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Critical P, K and S Concentrations in Soil and Shoot Samples for Optimal Tedera Productivity and Nodulation

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Abstract: Tedera is a forage legume that can provide out-of-season green feed in Mediterranean climates. To date, growers have had no comprehensive soil nutrition guidelines to optimise tedera production. We undertook field and glasshouse studies to understand tedera's macronutrient requirements. Three field experiments were sown with tedera cv. Lanza® at Cunderdin, Dandaragan and Three Springs in Western Australia. These experiments evaluated seven levels of phosphorus (P) (0–30 kg ha^{-1}) and potassium (K) (0–80 kg ha^{-1}) and two combined treatments with P and K. Glasshouse pot experiments were conducted using tedera cultivars Lanza® and Palma and lucerne cultivar SARDI Grazer. Ten concentrations of added P (0–256 mg kg^{-1}), ten of K (0–256 mg kg^{-1}) and ten of sulphur (S) (0–16 mg kg^{-1}) were tested. There was no significant response to P or K in field soils at Cunderdin or Three Springs. There was no response to K at Dandaragan, but P produced a positive response in the July and October growing season cuts. In the glasshouse, tedera cultivars reached peak productivity at lower soil Colwell P (7.6 to 12 mg kg^{-1}) than lucerne (22 mg kg^{-1}). Lanza® had a moderate biomass response, and Palma did not show a significant response to Colwell K (0.8 to 142 mg kg^{-1}) or soil S (1.3 to 12.5 mg kg^{-1}). Nodulation was greatly reduced at the extremes in P and K treatments. For the first time, these field and glasshouse results have allowed us to establish guidelines for optimal soil nutrition for tedera that growers can use to benchmark the soil or shoot nutrient status of their tedera pastures and assess the economic benefit of correcting deficiencies.

Keywords: tedera; *Bituminaria bituminosa*; macronutrient; perennial pasture; phosphorus efficiency

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1. Introduction

The herbaceous perennial forage legume tedera (*Bituminaria bituminosa* C.H. Stirton var. *albomarginata*) is bred in Western Australia (WA) for direct grazing in Mediterranean-like environments across southern Australia [1,2]. Tedera's main distinctive attribute is its ability to remain green during summer and autumn with minimal leaf drop when grown in the medium to low rainfall zones of Western Australia [3], thereby providing highly valuable fodder for livestock systems in Mediterranean-like climates [4,5]. The first cultivar in the world, T15-1218 Lanza® was released by the Department of Primary Industries and Regional Development (DPIRD) and Meat & Livestock Australia (MLA) for commercial use in Australia in 2019 [6,7]. Tedera breeding is ongoing, and new cultivars are in the breeding pipeline. Cultivar Palma reported in this paper is a new release with improved cold tolerance [8]. During domestication and breeding of tedera, parallel programs developed the animal production and agronomy packages. The animal production research concluded that: (a) grazing tedera did not cause any ill-effect to the grazing

animals even when grazed as a sole diet or in mixtures at different times of the year [9-11] and (b) tedera proved to be a valuable summer and autumn feed for sheep in Mediterranean-like climates [5]. The agronomy package for a newly domesticated species needs to cover all aspects of crop production, such as establishment techniques [12], herbicide tolerance [13], harvesting technologies under local conditions and an understanding of the P, K and S requirements for optimum production.

Phosphorus is an essential macronutrient for plant growth. It is a key component of ATP, the energy currency in biological organisms, but is also a component of DNA, RNA, catalysts, plant membranes and plant structural compounds [14]. Most natural Australian soils are highly weathered and deficient in P when first cleared for agriculture, and phosphorus application has been required to overcome P limitation in major agricultural plant species. Appropriate P application requires evidence-based management through soil testing to provide suitable P levels while avoiding losses via leaching from sandy soils, overaccumulation in clay soils and limiting losses via erosion [15]. Plant biomass removal is another significant factor of P dynamics in soils [16].

While there is evidence that tedera may be more P-efficient than other comparable perennial forage legumes, critical soil or shoot values for P deficiency or toxicity have not been established. Pang, *et al.* [17] showed that tedera had excellent P-response efficiency and had the highest biomass response among the perennial legumes tested when a small amount of P was added to deficient soil. Tedera also reached maximum production potential at a lower rate of added soil P than lucerne (*Medicago sativa* L.) [17]. In the low P treatments, tedera was better nodulated than many other species and was among the species with the highest root length. In a study by Nazeri, *et al.* [18], adding P to a soil with a moderate level of P did not affect the biomass of any of the pasture species studied, including tedera. In terms of toxicity, there are indications that high levels of P will elicit reduced biomass in tedera in a similar manner to comparable pasture legumes [17,19], and high rates of added P also resulted in a decrease in total and individual nodule biomass [20]; however, the levels at which P oversupply limits nodulation or growth have not been established.

Potassium is another essential macronutrient that plays a role in osmotic regulation, stomatal function, the transport of water and photosynthates around the plant, and enzyme activity [14]. Potassium is released from weathering soils and rocks, and while K is not typically deficient in heavier soils in Australia, it can be limiting where sandy soils and high rainfall contribute to leaching or where soil K has been depleted by repeated cropping [21,22]. Potassium toxicity is not common in agricultural plants and soils, but excess K in pasture plants can lead to animal health issues [23]. The K requirements of tedera have not been compared to perennial pasture legumes, but a comparison between Lanza® tedera and the annual legume sub-clover cv. Narrikup (*Trifolium subterranean* L.) found that tedera required substantially less K to attain optimal yield [19].

Sulphur is, again, a critical component of amino acids and proteins, and it is necessary for chlorophyll formation and effective nodulation in legumes [14]. Sulphur deficiency has become increasingly important, especially in lighter soils with low organic matter, which are common in WA's southwest [24]. Superphosphate, a commonly used P fertiliser, contains significant amounts of sulphur, and so, regular use of superphosphate can mask S deficiencies. Sulphur does not reach toxic levels until soil levels are extremely high (ca. 500 mg kg^{-1} S in cucumber and tomato [25]), and toxicity is rare in agricultural soils [26]. Hardy, Brennan and Real [19] found that the sulphur requirement of tedera appeared to be low. Despite being considered nutrient-deficient in an agricultural context, the field soil they used was apparently high enough in S (3.5 mg kg^{-1}) for maximum production in tedera.

The results of these articles indicate that the biology of tedera enables it to provide peak productivity in soils with lower concentrations of P compared to lucerne and K compared to sub-clover. This aspect of tedera's adaptation is worthy of further research, as the cost of fertilisers continues to rise dramatically, and there is increasing awareness of

the environmental impacts of over-using fertiliser. Critical soil or shoot values for P, K or S deficiency or toxicity have not been established.

For growers to ensure that tedera swards have sufficient nutrition, without wasting fertiliser, growers require information on critical soil nutrient status and plant nutrient concentrations that provide peak productivity. These critical concentrations can be compared to samples of soil or plant material taken from growing areas. While Hardy, Brennan and Real [19] provide some information on ideal tissue nutrient concentrations for P, K and S, they do not provide information on soil nutrient concentrations that allow for this productivity, and scarce information on nutrient excess is only available for P. Similarly, Pang, Ryan, Tibbett, Cawthray, Siddique, Bolland, Denton and Lambers [17],Pang, Tibbett, Denton, Lambers, Siddique and Ryan [20] did not report the Colwell extractable P in their treatments after the addition of P, and the first lowest level of added P was rather high, so the application of this information to field soils is difficult. In addition, there is no information on the productivity response of tedera to fertiliser in an agricultural context; hence, this article seeks to address these gaps by fertilising tedera swards in field conditions and testing a very wide range of soil nutrient concentrations in a glasshouse, seeking to include both deficient and toxic levels of P and K.

In this article, the fertilisation response to three macronutrients, P, K and S, was studied. Research results are presented for field experiments at three sites in WA for P and K and for a glasshouse experiment for P, K and S, with lucerne as a control. It is hypothesized that: (1) tedera will achieve maximum productivity at lower levels of P, K and S than lucerne in the glasshouse experiment; (2) field soils are generally deficient in P and K, and so tedera will have a response to P and K in the field and best biomass yields will be produced with the higher P and K rates; (3) the nodulation of tedera will be affected by soil nutrient levels.

2. Materials and Methods

2.1. Field Experiments 2017–2019

Three field experiments were conducted in WA at Dandaragan, Three Springs and Cunderdin using tedera cv. Lanza®. As indicated in the introduction, Lanza® is the first cultivar of tedera ever released for commercial sale. The sites' location, characterisation and soil analysis are presented in Table 1.

Fertiliser was applied at sowing time and four weeks after sowing in a randomised complete block design with seven levels of P (0, 5, 10, 15, 20, 25 and 30 kg ha^{-1}), seven levels of K (0, 5, 10, 20, 40, 60 and 80 kg ha^{-1}) and two treatments with P and K at medium (P 15 + K 20) and high levels (P 30 + K 80). P fertiliser was applied as triple super phosphate, K was applied as muriate of potash (KCl) and other fertilisers applied were urea, copper 25 oxysulphate, zinc sulphate, manganese sulphate, sodium molybdate and gypsum to add nutrients or to balance them (Table 2). All field experiments used a row spacing of 44 cm, 2 cm sowing depth, 10 kg seed ha^{-1}, 4 replicates and plots of 1.54 m × 10 m.

Table 1. Site location, characterisation and soil analysis for Dandaragan, Three Springs and Cunderdin field sites.

Site	Dandaragan	Three Springs	Cunderdin
Latitude	30°50′14″ S	29°36′98″ S	31°37′34″ S
Longitude	115°45′44″ E	115°44′90″ E	117°13′14″ E
Annual average rainfall (mm)	480	380	310
Paddock history:			
2015	Wheat	Wheat	Wheat
2016	Lupins	Wheat	Field Peas
Sowing dates 2017	30 May	25 May	4 July
Soil texture	Sandy Loam	Loamy sand	Loam
	0–10 cm		

Soil pH($_{CaCl2}$)	6.8	5.4	7.6
Electrical conductivity (dS m^{-1})	0.143	0.225	0.139
Organic carbon (%)	2.03	0.75	1.45
NO_3 (mg kg^{-1})	36	8	10
NH_4 (mg kg^{-1})	3	1	0
Colwell P (mg kg^{-1})	30	35	22
Phosphorus buffering index (PBI)	19	23	120
Colwell K (mg kg^{-1})	47	170	291
S (mg kg^{-1}) KCl 40	12	19	23
	11–30 cm		
Soil pH($_{CaCl2}$)	5.1	5.2	5.7
Electrical conductivity (dS m^{-1})	0.040	0.230	0.073
Organic carbon (%)	0.77	0.48	1.38
NO_3 (mg kg^{-1})	7	5	19
NH_4 (mg kg^{-1})	0	0	2
Colwell P (mg kg^{-1})	11	18	6
PBI	26	20	49
Colwell K (mg kg^{-1})	18	247	414
S (mg kg^{-1}) KCl 40	16	17	15

Rainfall during the field trials was much lower than the 30-year average, with total rainfall and rainfall percentiles from sowing in mid-2017 up to the end of June 2020 for each site being: Dandaragan 1450 mm and 2%, Three Springs 900 mm and 4% and Cunderdin 850 mm and 11%.

The three experimental sites were assessed for the first time at the end of the first summer in April 2018 and then every three months. Dandaragan had nine defoliations up to July 2020, when the experiment was terminated. Cunderdin had five defoliations up to October 2019. In June 2019, just prior to the scheduled evaluation cut, the whole site was accidentally heavily grazed by livestock; therefore, no measurements were taken in July 2019. Three Springs had only four defoliations due to the extremely dry conditions. Recovery after the January 2019 cut was very poor, and the experiment was terminated due to the low number of surviving plants.

Dry matter (DM) production was evaluated by cutting a strip with a 21-inch-wide self-propelled lawn mower at a height of 5 cm for the full length of the tedera plots (10 m). The biomass produced per plot was measured by weighing the total biomass collected in the mower bag and a subsample from the mowed biomass. The subsamples were oven-dried for 72 h at 60 °C and weighed to calculate the DM percentage. Results were converted to DM kg ha^{-1} for each plot. After cutting, the remainder of the plot was also mowed to 5 cm of height and the cut biomass removed.

Table 2. Nutrients (kg ha^{-1}) applied at sowing time or four weeks after sowing to generate the seven levels of P, seven levels of K and the two P and K treatments.

	At sowing Time (kg ha^{-1})								**Four Weeks After Sowing (kg ha^{-1})**		
Treatment	P	S	Ca	N	Cu	Zn	Mn	Mo	K	Ca	S
P 0	0.0	3.5	0.0	20.6	1.0	0.7	5.1	0.1	61.7	30.2	24.2
P 5	5.1	3.9	4.2	20.6	1.0	0.7	5.1	0.1	61.7	30.2	24.2
P 10	10.3	4.3	8.5	20.6	1.0	0.7	5.1	0.1	61.7	30.2	24.2
P 15	15.4	4.6	12.7	20.6	1.0	0.7	5.1	0.1	61.7	30.2	24.2
P 20	20.6	5.0	17.0	20.6	1.0	0.7	5.1	0.1	61.7	30.2	24.2
P 25	25.7	5.4	21.2	20.6	1.0	0.7	5.1	0.1	61.7	30.2	24.2
P 30	30.8	5.8	25.4	20.6	1.0	0.7	5.1	0.1	61.7	30.2	24.2
K 0	25.7	5.4	21.2	20.6	1.0	0.7	5.1	0.1	0.0	30.2	24.2

K 5	25.7	5.4	21.2	20.6	1.0	0.7	5.1	0.1	5.1	30.2	24.2
K 10	25.7	5.4	21.2	20.6	1.0	0.7	5.1	0.1	10.3	30.2	24.2
K 20	25.7	5.4	21.2	20.6	1.0	0.7	5.1	0.1	20.6	30.2	24.2
K 40	25.7	5.4	21.2	20.6	1.0	0.7	5.1	0.1	41.1	30.2	24.2
K 60	25.7	5.4	21.2	20.6	1.0	0.7	5.1	0.1	61.7	30.2	24.2
K 80	25.7	5.4	21.2	20.6	1.0	0.7	5.1	0.1	82.2	30.2	24.2
P 15 and K 20	15.4	4.6	12.7	20.6	1.0	0.7	5.1	0.1	20.6	30.2	24.2
P 30 and K 80	25.7	5.8	25.4	20.6	1.0	0.7	5.1	0.1	82.2	30.2	24.2

2.2. Glasshouse Experiment 2021

Two tedera cultivars (Palma and Lanza®) were grown alongside lucerne cv. SARDI Grazer in an air-conditioned glasshouse at DPIRD, South Perth (latitude: 31°59′22″ S; longitude: 115°53′2.0″ E) between 31 August 2021 and 30 November 2021. The tedera cultivars Lanza® and Palma are the first two commercial tedera cultivars ever developed worldwide. Lucerne SARDI Grazer was developed in South Australia for higher persistence under heavy grazing than previous lucerne cultivars. Plants were grown in nutrient-deficient washed play sand (Richgro) with nutrients added to provide basal nutrients and 30 treatments with ten levels of P, K and S. The play sand had a phosphorus buffering index (PBI) of 2.5. Nutrients added to the play sand for the 30 treatments are provided in Table 3. Each nutrient by genotype treatment combination was replicated twice, and the experiment was managed as two randomised blocks, with all pots completely randomised every two weeks within each block.

Table 3. Quantity of nutrients added to play sand to create the 30 treatments of P, K and S. All values are in mg kg^{-1}.

	P (mg kg^{-1})			**K (mg kg^{-1})**		**N (mg kg^{-1})**		**S (mg kg^{-1})**		
P treatments	0, 1, 2, 4, 8, 16, 32, 64, 128, 256			24.3		18.9		23.7		
K treatments	23.8			0, 1, 2, 4, 8, 16, 32, 64, 128, 256		18.9		111-119		
S treatments	26.6			41.4–72.8		17.4		0, 0.06, 0.13, 0.25, 0.50, 1.01, 2.01, 4.02, 8.04, 16.1		
mg kg^{-1}	Mg	Na	Ca	Cl	Cu	Zn	Co	Mn	Mo	B	Fe
P treatments	7.39	0.54–190	15.3	20.1	0.74	0.57	0.035	2.33	0.53	0.012	1.01
K treatments	7.39	18.23	137–15.3	20.1	0.74	0.57	0.035	2.33	0.53	0.012	1.01
S treatments	15.1	0.54	12.8	31.6–24.5	0.74	0.57	0.035	2.33	0.53	0.012	1.01

The pots used were an 8 L sealed pot measuring 250 mm in height and 250 mm in diameter, and each pot contained 6 kg of dry sand. Basal and treatment nutrients were added from stock solutions and mixed in a cement mixer in batches large enough to fill the six pots needed for each nutrient treatment (three genotypes × two replicates). No further nutrients were provided during the experiment. Samples of un-amended soil and soil prepared with nutrients prior to planting were analysed for colour, texture, ammonium and nitrate nitrogen (Rayment and Lyons Method 7C2b [27]), Colwell P and K [28], KCl 40 S [29] (hereafter called soil S), organic carbon [30], and electrical conductivity and pH (Rayment and Lyons Method 4A1 (pH water); 4B4 (pH CaCl2); 3A1 (conductivity)) by CSBP Laboratories (Bibra Lake, WA, CSBPlab.com.au). Results of un-amended sand and sand prepared with nutrients are given in Table 4.

Table 4. Nutrient content analysis and estimated nutrient concentrations based on smoothing models (see statistical analysis section for details) for all treatments. All values are in mg kg^{-1}.

	Play Sand	P Treatments	K Treatments	S Treatments
P Colwell	<2	<2, <2, 4, 6, 12, 20, 32, 39, 87, 147	33.3	22.9
Smoothed P Colwell		0, 2, 4, 7, 11, 18, 30, 50, 83, 138		
K Colwell	<15	18.3	Levels 1 to 6 < 15, 20, 39, 77, 138	23–69
Smoothed K Colwell			0, 1, 2, 3, 6, 11, 20, 39, 74, 142	
NH_4 N	<1	3.7	4.0	<1
Available Soil N	<2	7.8	8.9	4.7
Soil S	1.35	13.5	254	1.1, 1.2, 1.1, 0.8, 1.6, 1.8, 2.8, 3.8, 6.6, 13
Smoothed Soil S				1.3, 1.4, 1.4, 1.5, 1.7, 2.0, 2.7, 4.1, 6.9, 12.5
Conductivity (dS m^{-1})	<0.01	0.052	0.37	0.032
pH (CaCl2)	6.1	5.6	6.3	6.1

The glasshouse was set to cool the environment to 24 °C during the daytime (6 am to 6 pm) and 20 °C at night. Glasshouse temperatures exceeded the cooling capacity of the air conditioners on several occasions in late November, but temperatures did not exceed 35 °C at any time.

Seeds were prepared for sowing by scarification and inoculation with appropriate strains of root nodule bacteria. Lucerne received Group AL inoculum, and WSM 4083 was used for tedera. Approximately 15 seeds of the allocated genotype were initially sown in each pot, and seedlings were thinned to five healthy and uniform plants within three weeks of germination. Pots were watered to 100% field capacity weekly, with additional unweighed top-up watering every two to three days. High-density polyethylene beads (200 g) were added to the surface of each pot in week 4 of the experiment, providing a layer approximately 15 mm deep to limit evaporation.

Plant components were separated at harvest for measurement of dry biomass and, later, nutrient analysis. After thoroughly washing all sand from roots, the above- and below-ground components were separated. Above-ground shoots were treated on a per-plant basis, and the leaf, stem and flower components were separated for individual drying and measurement. The nodulation of plants at harvest was scored according to the rating system of Yates, et al. [31]. Leaf, stem and flower samples were recombined on a pot basis and analysed for nutrient content by CSBP Laboratories. Chloride and nitrate were analysed via a colourimetric method; P, K, S, Cu, Zn, Mn, Na, Fe and B were measured using inductively coupled plasma (ICP) [32]; and total N was measured using Rayment and Lyons Method 9G2 [27]. In some cases, the weight of plant samples was insufficient to perform all tests.

2.3. Statistical Analyses

For the field experiments, cuts from the dry seasons (January and April) and the growing seasons (July and October) were bulked and analysed by two-way ANOVA (R Studio ver. 2022.02.1) with site and nutrient level as factors. Where there was a significant site effect or interaction between site and nutrient level, sites were analysed separately by one-way ANOVA at each measurement time. Significant ($p < 0.05$) site and nutrient combinations were plotted, and Mitscherlich models were fitted and plotted using the nls function (R Studio ver. 2022.02.1).

For the glasshouse experiment, analysis of soil nutrients in experimental soils (one sample from each nutrient treatment) showed some variation, and, to provide a reliable measure for our independent variable, models were created to smooth the variability in the results by regressing soil nutrient concentration against the amount of nutrients added to soil. In the case of lower levels of P and K, the testing returned results below the minimum detectable limit, and so the smoothing models were extrapolated to provide estimated soil nutrient concentrations in these lower levels. Both the original results and the estimated soil nutrient levels are presented in Table 4.

Glasshouse plant responses in shoot biomass, shoot nutrient concentrations and nodulation in response to soil nutrient levels were summarized using quadratic models. Quadratic models were used for simplicity for all glasshouse results as Mitscherlich curves could not model toxicity responses at higher nutrient levels. Soil nutrient concentrations were $\log_{10}$ transformed prior to fitting. The lowest levels for P and K were disregarded to enable the log transformation. One P treatment level (8 mg kg^{-1} added P) was disregarded due to a mistake in preparing this fertiliser/sand mixture. Where a significant relationship with shoot biomass or shoot concentration existed, soil nutrient levels at which the models predicted >90% peak biomass production and the coincident shoot nutrient concentrations were extracted from the models and tabulated.

3. Results

3.1. Field Experiment

The site effect was significant for all nutrients and in all measurements in the two-way analyses (Table 5). In one-way analyses of nutrient effects at separate sites, there were no significant responses for Lanza® tedera to either P or K or combined P and K for any of the biomass cuts above 5 cm at Three Springs or at Cunderdin. There was also no response to K or combined P and K at Dandaragan, but the P response at Dandaragan was significant for the cumulative growing season cuts (July and October) in 2018 (p = 0.027) and 2019 (p = 0.009) (Table 5 and Figure 1).

Table 5. ANOVA of added P and K effects on harvested biomass in dry seasons of 2018 and 2019 and growing seasons in 2018 and 2019 at three field sites. Analyses were two-way ANOVAs of both nutrient level and site effects and one-way ANOVAs of the nutrient level effect at separate sites. Measures were not available in the 2019 growing season at Cunderdin or Three Springs.

Two-Way ANOVA of Nutrient Level across Site				
Biomass Measure	**Nutrient**	**Nutrient Effect Pr (>F)**	**Site Effect Pr (>F)**	**Interaction Pr (>F)**
2018 Dry Season	K	0.148	0.001	0.077
2018 Growing Season	K	0.997	0.003	0.594
2019 Dry Season	K	0.788	0.000	0.781
2018 Dry Season	P	0.335	0.004	0.298
2018 Growing Season	P	0.051	0.013	0.020
2019 Dry Season	P	0.356	0.000	0.140
2018 Dry Season	P & K	0.523	0.014	0.226
2018 Growing Season	P & K	0.176	0.035	0.156
2019 Dry Season	P & K	0.151	0.000	0.143
One-way ANOVA of Nutrient Level at Separate Sites				
		Nutrient effect Pr (>F)		
Biomass Measure	**Nutrient**	**Cunderdin**	**Dandaragan**	**Three Springs**
2018 Dry Season	K	0.774	0.055	0.730
2018 Growing Season	K	0.526	0.560	0.603
2019 Dry Season	K	0.610	0.711	0.494
2019 Growing Season	K	-	0.933	-
2018 Dry Season	P	0.715	0.170	0.703

2018 Growing Season	P	0.555	0.027	0.677
2019 Dry Season	P	0.810	0.173	0.464
2019 Growing Season	P	-	0.009	-
2018 Dry Season	P & K	0.685	0.225	0.279
2018 Growing Season	P & K	0.653	0.167	0.765
2019 Dry Season	P & K	0.866	0.139	0.932
2019 Growing Season	P & K	-	0.107	-

Mitscherlich models indicated that growing season productivity in both years in the absence of added P was similar at just over 1500 kg ha^{-1}, and the addition of P raised productivity by over 900 kg ha^{-1} in 2018 and over 500 kg ha^{-1} in 2019, up to a maximum of around 2500 kg ha^{-1} in 2018 and 2100 kg ha^{-1} in 2019.

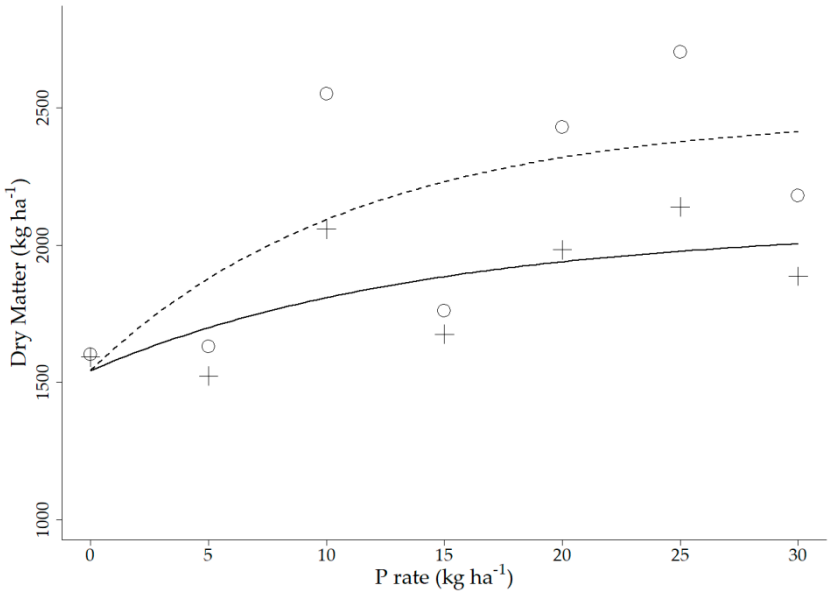


Figure 1. Lanza® tedera shoot biomass response (kg dry matter ha^{-1}) to P application over two growing seasons (cumulative biomass above 5 cm from July and October cuts) at Dandaragan: 2018 data are open circles and dotted line, and 2019 data are crosses and solid line. Fitted models are Mitscherlich functions (2018 DM = 2479 − 934e$^{-0.09x}$; 2019 DM = 2069 − 525e$^{-0.07x}$).

3.2. Glasshouse Experiment 2021

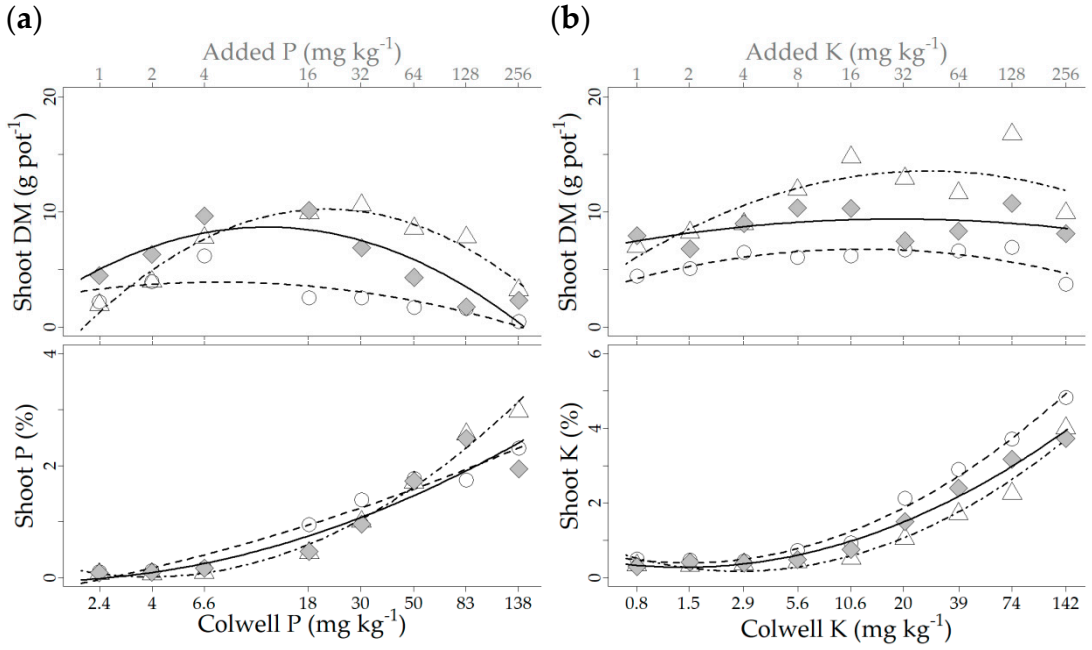
Details of quadratic models of plant responses in shoot biomass, shoot nutrient concentrations and nodulation in response to soil nutrients are presented in Table 6. In tedera Palma, shoot biomass did not show a strong response to Colwell K (p = 0.25) or soil S (p = 0.15). Additionally, no response was found between nodulation and Colwell K in lucerne (p = 0.5) and nodulation and soil S in Lanza® (p = 0.33).

Table 6. Model descriptions for curves fitted to shoot biomass (BM), shoot nutrient concentrations (K, P, or S) and nodulation score in response to soil test results (Colwell P, Colwell K, Soil S). Model fits were quadratic ($y = ax^2 + bx + c$); we used a log10 transformation of soil fertility as the × variable, and its significance (Pr (>F)) is presented. In two cases, no significant fit (NS) was available for shoot BM in response to soil nutrient levels, and, in two cases, there was no significant fit for nodulation score.

Variable (y)	Genotype	× (Log_{10} Transformed)	Model Terms			Model Fit Pr(>F)
			Quadratic (a)	Linear (b)	Constant (c)	
Shoot BM	Lanza®	Colwell P	−2.42	4.28	2.00	4.1×10^{3}
Shoot BM	Lucerne	Colwell P	−9.83	26.3	−7.30	3.2×10^{-7}

Shoot BM	Palma	Colwell P	−7.41	16.1	−0.027	2.6×10^{-4}
Shoot BM	Lanza®	Colwell K	−1.83	3.98	4.59	8.1×10^{-3}
Shoot BM	Lucerne	Colwell K	−3.22	9.20	6.97	1.3×10^{-3}
Shoot BM	Palma	Colwell K	−1.03	2.60	7.72	2.5×10^{-1} NS
Shoot BM	Lanza®	Soil S	0.52	1.50	3.49	4.1×10^{-2}
Shoot BM	Lucerne	Soil S	−9.22	17.4	3.80	4.0×10^{-5}
Shoot BM	Palma	Soil S	4.20	−3.42	9.20	1.5×10^{-1} NS
Shoot [P]	Lanza®	Colwell P	0.25	0.70	−0.34	3.40×10^{-8}
Shoot [P]	Lucerne	Colwell P	1.31	−1.55	0.47	1.45×10^{-12}
Shoot [P]	Palma	Colwell P	0.58	−0.080	−0.070	1.02×10^{-6}
Shoot [K]	Lanza®	Colwell K	1.16	−0.39	0.43	6.37×10^{-14}
Shoot [K]	Lucerne	Colwell K	1.20	−1.06	0.41	4.44×10^{-12}
Shoot [K]	Palma	Colwell K	0.91	−0.27	0.30	2.47×10^{-13}
Shoot [S]	Lanza®	Soil S	0.0012	0.12	0.12	5.24×10^{-5}
Shoot [S]	Lucerne	Soil S	0.23	−0.055	0.074	6.54×10^{-9}
Shoot [S]	Palma	Soil S	0.058	0.15	0.076	1.09×10^{-12}
Nodulation	Lanza®	Colwell P	−4.13	8.61	0.37	2.45×10^{-16}
Nodulation	Lucerne	Colwell P	−4.33	10.5	0.62	7.22×10^{-9}
Nodulation	Palma	Colwell P	−6.57	15.0	−0.30	1.05×10^{-20}
Nodulation	Lanza®	Colwell K	−1.87	3.46	4.03	1.27×10^{-5}
Nodulation	Lucerne	Colwell K	−0.14	0.55	6.00	4.97×10^{-1} NS
Nodulation	Palma	Colwell K	−0.74	1.21	5.98	1.98×10^{-2}
Nodulation	Lanza®	Soil S	−0.67	1.59	3.64	3.26×10^{-1} NS
Nodulation	Lucerne	Soil S	−6.205	9.94	3.33	8.15×10^{-10}
Nodulation	Palma	Soil S	−1.39	2.43	5.82	3.34×10^{-2}

The shoot biomass and shoot concentration in response to soil nutrient levels of P, K or S for Lanza®, Palma and lucerne are presented in Figure 2. Critical nutrient concentrations in soil and shoots, where productivity was 90% of the peak, and the concentrations at which peak productivity occurred are given in Table 7. Images of pots from the different treatments, taken at 12 weeks of age, are shown in Figures 3–5.



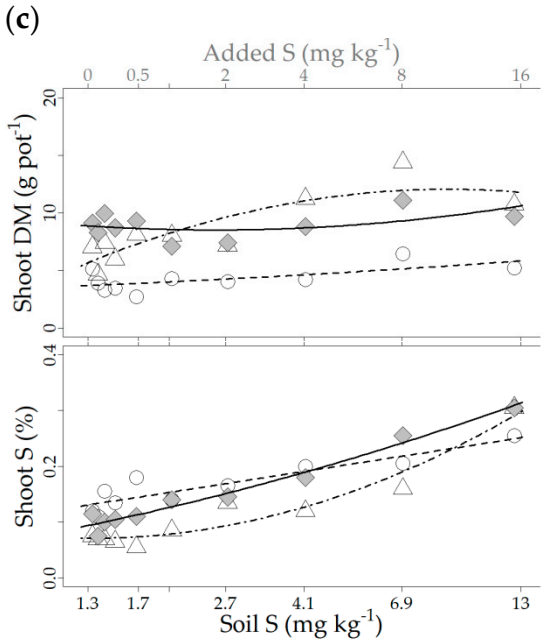


Figure 2. Shoot biomass response (g pot^{-1}) and shoot P, K or S concentration (%) in response to increasing levels of (**a**) Colwell P, (**b**) Colwell K or (**c**) Soil S. Circles, triangles, and diamonds represent Lanza®, lucerne and Palma, respectively. Dashed, dotted and solid lines are fitted models for Lanza®, lucerne and Palma, respectively. The scale on the X-axis is a log scale, and the grey text at the top of the panels is the level of nutrient added to treatments. Levels of Colwell P less than 4 and Colwell K less than 20 were below the detectable limits of the soil tests and have been estimated from smoothed models of soil test results vs. added nutrients.

Table 7. P, K and S nutrient concentrations in soils (mg kg^{-1}) and shoots (%) at which two tedera genotypes and lucerne SARDI Grazer production reached and then dropped to 90% of peak biomass based on quadratic models. Measurements outside these figures could indicate deficiency or toxicity.

	Lanza®			**Palma**			**Lucerne**		
	≥90%	**Peak**	**≤90%**	**≥90%**	**Peak**	**≤90%**	**≥90%**	**Peak**	**≤90%**
Colwell soil P (mg kg^{-1})	3.0	7.6	19	5.5	12	26.6	10	22	46
Shoot P (%)	0.06	0.48	0.98	0.19	0.52	0.99	0.24	0.74	1.5
Colwell soil K (mg kg^{-1})	3.0	12	50	NS [B]	NS	NS	6.0	27	120
Shoot K (%)	0.50	1.36	3.1	NS	NS	NS	0.31	1.3	3.4
Soil S (mg kg^{-1})	7.4	12 [A]	No max	NS	NS	NS	3.8	8.8	20 [C]
Shoot S (%)	0.22	0.25 [A]	No max	NS	NS	NS	0.12	0.23	0.39 [C]

[A] Peak productivity was not reached within the soil nutrient concentrations tested (No max), and so the peak productivity level is taken as the maximum productivity. [B] NS indicates the model fitted did not show a significant fit between soil nutrient levels and shoot biomass or shoot nutrient concentration. [C] These figures are extrapolated from beyond the range of tested soil S concentrations.

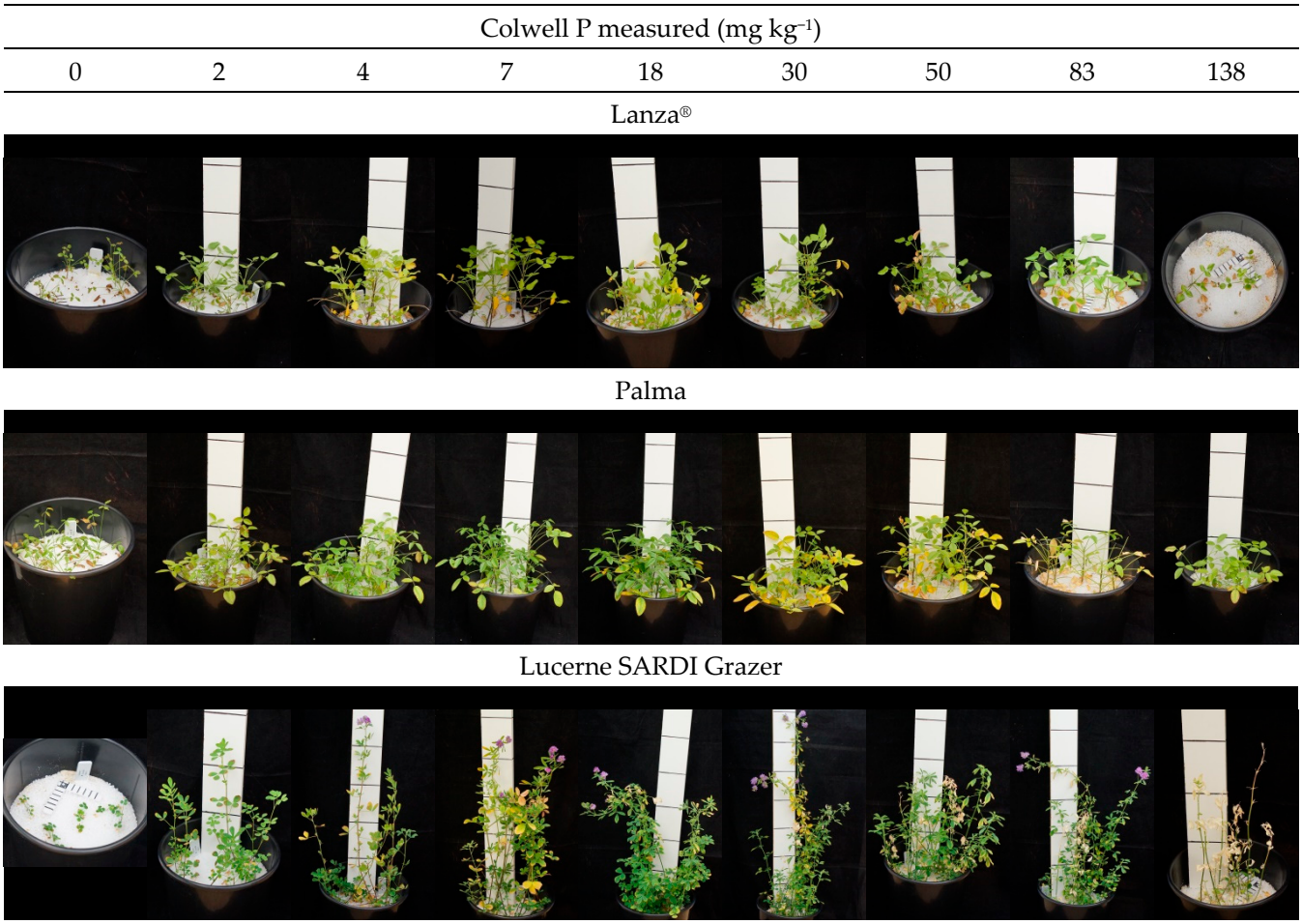


Figure 3. Images of pots containing 12-week-old Lanza® (top), Palma (middle), and lucerne (bottom) grown in the glasshouse with differing levels of soil Colwell P. Black lines on large scale bars are 10 cm intervals.

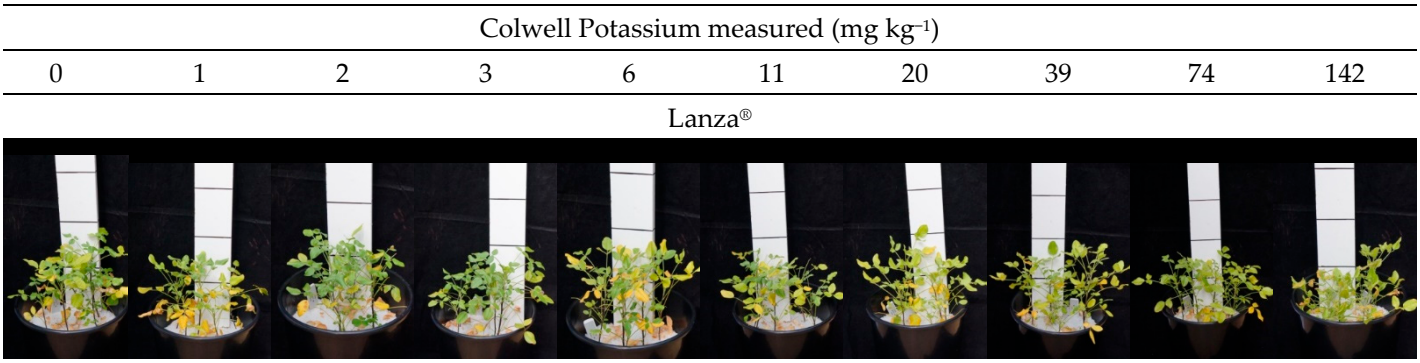


Figure 4. Images of pots containing 12-week-old Lanza® (top), Palma (middle), and lucerne (bottom) grown in the glasshouse with differing levels of Colwell K in soil. Black lines on large scale bars are 10 cm intervals.

All three genotypes showed similar shoot biomass responses to Colwell P, with a rise in productivity as Colwell P increased and then a fall in productivity as Colwell P and shoot P concentration reached higher levels and shoot P toxicity occurred (Figures 2a and 3). However, the two tedera genotypes reached 90% peak productivity with lower Colwell P levels (3–19 mg kg^{-1} for Lanza® and 5–27 mg kg^{-1} for Palma) compared to lucerne (10–45 mg kg^{-1}) (Table 7). The tedera genotypes showed a more severe toxicity response to high P compared to lucerne, with the productivity of tedera being reduced to almost zero, whereas lucerne productivity at the highest P level was reduced to roughly 50% of peak productivity. All three genotypes had similar responses in shoot P concentration for the P treatments. Shoot P concentration rose from very low levels (<0.1%) to levels ca. 2.5% in the highest P treatment. Lanza®, lucerne and Palma reached 90% peak productivity, with shoot P concentrations of 0.06, 0.24 and 0.19%, respectively. For both tedera genotypes, the decline to 90% of peak productivity set in with shoot P concentrations just below 1.0%, whereas lucerne tolerated a higher internal P, dropping below 90% productivity at 1.5% shoot P.

Soil Sulphur (mg kg^{-1})									
1.3	1.4	1.4	1.5	1.7	2.0	2.7	4.1	6.9	13

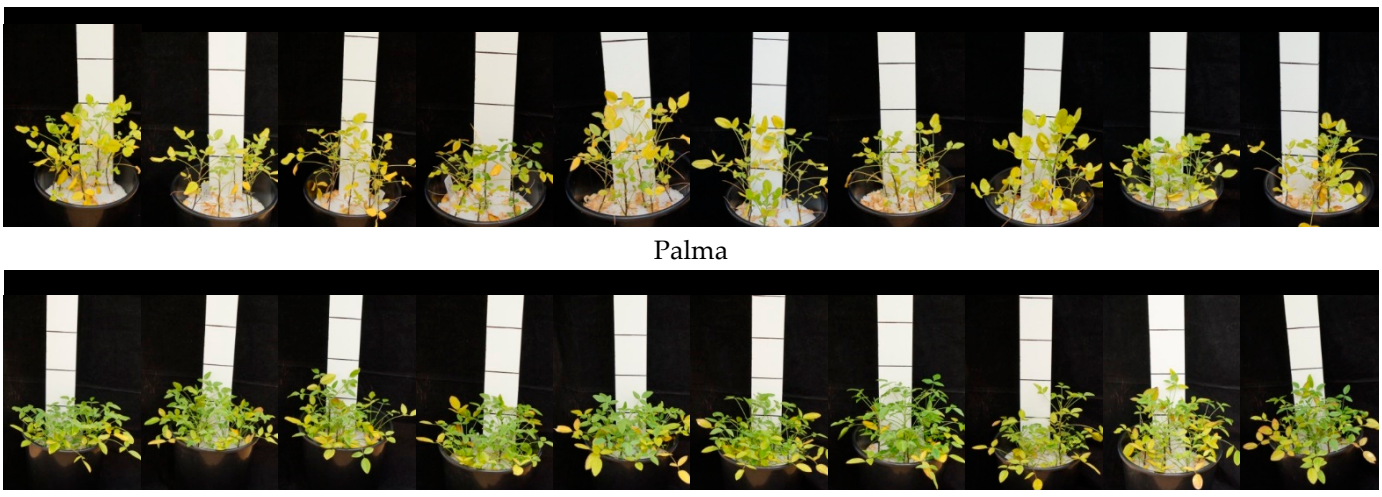


Figure 5. Images of pots containing 12-week-old Lanza® (top), Palma (middle), and lucerne (bottom) grown in the glasshouse with differing levels of soil S. Black lines on large scale bars are 10 cm intervals.

Phosphorus deficiency in tedera was expressed as a combination of several characteristics. First, leaf margins developed dark necrotic lesions surrounded by a small ring of chlorosis (Figure 6a,b,d). Second, some leaves developed widespread mottling with a purple hue (Figure 6d). Third, leaf size was markedly reduced compared to healthy leaves (Figure 6c,e). P deficiency damage to leaf margins did not include bleaching. Phosphorus-deficient plants dropped old leaves once severely affected. Overall, plants growing under extreme P deficiency failed to grow beyond a few leaves once seed reserves were exhausted, although plants did survive until the end of the experiment. Lucerne also expressed marginal necrosis (dark brown) and marginal chlorosis in low P treatments.

Phosphorus toxicity symptoms in tedera were consistent among the two genotypes (Figure 7) and, as severity increased, the symptoms progressed through obvious interveinal chlorosis, bleaching on the leaf margins (Figure 7a,c), extensive bleaching across the entire lamina, and leaf drop that left the petioles attached to stems (Figure 7b). In extreme cases, the growing tips of plants were killed and entire plants died. The lightly coloured bleaching symptoms were distinct from P deficiency and K imbalance in the lack of dark necrotic lesions or margins.

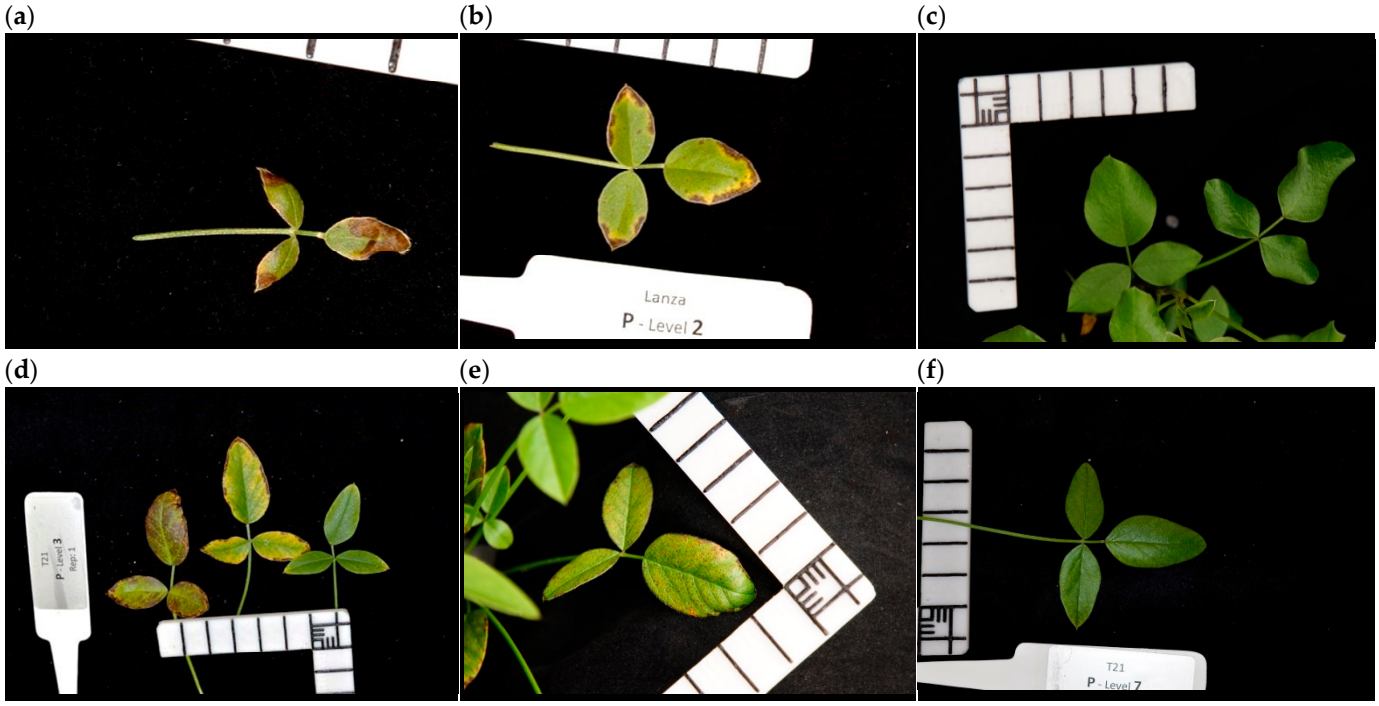


Figure 6. Images of leaves taken from Lanza® tedera (**a**–**c**) and Palma (**d**–**f**) grown in soils with deficient levels of phosphorus (**a**,**b**,**d**,**e**) and adequate phosphorus (**c**,**f**). Symptom progression from younger to older leaves (R to L) is shown in panel (**d**).

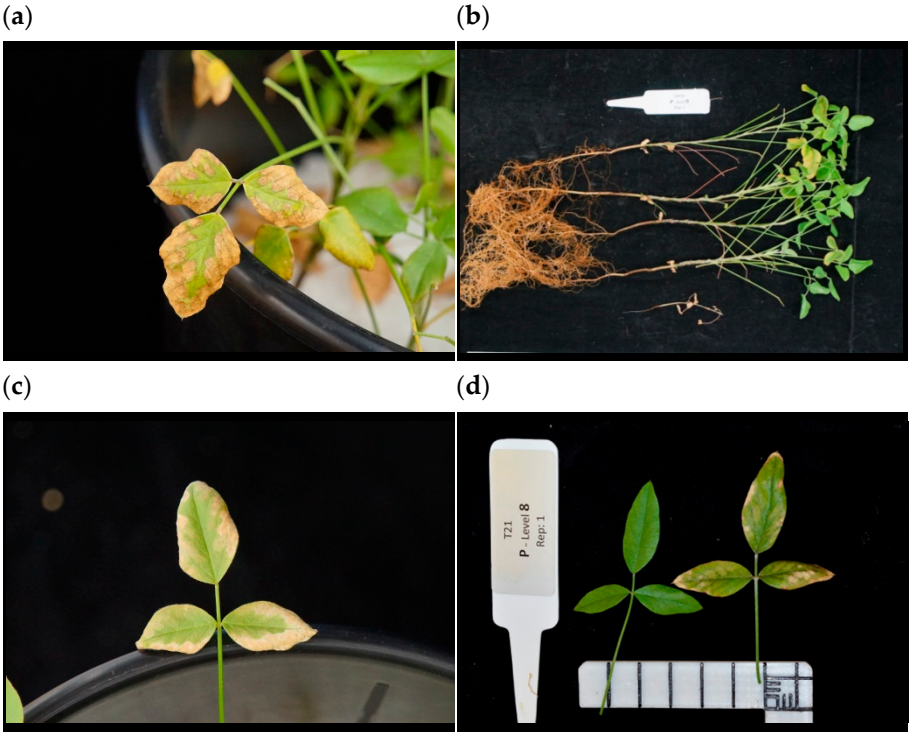


Figure 7. Images of leaves taken from Lanza® tedera (**a**), whole Lanza® plants (**b**) and Palma (**c**,**d**) grown in soils with toxic levels of soil phosphorus (ca. 80 mg kg^{-1} Colwell P).

Lanza® and lucerne showed significant responses to added K, with 90% of peak productivity occurring across a broad range of Colwell K for both genotypes (Figure 2b), although lucerne did require a higher Colwell K (3–50 mg kg^{-1} for Lanza® and 6–119 mg kg^{-1} for lucerne). The overall biomass benefit of K in Lanza® was less compared to lucerne. In Lanza®, peak productivity was 6.8 g pot^{-1} at 12.2 mg kg^{-1} Colwell K, roughly a 60% improvement on the productivity at 0.8 mg kg^{-1} Colwell K. For comparison, lucerne produced 13.5 g pot^{-1} at peak Colwell K (27 mg kg^{-1}), which was roughly a 125% productivity improvement compared to biomass production in the 0.8 mg kg^{-1} Colwell K treatment. The shoot K concentration response of all three genotypes to added K followed a similar curve, and a similar shoot K concentration was required to obtain 90% peak productivity (0.5–3.1% Shoot (K) for Lanza® and 0.3 to 3.4% Shoot (K) for lucerne). Palma appeared to be insensitive to low and high soil K as it did not show a significant relationship between shoot biomass and K added to soil. Palma was also more productive overall than Lanza® at all levels of added K.

In both tedera genotypes, leaves presented with marginal necrosis and bleaching in response to low soil K, with a dark margin between healthy and damaged tissue (Figure 8a,b,d). In Lanza®, the damaged tissue had a very distinct transition to undamaged tissue, but the margins between healthy and damaged tissue in tedera T21 were less distinct, and some leaves had widespread dark spots (Figure 8e). Leaves also showed general yellowing across the lamina in both species. As expected, the deficiency symptoms were most severe in older leaves, with the youngest leaves barely affected. Compared to healthy leaves (Figure 8c,f), K deficiency did not appear to affect leaf size, in contrast to the deficiency symptoms seen for P. As symptoms progressed, old leaves became completely necrotic and dropped readily. The deficiency images shown are taken from treatments with very low soil Colwell K (estimated <1.5 mg kg^{-1}).

Tedera leaves displayed marginal necrosis in response to high levels of K (~150 mg kg^{-1} Colwell K). However, in contrast to K deficiency, K toxicity symptoms included a distinct ring of chlorosis around the necrotic margins (Figure 9b,d), and the widespread spotting seen in tedera T21 due to K deficiency was not seen under toxicity conditions.

Symptoms of K toxicity were more pronounced in older leaves (Figure 9a,c), likely due to the accumulation of toxic K levels over time, and leaves dropped readily once badly affected.

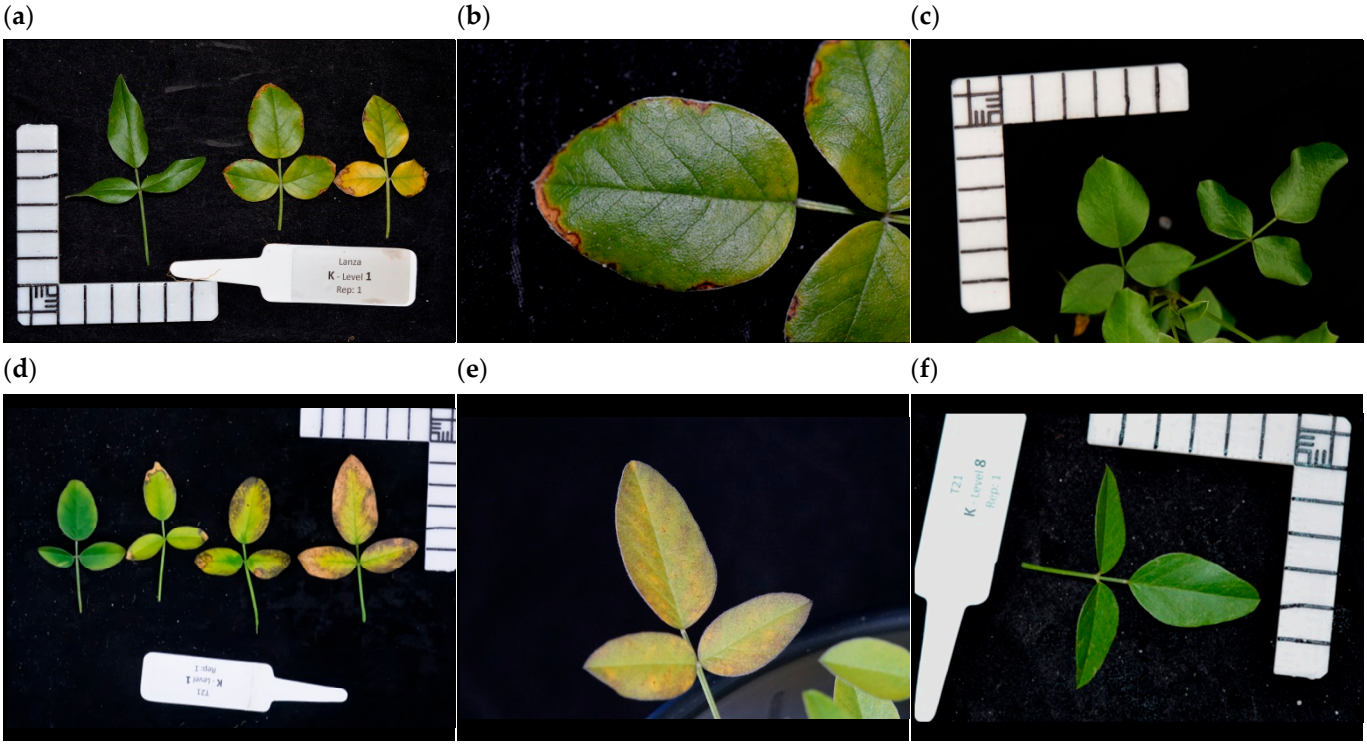


Figure 8. Images of leaves taken from Lanza® tedera (**a**–**c**) and Palma (**d**–**f**) grown in soils with deficient levels of potassium (**a**,**b**,**d**,**e**) and adequate potassium (**c**,**f**). Symptom progression from younger to older leaves (L to R) is shown in panels (**a**,**d**).

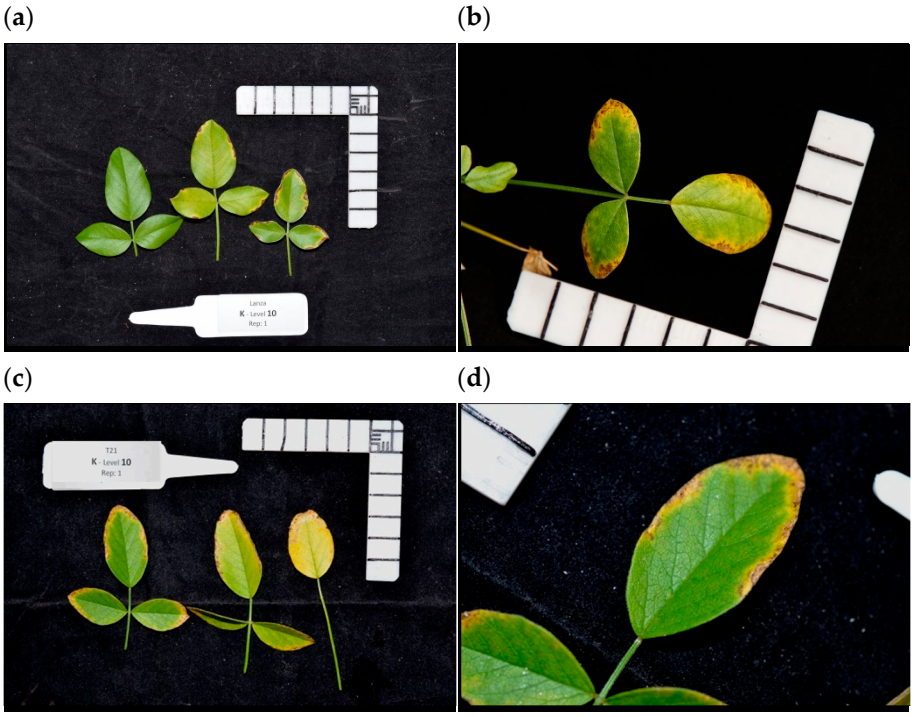


Figure 9. Leaf symptoms of potassium toxicity in Lanza® tedera (**a**,**b**) and Palma (**c**,**d**).

Sulphur deficiency was expressed in all genotypes as uniform chlorosis across the entire leaf lamina (Figure 10a,b), with mild black spotting and necrotic lesions in the worst cases (Figure 10b). Sulphur deficiency symptoms can be confused with N deficiency; however, in this experiment, S deficiency led to more uniform chlorosis across the leaf (not inter-veinal) and across the plant as growing tips were affected (Figure 10c,d).

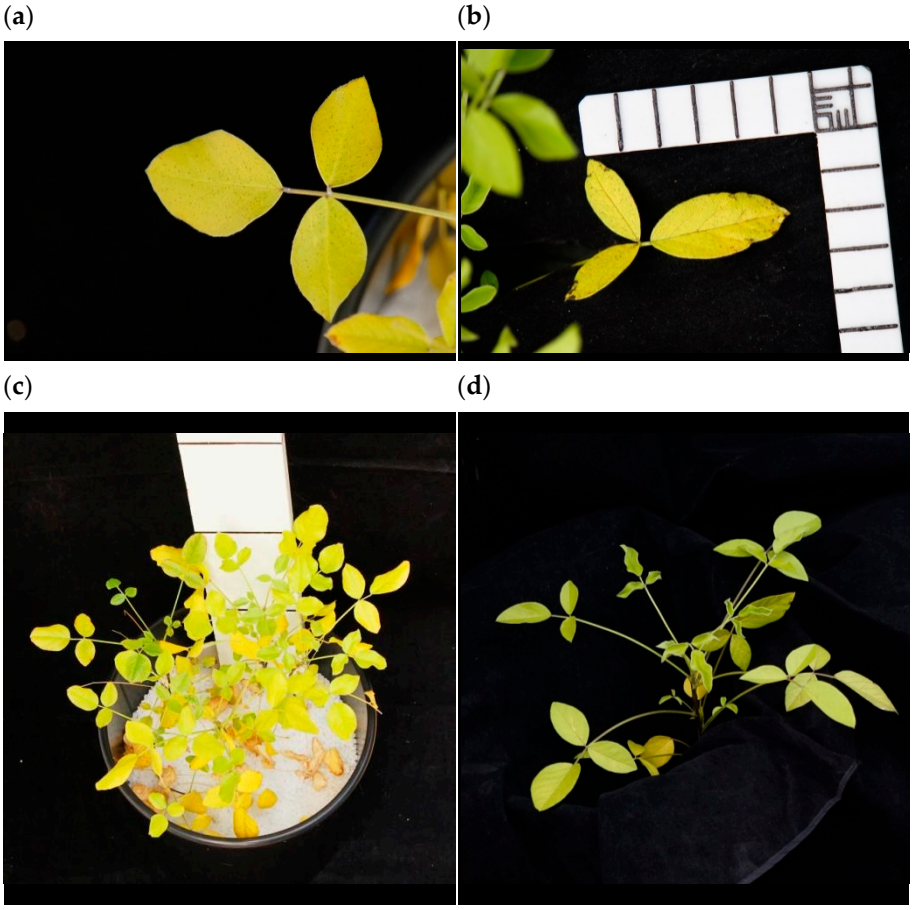


Figure 10. Leaf and whole plant symptoms of Lanza® tedera (**a**,**c**) and Palma (**b**,**d**) grown in soils with very low sulphur (<2 mg kg^{-1}). Photos were taken at 12 weeks old, except panel (**d**), taken at 8 weeks old.

Nodulation responses to soil nutrients are presented in Figure 11. Significant declines in nodulation were observed at low and high levels of P in all three genotypes and high levels of Colwell K in Lanza® and Palma. At the highest P concentration, nodulation of Lanza® was completely absent, and Palma scored less than 2 on average (nodules scored as 'scarce'). Low levels of Colwell K also reduced nodulation in Lanza®, and low soil S reduced nodulation in lucerne and Lanza®. Nodulation scores were lower overall for Lanza® compared to the other genotypes.

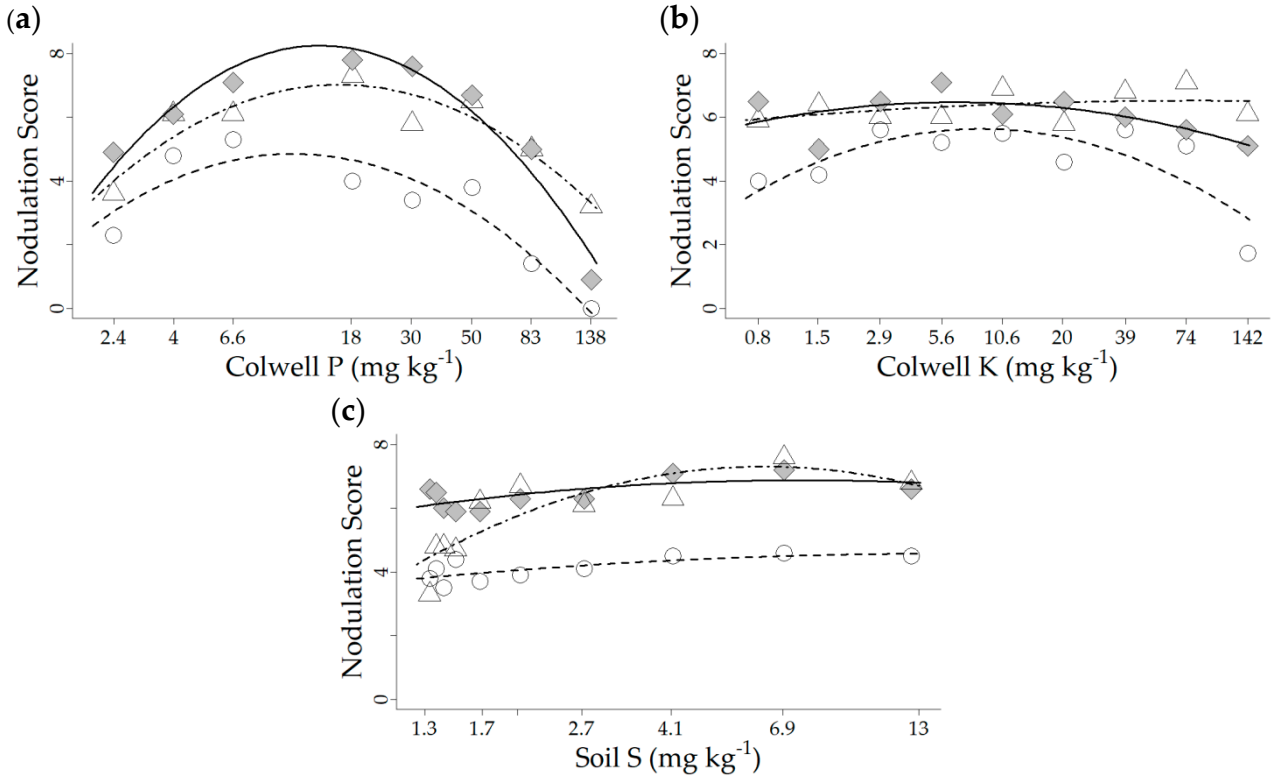


Figure 11. Nodulation scores (Yates, Abaidoo and Howieson [31]) in response to increasing levels of (**a**) Colwell P, (**b**) Colwell K or (**c**) Soil S. Circles, triangles, and diamonds represent Lanza®, lucerne and Palma, respectively. Dashed, dotted and solid lines are fitted models for Lanza®, lucerne and Palma, respectively. The scale on the X-axis is a log scale. Levels of Colwell P less than 4 and Colwell K less than 20 were below the detectable limits of the soil tests and have been estimated from smoothed models of soil test results vs. added nutrients.

4. Discussion

The most important outcome of this paper is further evidence that tedera genotypes are more P-, K- and S-efficient than comparable legume pastures; this finding is consistent with our first hypothesis that tedera will achieve maximum productivity at lower levels of P, K and S than lucerne. Our findings in the glasshouse trial were that tedera genotypes required substantially less soil P to achieve maximum production than lucerne (Table 7), and this is consistent with Pang, Ryan, Tibbett, Cawthray, Siddique, Bolland, Denton and Lambers [17], who were first to suggest that tedera reached maximum production potential at a lower rate of soil P than lucerne. The high P concentration required for optimum lucerne production that we observed is consistent with previous research in field trials that established critical values of P for lucerne in field soils of 45 mg kg^{-1} or more [33]. While these values are even higher than the values we observed, the field sites used in Sandral, Price, Hildebrand, Fuller, Haling, Stefanski, Yang, Culvenor, Ryan, Kidd, Diffey, Lambers and Simpson [33] had PBI values in the range of 40–80. With regards to K-efficiency, our results indicate that Lanza® reached peak productivity with soil K concentrations around half of that of lucerne. While Hardy, Brennan and Real [19] did not compare the K response of tedera to lucerne, they did find that tedera was more K-efficient than sub-clover cv. Narrikup. Tedera genotypes also appear to be relatively efficient at low levels of soil S, with small responses over an order of magnitude of soil S concentrations between 1.3 and 13 mg kg^{-1}. In our glasshouse experiment, adding between 0 and 16 mg kg^{-1} S to soil that contained around 1.5 mg kg^{-1} S did not lead to strong biomass responses in either tedera genotype (p = 0.042 for Lanza® and p = 0.15 for Palma), but lucerne did respond strongly ($p = 4.0 \times 10^{-5}$). This is, once again, a demonstration that tedera is less sensitive to S-deficient soils than lucerne and is consistent with the conclusions of Hardy,

Brennan and Real [19], who grew tedera in field soil with 3.5 mg kg^{-1} S and did not observe any response when adding up to 100 mg kg^{-1} S. The reduced requirements for P, K and S at peak production of tedera represent a major advantage over less efficient species, meaning that growers will require less fertiliser and input costs to maximise tedera biomass production. In many cases, existing soil fertility is likely to support optimal tedera production without additional nutrient input for a period of time. Soil or shoot testing should identify when further nutrient additions are required.

Our second hypothesis was that tedera would respond to added P and K in agricultural field soils, and this hypothesis was only partly supported by our results. We did not observe a strong biomass response from tedera when up to 80 kg ha^{-1} of K was added to any of the three field sites. This may reflect the relatively high Colwell K status of the soils prior to nutrient addition, with the sites containing from 47 to 291 mg kg^{-1} Colwell K in the topsoil, along with Colwell K levels from 18 to 414 mg kg^{-1} in the subsoil. These values are all in excess of the Colwell K concentrations at which peak productivity occurred for Lanza® in the glasshouse experiment. Indeed, some sites had Colwell K concentrations that were well into the range where a toxicity response was seen in the glasshouse experiment, although the PBIs in the field soils were higher than in the glasshouse soil and this likely played a role in buffering the toxic effects of K. A productivity benefit was seen at Dandaragan when up to 30 kg ha^{-1} of P was added, but only during the cooler, wetter growing seasons, whereas no benefit was seen at Cunderdin or Three Springs or at Dandaragan during the warmer and drier seasons. The different responses to P at the three sites are not explained by initial P concentrations in the soil, as all sites had relatively similar levels of Colwell P (between 22 and 30 mg kg^{-1} in topsoil and 6 and 18 mg kg^{-1} in subsoil), with the lowest P occurring at Cunderdin. Instead, it is likely that the difference in sites is best explained by rainfall and the overall productivity potential of the sites. Dandaragan received more than 1.6 times the rainfall at either Three Springs or Cunderdin, and productivity at this site was, therefore, substantially higher. Similarly, the added P was only of benefit during the more productive cooler and wetter growing seasons at Dandaragan and not in the drier and warmer seasons, when high temperatures and moisture stress are limiting growth.

Our final hypothesis, that nodulation in tedera would be affected by soil nutrient status, was strongly supported. The most significant reductions in effective nodulation were seen when P levels were either low or high, and this was consistent among the three genotypes studied in the glasshouse experiment. Pang, Tibbett, Denton, Lambers, Siddique and Ryan [20] also demonstrated that tedera nodulation could be detrimentally affected by extremes in soil P and speculated that the causes could relate to the high demand for P in nodules or reduced carbohydrate supply to nodules due to P deficiency or toxicity, affecting shoot growth. The second of these explanations is most consistent with our findings, as we observed marked impacts on nodulation at high soil P concentration, although the first explanation could apply to nodulation impacts in P-deficient plants. Our results are the first evidence of soil K or S levels affecting nodulation in tedera. For Lanza®, we observed marked decreases in nodulation at low and high Colwell K and some detrimental effects at low soil S levels. Among crop and pasture legumes, tedera is most closely related to soybean (*Glycine max* Merrill.) [34,35], and it has long been recognised that effective nodulation in soybean requires a suitable K supply [36-38]. Similarly, S supply is also important for adequate soybean nodulation [39]. It will be particularly important for growers to be mindful of the strong detrimental effects of high K on tedera nodulation that were observed in our study if tedera is grown in agricultural soils with a history of high K applications.

Given that our experimental design included the inoculation of all plants with rhizobia, we cannot empirically disregard the potential that the observed interaction between nodulation and biomass was the primary cause of the biomass effects, as opposed, per se, to the effect of soil nutrition on plant nutrient status. However, there are aspects that support the argument that the nodulation effects were, at most, merely a contribution to

biomass effects. First, all treatments were supplied with between 17 to 19 mg kg^{-1} N at the start of the experiment (Table 3), equivalent to approximately 55 kg N ha^{-1} in an agricultural context. Second, plants were carefully examined for symptoms of nutrient deficiency and toxicity throughout the experiment, with images presented in Figures 6–10, and there was no evidence of typical N-deficiency symptoms of widespread interveinal chlorosis in older leaves. Thirdly, there were two cases where the nodulation effect was not significant, but the biomass effect was (lucerne vs. K and Lanza® vs. S) and two cases where the biomass effect was not significant despite significant nodulation effects (Palma vs. K and vs. S). These examples of a disconnect between the effect of soil nutrition on nodulation and plant biomass indicate that nodulation was not the sole reason for the biomass effects. Nevertheless, our results are an appropriate comparison to the effects of soil nutrition on plant performance in the field, whether related solely to plant nutrient status per se or to the combined effects of plant nutrition and nodulation as the appropriate establishment of both tedera and lucerne in an agricultural context will include inoculation with nitrogen-fixing rhizobia.

The final high-impact outcome of this study is the identification of critical soil and shoot concentrations of P, K and S required to maximise the productivity of tedera. This information will enable growers to better select paddocks that are suitable for tedera and to better manage ongoing fertiliser inputs to save input costs and reduce the risk of environmental pollution. The values identified in our study are consistent with previous studies. Peak production in both tedera and lucerne coincided with a shoot P concentration <0.3% in Pang, Ryan, Tibbett, Cawthray, Siddique, Bolland, Denton and Lambers [17], and this is consistent with our findings, where shoot P concentrations of 0.06%, 0.19% and 0.24% coincided with 90% of peak biomass for Lanza®, Palma and lucerne (Table 7). Hardy, Brennan and Real [19] also suggested that an internal P of 0.28% was the critical concentration for tedera, which is within the range of 90% to 100% production of both genotypes in this glasshouse experiment. We identified that peak productivity in Lanza® occurred with a shoot K concentration of 1.36%, and this is comparable to the level at which Hardy, Brennan and Real [19] found Lanza® produced 90% of peak productivity (1.44% shoot [K]).

5. Conclusions

Tedera cultivars (Lanza® and Palma) are more P-, K- and S-efficient than the comparable legume pasture species, lucerne, reaching peak production at lower levels of the three macronutrients and, therefore, requiring less input cost to maximise tedera biomass production. The tedera genotypes showed a more severe toxicity response to high P compared to lucerne, with the productivity of tedera being reduced to almost zero, whereas lucerne productivity at the highest P level was reduced to roughly 50% of peak productivity.

In the low PBI soil type used in the glasshouse, tedera productivity was maximised with Colwell P values between 3 and 26 mg kg^{-1}, Colwell K values between 3 and 50 mg kg^{-1}, and soil S values above 7.4 mg kg^{-1}. Low and high levels of P and K reduced nodulation in tedera, and it is likely that this could be an additional cause of reduced biomass production in field conditions. With this comprehensive analysis of both shoot and soil nutrient concentrations, growers will be able to sample soils in potential tedera growing areas prior to establishment to identify the best-suited soil types and sample shoot biomass once tedera swards are established for ongoing monitoring of plant nutrient status to ensure optimum productivity is achieved with minimal fertiliser inputs.

This study also provides the first description of foliar symptoms of P and K deficiency and toxicity and S deficiency in tedera, which will be useful for identifying factors constraining tedera productivity in the field.

Author Contributions: Conceptualisation, D.R. and D.M.W.; methodology, D.R., D.M.W., R.G.B. and N.K.N.; formal analysis, R.G.B.; resources, D.R.; data curation, R.G.B.; writing—original draft

preparation, D.R. and R.G.B.; writing—review and editing, all authors; project administration, D.R.; funding acquisition, D.R. All photographs by R.G.B. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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