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Growth, Rhizosphere Carboxylate Exudation, and Arbuscular Mycorrhizal Colonisation in Temperate Perennial Pasture Grasses Varied with Phosphorus Application

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Abstract: Phosphorus (P) fertiliser is applied regularly to the nutrient-poor sandy soils in southwestern Australia to elevate and/or maintain pasture production. This study aimed to characterise differential growth, root carboxylate exudation, and mycorrhizal responses in three temperate perennial pasture grasses at variable P supply. Tall fescue (*Festuca arundinacea* L. cv. Prosper), veldt grass (*Ehrharta calycina* Sm. cv. Mission), and tall wheatgrass (*Thinopyrum ponticum* L. cv. Dundas) with five P rates varying from 0 to 100 mg P kg kg^{-1} soil were evaluated in a controlled environment. Rhizosphere carboxylate exudation and mycorrhizal colonisation were assessed. Veldt grass produced the maximum shoot dry weight, highest agronomic phosphorus-use efficiency at low P supply, as well as the highest specific root length and shoot P content at all P rates. Across species, the maximum shoot weight was obtained at 20 and 50 mg P kg kg^{-1} soil, which differed significantly from the two lowest P rates (0 and 5 mg P kg kg^{-1} soil). Phosphorus application influenced carboxylate exudation, with plants exuding acetate only in the zero P treatment, and citrate and malonate in the P-supplemented treatments. In all three species, acetate and malonate were the major carboxylates exuded (37–51% of the total). Only tall wheatgrass released *trans*-aconitate. Citrate and malonate concentrations in the rhizosphere increased with P supply, suggesting their important role in P acquisition. Phosphorus applications reduced arbuscular mycorrhizal colonisation and increased root diameter as the P rate increased. Root carboxylate exudation in low-P soil played a role in mobilisation of P via P solubilisation, but the role of exuded carboxylate in soils well supplied with P might be diminished.

Keywords: nutrient uptake; phosphorus-use efficiency; root morphology

1. Introduction

Most soils in southwestern Australia are sandy, with low nutrient availability [1]. These soils also have a moderate- to high sorption capacity for elements such as phosphorus (P) [2], leading to inefficient fertiliser use [3]. Consequently, the growth of cereals and pastures is constrained, and growers resort to adding inorganic fertilisers to lift and/or maintain production [4].

Phosphorus accessibility by plants is an issue because most (>80%) of the P in soil is unavailable because of precipitation, low mobility, and conversion to organic forms [5]. Thus, an effective use of P fertilisers is imperative as they represent a high cost to farmers, particularly in developing countries [6];

moreover, P is a finite resource [7]. In Australian agriculture, P fertilisers represent on average 47% of all fertilisers used on an elemental basis [8].

Considering the significance of P in modern agriculture, different approaches were formulated to increase P-use efficiency through improved fertiliser management practices, changes in fertiliser technology, and improved soil biology [8]. An additional and equally important strategy in this endeavour is the selection of P-efficient species and cultivars that have higher nutrient acquisition and use efficiencies in low-P soils to outperform standard varieties [9,10]. Such cultivars were identified in different pasture grasses and legumes [4,11]. Hill et al. [12] characterised different temperate pasture species into competitive, stress-tolerant, and ruderal functional types based on their critical P requirement. Phalaris (*Phalaris aquatica* L.) was classified as a stress-tolerant competitor because of its abundance in the non-fertilised grazing systems. Its dominance was attributed to its effective P acquisition from infertile soils. Another viable approach is to identify the optimal P requirement of a particular variety to maximise productivity and avoid either over- or under-fertilisation. The right amount of fertiliser application will have the dual effect of environmental conservation and achieving a P-efficiency objective [6].

Plants have evolved different mechanisms in response to nutrient deficiency, particularly regarding soil-immobile elements such as P. One mechanism is enhanced carboxylate exudation into the rhizosphere that facilitates the acquisition of sparingly soluble P [13], thereby increasing P fertiliser-use efficiency [14]. In certain *Lupinus* species, carboxylate exudation is considered more important for nutrient acquisition than a large root system [15]. Even though the potential for carboxylate exudation has been characterised in many cultivated crops and pastures [14,16] and native plant species [17], there is a paucity of such knowledge in temperate perennial pasture grasses. However, in two perennial pasture grass species, phalaris and cocksfoot (*Dactylis glomerata* L.), Kidd et al. [18] reported citrate and malate as the major carboxylates released into the low-P soils. Although it is often challenging to quantify the contribution to plant growth of soil P made available by carboxylates [18], a positive relationship between the quantity of root exudates and plant P content has been documented [19].

Other adaptations that plants have developed in response to low P availability include changes in root morphology [5,20] and arbuscular mycorrhizal (AM) associations [21,22]. Most pasture species respond to P deficiency by reducing the root diameter and increasing the specific root length [6,20]. Similarly, most plant species develop a mycorrhizal association that improves plant P uptake, whereby P is foraged from large volumes of soil by the hyphae and delivered to the root cortical cells [23].

In southwestern Australia, there is renewed interest in the diversification of pastures and cultivation of perennial pasture species owing to increased dryland salinity [24] and the potential of suitable perennial pasture grasses to occupy vacant "cold zones" [25]. Perennial pastures are generally more effective at extracting water from the deep soil profile than annual crop and pasture species [26]. Given that P fertilisation is needed as pasture grasses are introduced or their area expanded, the present study was aimed at characterising the yield response of three temperate perennial pasture grasses to different P rates and determining the amount and composition of rhizosphere carboxylates because there is limited information on root exudation by these species.

2. Materials and Methods

2.1. Plant Species and Soil Material

Three temperate perennial pasture grasses—tall fescue (*Festuca arundinacea* L. cv. Prosper), veldt grass (*Ehrharta calycina* Sm. cv. Mission), and tall wheatgrass (*Thinopyrum ponticum* L. cv. Dundas)—were chosen considering their importance in the Australian farming systems and positive responses to biofertiliser in our earlier experiment [27]. Tall fescue has a wide adaptability to diverse environments, and tall wheatgrass has a wide adaptability to the saline environments [28]. Veldt grass has high palatability and adaptability to nutrient-poor sandy soils [29]. The seeds were obtained from Heritage Seeds and Irwin Hunter Seeds based in Perth.

Surface soil of about 10 cm depth was collected from the Shenton Park Field Station (31°94′69′′ S, 115°79′53′′ E) that had no record of any external fertilisation. The soil was sandy and analysed by CSBP analytical laboratories at Bibra Lake, Western Australia, and contained (mg kg^{-1} soil) 2 nitrate N [30], 3 P, and 20 K [31]. The pH was 4.8 ($CaCl_2$) [32], organic carbon was 15 g kg^{-1}, and the P-buffering index was 21.

2.2. Experimental Treatments and Plant Growth Conditions

The soil was air-dried, sieved, and weighed 6 kg into long cylindrical PVC columns (10 cm diameter and 60 cm length) lined with a polyethylene bag. There were five P treatments (0, 5, 20, 50, and 100 mg P kg kg^{-1} soil) to provide conditions from P starvation to excess. $Ca(H_2PO_4)_2{\cdot}H_2O$ (monocalcium phosphate monohydrate) was used as the P source. In addition, each treatment received a basal dose of nutrients from stock solutions (mg kg^{-1} of soil): N (33), K (89), S (34), Ca (41), Cl (73), Mg (4), Mn (3), Zn (2), Cu (0.5), B (0.1), Co (0.1), and Mo (0.08). The treatments and basal nutrients were mixed thoroughly with the soil. Plants were top-dressed with N at the same rate as in the basal fertilisation at 4 weeks after sowing to ensure that plant growth was not limited by N supply. Iron-EDTA was not supplied to avoid interference with the high-performance liquid chromatography (HPLC) analysis of carboxylates [18,33], but we have never observed problems with iron supply in Western Australian soils.

Ten seeds were planted at 2 cm depth and thinned to six plants per pot 10 days after emergence. The pots were watered with deionised water to maintain 70% field capacity. Plants were grown in a glasshouse complex at The University of Western Australia (31°98′ S, 115°81′ E) with the mean day and night temperatures of 25 °C and 15 °C, respectively. There were four replications laid out in a randomised complete block design. Pots were re-randomised within blocks weekly to reduce the influence of environmental gradients.

2.3. Carboxylate Extraction and Analysis

Plants were harvested 10 weeks after sowing by cutting the shoots just above the soil surface. After the shoots were harvested, the plastic bags were removed from the cylindrical columns and cut open. The carboxylate extraction procedure followed an established method routinely used [14,18]. Briefly, plants with intact roots were lifted carefully and shaken gently to remove the bulk soil. Then, roots with the rhizosphere soil still attached were immersed into a 500 mL beaker containing 25 to 100 mL of 0.2 mM $CaCl_2$ depending on root size. The roots were kept in the solution for approximately one minute with frequent gentle shaking to maximise the detachment of rhizosphere soil. Then, the roots were taken out, and a subsample of the suspension was drawn and filtered (0.20 µm) into a 1 mL HPLC vial containing 20 µL concentrated orthophosphoric acid. The filled vials were placed on ice during extraction and stored at −20 °C until analysis.

The analysis was performed by HPLC as described [34]. The carboxylates were detected by relating the retention times and absorption spectra of samples with those of working standards (acetic, citric, fumaric, malonic, shikimic, *cis*-aconitic, and *trans*-aconitic acids).

2.4. Plant Harvest and Nutrient Measurements

The roots were washed with water to remove adhering soil particles and organic matter. A subsample of roots (after cutting up the root system and dispersing it in water) was collected for mycorrhizal colonisation and morphological assessments, transferred to 50% ethanol, and preserved at 4 °C prior until analysis [35]. The shoots and left over roots were oven-dried at 65 °C for 3 days and weighed.

The phosphorus concentration was determined by Inductively Coupled Plasma Optical Emission Spectrometry after digesting about 20 mg subsamples of finely ground shoots and roots in concentrated nitric and perchloric acids as detailed [4]. The P content was obtained as a product of P concentration and the corresponding dry biomass. Agronomic P-use efficiency was determined as an increase in shoot yield per unit of added P fertiliser [9].

2.5. Root Morphology

The root subsamples were spread in glass dishes containing water and scanned in greyscale at 400 dots per inch using a flatbed scanner (Epson Perfection V800 photo). A WinRHIZO Pro (v. 2009c Regent Instruments, Montreal, QC, Canada) analysed the root images as in other studies [33,36]. Specific root length was calculated as the root length per root dry mass.

2.6. Arbuscular Mycorrhizal Colonisation

The sub-sampled roots were dissected into approximately 0.5 to 1 cm length [37] and stained as described [38]. The mycorrhizal colonisation percentage was assessed by the gridline intercept method [37] through dispersing the roots in Petri dishes and observing under an optical microscope. At least 200 intersections per sample were counted.

2.7. Statistical Analyses

The two-way ANOVA was used to measure the effect of main factors (grass species and P rate) and their interaction on different measured variables in Genstat 19 statistical software (VSN International). The two-way interaction of grass species and P rates was presented when significant at $p \leq 0.05$. When there was no significant interaction, significant main effects were presented by pooling the data across the grass species or P rates. The differences in treatment were compared by Tukey's Honest Significant Difference test when ANOVA indicated significant main effects or interaction.

3. Results

The grass species and P rates interacted significantly ($p \leq 0.05$) to influence arbuscular mycorrhiza colonisation and agronomic P-use efficiency (Table 1). In contrast, the shoot and root dry weight, specific root length, average root diameter, citrate exudation, total carboxylates exuded, shoot P content, and root P concentration and content were influenced significantly by the both main effects. Acetate exudation, malonate exudation, and shoot P concentration were influenced significantly only by the P rates (Table 1).

Table 1. Analysis of variance of measured parameters with species and phosphorus (P) rates as the main factors.

Variable	Species	P Rates	Species x P Rates
Shoot dry weight (g plant^{-1})	***	***	NS
Root dry weight (g plant^{-1})	***	***	NS
Arbuscular mycorrhiza colonisation (%)	NS	***	***
Specific root length (m g^{-1})	***	***	NS
Average root diameter (mm)	***	*	NS
Acetate exudation (µmol g^{-1} dry root)	NS	***	NS
Citrate exudation (µmol g^{-1} dry root)	***	**	NS
Malonate exudation (µmol g^{-1} dry root)	NS	**	NS
Total carboxylates exuded (µmol g^{-1} dry root)	***	**	NS
Shoot P concentration (g kg^{-1} dry weight)	NS	***	NS
Shoot P content (mg plant^{-1})	***	**	NS
Root P concentration (g kg^{-1} dry weight)	**	***	NS
Root P content (mg plant^{-1})	***	**	NS
Agronomic PUE (g shoot dry weight g^{-1} P applied)	***	***	***

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; PUE, phosphorus-use efficiency; NS, not significant.

3.1. Plant Growth

Shoot production in the three species differed significantly ($p \leq 0.05$), with veldt grass producing significantly higher shoot dry weight than the other two species (Figure 1A). Tall wheatgrass produced significantly more root dry weight than the other two species (Figure 1A).

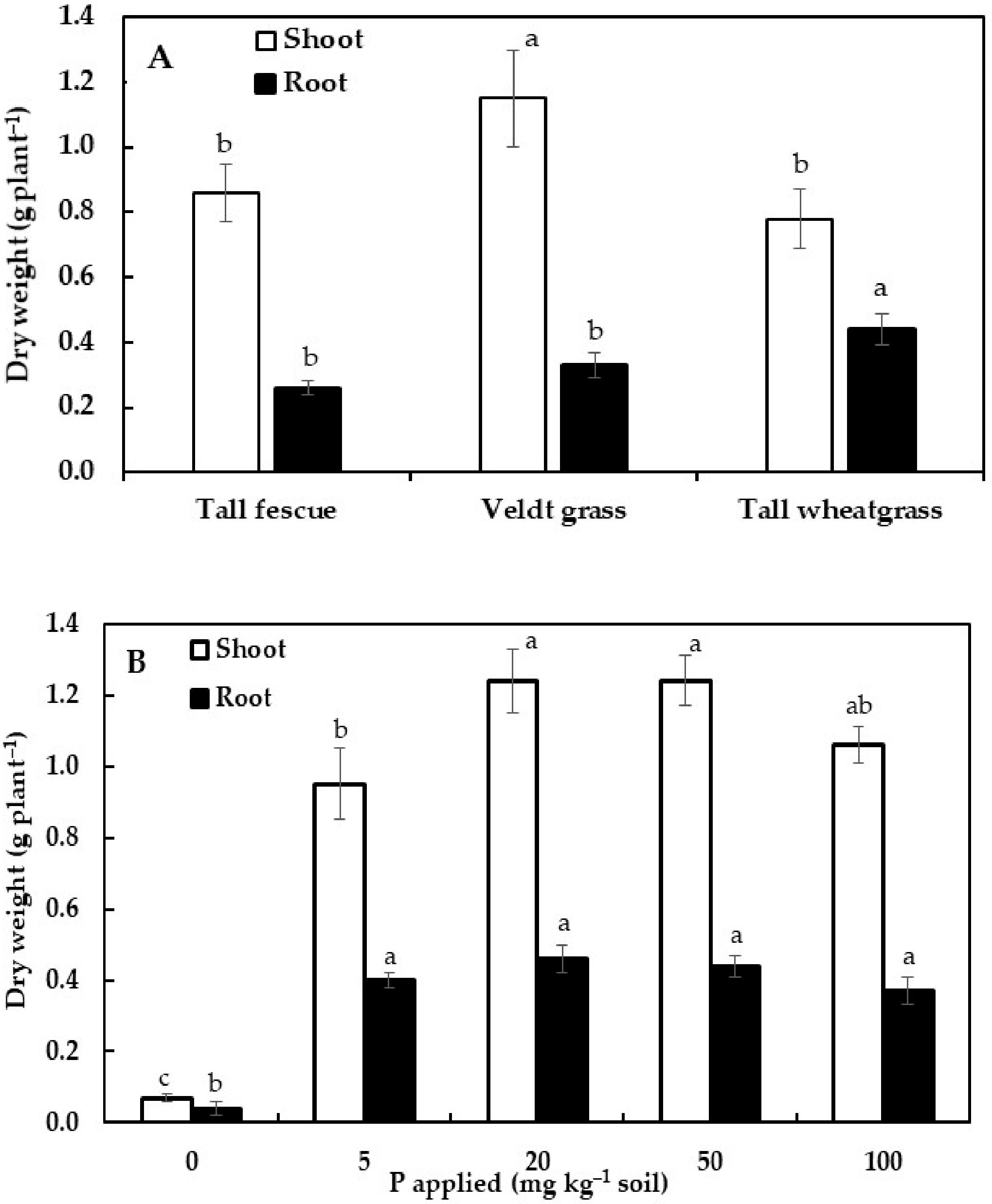

Figure 1. Shoot and root dry weights (**A**) in three temperate perennial pasture grasses and as influenced by different phosphorus (P) rates (**B**). Values for a given parameter indicated by different letters differed significantly ($p \leq 0.05$). ANOVA showed the significant main effects of grass species and P rates but not the interaction. The data on individual grasses were averaged across five P rates ($n = 20$), and the data on individual P rates were averaged across three pasture grasses ($n = 12$). Means ± SE.

The P rate had a significant effect ($p \leq 0.05$) on shoot and root dry matter production. Shoot weight at 20 and 50 mg P kg kg^{-1} soil differed significantly from the two lowest P rates (0 and 5 mg P kg kg^{-1} soil) (Figure 1B). Increases in P application rates beyond 20 mg P kg kg^{-1} soil did not improve shoot dry matter production. Regarding roots, significantly higher root dry weight was recorded at 5 mg P kg kg^{-1} soil and the higher rates than 0 mg P kg kg^{-1} soil (Figure 1B).

3.2. Arbuscular Mycorrhizal Colonisation

The grass species and P rates interacted significantly in influencing mycorrhizal colonisation. Tall wheatgrass and veldt grass without any added P had significantly higher AM colonisation ($p \leq 0.05$) than tall fescue, but there was no significant difference among the three species at other P rates (Figure 2). Furthermore, the percent of AM colonisation in all the three species decreased between non-fertilised control and the treatments with P fertilisation regardless of the rate applied, with the exception of tall fescue at 5 mg P kg kg^{-1} soil compared with 20 and 50 mg P kg kg^{-1} soil (Figure 2).

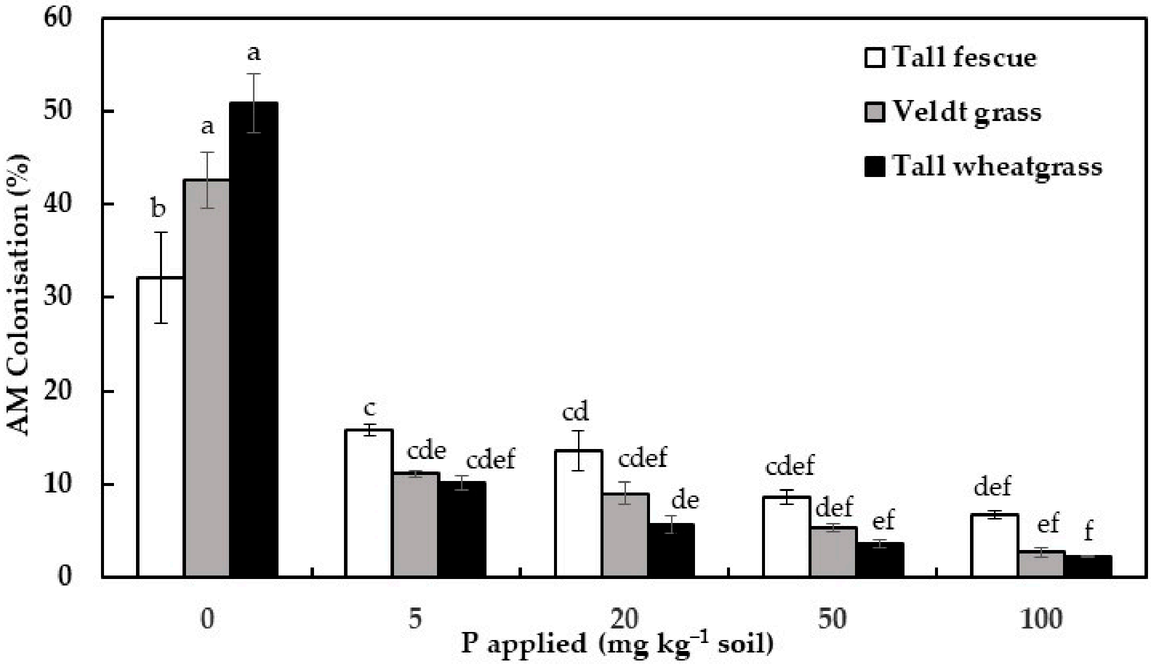

Figure 2. Effect of different phosphorus (P) rates on arbuscular mycorrhiza (AM) colonisation in three temperate perennial pasture grasses. Treatment values indicated by different letters differed significantly ($p \leq 0.05$). ANOVA showed a significant interaction between the grass species and P rates. Means ± SE. $n = 4$.

3.3. Specific Root Length and Diameter

Significant difference was observed between species and among P rates in specific root length and average root diameter (Table 1). Veldt grass had significantly higher specific root length ($p \leq 0.05$) than the other two species (Figure 3A), and tall wheatgrass had significantly higher average root diameter than the other two species (Figure 3C). The zero P treatment produced significantly higher specific root length than the two highest P rates (50 and 100 mg P kg kg^{-1} soil) (Figure 3B). Conversely, the zero P treatment produced significantly smaller average root diameter than the other P rates (Figure 3D).

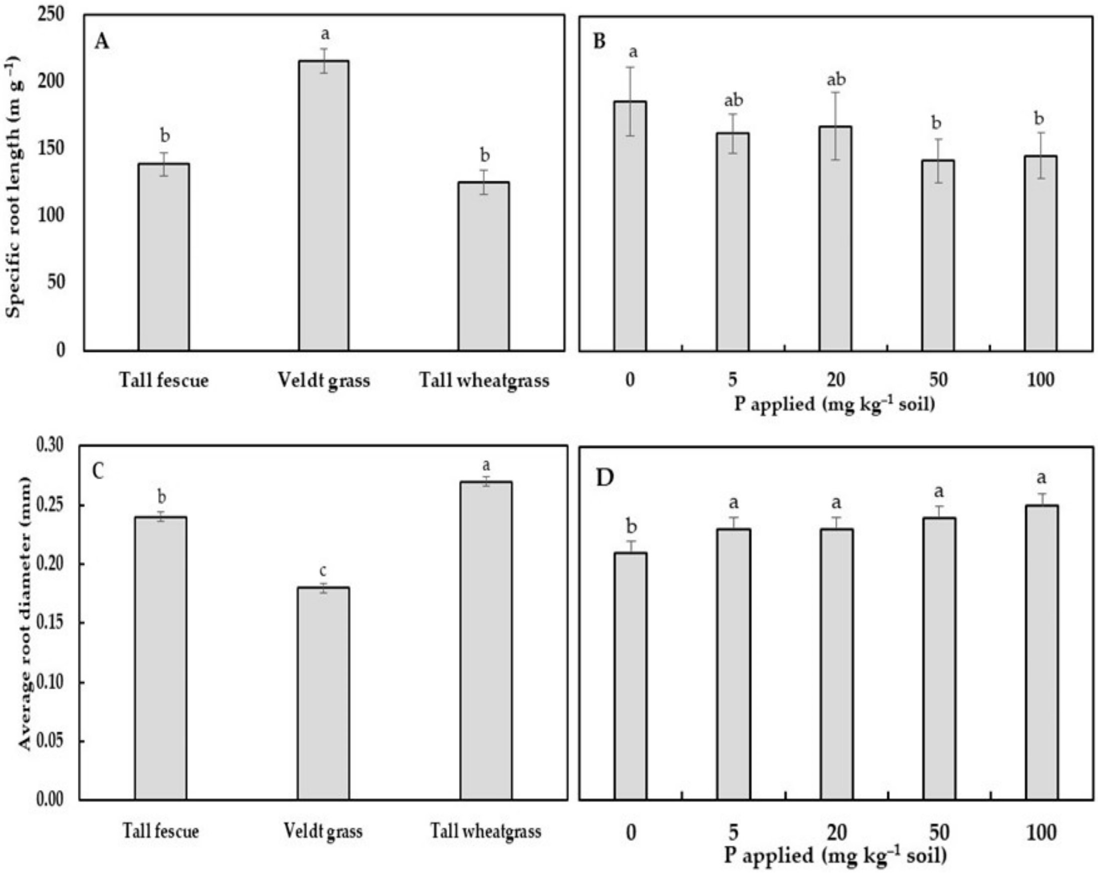

Figure 3. Specific root length (**A**) in three temperate perennial pasture grasses and as influenced by different phosphorus (P) rates (**B**). Average root diameter (**C**) in three temperate perennial pasture grasses and as influenced by different P rates (**D**). Treatment values indicated by different letters differed significantly ($p \leq 0.05$). ANOVA showed significant main effects of grass species and P rates, but not the interaction. The data on individual grasses were averaged across five P rates ($n = 20$) and the data on individual P rates were averaged across three pasture grasses ($n = 12$). Means ± SE.

3.4. Carboxylate Exudation

Species exerted a significant effect ($p \leq 0.05$) on citrate exudation. Tall wheatgrass exuded significantly more citrate than the other two species and significantly more total carboxylates than tall fescue (Figure 4A). The P rates also had significant effect on the exudation of both individual anions and the total amount. All the three species exuded acetate only at zero P treatment (Figure 4B). In contrast, citrate and malonate were released only in the P-supplemented treatments, with no significant difference among the P rates (Figure 4B). Malonate and acetate were the major anions exuded by all the three species, ranging from 37 to 51% of the total (Figure 4C). Tall wheatgrass was the only species that exuded *trans*-aconitate (Figure 4C).

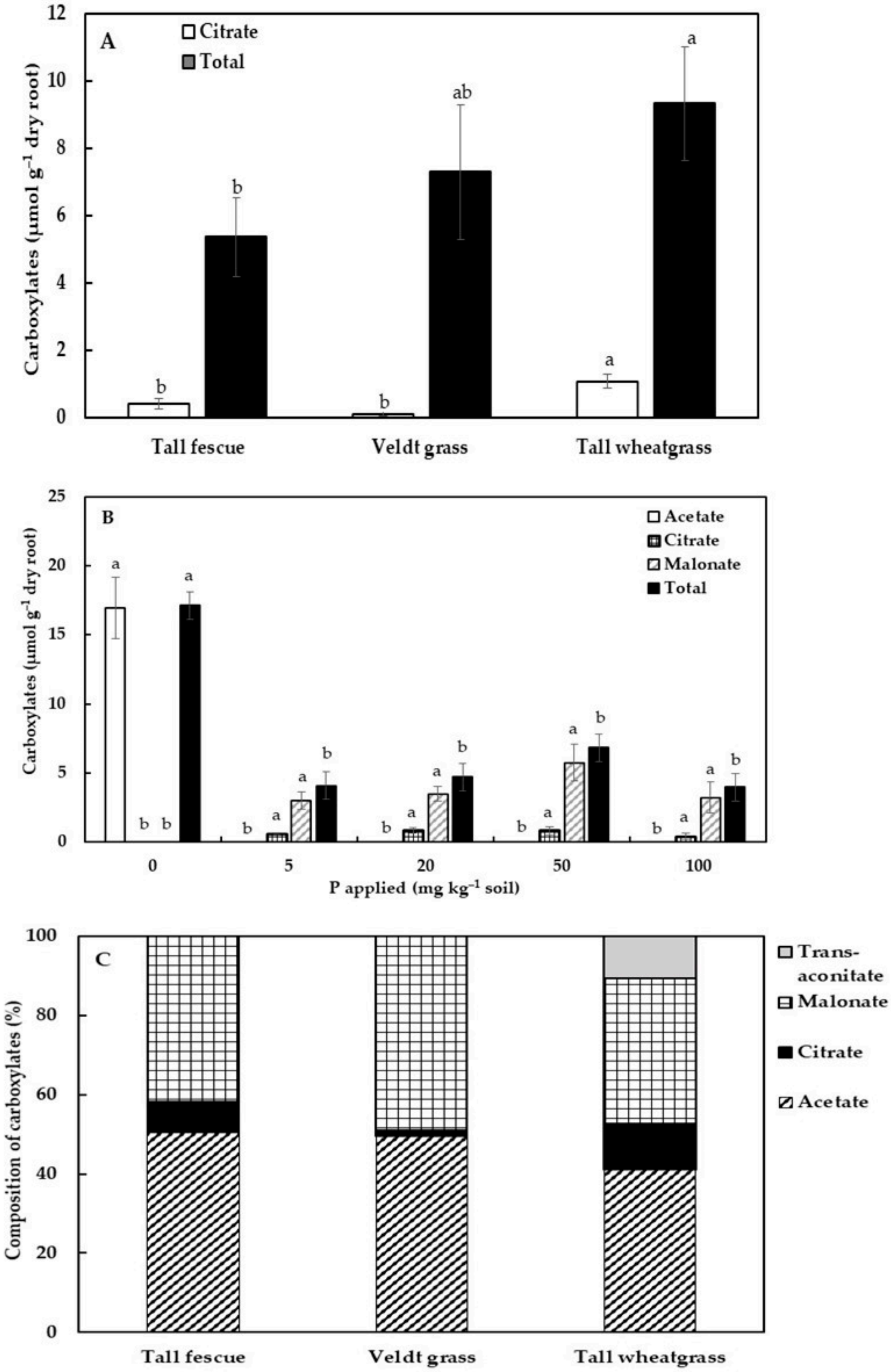

Figure 4. Carboxylate concentrations in the rhizosphere extracts of (**A**) three temperate perennial pasture grasses, effect of phosphorus (P) rates (**B**) on different carboxylate concentrations, and percentage composition of carboxylates in the rhizosphere extracts (**C**). Values for a given carboxylate concentration indicated by different letters differed significantly ($p \leq 0.05$). ANOVA showed significant main effects of grass species and P rates ($p \leq 0.05$) on different carboxylates shown in (**A**,**B**), but not the interaction. The data on individual grasses were averaged across five P rates ($n = 20$) and the data on individual P rates were averaged across three pasture grasses ($n = 12$). Means ± SE.

3.5. Shoot and Root P Accumulation

The P rate had a significant effect on shoot P concentration, whereas both main effects (species and P rate) had a significant effect on shoot P content. Veldt grass had significantly higher shoot P content than tall fescue and tall wheatgrass (Table 2). Shoot P concentration and content increased with increasing P rate, which was significantly higher at 100 mg kg^{-1} soil than the other P rates, except for P content at 50 mg P kg kg^{-1} soil (Table 2).

Table 2. Shoot and root P concentration and content in three temperate pasture grasses as influenced by the main effects of species and P rates. Means ± SE. Means within the columns followed by different letters differ significantly. Two-way ANOVA showed no significant interaction, but the main effects of species (except for shoot P concentration) and P rates were significant ($p \leq 0.05$). Data of individual species were averaged across five different P rates resulting in $n = 20$, whereas data of P rates were averaged across three species resulting in $n = 12$.

	Shoot P		**Root P**	
Species	**Concentration (g kg^{-1} Dry Weight)**	**Content (mg Plant^{-1})**	**Concentration (g kg^{-1} Dry Weight)**	**Content (mg Plant^{-1})**
Tall fescue	5.14	4.59 ± 0.7b	3.04 ± 0.5a	0.90 ± 0.2b
Veldt grass	5.38	6.71 ± 1.3a	2.61 ± 0.3ab	0.94 ± 0.1ab
Tall wheatgrass	4.68	4.50 ± 0.8b	2.24 ± 0.3b	1.19 ± 0.2a
P rates (mg kg^{-1} soil)				
0	2.54 ± 0.1d	0.43 ± 0.3d	1.29 ± 0.1d	0.04 ± 0.01e
10	3.05 ± 0.4d	2.7 ± 0.6c	1.26 ± 0.1d	0.49 ± 0.02d
20	4.87 ± 0.4c	6.08 ± 1.0b	2.29 ± 0.2c	1.06 ± 0.07c
50	6.36 ± 0.3b	7.96 ± 0.8ab	3.35 ± 0.2b	1.44 ± 0.05b
100	8.77 ± 0.5a	8.71 ± 0.6a	4.53 ± 0.3a	1.70 ± 0.2a

Species differed in root P concentration and content, with tall fescue having significantly greater P concentration than tall wheatgrass, whereas the reverse was true for P content (Table 2). The P rate also had a significant effect on root P concentration and content. Root P concentration and content increased significantly with increasing P rate, except for no difference in concentration between 0 and 5 mg P kg kg^{-1} soil (Table 2).

3.6. Agronomic Phosphorus-Use Efficiency

The species and P rates interacted significantly ($p \leq 0.05$) to influence agronomic phosphorus-use efficiency. Veldt grass had significantly higher agronomic phosphorus-use efficiency than the other species at 5 mg P kg kg^{-1} soil, but there was no significant difference among the species at other P rates. Furthermore, the agronomic phosphorus-use efficiency declined in all three species with P fertilisation, relative to 5 mg P kg kg^{-1} soil (Figure 5).

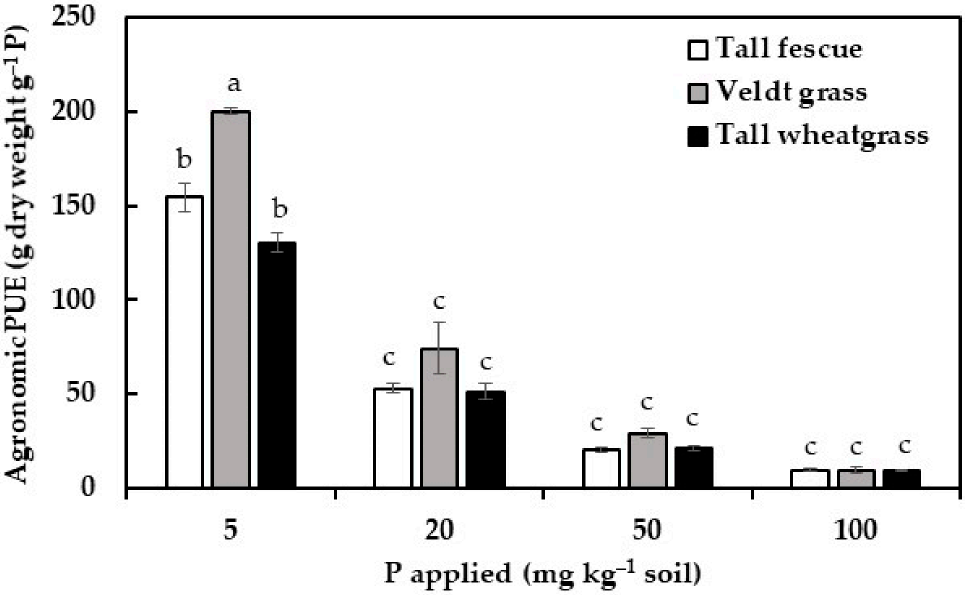

Figure 5. Effect of phosphorus (P) rates on agronomic P-use efficiency (PUE) in three temperate perennial pasture grasses with respect to the zero P control. Treatment values indicated by different letters differed significantly ($p \leq 0.05$). ANOVA showed a significant interaction between the grass species and P rates. Means ± SE. $n = 4$.

4. Discussion

This study characterised differential growth, rhizosphere carboxylate exudation, and mycorrhizal responses in three grasses at variable P supply. Of the P rates used in the current study, 20 and 50 mg P kg kg^{-1} soil produced the maximum shoot dry weight, with the P rate beyond 20 mg P kg kg^{-1} soil not significantly increasing shoot production (Figure 1B). A low critical P requirement has been reported for similar species; for example, phalaris at 16 mg P kg kg^{-1} soil [12]. Generally, temperate pasture grasses have a low critical nutrient requirement [6,39] as they possess longer, thinner, and more finely branched roots that can scavenge nutrients efficiently, particularly immobile elements such as P [40,41].

The effectiveness of species in acquiring P is also determined by root morphology (e.g., specific root length and root diameter) [22,42]. Even though species inherently differ in these root traits, environmental conditions such as P availability are also important. Species with high specific root length are considered more P-efficient because they can explore large soil volume per unit of root biomass and therefore increase P absorption [6,43]. Plants generally increase the specific root length and reduce the root diameter in response to P deficiency [44,45], but this is not a common response to low P supply [44]. In our study, the specific root length of the three species differed, being significantly higher in veldt grass compared with the other two species (Figure 3A). The three species adapted their root morphology to low P conditions (e.g., no added P) by producing high specific root length and small root diameter, as reported elsewhere [20,46].

The release of carboxylates is considered as a prime mechanism for plants to enhance the acquisition of P, particularly from soils with low P availability [15]. In the current study, carboxylate exudation differed significantly among the three pasture grasses. Similarly, species differences have been reported in cultivated crops and pastures [14,16] as well as native plant species [17]. In phalaris and cocksfoot, the major rhizosphere compounds were citrate and malate (approximately 40–55% of the total) [18], both of which are common carboxylates found in *Proteaceae* and other families [17,47]. However, we recorded citrate in low amounts (≈12%), whereas malonate and acetate were the major compounds in all three species (Figure 4C). Citrate and malonate are important carboxylates for nutrient acquisition in *Proteaceae* [17]. Furthermore, the exudation of specific carboxylates in the present study was species-specific, such as *trans*-aconitate recorded only for tall wheatgrass. Malonate and *trans*-aconitate carboxylates are not common outside *Proteaceae* [17], and the occurrence of these compounds in the *Poaceae* species tested here merits further investigation. Malonate may be involved in controlling mycorrhizal colonisation at relatively high P supplies treatments as malonate inhibited succinate dehydrogenase activity and microbial respiration [48].

Acetate was exuded in significant amounts only in the zero P treatment, and citrate and malonate were not found in that treatment. In general, P affects the amount of carboxylate release, with plants releasing more under P deficiency [14,47,49]. An increased release of carboxylates can lead to an increased concentration of P in soil solution and improved accessibility to plants due to a chelating effect of exuded compounds [13,50]. In rice (*Oryza sativa* L.), increased P uptake was attributed to its mobilisation by citrate [51]. However, plants tend to modify their exudate composition based on the amount of external P supplied. In grain legumes, Pearse et al. [14] reported that some species shifted from exuding citrate to malonate when the P supply was increased. Although not statistically significant, an increasing trend of malonate exudation was observed in our study as P rates increased from 0 to 50 mg P kg kg^{-1} soil (Figure 4B).

The species efficiency in acquiring P is also determined by its potential to form a symbiosis with the AM fungi, as the fungi increase the volume of soil that can be explored per unit root length [52]. However, the effectiveness of mycorrhizal association is determined by soil and shoot P concentration, with declining mycorrhizal colonisation as the P supply increases [8,53,54]. Even though the current study also observed a similar trend of decreased colonisation with increasing P supply, the relationship between yield and colonisation was not significant. The high shoot and root yields in the pasture grasses tested in the present study were obtained in treatments with low AM colonisation. Grasses are either poorly dependent or non-dependent on AM fungi because they do not contribute to nutrient uptake [53,54] as much as the fine roots and root hairs of grasses do [55]. Other studies also reported a greater significance of root morphology (fine root length density) in P uptake than the mycorrhizal symbiosis [56].

The current study noted a significant interaction between species and phosphorus rates in agronomic phosphorus-use efficiency. Despite the trend of high agronomic phosphorus-use efficiency at low P rates in all the species, veldt grass produced significantly higher agronomic phosphorus-use efficiency at 5 mg P kg kg^{-1} soil compared with other species at other P rates (Figure 5). A species with high agronomic phosphorus-use efficiency should produce high yields due to its responsiveness to increased P applications [3]. Further, species with high P acquisition efficiency absorb more P per unit root biomass, resulting in a low critical requirement for external P [57].

5. Conclusions

Veldt grass produced highest shoot dry weight likely due to higher agronomic phosphorus-use efficiency and specific root length. Plants exuded acetate only in the zero P treatment and citrate and malonate in the P-supplemented treatments, suggesting their significant roles in P acquisition. The total carboxylate exudation was higher in the low-P soil. Mycorrhizal colonisation also decreased with increasing P rates.

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