Complementary phosphorus acquisition strategies of interplanted subterranean clover and white lupin increase sward yield in a low phosphorus soil

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Abstract
Two pot experiments were conducted to assess whether growth of subterranean clover (*Trifolium subterranean*) increases when it was grown together with white lupin (*Lupinus albus*), a legume that can access fixed-phosphorus (P) in soil by secreting of organic acids from its roots. Total shoot dry mass of microswards of clover interplanted with lupin was significantly higher in P-deficient soil, compared to clover monocultures. The benefit to P-efficiency was greater when there was more lupin growing in the mixture. However, the clover component of the sward was depressed by the lupin. In a second experiment, subterranean clover and white lupin were interplanted with their shoots physically separated to assess whether mobilisation of P by the lupin directly improved the P nutrition of the interplanted clover. However, the shoot dry matter and P uptake by subterranean clover were generally lower when grown in interplanted treatments, compared with growth and P uptake in clover monocultures. This suggested that interplanted lupins had not “facilitated” improved P acquisition by the clover; instead the improved yield of mixed swards in low-P soil was more likely the result of “complementarity” or “niche differentiation”, with the companion species exploring different soil horizons and/or different soil P resources.

Keywords
Intercropped legumes, root interaction, phosphorus deficiency, *Trifolium subterraneum*, *Lupinus albus.*

Introduction
Phosphorus (P) fertiliser use efficiency is poor in temperate, legume-based pastures on soils that have moderate to high P-sorption capacity (Simpson et al. 2015). Some plant species can access fixed-P in soil by secreting organic acids from their roots; a P-acquisition strategy that is often equated to “mining” (e.g. cluster roots, organic acid release, rhizosphere acidification, etc; Lambers et al. 2012). There is evidence that these species when interplanted with less P-efficient plants can “facilitate” improved P nutrition and growth by the less efficient plants (Li et al. 2014). This may occur as a consequence of mobilising less-accessible P resources in the soil or other positive interactions (e.g. rhizosphere pH modification) that enable one of the species to increase soil P availability for the benefit of both. In other instances, if access to P by interplanted species is mutually exclusive (e.g. when the companion species explore different soil horizons or deplete different pools of soil P), a net P-acquisition benefit can occur as a result of “complementarity” (i.e. reduced competition for P) (Hinsinger et al. 2011).

Subterranean clover (*Trifolium subterraneum* L.) is the dominant annual pasture legume used on acid soils in southern Australia. It has a relatively high requirement for P-fertiliser to achieve maximum growth. The pastures in which it is used are interplanted mixtures of grasses, legumes and forbs. These plants acquire P by exploring soil and proliferating roots in nutrient-rich soil patches (a “foraging” strategy). We wished to test the hypothesis that pastures comprised of “P-foraging” and “P-mining” species would be able to access the residual P in fertilised soils, leading to an overall improvement in the P-efficiency of grazing systems.

Methods
Experiment 1. A sandy loam soil (8.3 mg kg^{-1} Colwell-extractable P, PBI=50) was steam pasteurised to reduce levels of disease inoculum, sieved to < 2 mm, and mixed with lime (to raise pH$_{(CaCl_2)}$ to 5.2) and P-free basal nutrients. Pots (190 mm height; 86 mm internal diameter) were filled with a bottom layer of 0.9 kg soil that was not fertilised with P (subsoil), followed by a top layer of 0.3 kg of P-fertilised soil (topsoil). The resultant P-application rates were 0, 4.5, 9.0, 21.0, 40.5 and 75.0 mg P pot^{-1}. Subterranean clover (cv. Leura) was planted at a constant density per unit ground area with white lupin (*Lupinus albus* L. cv. Luxor) interplanted at increasing densities. The clover monocultures had a clover:lupin ratio = 10:0 plants pot^{-1}; interplanted treatments had 10:1, 10:2 or 10:4 (clover:lupin) plants pot^{-1} (n = 5 replicates). Both legumes were inoculated with appropriate rhizobia after planting and were well nodulated when harvested. The high-

P treatment was sufficient to achieve maximum subterranean clover yield. The two layers of soil were used to mimic the stratification of P within the soil profile that occurs in fields when P fertiliser is broadcast onto the soil surface.

Plants were grown in a controlled environment cabinet for five weeks (15-20°C; photon flux density 620 µmol m^{-2} s^{-1}; 12 h light/dark) and watered daily to maintain soil moisture at ~80 % of field capacity. Reflective sleeves were fitted to the outside of the pots and raised with plant height each day to reproduce the light conditions in a pasture sward (Figure 1). Pots were arranged in a randomised block design.

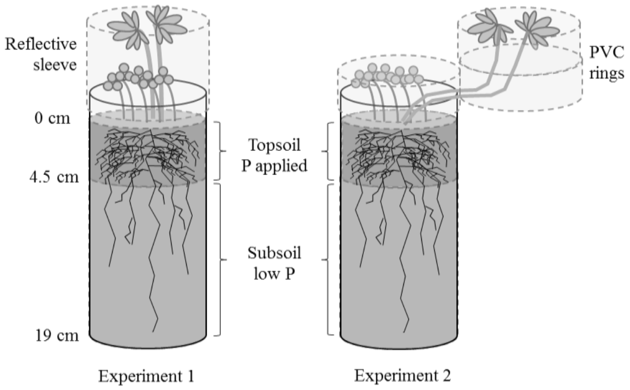

Figure 1. Schematic diagram illustrating the arrangement of the clover and lupin leaves in the Experiment 1 and Experiment 2 microswards. In Experiment 1, the reflective sleeves were raised daily to equal the height of the leaf canopy. In experiment 2, white PVC rings (each 5 cm in height) were added as the clover or lupin leaf canopy reached the top of the existing PVC ring.

Experiment 2. Plant growth and P supply conditions were the same as those in Experiment 1. However, shoots were separated (Figure 1), so that the plants only competed for soil resources. Subterranean clover and white lupin were both grown as monocultures at densities of 10 and 2 plants pot^{-1}, respectively, or were interplanted at proportions of 10:1 and 10:2, (n = 5 replicates). Data from the 10:1 treatment are not presented for simplicity; the responses in this treatment were intermediate between those of the monocultures and the 10:2 treatment. Light capture by single canopies (monocultures) and dual canopies in each interplanted treatment was managed using stackable white PVC rings (5 cm height) instead of reflective sleeves. As canopy height approached the top of each ring, another ring was added to achieve light conditions similar to those in a pasture sward.

Analyses

Shoots were cut at the soil surface and dried at 70°C for dry mass determination, and (in Experiment 2) total P analysis. Data were subjected to two-way ANOVA with pasture treatment and P as factors using R statistical software.

Results

Experiment 1. Shoot dry weight of subterranean clover was increased by increasing soil P supply in every treatment (Figure 2). In contrast the shoot yield of the lupin component in each interplanted treatment was only marginally improved by application of P to the soil. There was substantial competitive interference between lupins and subterranean clover in the mixed swards. Increased densities of lupin decreased the contribution of clover to total shoot yield, whilst increasing the yield of the lupin component. However, the net result of interplanting subterranean clover with white lupin was to significantly improve the total shoot yield of the microswards growing in low-P soil (Figs. 2b, c, d). For example, in very low P soil (0 mg P pot^{-1}), the addition of 1, 2 and 4 white lupins raised the total sward yield by 2.3-, 3.1- and 3.5-fold, respectively, compared with the clover monoculture. The more lupins contributed to sward mass, the greater the yield advantage in low-P soil. However, in high P soil (e.g. at 75 mg P pot^{-1}) the total shoot yields in all treatments were similar. Thus, interplant treatments promoted increases in total shoot dry weight at levels of P addition that were deficient for subterranean clover growth.

Experiment 2. In this experiment, shoots were prevented from contributing to competition between the companion species in the interplanted treatments. The shoot biomass and shoot P content of the subterranean clover in monocultures, and when interplanted with lupin, were stimulated significantly by increased soil P supply (Figs. 3a, c). Maximum monoculture yield was achieved at 20-40 mg P pot^{-1}. Lupin shoot P content was increased by application of P to the soil, but in contrast to the clover, lupin shoot yields were insensitive to soil P supply (Figs 3b, d). The yield and shoot P contents of both species were reduced by interplanting. On average the reduction in shoot yield due to competitive interference in the rhizosphere was ~13% and ~16% for the clover and the lupin, respectively. Similarly, shoot P contents were reduced on average by ~20% and ~17%, for clover and lupin, respectively.

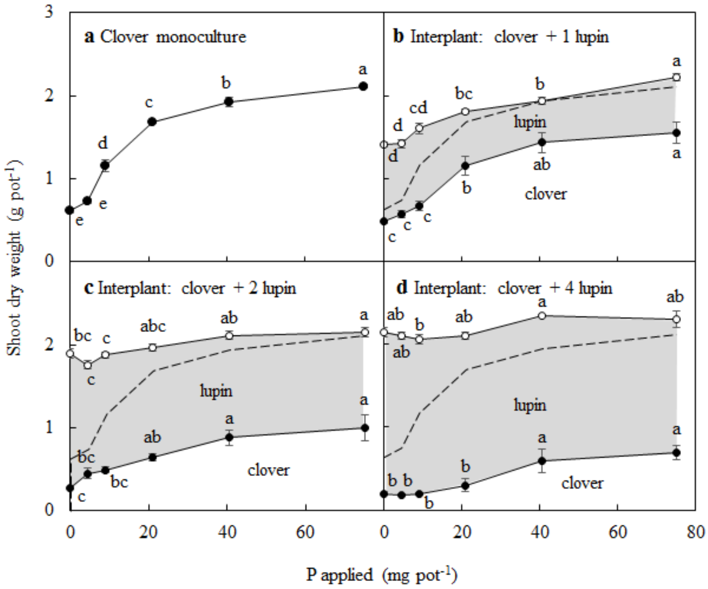

Figure 2. Experiment 1: shoot dry weight of the *Trifolium subterraneum* monoculture (a) and *T. subterraneum* inter-planted with *Lupinus albus* (b-d) in response to phosphorus applied to the topsoil. Shown (b-d) is shoot dry weight for clover plants and total sward (clover + lupin) dry weight. The dashed line in panels b, c and d shows the yield of the subterranean clover monoculture to assist comparisons. Bars are the standard error of means (n=5). Different letters indicated significant differences between P rates for the clover component and the total sward yield (P < 0.05).

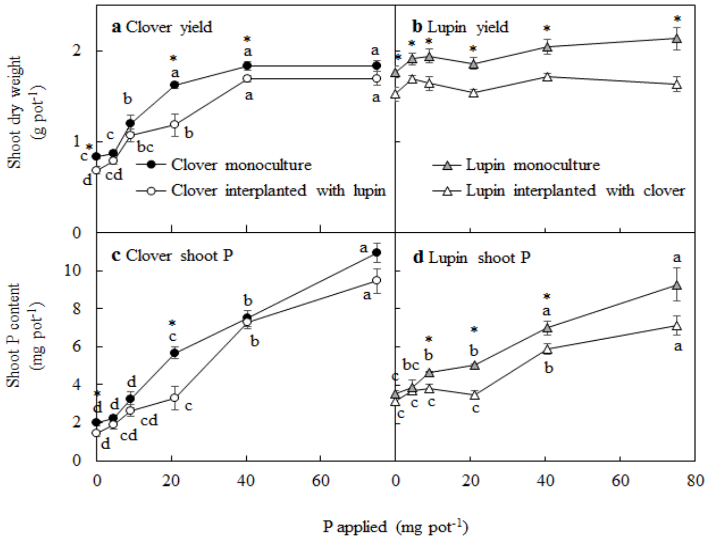

Figure 3. Experiment 2: Shoot dry weight and shoot P content of *Trifolium subterraneum* (a, c) and *Lupinus albus* (b, d) supplied with phosphorus applied in the topsoil layer. Subterranean clover and white lupin were grown as monoculture at densities of 10 and 2 plants pot^{-1}, respectively, or were interplanted at proportions 10:2. Bars are the standard error of means (n=5). Different letters indicated significant differences between P rates (P < 0.05). Asterisks indicate significant differences between treatments at each P rates (P < 0.05).

Discussion

Experiment 1 demonstrated that a significant dry matter yield benefit can be achieved at low and intermediate levels of soil P-supply when subterranean clover is interplanted with white lupin. However, this was at the expense of the subterranean clover component of the sward, suggesting strong competition between subterranean clover and white lupin. The major factor was competition for light, with the large leaved and tall lupins outcompeting the clover. When subterranean clover and white lupin shoots were separated and grown in mutually exclusive compartments (Experiment 2), the species were still competing for below-ground resources, but on much more equal terms (i.e. shoot yield and P uptake was suppressed by up to 20% for both species).

Experiment 2 also appeared to indicate that the clover did not gain a P-acquisition benefit when interplanted with the lupin or, if it did, other factors must have suppressed its ability to use any “facilitated” P release because neither clover yield nor P acquisition were improved in the low P soil. This is in direct contrast to many studies of P acquisition by cereals in cereal-legume intercrops, which report increases in plant growth and P uptake by the cereal when compared to cereal monocultures (Li et al. 2014). There were clear net benefits in dry matter yield by the interplanted treatments in Experiment 1. However, the results of Experiment 2 indicate that this is more likely to be a result of “complementarity” where enhanced productivity of a multi-species agroecosystem (compared with that of a monospecific system) is explained by a decrease in interspecific competition and/or competitive exclusion that improves resource partitioning among the interplanted species (Hinsinger et al. 2011). In the present case, white lupin may have improved net access to soil P in the interplanted treatments by (i) exploring different soil horizons and/or (ii) depleting different (i.e. sparingly-available) pool(s) of soil P.

Conclusion

The two experiments support the hypothesis that introducing root traits that enable P acquisition by “mining” to an agronomic system that relies on “P-foraging” strategies, can potentially increase the yield and P-efficiency of the production system. Experiment 1 also demonstrated that companion species must also exhibit equivalent shoot competition if stable botanical composition is to be achieved. Interplanted white lupins were used in the present experiments as a tool for hypothesis testing. However, species of lupins are used both as forages and as naturalised components of pastures, particularly on nutrient impoverished soils where they are known to have a low P requirement (Burtt and Hill 1990). The problem for applying these concepts to other pasture systems is that there are few convincing examples of pasture species with “P-mining” traits equivalent to those deployed by the white lupin. Gerke (2015), for example, has reported that red clover exudates protons and carboxylates (in particular citrate), but the field-relevance of this, or any other pasture species for P mobilisation is untested.

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