

Article Potential Nitrogen Contributions by Tropical Legume Summer Cover Crops in Mediterranean-Type Cropping Systems

Shahnaj Parvin^{1,2,3,*}, Jason Condon^{2,3}, and Terry J. Rose^{1,2,4}

- ¹ Faculty of Science and Engineering, Southern Cross University, Lismore, NSW 2480, Australia
- ² Cooperative Research Centre for High Performance Soils, Callaghan, NSW 2308, Australia
- ³ The Gulbali Institute of Agriculture, Water and Environment, Charles Sturt University and NSW Department of Primary Industries, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
- ⁴ Southern Cross Plant Science, Southern Cross University, Lismore, NSW 2480, Australia
- * Correspondence: shahnaj.parvin@scu.edu.au; Tel.: +61-469-064-458

Abstract: Legume cover crops in temperate cropping systems can fix substantial amounts of nitrogen (N) and reduce N fertiliser requirements for subsequent crops. However, little is known about potential biological N₂ fixation by summer cover crop legumes in the short summer fallow in Mediterranean-type cropping systems. Six legume species (balansa clover, barrel medic, mung bean, sunn hemp, lablab and cowpea) were grown for 8-9 weeks in the field in semi-arid southern Australia during the summer fallow, and in a glasshouse experiment, to estimate N2 fixation using the ¹⁵N natural abundance method. Cowpea, sunn hemp and lablab produced 1.2-3.0 t ha⁻¹ biomass in the field while balansa clover and barrel medic produced < 1.0 t ha⁻¹. The percent of N derived from the atmosphere (%Ndfa) in the field ranged from 39% in barrel medic to 73% in sunn hemp, but only 15% (balansa clover) to 33% (sunn hemp) in the glasshouse experiment, likely due to higher soil mineral N availability in the glasshouse study. Biological N2 fixation of cowpea and sunn hemp in the field was 46–55 kg N ha⁻¹, while N₂ fixation in lablab and mung bean was lower (around 26 kg N ha⁻¹). The N_2 fixation in cowpea and sunn hemp of around 50 kg N ha⁻¹ with supplementary irrigation in the field trial likely represents the upper limit of N contributions in the field in typically hot, dry summer conditions in Mediterranean-type climates. Given that any increase in summer cover crop biomass will have implications for water balances and subsequent cash crop growth, maximising N benefits of legume cover crops will rely on increasing the %Ndfa through improved rhizobium strains or inoculation technologies. This study provides the first known estimates of biological N2 fixation by legume cover crops in the summer fallow period in cropping systems in Mediterranean-type environments, providing a benchmark for further studies.

Keywords: cover crops; short duration summer legumes; %Ndfa; B-value'; biological N₂ fixation

1. Introduction

The use of legumes in cover crops, either as monocultures or as a component of a mixed species cover crop, can provide substantial nitrogen (N) inputs into temperate cropping systems. For example, fixed N contributions >100 kg ha⁻¹ have been reported for field peas (*Pisum sativum*), hairy vetch (*Vicia villosa*) and faba bean (*V. faba*) grown as winter cover crops in temperate cropping systems [1,2]. While slow decomposition rates due to low temperatures may limit the proportion of the fixed-N available to the subsequent cash crop [3], continual contributions from legume cover crops over time will likely reduce the synthetic N fertiliser requirements of cash crops over the longer term [4].

In water-limited Mediterranean-type climates, annual cash crops are grown during the mild, wet winter period with a short 4–6-month fallow period over the hot, dry summer months. Water conserved in the soil over the summer fallow can contribute up to 2 t ha⁻¹ grain yield in subsequent wheat (*Triticum aestivum* L.) crops [5]. Therefore, cover cropping



Citation: Parvin, S.; Condon, J.; Rose, T.J. Potential Nitrogen Contributions by Tropical Legume Summer Cover Crops in Mediterranean-Type Cropping Systems. *Nitrogen* 2022, *3*, 592–599. https://doi.org/10.3390/ nitrogen3040038

Academic Editor: Germán Tortosa

Received: 15 September 2022 Accepted: 13 October 2022 Published: 21 October 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). is not practiced regularly in these environments because of the potential negative consequences of cover crop water use on subsequent cash crop grain yields. In addition, a lack of regular rain events over summer provides a risk to the establishment of cover crops in some seasons [6]. There may be a role for strategic use of cover crops in Mediterranean-type climates where hay crops are cut and ground cover leading into summer is low; following wet seasons where the rainfall forecast for the subsequent season is above average [6] or in environments where fallow efficiency is low and in-crop rainfall (winter season) is reliable [7]. However, even when used strategically, summer cover crops are likely to be grown for <2 months in these environments to avoid excessive moisture use.

We are unaware of any published studies on fixed-N₂ contributions of short-term (<2 months) summer legume cover crops in Mediterranean-type environments with 4–6-month fallows over the hot, dry summer period. However, tropical legumes are able to fix up to 25 kg N ha⁻¹ from the atmosphere when grown for 3 months in long fallow systems in semi-arid humid regions of Australia [8]. While this quantity of fixed atmospheric N appears relatively low, the average amount of N applied to Australian grain crops is only around 45 kg N ha⁻¹ [9]. Thus, summer-grown legumes may be able to offset a substantial proportion of crop fertiliser-N requirements in these environments. The aim of the present study was therefore to investigate potential fixed-N₂ contributions from a range of summer-growing legumes when integrated as short-term cover crops in the winter cash crop production systems of southern Australia.

2. Materials and Methods

A field trial and glasshouse trial were conducted over summer in Wagga Wagga, NSW, Australia to investigate N_2 fixation in a range of legumes. Six legume species were trialled: balansa clover (*Trifolium michelanium* L.) cv. Paradana, barrel medic (*Medicago trunculata* L.) cv. Paraggio, cowpea (*V. unguiculata* L.) cv. Red Caloona, lablab (*Dolichos lablab* L.) cv. Highworth, sunn hemp (*Crotalaria juncea* L.) cv. Global sunn and mung bean (*V. radiata* L.) cv. Crystal. While most of the species selected were summer-active species, two winteractive species, barrel medic and balansa clover, were included as experimental treatments to examine their biomass accumulation and N_2 fixation over summer. A separate glasshouse experiment was undertaken to determine B values for estimation of biological N fixation using the ¹⁵N natural abundance method [10].

2.1. Field Trial

The field trial was conducted January–March (summer) 2020 at the Charles Sturt University research farm, Wagga Wagga, NSW, Australia, on a clay loam soil. The soil is classified as a Red Kandosol in the Australian Soil Classification System [11] or Profondic Lixisol according to the World Reference Based (WRB) classification [12]. Physiochemical properties of the 0–10 cm layer of soil are shown in Table 1. Wagga Wagga has a long-term average annual rainfall of 571 mm, with growing season rainfall of 399 mm (April–November).

Table 1. Selected soil physical and chemical properties in the 0–10 cm soil layer.

Soil Property	Values	
Soil texture	Loam	
Soil colour	Brownish	
Organic C (%)	1.73	
Organic matter (%)	4.0	
Bulk density (g cm $^{-3}$)	1.11	
Electrical conductivity (dS m^{-2})	0.11	
pH (H ₂ O)	6.11	
Total C (%)	2.3	
Total N (%)	0.24	

The six legume treatments were part of a larger study investigating a range of leguminous and non-leguminous cover crops, with the experiment laid out in a randomised complete block design with four replicate plots per treatment. Plots (6 m long \times 1.5 m with 6 sowing rows) were sown with the inoculated cover crop legumes on 29 January 2020 with a row spacing of 24 cm. Legumes seeds were inoculated with appropriate rhizobia (Nodule NTM, New Edge Microbials PTY Ltd., Albury, NSW, Australia) as indicated in Table 2. Seeds of balansa clover, barrel medic, cowpea, lablab, mung bean and sunn hemp were sown at 8, 20, 20, 30, 30 and 10 kg ha⁻¹, respectively. Superphosphate fertiliser was banded with seeds during sowing at a rate of 9 kg P ha⁻¹ and 11 kg S ha⁻¹.

Table 2. Cultivars, inoculant groups and B values of six legume species grown in the field and glasshouse trials.

Species	Cultivar	Inoculant Group	Shoot B Value (δ ¹⁵ N; ‰)
Balansa clover	Paradana	Group C	-0.43 ± 0.07
Barrel medic	Paraggio	Group AM	-0.54 ± 0.04
Mung bean	Crystal	Group I	-1.05 ± 0.08
Sunn hemp	Global Sunn	Group M	-0.73 ± 0.05
Lablab	Highworth	Group J	-1.26 ± 0.07
Cowpea	Red Caloona	Group I	-1.85 ± 0.05

Precipitation at the site was recorded with a rain gauge. Irrigation was applied (50 mm in total) to ensure adequate cover crop growth. Rainfall and supplementary irrigation over the duration of the trial are shown in Figure 1. Total rainfall over the summer cover crop growing duration (February–March) was 96 mm.

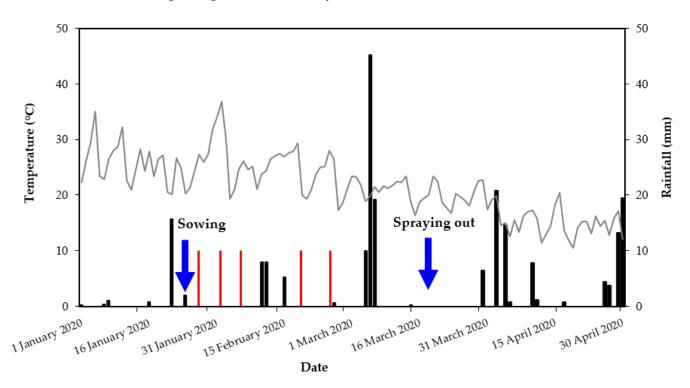


Figure 1. Rainfall and average maximum daily temperature at Charles Sturt University field site, Wagga Wagga, over the duration of the trial. Red bars indicate timing and magnitude of irrigation events while blue arrows indicate time of sowing and termination of the summer cover crops.

After 9 weeks, cover crops were sprayed out with glyphosate (a.i. $2 L ha^{-1}$ with wetter surfactant TX) on 25 March. Shoot biomass production was quantified by cutting shoots at ground level within a $1 m^2$ quadrat of each plot. Buckwheat (*Fagopyrum esculentum*) and

radish (*Raphanus sativus*) grown in neighbouring plots were used as non-N-fixing reference plants. Shoot material was oven-dried at 65 °C for 72 h before weighing, and then ground to a fine powder (<0.05 mm) using a ball mill (Retsch, ZM 200, GmbH, Germany).

Finely ground shoot material (legumes and reference plants) was analysed for N concentration using a LECO TruMac Analyser (LECO Corporation, MI, USA) and δ^{15} N values using a Thermo Delta V plus isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) after combustion on a Thermo Flash EA 1112 elemental analyser (Thermo Scientific, Bremen, Germany). Legume shoot N content was calculated by multiplying the shoot biomass by the respective shoot N concentration. The percentage of N₂ derived from the atmosphere (%Ndfa) was determined using the ¹⁵N natural abundance method [10]:

% Ndfa =
$$[(\delta^{15}N \text{ reference plant} - \delta^{15}N \text{ legume})/(\delta^{15}N \text{ reference plant-B})] \times 100$$
 (1)

where $\delta^{15}N$ reference plant refers to the isotopic signature of non-legume plants grown adjacent to the legume summer cover crop plots in each block (buckwheat and radish in this case). $\delta^{15}N$ legume refers to the isotopic signature of legume summer cover crop species that were tested in this study. The 'B' value describes the isotopic N fractionation for a particular legume species/cultivar × rhizobium combination. The B value for each legume cultivar and rhizobium combination used is given in Table 2 and was determined by growing three replicate pots of each legume, inoculated with the appropriate rhizobium, in sand watered with N-free nutrient solution as per Rose et al. [13] for 8 weeks.

Fixed-N in shoot biomass was calculated as follows:

Fixed N = Shoot N content
$$\times$$
 %Ndfa/100 (2)

2.2. Cover Crop Glasshouse Trial

The glasshouse experiment was conducted at Charles Sturt University, Wagga Wagga, from August–September 2020, with daily maximum temperatures ranging from 25–32 °C, similar to the temperatures recorded in the field trial (Figure 1).

Soil was collected from the 0–10 cm layer of the field site described above. The soil was air-dried and passed through a 5-mm sieve. Field capacity of the soil was determined in the laboratory using the tension table method as described by McKenzie et al. [14]. PVC columns (15 cm diameter \times 60 cm high) were filled with 15 kg air-dried soil, and soil was then wet to 80% field capacity. Each column had removable caps attached to the bottom with a 2-mm-diameter hole in the centre of the cap to allow drainage.

The six legume species grown in the field trial were grown in the glasshouse in a randomised complete block design with three replicates. Seeds were inoculated with commercial crop specific peat-based inoculum before sowing as per Table 2. Balansa clover and barrel medic were sown at 50 seeds per column, while all other species were sown at five seeds per column. Tropical legumes were thinned to two plants per column after emergence while all plants were retained for balansa clover and barrel medic.

Plants were harvested 9 weeks after sowing. Shoots were cut at the soil surface, oven dried at 65 °C for 72 h, then weighed. Columns were cut vertically with a saw to open the column and roots were washed out of the soil with tap water over a 2-mm sieve, then manually collected with tweezers and dried at 70 °C for 72 h and weighed.

Shoot and root N concentrations and content were calculated as described above for the field trial, and%Ndfa and fixed N content in shoots were also determined as per the field experiment. Fixed-N in roots could not be quantified but was estimated by multiplying the root N content by shoot%Ndfa as per Kearney et al. [15]. Total plant fixed N was then calculated by summing the shoot fixed-N with the estimated root fixed-N.

2.3. Statistical Analysis

All data were analysed using R version 3.4.1 (R Core Team, 2020). One-way analysis of variance (ANOVA) was performed using a linear mixed-effect model fit by REML using R package "nlme" [16] considering treatments as fixed effect and replications as random

effect. When the treatments effects were significant (p < 0.05), least significant difference (LSD) test was performed to assess significant differences among the treatments mean using the R package "predictmeans" [17].

3. Results

3.1. Glasshouse Experiment

Shoot biomass production significantly differed between each species in the order of lablab (9.85 g plant⁻¹) > cowpea (8.7 g plant⁻¹) > mung bean (7.6 g plant⁻¹) > sunn hemp (6.9 g plant⁻¹), with winter/spring-growing legumes barrel medic and balansa clover producing only 3.0 and 0.4 g plant⁻¹, respectively (Table 3).

Table 3. Shoot biomass production, N content and biological N_2 fixation of six legume summer cover crops grown for 9 weeks in the glasshouse. Means not followed by a common letter are significantly different at p < 0.05.

Species	Shoot Biomass (g Plant ⁻¹)	Shoot N%	Shoot N Content (mg N Plant ⁻¹)	Shoot %Ndfa	Shoot N ₂ Fixation (mg N Plant ⁻¹)	Root N Content (mg N plant ⁻¹)	Estimated Whole Plant N_2 Fixation (mg N plant ⁻¹)
Balansa clover	$0.4\pm0.0~{ m f}$	5.2 ± 0.1 a	$22 \pm 1 \text{ e}$	$15\pm2\mathrm{b}$	$3\pm 1d$	$5.8\pm1.1~{ m c}$	$4\pm1\mathrm{c}$
Barrel medic	$3.0\pm0.1~\mathrm{e}$	5.2 ± 0.2 a	$151 \pm 7 d$	30 ± 1 ab	$47\pm17~{ m c}$	23.2 ± 1.2 b	$54\pm10~{ m b}$
Mung bean	$7.6\pm0.1~{ m c}$	4.7 ± 0.1 ab	358 ± 12. b	32 ± 1 a	116 ± 5 a	$20.5\pm3.1~\mathrm{b}$	122 ± 5 a
Sunn hemp	$6.9 \pm 0.2 \text{ d}$	4.7 ± 0.3 ab	$324\pm14~{ m c}$	33± 3 a	108 ± 12 a	$22.5\pm1.7~\mathrm{b}$	116 ± 13 a
Lablab	9.9 ± 0.4 a	$4.2\pm0.22\mathrm{b}$	405 ± 4 a	19 ± 1 ab	$75\pm9\mathrm{b}$	33.3 ± 4.9 a	$81\pm8\mathrm{b}$
Cowpea	8.7 ± 0.4 b	4.6 ± 2.1 ab	411 ± 22 a	$16\pm 2b$	$67 \pm 6 \text{ bc}$	$35.8 \pm 2.5 \text{ a}$	$73\pm15\mathrm{b}$
p-value	< 0.001	0.011	< 0.001	0.006	< 0.001	< 0.001	< 0.001
LSD _{0.05}	0.72	0.54	39	10	30	8.7	31

Shoot N concentration did not differ significantly between tropical legume species (range of 4.2–5.2%) (Table 3). Shoot N content followed a similar trend to shoot biomass production, with the highest shoot N content observed in cowpea and lablab (both >400 mg N plant⁻¹). The low shoot N accumulation in balansa clover (22 mg plant⁻¹) and barrel medic (151 mg plant⁻¹) was reflective of their poor biomass production. The%Ndfa ranged 15–33% across all species (Table 3) and resulted in shoot N fixation being significantly greater in mung bean (116 mg N plant⁻¹) and sunn hemp (108 mg N plant⁻¹) than all other species, with a similar trend observed for whole plant fixed-N (Table 3).

3.2. Field Trial

Cowpea produced significantly more biomass (3.3 t ha^{-1}) than all other species, while balansa clover (0.2 t ha^{-1}) produced significantly less biomass than all other species (Table 4). Lablab, sunn hemp and mung bean biomass production ranged from 1.2–2.0 t ha^{-1} , with no significant difference between the species.

Table 4. Shoot biomass production, N concentration, N content and biological N₂ fixation of six legume summer cover crops grown for 9 weeks in the field. Means not followed by a common letter are significantly different at p < 0.05.

Treatments	Shoot Biomass (t ha ⁻¹)	Shoot N%	Shoot N Content (kg ha ⁻¹)	Shoot %Ndfa	Shoot N Fixation (kg ha ⁻¹)
Balansa clover	$0.2\pm0.1~{ m d}$	3.1 ± 0.2 b	$8\pm1d$	72 ± 10 a	$6\pm1\mathrm{c}$
Barrel medic	$1.0\pm0.5~{ m cd}$	3.1 ± 0.2 b	$32\pm16~{ m cd}$	39 ± 6 b	$15\pm9~{ m c}$
Mung bean	2.0 ± 0.2 b	$3.2\pm0.1\mathrm{b}$	63 ± 6 bc	$42\pm10~\mathrm{b}$	$26\pm7\mathrm{bc}$
Sunn hemp	1.9 ± 0.2 b	$3.8\pm0.3~\mathrm{ab}$	$75\pm14\mathrm{b}$	73 ± 3 a	55 ± 10 a
Lablab	$1.2\pm0.3~{ m bc}$	4.1 ± 0.5 a	$49\pm13~{ m bc}$	50 ± 4 b	$26\pm8\mathrm{bc}$
Cowpea	3.3 ± 0 a	3.3 ± 0.2 b	114 ± 13 a	$40\pm5\mathrm{b}$	$46\pm10~\mathrm{ab}$
<i>p</i> -value	< 0.001	0.007	< 0.001	< 0.001	0.001
LSD	0.78	0.57	32	17	21

Shoot N content was higher in cowpea (114 kg ha⁻¹) than all other species, while shoot N content was not significantly different among lablab (49 kg ha⁻¹), sunn hemp (75 kg ha⁻¹) and mung bean (63 kg N ha⁻¹). Shoot N content of balansa clover was the lowest at 8 kg N ha⁻¹ (Table 4).

Balansa clover and sunn hemp had significantly higher%Ndfa (72–73%) than all other species (range from 39–50%). Shoot N₂ fixation was the greatest for sunn hemp (55 kg N ha⁻¹) followed by cowpea (46 kg N ha⁻¹), while shoot fixed-N in balansa clover was only 6 kg N ha⁻¹ (Table 4).

4. Discussion

Limited rainfall and high temperatures can limit cover crop establishment and growth over summer in many regions with Mediterranean-type climates, and where subsoil moisture is available over summer, excess moisture use by summer cover crops will typically reduce the yields of subsequent cash crops [6]. Biological N₂ fixation from cover crops comprising legume species provides a system benefit to offset the risk of cash crop yield penalty from water use by cover crops. Notably, the winter/spring legumes balansa clover and barrel medic produced limited biomass over the hot summer, and the discussion herein focusses on growth and N₂ fixation by tropical legumes.

Given potential trade-offs between summer cover crop biomass (water use) and winter cash crop yields in Mediterranean-type environments, maximising N benefits of legume cover crops may rely on fixing more atmospheric N per kg dry matter produced as opposed to increasing biomass production. The %Ndfa for tropical legumes in the field trial ranged from around 40% in cowpea and mung bean to 72% in sunn hemp. Lower %Ndfa values were obtained in the glasshouse study, likely due to higher mineral N in the columns because only topsoil was used, and potentially due to N mineralisation as a result of drying and sieving the soils then prewetting soil columns prior to sowing [18]. Biomass rankings between crops also differed between the field and glasshouse trials, but we note that the data sets are not directly comparable because glasshouse data were expressed as biomass per plant (two plants per column) while field data were expressed as biomass per ha. Differences in plant density and competition between plants for each crop species in the field are a likely reason for the differences in rankings for biomass between the two trials.

Previous studies using the same cultivars for sunn hemp (cv. Global sunn) and mung bean (cv. Crystal) reported %Ndfa in field trials of around 47% for sunn hemp [19] and 56–68% in mung bean [20] in 3–4-month-old plants in the subtropics and tropics. Field studies on mung bean across a range of soil types in semi-arid Australia indicated low %Ndfa (25–30%) using the ¹⁵N natural abundance method [21], with the authors suggesting that ineffective inoculation may have contributed to the low %Ndfa. Given that %Ndfa in the field at Wagga Wagga was < 50% for all summer legumes except sunn hemp (72%), we suggest that improvements in inoculant strains or advances in technologies for the delivery of inoculum would be beneficial for maximising the value of summer legume cover crops in these water-limited environments.

We acknowledge that calculation of biological N₂ fixation using the ¹⁵N natural abundance technique provides an estimate only, due to numerous assumptions in the method. In the present study, we determined the B values for tested legume species using the same genotypes and inoculant strains as the field and glasshouse experiments, and at the same growth stage that plants in the field were terminated. B values obtained for 9-week-old legumes can be inaccurate due to the relatively high contribution of seed N to total plant N; however, the degree of error is minimised when $\delta^{15}N$ signatures of the reference (non-N₂-fixing) plants is >+4 ‰ [13]. Given that the mean $\delta^{15}N$ signature of non-N₂-fixing reference plants (buckwheat and radish) in the field at Wagga Wagga was +7.6 ‰, we believe our estimates provide a reasonable estimation of%Ndfa and fixed N for summer legume crops under optimal (wet summer) conditions as a reference point for future studies.

Legume performance at the Wagga Wagga site with supplementary irrigation over the 2-month period likely represents the upper range for growth and N fixation by legume cover crops in annual cropping systems in Mediterranean-type climates. The potential biological N₂ fixation by legume summer cover crops in these environments of around 50 kg N ha⁻¹ is substantially lower than the 100–150 kg N ha⁻¹ accumulated in sunn hemp over 3-4 months in temperate environments in USA [22] or 100-200 kg fixed-N accumulated in sunn hemp, cowpea and lablab over 10 weeks in the Australian wet tropics [19]. While cowpea produced 3.2 t biomass ha⁻¹ at the Wagga Wagga site with supplementary irrigation, the highest yield of cowpea as a summer cover crop in the Mediterranean-type climate of the wheatbelt of Western Australia over a 3-yr (dryland) trial was 0.9 t ha^{-1} after 10 weeks growth, reflecting the hot, dry summer conditions [7]. Thus, while 50 kg fixed-N ha⁻¹ may be achievable in summer legume crops in seasons with wet summers, 15–20 kg fixed-N ha⁻¹ may be more realistic for legume cover crops under typical hot, dry summer conditions. Nonetheless, fixed-N contributions of 15–20 kg fixed-N ha⁻¹ by summer cover crops are valuable in Australian dryland cropping systems, where the average amount of fertiliser-N applied to winter cereal and oilseed crops is only 45 kg N ha⁻¹ [9]. These contributions may become more valuable over the coming years if synthetic N fertiliser prices remain high [23], provided that the summer cover crop moisture use does not impact on the yield of subsequent cash crops.

5. Conclusions

This study demonstrated that under optimal growing conditions, tropical legumes can fix up to 50 kg N ha⁻¹ in the short summer fallow period in cropping systems in Mediterranean-type environments. Any improvements in fixed-N contributions by cover crops legumes in these environments will likely arise from enhanced rhizobium strains or inoculation techniques, since any increase in legume biomass production will use more stored soil water and may have consequences for subsequent winter cash crops.

Author Contributions: S.P.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing—original draft, Writing—review and editing, Finalizing the draft. J.C.: Conceptualization, Resources, Writing—review and editing. T.J.R.: Conceptualization, Funding acquisition, Methodology, Supervision, Writing—original draft, Writing—review and editing, Finalizing the draft. All authors have read and agreed to the published version of the manuscript.

Funding: This research has been supported by the Cooperative Research Centre for High Performance Soils (PROJECT: 4.1.002—Plant based solutions to improve soil performance through rhizosphere modification) whose activities are funded by the Australian Government's Cooperative Research Centre Program.

Data Availability Statement: All data reported in this article will be available on request.

Acknowledgments: This work has been supported by the Cooperative Research Centre for High Performance Soils whose activities are funded by the Australian Government's Cooperative Research Centre Program. We are thankful to Graeme Heath (Charles Sturt University) for his support in managing the glasshouse trial and Lee Kearney (Southern Cross University) for nitrogen and ¹⁵N isotopic analysis.

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

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