Differences in subsoil P acquisition by two subterranean clover cultivars in a P deficient soil

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Abstract
Phosphorus (P) is usually concentrated in the uppermost layers of the soil profile under pasture, hence topsoil root allocation is important for maximising P acquisition. However, total root length was recently found to be a marginally better predictor of variation in P uptake among twenty-six genotypes of subterranean clover (*Trifolium subterraneum* L.) when compared to topsoil root length alone. This result prompted a preliminary assessment of P acquisition by subsoil roots. Micro-swards of two cultivars were grown with a topsoil layer that was either P-deficient or amended with P for improved plant growth, overlying a low-P subsoil that contained ^{32}P-labelled phosphate. Both cultivars produced less shoot dry mass under P constraint, and the cultivar that allocated more root length to the subsoil layer produced a larger shoot dry mass in the P-deficient soil. This cultivar also recovered more ^{32}P-labelled phosphate from the subsoil layer in both P treatments. Therefore, variation exists for subsoil P acquisition and this trait may be important for determining shoot yield in P-deficient soil.

Key Words
Trifolium subterraneum L., ^{32}P-radioisotope, phosphorus acquisition, root system distribution

Introduction
Phosphorus is commonly stratified within the soil profile under pasture because most P fertiliser is broadcast (McLaughlin *et al.* 2011). Allocation of roots to the nutrient-rich surface layer is therefore important for maximising P acquisition (Lynch and Brown 2001). Cultivars of subterranean clover that achieve greater length of nutrient foraging roots in the P-enriched topsoil layer can acquire more P and increase shoot yield when growing in P-deficient soil (Haling *et al.* 2018). McLachlan *et al.* (2019) have shown that differences in topsoil root length among twenty-six genotypes of subterranean clover explained 72% of variation in P uptake. However, variation in total root length (topsoil and subsoil root length combined) explained 78% of this variation. Among the subterranean clover genotypes, there were differences in the proportion of roots allocated to the nutrient-enriched topsoil and the lower-P subsoil.

Subsoil roots are beneficial for increasing water acquisition in many agroecosystems (Lynch and Wojciechowski 2015). It is surmised that some genotypes of subterranean clover may have evolved in dry environments where water acquisition from depth was important or where periods of dry weather reduce surface P availability. In contrast to soil moisture, both P availability and arbuscular mycorrhizal fungi colonisation generally decrease with depth (Lynch and Wojciechowski 2015). Consequently, subsoil roots are expected to contribute minimally to total plant P uptake. The above observation that P acquisition from a P-deficient soil was improved by accounting for both topsoil and subsoil root length (McLachlan *et al.* 2019) prompted us to examine P acquisition by subsoil roots.

Methods
Soil treatments and plant growth
Shoot yield, root morphological traits and P uptake parameters were assessed for two cultivars of subterranean clover (*Trifolium subterraneum* L. cv. Losa and cv. Napier) in a controlled-environment pot trial. A sandy loam soil was collected near Armidale, Australia and separated into topsoil (2.5–10 cm) and subsoil (10–20 cm) layers. The soil layers were sieved (<5 mm) and a basal nutrient solution was applied. Two P treatments (0 and 40 mg P kg^{-1}; P0 and P40) were established in the topsoil by adding KH_2PO_4 to the basal nutrient solution before it was applied. This resulted in Colwell extractable P concentrations of 21.3 and 83.5 mg kg^{-1} (Colwell 1963), respectively. The subsoil layer contained 8.7 mg Colwell P kg^{-1} of native P and was labelled with 2.8 MBq pot^{-1} of $KH_2{}^{32}PO_4$ solution which provided an additional 0.015 mg P kg^{-1}. The amended topsoil and subsoil were then incubated separately for two weeks with occasional mixing. Based on the topsoil and subsoil Colwell extractable P concentrations achieved in a previous experiment (Haling *et al.* 2018), the P0

and P40 treatments were expected to provide soil-P conditions that were deficient and sufficient for clover growth, respectively.

Cylindrical PVC pots (87 mm internal diameter; 200 mm height) were filled with a bottom layer of 0.9 kg oven dry 'subsoil' followed by a top layer of 0.3 kg oven dry 'topsoil' of the amended soils after incubation. This mimicked the stratification of P that commonly occurs within the soil profile under pasture (McLaughlin *et al.* 2011). Micro-swards of the two subterranean clover cultivars (Losa and Napier) were established at a density of 6 plants pot^{-1}. Plants were grown under natural lighting in a glasshouse (12–20°C) between 5th October 2018 and 9th November 2018 at Armidale. The plants were inoculated with a commercially available symbiotic effective strain of *Rhizobium leguminosarum* bv. *trifolii* five days after planting. The soil was maintained at 80% field capacity and pots were watered to reach 100% field capacity once per week. Reflective sleeves were fitted and raised as canopy height increased to mimic the light conditions of a pasture sward.

Harvest and analysis

Shoots and roots from both soil layers were harvested after five weeks' growth. Topsoil and subsoil root samples were scanned to determine root length and average root diameter using winRHIZO (Reagent Instruments Inc.). Shoot and root samples were then dried at 80°C to determine dry mass. Additional topsoil and subsoil root samples were assessed for root hair length, root hair coverage and arbuscular mycorrhizal fungi colonisation. Total plant P uptake was determined by digesting the dried shoot and root samples using nitric acid, with subsequent analysis of the solution P concentration using malachite green. Digested shoot and root samples were also analysed for ^{32}P-radioiostope activity using liquid scintillation counting. Measured traits were used to derive parameters including topsoil and subsoil root length, total plant ^{32}P activity (total ^{32}P-activity in the shoots and roots), and total plant ^{32}P activity per unit subsoil root length. Data was analysed using a general analysis of variance in R (R Core Team 2018).

Results

Both cultivars of subterranean clover produced an equivalent shoot dry mass in the P40 treatment. However, cv. Napier produced 33% more shoot dry mass than cv. Losa in the P0 treatment ($P < 0.05$). In this low-P treatment, cv. Losa and cv. Napier produced relative shoot yields (i.e. relative to the average shoot yield in the P40 treatment) of 44% and 59%, respectively (Fig. 1). Total plant P uptake was 30% higher for cv. Napier than cv. Losa in the P0 treatment when compared using a 10% level of significance ($P = 0.07$) (data not shown).

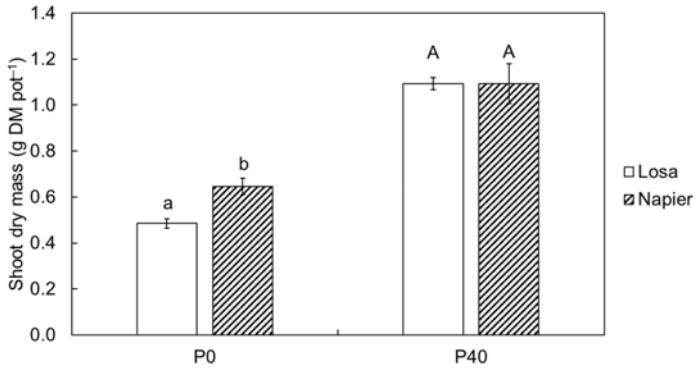

Figure 1. Shoot dry mass of two subterranean clover cultivars grown with either 0 or 40 mg P kg^{-1} applied in the topsoil layer of a pot. Values show mean ± standard error (n = 3). Cultivars of the same phosphorus treatment with the same letter are not significantly different at $P = 0.05$.

The topsoil root length of both cultivars was comparable in the P0 and the P40 treatments ($P > 0.05$). In contrast, significant variation was observed between the cultivars for subsoil root length. In the P0 treatment, cv. Napier produced 19% longer subsoil roots than cv. Losa ($P < 0.01$), while cv. Losa produced 32% longer subsoil roots than cv. Napier in the P40 treatment ($P < 0.01$) (Fig. 2). Although variation was observed for subsoil root length, total root length was not significantly different between cultivars in either P treatment ($P > 0.05$) (data not shown).

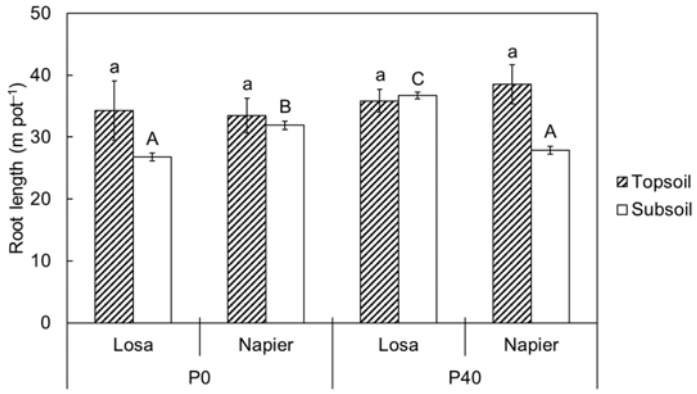

Figure 2. Topsoil and subsoil root length of two subterranean clover cultivars grown with either 0 or 40 mg P kg^{-1} applied in the topsoil layer of a pot. Values show mean ± standard error (n = 3) for each soil layer. Cultivars and phosphorus treatments of the same soil layer with the same letter are not significantly different at *P* = 0.05.

Total plant ^{32}P activity was 4.4-fold higher for cv. Napier than cv. Losa in the P0 treatment ($P < 0.01$). No difference was observed between cultivars in the P40 treatment ($P > 0.05$) (data not shown). Total plant ^{32}P activity per unit subsoil root length was 3.7-fold higher for cv. Napier than cv. Losa in the P0 treatment ($P < 0.05$), and 2.1-fold higher for cv. Napier in the P40 treatment ($P < 0.05$) (Fig. 3).

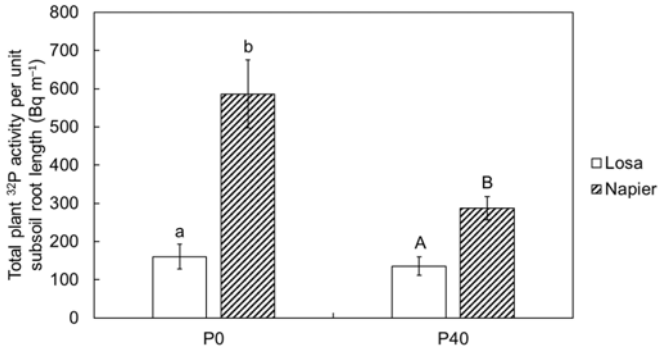

Figure 3. Total plant ^{32}P activity per unit subsoil root length of two subterranean clover cultivars grown with either 0 or 40 mg P kg^{-1} applied in the topsoil layer of a pot. Values show mean ± standard error (n = 3). Cultivars of the same phosphorus treatment with different letters are significantly different at *P* = 0.05.

To estimate the proportion of P derived from the subsoil it was assumed that: i) the ^{32}P-radioisotope had effectively labelled the extractable Colwell P fraction of the subsoil layer, and ii) the Colwell P fraction approximated the plant available P pool. Accordingly, the percentage of total plant P derived from the subsoil was estimated to be 3.3-fold higher for cv. Napier (26% of total plant P) than cv. Losa (8% of total plant P) in the P0 treatment ($P < 0.01$) (Fig. 4).

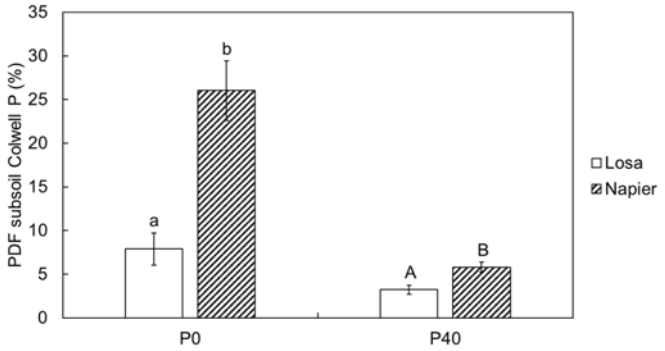

Figure 4. Estimated potential percentage of total plant phosphorus derived from the subsoil extractable Colwell P fraction (PDF subsoil Colwell P) for two subterranean clover cultivars grown with either 0 or 40 mg P kg^{-1} applied in the topsoil layer of a pot. Values show mean ± standard error (n = 3). Cultivars of the same phosphorus treatment with different letters are significantly different at *P* = 0.05.

Discussion

Both cultivars of subterranean clover produced less shoot dry mass under a P constraint, but the cultivar that produced longer subsoil roots in the low-P treatment recovered more P from the subsoil layer and produced a larger shoot yield. In comparison, the amount of ^{32}P-labelled phosphate acquired from the subsoil layer was significantly less in the P40 treatment and differences were not observed between cultivars for shoot yield. This result demonstrated that some cultivars of subterranean clover allocate more root length to the subsoil despite differences in the relative P nutrition status of the topsoil and subsoil layers. Similar results have been observed in common bean (*Phaseolus vulgaris* L.), where some genotypes responded to topsoil P availability by increasing the number of shallow basal roots, whereas a smaller number of genotypes increased the number of deeper basal roots (Bonser *et al.* 1996).

Topsoil foraging is expected to maximise P acquisition from soil (Lynch and Brown 2001). This result has been demonstrated regularly in genotypes of subterranean clover (McLaughlin *et al.* 1990; Haling *et al.* 2018). However, the current experiment indicates that roots allocated to the low-P subsoil layer may contribute substantively to total plant P uptake when clover is grown in P-deficient soil. For example, the estimates derived by assuming Colwell extractable P equalled the plant-available P fraction suggested that, under P-deficient growth conditions, cv. Napier acquired up to 26% of its total plant P from the subsoil, whilst cv. Losa may have acquired ~8% of its total plant P from this layer. Phosphorus uptake rate from the subsoil layer was not dependent on root length allocated to that layer alone, because cv. Napier achieved significantly higher levels of total plant ^{32}P activity per unit subsoil root length. This result indicated that other root morphology or physiological factors may be important for P acquisition differed between these cultivars.

Conclusion

Two contrasting cultivars of subterranean clover were shown to acquire some P from the subsoil, with the variety that allocated more roots to this layer achieving a larger shoot yield. However, root length allocation was not the only factor that was involved because this cultivar also achieved a higher rate of P acquisition per unit root length. Further experiments are required to quantify the relative contribution of P acquisition from subsoil layers for total plant P uptake, and to determine the mechanisms responsible for different rates of P uptake by cultivars of subterranean clover.

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References

Bonser, AM, Lynch, J, Snapp, S (1996) Effect of phosphorus deficiency on growth angle of basal roots in Phaseolus vulgaris. *New Phytologist* **132**, 281-288.

Colwell, JD (1963) The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales by soil analysis. *Australian Journal of Experimental Agriculture* **3**, 190-197.

Haling, RE, Brown, LK, Stefanski, A, Kidd, DR, Ryan, MH, Sandral, GA, George, TS, Lambers, H, Simpson, RJ (2018) Differences in nutrient foraging among *Trifolium subterraneum* cultivars deliver improved P-acquisition efficiency. *Plant and Soil* **424**, 539-554.

Lynch, JP, Brown, KM (2001) Topsoil foraging - an architectural adaptation of plants to low phosphorus availability. *Plant and Soil* **237**, 225-237.

Lynch, JP, Wojciechowski, T (2015) Opportunities and challenges in the subsoil: pathways to deeper rooted crops. *Journal of Experimental Botany* **66**, 2199-2210.

McLachlan, JW, Haling, RE, Simpson, RJ, Li, X, Flavel, RJ, Guppy, CN (2019) Variation in root morphology and P acquisition efficiency among *Trifolium subterraneum* genotypes. *Crop and Pasture Science* **submitted**,

McLaughlin, MJ, Baker, TG, James, TR, Rundle, JA (1990) Distribution and forms of phosphorus and aluminium in acidic topsoils under pastures in south-eastern Australia. *Australian Journal of Soil Research* **28**, 371-385.

McLaughlin, MJ, McBeath, TM, Smernik, R, Stacey, SP, Ajiboye, B, Guppy, C (2011) The chemical nature of P accumulation in agricultural soils - implications for fertiliser management and design: an Australian perspective. *Plant and Soil* **349**, 69-87.

R Core Team (2018) 'R: A Language and Environment for Statistical Computing.' (R Foundation for Statistical Computing: Vienna, Austria)