Competitive interactions between serradella (*Ornithopus* spp.) and subterranean clover (*Trifolium subterraneum*) in mixed pasture swards

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Abstract
Serradellas (*Ornithopus* spp.) are alternative, annual legumes for pastures in temperate Australia. They have several advantages relative to subterranean clover (*Trifolium subterraneum*) including a lower critical external phosphorus (P) requirement. However, persistent serradella pastures may depend on their ability to compete with clovers that will inevitably germinate from the soil seed bank. In a controlled-environment experiment, monoculture and mixed swards of French serradella (cv. Margurita) and subterranean clover (cv. Denmark) were grown in six different levels of soil P fertility. When grown as 50:50 mixtures, serradella had a competitive advantage over subterranean clover (i.e. produced more shoot biomass), especially at the intermediate levels of soil P fertility. However, the competitive advantage of serradella in the mixed sward declined under high soil P fertility. Management of soil P fertility at levels close to the critical soil test P requirement of serradella may be a useful management tool for maintaining serradella dominance in pastures that also contain subterranean clover.

Keywords
Legumes, pasture, persistence, P-acquisition, root competition.

Introduction
Serradellas (*Ornithopus* spp.) are annual pasture legumes suitable for use in acid soils and the same climatic zone as subterranean clover (Hill 1996). They have a number of useful agronomic attributes (productive, deep rooted, high acid-soil tolerance, low bloat risk) and a lower critical phosphorus (P) requirement for maximum yield (Sandral et al. 2019) making them more productive than subterranean clover (*Trifolium subterraneum*) in low-P soils (Paynter 1990; Haling et al. 2016a; Sandral et al. 2019). A serradella-based pasture is estimated to be maintained with up to 30% less P-fertiliser than a subterranean clover-based pasture, without compromising yield (Simpson et al. 2014).

Subterranean clover has been sown over ~29M ha in southern Australia and is the most widely grown annual legume (Nichols et al. 2013). If serradellas were to be sown in place of subterranean clover to capture their P-efficiency advantage, it is inevitable that they will be sown into soils that have an existing subterranean clover seed bank and the two species will eventually co-exist and compete for common resources. We hypothesised that the serradellas would have a competitive advantage over subterranean clover in P-deficient soils due to their root morphology characteristics which enable greater P acquisition in low P soil, and a lower critical external P requirement (Haling et al. 2016b). However, it is unknown whether the root morphology of serradella is affected when competing with subterranean clover. Many plant traits are influenced by the presence of neighbours and it is possible that root traits may be modified as a consequence of competitive interactions leading to unexpected outcomes in mixed swards (Novoplansky 2009).

This experiment investigated the potential consequences for botanical composition and yield in a serradella sward with subterranean clover "volunteers" at different proportions.

Methods
Soil and phosphorus supply: A sandy loam soil (Yellow Chromosol) with a low concentration of extractable P (5.63 mg kg^{-1} P; Colwell 1963) was collected from near Hall, ACT (35°10′34.5″S, 149°02′37.5″E). The soil was steam pasteurised at 60-65 °C for 2 hours to reduce disease inoculum

and then mixed with lime (1.5 g $CaCO_3$ kg^{-1}) to raise pH to 5.5 (1:5 *w/v*; 0.01 M $CaCl_2$). Basal nutrients and starter N were incorporated to ensure that only soil P and N would be limiting for plant growth.

Six P-fertilised topsoil treatments (0, 20, 40, 80, 120, and 300 mg P kg^{-1} oven dry soil; hereafter referred to as P0, P20, P40, P80, P120 and P300) were created by mixing KH_2PO_4 with subsamples of the limed and basal nutrient-amended, low-P soil. The P-fertilised soil was then used as the topsoil for the P treatments. Preliminary experiments indicated that the P80 treatment would be close to the critical P requirement of serradella (P sufficient to support 90% max. growth) but would be deficient for the growth of subterranean clover (data not shown).

Pots (cylindrical PVC; 87 mm internal diameter, 190 mm soil height) were filled with the equivalent of 0.9 kg of oven dry soil using the basal nutrient and lime-amended soil to create a subsoil layer with low P fertility. A suitable topsoil layer for each P treatment (0.3 kg, oven dry equivalent) was then added. Creating a P-fertilised topsoil layer in this way was intended to mimic the stratification of P in soil profiles that occurs when P-fertiliser is broadcast onto pasture.

Plant material: French serradella (*O. sativus,* cv. Margurita) and subterranean clover (cv. Denmark) were selected based on results from preliminary experiments that indicated these cultivars would achieve comparable monoculture yields when supplied with adequate P. The five sward composition treatments were: serradella monoculture; subterranean clover monoculture; 25:75, subterranean clover: serradella mixture; 50:50, subterranean clover: serradella mixture; and 75:25, subterranean clover: serradella mixture. All sward treatments were sown in an even planting pattern (total plant density = 12 plants pot^{-1} [2,020 plants m^{-2}]). Because serradella germinates slowly, the French serradella was sown first, and after 50% of the seedlings had emerged (i.e. 6 days after sowing), subterranean clover was over-sown. Subterranean clover emerged within 2 days after sowing. Each legume was inoculated with their specific rhizobium inoculum: Group C (strain WSM 132, clover), Group S (strain WSM 471, serradella).

Plant growth conditions: The micro pasture swards were grown in a controlled-environment growth cabinet with daily watering. The experiment was a randomised block design with 6 replicates per treatment combination. Days had 12 hours light (720 µmol $quanta^{-1}$ m^{-2} s^{-1} at canopy height) and 12 hours dark, at 24/15 °C (at plant height), respectively. Sleeves with reflective interiors were fitted to each pot and raised daily to equal canopy height to mimic the light conditions within a pasture sward.

Shoot yield: The micro pasture swards were harvested five weeks after the emergence of the subterranean clover. Shoots were cut at the soil surface and separated into the component species before being dried (70°C) and weighed to determine dry mass.

Root growth and morphology: Soil was removed from each pot as an intact core and cut at the interface of the fertilised topsoil (0-45 mm depth) and the subsoil (45-190 mm depth). Roots were washed from the soil and floated in water so that they could be gently teased apart with minimal damage. The root systems of the two legumes were differentiated using root morphology and colour (serradella roots, fine root system and white in colour; clover roots, relatively coarse and off-white in colour). The topsoil and subsoil roots of the component species in all mixtures were able to be separated. The separated root samples were scanned (400 dpi) and analysed for root length and root diameter using WinRHIZO software (Regent Instruments Inc., Quebec City, Canada). Following scanning, the roots were dried at 70°C to determine root dry mass. Root hair length was assumed to be equivalent to that measured on these species by Yang et al. (2017): i.e. 0.25 mm for subterranean clover and 0.7 mm for French serradella. The root morphology data were used to calculate the surface area of the root hair cylinder (SARHC); a measure that approximates the root-soil interface for P acquisition by a pasture legume (Haling et al. 2016b):

$$\text{SARHC} = \text{total root length} \times \pi \times (\text{average root diameter} + 2 \times \text{root hair length})$$

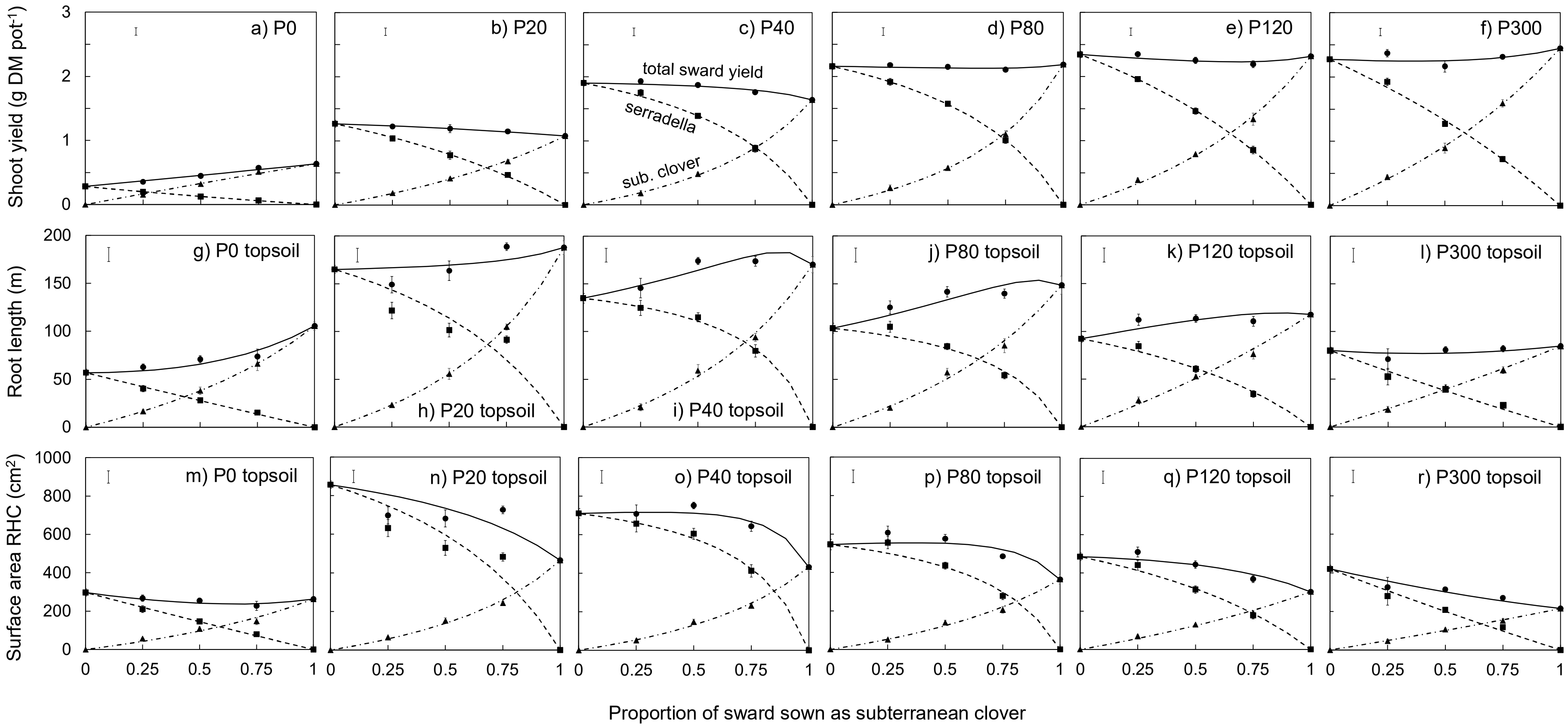

Figure 1. Shoot yields (a – f), topsoil root length (g – l) and topsoil surface area of the root hair cylinder (SARHC; a measure of the size of the root-soil interface for P acquisition) (m-r) of the subterranean clover (dash-dot lines) and French serradella (dashed lines) components of mixed pasture swards grown at 6 levels of phosphorus supply. Total yield, total root length and total SARHC of the mixed swards is shown by the solid lines. Error bars associated with symbols = 2x SE. Bars at the top of each pane of the figure show the LSD (*P*=0.05) for the 'phosphorus treatment x sward composition' interaction.

Results and Discussion

Competitive interactions between the species were examined by graphing the shoot yield outcomes for each species when sown in mixtures (Figs.1a-f). In general, the species component in a mixed sward that was present in the larger proportion achieved a larger shoot mass. When the proportion of serradella plants in a sward was 50% or more, the yield of the serradella component exceeded that of the subterranean clover (1.4 to 9.6-fold). The only exception being when no P was applied (P0). However, the proportion of clover plants usually needed to be ≥75% in the mixed swards for the clover to produce similar (e.g. at P40 and P80) or more shoot yield (at P120, P300) than the French serradella component. The serradella component of the 50:50 mixed swards achieved shoot yields 30-70% larger than the subterranean clover component at most P levels >P0 ($P<0.05$). However, the largest competitive advantage for French serradella over subterranean clover occurred at intermediate P levels (P40, P80). Serradella's competitive advantage was reduced at high P levels (P120, P300).

Both species proliferated topsoil root length in low P soil (a typical acclimation response to P deficiency), reaching lengths in the P20 treatment that were ~2-fold that grown at P300 (Figs.1g-l). However, this was not maintained in P0 soil. When grown as a monoculture, the clover proliferated longer roots than the serradella. However, the serradella consistently produced longer roots than the clover in the 50:50 mixed swards with intermediate P levels (P20-P80). Neither species proliferated root length (in response to surface P treatment) in the low-P subsoil (data not shown). When root hair length was accounted for by calculating the SARHC (Figs.1m-r), it was clear that the long root hairs of serradella enabled it to develop a larger root-soil interface for P acquisition in monocultures and in the mixed pastures. However, this root morphology advantage was lost when the mixed swards and monocultures were grown in high P soil (P120, P300). In general, the relative root morphology advantage (or lack thereof) revealed by the SARHC estimates reflected the competitive shoot yield outcomes achieved by each of the species.

Conclusion

The fecundity of these annual legumes will have a strong, perhaps overriding, influence on their competitiveness in mixed legume swards irrespective of soil P fertility; the species present in the largest proportion in a mixture generally achieved the largest shoot mass. However, serradella had a root morphology advantage for P acquisition when grown at intermediate soil P levels. This indicated that management of soil P levels close to the lower critical soil test P requirement of serradellas may be a useful tool for supporting serradella dominance in pastures that also contain subterranean clover.

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