorus in semi-natural grasslands: assessment methods and community tolerances. *Biological Conservation*, *142*(5), 1074-1083.
9. Lelei, J. J., & Onwonga, R. N. (2014a). White lupin (*Lupinus albus L*. cv. Amiga) increases solubility of Minjingu phosphate rock, phosphorus balances and maize yields in Njoro Kenya. *Sustainable Agriculture Research*, *3*(526-2016-37859).
10. Shane, M. W., Dixon, K. W., & Lambers, H. (2005). The occurrence of dauciform roots amongst Western Australian reeds, rushes and sedges, and the impact of phosphorus supply on dauciform-root development in Schoenus unispiculatus (Cyperaceae). *New Phytologist*, *165*(3), 887-898.
11. Gardner, W. K., & Boundy, K. A. (1983). The acquisition of phosphorus by *Lupinus albus L*. IV. The effect of interplanting wheat and white lupin on the growth and mineral composition of the two species. *Plant and Soil*, *70*, 391-402.
12. Dinkelaker, B., Römheld, V., & Marschner, H. (1989). Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (Lupinus albus L.). *Plant, cell & environment*, *12*(3), 285-292.
13. Shane, M. W., De Vos, M., De Roock, S., & Lambers, H. (2003). Shoot P status regulates cluster-root growth and citrate exudation in *Lupinus albus* grown with a divided root system. *Plant, Cell & Environment*, *26*(2), 265-273.
14. Handreck, K. A. (1997). Phosphorus requirements of Australian native plants. *Soil Research*, *35*(2), 241-290.
15. Johnston, S., & Ryan, M. (2000). Occurrence of arbuscular mycorrhizal fungi across a range of alpine humus soil conditions in Kosciuszko National Park, Australia. *Arctic, Antarctic, and Alpine Research*, *32*(3), 255-261.
16. Islam, M., Turner, D. W., & Adams, M. A. (1999). Phosphorus availability and the growth, mineral composition and nutritive value of ephemeral forbs and associated perennials from the Pilbara, Western Australia. *Australian Journal of Experimental Agriculture*, *39*(2), 149-159.
17. Ryan, M. H., Ehrenberg, S., Bennett, R. G., & Tibbett, M. (2009). Putting the P in Ptilotus: a phosphorus-accumulating herb native to Australia. *Annals of Botany*, *103*(6), 901-911.
18. Douglas, G. B. (2002). *U.S. Patent No. 6,350,383*. Washington, DC: U.S. Patent and Trademark Office.
19. Geurts, J. J., van de Wouw, P. A., Smolders, A. J., Roelofs, J. G., & Lamers, L. P. (2011). Ecological restoration on former agricultural soils: Feasibility of in situ phosphate fixation as an alternative to top soil removal. *Ecological Engineering*, *37*(11), 1620-1629.
20. Olsen, S. R. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate* (No. 939). US Department of Agriculture.
21. Minitab, I. (2014). MINITAB release 17: statistical software for windows. *Minitab Inc, USA*, *371*.
22. Pang, J., Ryan, M. H., Tibbett, M., Cawthray, G. R., Siddique, K. H., Bolland, M. D.,& Lambers, H. (2010b). Variation in morphological and physiological parameters in herbaceous perennial legumes in response to phosphorus supply. *Plant and Soil*, *331*, 241-255.
23. Lelei, J. J., & Onwonga, R. N. (2014b). Response of microbial populations, soil available, P and yield of Lupin (*Lupinus albus L* cv. Amiga) to application of Minjingu phosphate rock-A greenhouse study. *International Journal of Current Microbiology and Applied Sciences*, *3*(4), 671-684.
24. Pang, J., Tibbett, M., Denton, M. D., Lambers, H., Siddique, K. H., Bolland, M. D. & Ryan, M. H. (2010a). Variation in seedling growth of 11 perennial legumes in response to phosphorus supply. *Plant and Soil*, *328*, 133-143.
25. Haghseresht, F. (2005). A revolution in phosphorous removal. *Phoslock Water Solutions Ltd. In*, *21*.