Final report

Extending the boundaries of legume adaptation through better soil management

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Abstract

This project arose in recognition of the fact that nitrogen (N) remains a key limitation to pasture productivity in the ‘high-rainfall’ permanent pasture regions of south-eastern Australia and that legumes, the key source of N in those systems, generally lack persistence, especially under drought. This project examined the extent to which improved soil fertility could improve legume persistence.

The project employed a component approach, involving a range of discreet experiments, including a network of legume evaluation experiments, a farmer survey to assess factors essential to white clover success, glasshouse experiments to assess mechanisms driving legume persistence under drought, a detailed study assessing N-fixation in legumes and nutrition experiments assessing legume response to lime or fertiliser in mixed pastures. The project was book-ended by a review of literature and an economic analysis.

Improving soil fertility, especially elevated levels of pH and soil phosphorus (P), improved legume productivity and persistence. However, in white clover this was not due to improved survival as white clover died just as quickly in pots under drought in fertilised soil compared to untreated soil. Rather, increased persistence in white clover was associated with increased seedling recruitment. Improved gross margins supported the increased investment in lime and P fertiliser in the target environments.

Executive summary

Background

Nitrogen (N) deficiency remains a key limitation to livestock production in south-eastern Australia, reducing biomass production and forage quality of pastures. Legume production in the 'high-rainfall' permanent pasture region is constrained by an over-reliance on winter-growing annual species, such as subterranean clover, which are unable to utilise warmer temperatures and rainfall over summer. Yet, perennial legume options typically lack the persistence required in permanent pastures, due to a sensitivity to periodic drought. With increased incidence of summer rainfall predicted under future climate scenarios, there was a need to explore ways to improve perennial legume persistence, to improve pasture resilience and increase livestock productivity across the vast permanent pasture region of south-eastern Australia.

Increased soil fertility was suggested as a viable means to improve productivity in the near-medium term. Fertilisers are already available and broadly used by farmers and have long been recognised as a key ingredient in developing more productive pastures. However, the extent to which soil fertility could improve the persistence of perennial legumes was not well understood.

Objectives

The project achieved all seven stated objectives, listed below. Following each objective is a brief statement describing the evidence presented in this report supporting this objective.

1. A financial risk analysis detailing the benefits and risks to farm businesses of increasing inputs to soil. Evidence:
    a. An analysis, using the Sustainable Grasslands Model, was undertaken by Dr Karl Behrendt of Harper Adams University.
2. Provide producers and advisers with management guidelines to improve the productivity and persistence of pasture legumes in the HRZ which address total feedbase production and feedgap issues. Evidence:
    a. Management guidelines include informed species choice for improved persistence.
    b. Practices (inoculation and cultivar choice) to improve nodulation when establishing perennial legumes.
    c. Project findings support existing guidelines around use of lime and P fertiliser, highlighting financial advantages to producers in investing in better soil fertility.
3. Collation of objective data that defines the potential productivity of key legume species and the mechanisms that increase legume persistence. Evidence:
    a. Glasshouse experiments defining the response of white clover, strawberry clover and lucerne under drought.
    b. Field evaluation experiments comparing productivity of different species over time in contrasting environments.
    c. Farmer survey highlighting the importance of seedling recruitment for white clover persistence under drought.
4. Objective data defining the impact of changed soil nutrition on herbage mineral concentration, and likely impacts on livestock productivity and health based on comparison with established thresholds. Evidence:
    a. Data from field experiments showing the changes in herbage mineral concentration associated with lime, P, potassium (K) or sulfur (S) fertiliser.
    b. Data from glasshouse studies showing the changes in white clover herbage mineral concentration associated with lime or P, K and S fertiliser.

    c. Estimated incidence of metabolic disorders in livestock, based on calculation of important ratios and indices, such as Tetany index, K:Na+Mg and dietary cation-anion difference (DCAD).
5. Objective data defining the impact of elevated levels of soil nutrition on symbiotic nitrogen fixation by legumes.
    a. Data from legumes in field experiments estimating N-fixation using the natural abundance technique.
    b. Data from white clover in glasshouse experiments, also estimating N-fixation using the natural abundance technique.
6. Quantified thresholds for soil nutrients that improve legume persistence under moisture stress.
    a. Data from farmer survey relating legume presence with soil test results.
    b. Data from field experiments, also relating legume presence with soil test results.
7. Support for post-graduate student projects, in conjunction with a collaborating university, to undertake related research and develop capacity in the field of legume adaptation and nutrition.
    a. Two post graduate research projects conducted in conjunction with this project, including one Honours project (completed) in conjunction with the University of New England, and one PhD project (ongoing), in conjunction with the University of Tasmania.

Methodology

There was emphasis on perennial legume options in this work, in recognition of the importance of summer rainfall for pasture growth in much of the target region. White clover featured prominently not only because of its existing importance to significant areas of grazing land in parts of southern Australia and internationally, but also because of its known sensitivity to moisture stress.

The project employed a component approach, involving a range of discreet experiments, including:

- Review of literature
- Series of field experiments evaluating perennial legume persistence
- Survey of farmers and pastures on the Northern Tablelands of NSW
- Detailed study on N-fixation in perennial legumes
- Series of glasshouse experiments examining mechanisms of drought tolerance in key legume species
- Series of field experiments examining legume response to lime and P, K and S fertiliser when grown in mixtures with phalaris, and
- An economic analysis assessing the costs and benefits of increased fertiliser inputs

Results/key findings

White clover remains the most promising perennial legume species across the ‘high rainfall’ permanent pasture region of south-eastern Australia, where soils are too acidic, shallow and low in fertility to support species such as lucerne. However, white clover lacks the persistence to withstand periodic drought inevitable across most environments. In contrast to lucerne or even strawberry clover, white clover exhibits few mechanisms to conserve water under periods of drought meaning that no management strategy, other than irrigation, will improve its longevity. Rather, white clover persistence in the face of drought is through seed production and seedling recruitment, a trait that has hitherto received little research attention. Further work is recommended to improve reliability of seedling recruitment in white clover, using subterranean clover as a model ideotype, an approach which promises to extend white clover adaptation well beyond its current niche. Improved soil fertility is a critical component of improved white clover performance under drought, to increase its productivity and seed production.

Benefits to industry

The project reinforces the benefits of managing soil pH and P-fertility and demonstrates improved legume persistence, increasing the resilience of permanent pastures and, as a consequence, the profitability of livestock production enterprises. The importance of white clover across this region is emphasised, and the use of cultivars such as cv. Haifa that exhibit compatibility with background rhizobia is encouraged. The best way for farmers to increase N inputs to their pasture through biological N-fixation is to increase legume biomass, which is reliably achieved with better soil fertility. By applying these basic principles, farmers will improve the resilience of their pastures under drought and increase the productivity and profitability of their livestock enterprise.

An unanticipated benefit to industry was the rediscovery of sainfoin rhizobial inoculum, which was found to be no longer available in Australia. As of 2022 and as a direct result of efforts from this Project in collaboration with the Australian Inoculants Research Group and the South Australian Research and Development Organisation (SARDI), rhizobial strain CC1099 is once again commercially available in Australia.

Future research and recommendations

Seedling recruitment was identified as the key drought-adaptation mechanism employed by white clover. Seedling recruitment is a key drought-avoidance mechanism employed by a range of annual legume species that have proven adaptation to very dry environments and we see advantages in developing a species such as white clover which exhibits a facultative perennial habit, enabling it to use summer rainfall when conditions are favourable.

Further research is also warranted to better understand the impact to livestock productivity and meat quality of more nutrient dense forages associated with the increased use of fertilisers.

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1 Background

1.1 Rationale

The poor adaptation of legumes in the south-eastern high rainfall zone (HRZ) has long been recognised as a significant constraint to production of permanent pastures. Nitrogen deficiency remains a key limitation to pasture productivity, perhaps explaining 60% of the gap between actual and potential yield (Cocks 1980). The legume component in many regions in the 600-850mm rainfall zone is predominantly reliant upon subterranean clover, a winter growing annual species with little ability to utilise summer rainfall, and there are relatively few perennial legume species used in this region. As climates continue to change, it is predicted that the incidence of summer rainfall will increase and the growing season will change from being 'cool-season dominant' to a climate where well-adapted plants must have the ability to grow throughout any season of the year, including in summer. This further reduces the reliability of annual cool-season legumes in permanent pasture systems.

Soils across the HRZ are commonly shallow, acidic and naturally low in fertility. Legume species in general are much more sensitive to low fertility and acidity compared with other sward components such as grasses. When combined with the normal stresses imposed in commercial paddocks such as grazing, insect burden or drought, the persistence of the legumes is more vulnerable to failure than any other sward component.

Previous research has addressed the poor persistence of legumes in permanent pastures by focussing on particular aspects of the problem. For example, there has been significant prior investment in understanding acidic soils and managing them with lime. Likewise, plant breeders have sought to address the paucity of perennial legume options available to this region through developing novel species. The present project proposes to build upon the fundamental pillars laid down by previous research with the aim of improving legume performance through a combined approach of advanced plant genetics and enhanced soil nutrition. It was the combination of lime and deep-rooted perennial species which enabled 27 mm more water to be extracted from the subsoil in a pasture study conducted under drought conditions in southern NSW (Hayes *et al.* 2016).

1.1.1 Identifying the knowledge gap

This project arose largely from a Tablelands Farming Systems Group (TFS) Soil Club field day near Crookwell in September 2015. The field day, attended by farmers and advisors associated with the TFS, involved an in-depth overview of acidic soil management in permanent pasture environments, including a local case study, followed by a farmer-led discussion about the practical constraints of managing soil acidity. The discussion included some analysis of pilot studies that demonstrated increased plant water availability as well as increased legume composition of mixed swards associated with the use of soil amendments. The group identified that unreliable legume persistence and nitrogen fixation were a key barrier to increased pasture production in their grazing systems, and that opportunity existed to further examine the potential of better soil management to enhance legume resilience under drought. The group felt that soil amendments and fertilisers could be a practical means of increasing legume abundance in pastures, but that demonstrating the economic viability of increased inputs would be essential for them to adopt the practice.

1.1.2 The research questions

The P.PSH.1030 *Extending the boundaries of legume adaptation through better soil management* project (the Project) was designed to answer some practical questions to bridge the knowledge gaps that the farmers and advisors had identified. Specifically, are there better legume options available to farmers? Will legume persistence be improved with better soil fertility?

1.1.3 The target audience

The Project focused on the 'Tablelands' environments of NSW, which includes the Northern, Central and Southern Tablelands, and the alpine Monaro region. Collectively, these regions are thought to include grasslands covering approximately 7.7 M ha that are thought to run approximately 6.7 dry sheep equivalent (DSE)/ha, or a total of 51.6 M DSE (Donald 2012). The research was targeted at the applied level to provide solutions for farmers and their advisors, and where appropriate, promote adoption of existing technologies.

2 Objectives

2.1 Contracted objectives

Outputs:

1. A financial risk analysis detailing the benefits and risks to farm businesses of increasing inputs to soil.
2. Provide producers and advisers with management guidelines to improve the productivity and persistence of pasture legumes in the HRZ which address total feedbase production and feedgap issues.
3. Collation of objective data that defines the potential productivity of key legume species and the mechanisms that increase legume persistence.
4. Objective data defining the impact of changed soil nutrition on herbage mineral concentration, and likely impacts on livestock productivity and health based on comparison with established thresholds.
5. Objective data defining the impact of elevated levels of soil nutrition on symbiotic nitrogen fixation by legumes.
6. Quantified thresholds for soil nutrients that improve legume persistence under moisture stress.
7. Support for post-graduate student projects, in conjunction with a collaborating university, to undertake related research and develop capacity in the field of legume adaptation and nutrition.

2.2 Success in meeting objectives

2.2.1 Financial risk analysis

This objective was met and is detailed in Appendix 7. This activity was subcontracted to Harper Adams University, UK, and undertaken by Dr Karl Behrendt using the Sustainable Grasslands Model, a stochastic whole-farm bioeconomic model that has been used in previous analysis of management practices in grasslands (Behrendt *et al*. 2013; 2020c).

2.2.2 Management guidelines

This objective was met. Results from this project support adoption of existing best management practices relating to species choice and fertiliser management, including seed inoculation practices.

Specifically, the Project confirms that there is only a narrow range of viable legume species available to farmers in the target environments (Appendix 2). Although white clover offers potential beyond its existing zone of adaptation, the seed characteristics have not been sufficiently developed to justify modifying guidelines of where that species could be sown. Nevertheless, the Project demonstrated the vulnerability of white clover to moisture stress and highlighted the crucial role that seedling regeneration plays in ensuring the persistence of that species in the face of periodic moisture stress. This is flagged as a priority of future research.

In terms of fertiliser, the Project provides supporting evidence for the maintenance of adequate soil pH and phosphorus (P) nutrition to improve legume persistence but there was nothing to prompt a revision of existing guidelines. Results did not support a change to guidelines regarding broadscale application of potassium (K) fertiliser but did allay concerns about the effect of some fertilisers on forage quality of pastures. There was no evidence that the application of K or S fertiliser would increase the risk of metabolic disorder in livestock, by pushing mineral concentrations beyond established thresholds.

The Project provides supporting evidence for proper inoculation of legume seed prior to sowing. Evidence is presented that advocates the use of legume cultivars that exhibit high levels of compatibility with background strains of rhizobia, such as white clover cv. Haifa or strawberry clover cv. Palestine. Choosing cultivars such as these provides the grower with some insurance against inoculation failure in soil environments where rhizobia strains similar to the subterranean clover strain (WSM 1325) dominate. The project highlights the importance of good inoculation practice, especially where novel species are being sown into soil with high background rhizobial populations.

The extension of these messages has now commenced, in a series of face-to-face forums between researchers and growers or advisors. The extension component remains ongoing in a staged process, linked with the delivery of key scientific outputs of this project.

2.2.3 Potential productivity of key legume species

The Project saw the delivery of a major scientific output, a comprehensive study reporting persistence of pasture legumes across the Tablelands and Monaro of NSW (Appendix 2). This cornerstone paper combines results from 20 separate field experiments conducted since 2012, which includes the dozen perennial legume evaluation experiments undertaken directly by this project as well as a number of experiments conducted either under the P.PSH.1048 'Perennial pasture and forage combinations to extend summer feed for southern NSW' project, or the previous P-efficient pasture projects, Phosphorus-Efficient Legume Pasture Systems' (B.PUE.0104) and 'RnD4P-15-02-016 Phosphorus Efficient Pastures'. That study highlighted the significant positive relationship between productivity and persistence of legume species.

2.2.4 Herbage mineral concentration

This objective was met. The network of 'nutrition' field experiments comprises 7 sites sown to mixtures of phalaris and white clover, with lime and fertiliser P, K and S applied alone or in various combinations. Herbage samples were collected in spring each year and sent to Incitec Pivot laboratories for analysis of herbage mineral concentration, including calcium (Ca), magnesium (Mg), sodium (Na), K, P, Chlorine (Cl), S, manganese (Mn), boron (B), molybdenum (Mo), copper (Cu), zinc (Zn), and nitrogen (total N), as well as nitrate and ammonium. For the sites established in 2019 (Gunning, Mandurama, and Orange) there are three sampling times for herbage quality. There are two sampling times for the remaining experiments at Paling Yards, Glen Innes and Dry Plain. At each sampling time, the phalaris was sampled separately to the legume, which was either white clover or a background legume where there was insufficient white clover for sampling. Those samples were also provided to the LPP NIR project to assist in the development of NIR calibration curves.

2.2.5 Impact of soil nutrition on nitrogen fixation

This Objective was met. The response of white clover to applications of lime, P, K and S in a glasshouse environment was observed and nitrogen fixation using the natural abundance technique was assessed at the end of that experiment. Nitrogen fixation was also assessed in 2020 at five of the nutrition experiments described above, using the natural abundance technique.

In addition, a detailed assessment nodulation and nodule occupancy was undertaken to inform management practice at sowing (Rigg et al. 2021, Appendix 5). In the course of this activity it was identified that the rhizobial strain used to inoculate the perennial legume, sainfoin (*Onobrychis viciifolia*), was no longer available in Australia. The Project, in collaboration with the Australian Inoculants Research Group (AIRG) and the South Australian Research and Development Institute (SARDI), set about to identify and re-establish the commercial source of inoculum for industry (Appendix 6).

2.2.6 Quantified thresholds for soil nutrients that improve legume persistence

This objective was met by an examination of the farm survey activity from Northern NSW. The Project surveyed more than 50 paddocks on six farms on the Northern Tablelands in 2019 and revisited those same paddocks throughout the project period (Appendix 3). There was a positive relationship between Colwell P and white clover prevalence across that site.

However, the Project was unable to define thresholds for other soil nutrients, specifically S and K. Initially, it was hypothesised that improved K nutrition would lead to improved osmotic regulation in white clover, leading to greater survival. A detailed glasshouse experiment conducted in 2020 proved the null hypothesis, indicating that additional K and S (or P and lime for that matter) did not improve osmotic adjustment. When results from the glasshouse experiment and farmer survey are taken together, we conclude that improvements in white clover abundance observed in the field are not due to improved survival but rather, improved seedling regeneration.

2.2.7 Support for post-graduate students

This objective was met.

Andrew Faithful completed his Honours degree at the University of New England (UNE) using some of the data collected early in the farmer survey of the Northern Tablelands in his dissertation, titled *The key drivers of sown pasture persistence on the Northern Tablelands of NSW: A survey*. Carol Harris was one of Andrew's supervisors and provided extensive support to his studies. Andrew still works with Carol and the team at NSW DPI at Glen Innes on a part-time basis and continues to develop his pasture research skills.

Huan Huu Lee has just completed his first year as a PhD candidate at the University of Tasmania (UTAS). He is co-supervised by Dr Richard Hayes along with Dr Rowan Smith (Tasmanian Institute of Agriculture), Dr Beth Penrose (UTAS) and Dr Chris Guppy (UNE). His post-graduate studies examining methods to improve establishment in perennial legumes reflects the collaboration between the current project and another MDC-funded project, P.PSH.2052 *Growing red meat productivity through the selection and establishment of perennial legumes*.

The Project advertised for additional PhD candidates through UTAS in 2019 and 2020, but no suitable applications were received.

3 Methodology

3.1 Review of literature

A review of literature was undertaken in 2018 at the commencement of the project to identify knowledge gaps, acknowledging substantial research efforts over many decades on the topic of legume persistence and drought. The review was particularly interested to find evidence of improved legume survival associated with elevated soil fertility, and focused on white clover due to its known sensitivity to drought and relative importance to grazing systems both in Australia and internationally. Eleven co-authors contributed to this review, including five from either of two other Livestock Productivity Partners, CSIRO and the Tasmanian Institute of Agriculture (TIA).

3.2 Perennial legume evaluation

The perennial legume evaluation experiments are reported in detail in a manuscript titled ‘Legume persistence for grasslands in south-eastern Australia’ submitted to Crop and Pasture Science for inclusion in a special issue, fostered by the upcoming Australian Grasslands Association (AGA) symposium (Hayes et al. 2022; Appendix 2). The manuscript included eleven co-authors from NSW DPI (7) and CSIRO (4) and combines results from this and three other MLA-sponsored projects, including B.PUE.0104 ‘Phosphorus-Efficient Legume Pasture Systems’, RnD4P-15-02-016 ‘Phosphorus Efficient Pastures’ and P.PSH.1048 ‘Perennial pasture and forage combinations to extend summer feed for southern NSW’. By combining results of the four projects, we gain a comprehensive understanding of the viable legume options available to farmers in the high-rainfall permanent pasture region of NSW through an assessment of annual and perennial legume persistence, encompassing twenty field experiments.

3.2.1 Perennial legume experimental sites

The evaluation of perennial legumes in the present project was undertaken at ten field sites from 2018, which were monitored for up to four years. Five experiments were located on ‘high fertility’ soils near Glen Innes (-29.69°S, 151.69°E; sown 30 May 2019) on the NSW Northern Tablelands; Mandurama (-33.63°S, 149.05°E; 2 Apr 2019), Orange (-33.33°S, 149.08°E; 10 May 2018) and Paling Yards (-34.17°S, 149.73°E; 29 May 2018) on the NSW Central Tablelands; and Dry Plain (-36.12°S, 148.90°E; 26 Mar 2019) on the Monaro. Five additional experiments were located on ‘low fertility soils’ near Guyra (-30.18°S, 151.54°E; 26 May 2020) and Wandsworth (-30.07°S, 151.52°E; 20 Jun 2018) on the NSW Northern Tablelands; at Merrill (-34.64°S, 149.33°E; 25 May 2018) and Middle Arm (-34.58°S, 149.72°E; 31 May 2018) on the NSW Southern Tablelands; and at Bombala (-36.83°S, 149.11°E; 3 Apr 2018) on the Monaro. The term ‘low fertility soil’ is used to describe soils with a combination of low-moderate extractable phosphorus (P) concentration (< 30 mg Colwell P/kg soil), moderate-high acidity (pH_{Ca}<4.7) and a low (<5 cmol(+)/kg) effective cation exchange capacity based on initial soil samples at 0-0.1 m.

Multi-site analysis also included two experiments established and managed under a separate Livestock Productivity Partnership project (P.PSH. 1048). Both were located on fertile soils, one at

Tirrannaville (-34.93°S, 149.68°E; 5 Sep 2018) on the NSW Southern Tablelands; and the other at Bombala (-36.85°S, 149.15°E; 26 Mar 2019) on the Monaro. This Bombala site was located less than 4 km and on an adjoining property to the Bombala site described above. They are distinguished in this report by soil fertility, the former grouped with the lower soil fertility experiments. A full list of experiments reported in Hayes et al. (2022) is listed in Table 1. It is only the Series 3 and 4 experiments that were conducted under the present project.

3.2.2 Treatments

Experiments comprised 12-20 legume species/cultivars sown as pure stands, replicated three times (Table 2). Plots were 7.5 × 2 m or 6 × 2 m, depending on the seeder used, and were sown with a cone seeder set at 15 cm row spacings. Lime was surface applied at 3.5 t/ha to all sites immediately prior to sowing and molybdised superphosphate was applied at 150 kg/ha at sowing to all sites, except Tirrannaville where it was top-dressed in autumn of year 2 and at Bombala where seed was instead sown with 200 kg/ha of granulated (2-6 mm granules) $CaCO_3$ (Calciprill, Omya Australia Pty Ltd, Lindfield). All seed was inoculated with rhizobium strain TA1 (Group B) prior to sowing, except for Caucasian clover (strain CC283b), lucerne (RRI128), birdsfoot trefoil (SU343), subterranean clover (WSM1325) and serradella (WSM471). Cultivars Nomad and Tribute white clover, as well as SARDI Grazer lucerne, were sown as pre-coated seed without re-inoculation. Sowing rates were based on local recommendations where available (Lattimore & McCormick, 2012) and adjusted for seed quality to deliver 2 kg/ha of germinable seed for white clover; 4 kg/ha birdsfoot trefoil, strawberry clover (*T. fragiferum*) and Talish clover (*T. tumens*); 5 kg/ha red clover, 6 kg/ha Caucasian clover; 8 kg/ha lucerne; 10 kg/ha subterranean clover, sulfur clover (*T. ochroleucum*) and serradella, and 25 kg/ha sainfoin (*Onobrychis viciifolia*). The same seed source was used for each cultivar across all sites within a given sowing year. Poor seedling emergence at Tirrannaville, Mandurama and Dry Plain required these sites to be resown. Seedlings and weeds were removed by spraying glyphosate at 2.0 L/ha and the treatments were resown the following year, or in spring 2018 at the Tirrannaville site.

3.2.3 Measurements

Seedling density was determined within ~12 weeks of sowing by randomly placing a quadrat of known size in the plot and counting all seedlings within the quadrat. This was repeated 4 times per plot and the average of all counts converted to plants/m^2. Beyond the initial count, plant frequency was used as the measure of legume persistence by placing a quadrat at two fixed locations in each plot and counting the number of cells containing the base of a sown plant. The quadrat size was 1 m × 1 m with 0.1 m × 0.1 m cells (n = 100) for all sites except at the Tirrannaville and Bombala sites where quadrat sizes were 1.0 × 0.75 m, divided into 0.1 m × 0.15 m cells (n = 50). The initial measurement occurred in late spring in year 1 and repeated each year at each site, usually occurring in late autumn or winter when herbage was short and live shoots of each species easily distinguished.

Table 1. Baseline soil test values for pH (in $CaCl_2$), available phosphorus (Colwell P; mg/kg), phosphorus buffer index (PBI), effective cation exchange capacity (ECEC; cmol(+)/kg) and exchangeable aluminium (Al) concentration (%) in the 0-0.10 m depth at each of the experimental sites used to assess legume persistence in grasslands of the high-rainfall permanent pasture zone. Only the perennial legume evaluation experiments (Series 3 & 4) were conducted under the present project. (Source: Hayes et al. 2022).

Site (year of sowing)	pH_{Ca}	Colwell P (mg/kg)	PBI	ECEC (cmol(+)/kg)	Al saturation (%)	Comment
Series 1; annual legume evaluation						
Yass (2012)	5.4	4	52	4.8	2	Granite soil, poorly drained, level area
Burrinjuck (2013)	6.0	9	65	7.2	2	Granite soil, westerly aspect, poorly drained
Middle Arm (2013)	5.1	16	79	4.7	2	Shaley soil, very stony, easterly aspect
Series 2; cultivar evaluation, serradella and subterranean clover						
Bigga (2017)		22	30	ND	ND	Granite soil, level area on a mid-slope
Bombala (2017)	4.7	21	99	4.9	4	Red basalt soil, westerly aspect, stony
Middle Arm (2017)	4.6	17	74	4.7	2	Shaley soil, stony, south-easterly aspect
Yass (2018)	5.1	29	52	4.8	2	Granite soil, poorly drained, level area
Merrill (2018)	4.3	22	97	9.9	17	Westerly aspect, deep, v. acidic subsoil to >1m
Series 3; Perennial legume evaluation						
Glen Innes (2018)	5.5	40	165	9.3	< 1	Basalt soil, dark brown, level area
Orange (2018)	5.1	24	88	5.5	< 1	Lower slope, level area
Paling Yards (2018)	5.2	123	153	15.2	< 1	Red basalt, upper slope, many boulders
Bombala (2019)	4.5	24	62	5.7	5	Basalt soil, lower slope, deep, level area
Dry Plain (2019)	4.6	47	69	6.9	4	Dark brown soil, deep, basalt influence
Mandurama (2019)	4.8	12	59	6.8	2	Granite, mid-low slope, easterly aspect
Tirrannaville (2019)	5.8	38	50	7.5	0	Deep sedimentary soil, many quartz stones
Series 4; Perennial legume evaluation, low fertility soils						
Bombala (2018)	4.7	21	95	4.9	4	Red basalt, westerly aspect, upper slope
Middle Arm (2018)	4.6	17	74	4.7	2	Shaley soil, stony, south-easterly aspect
Merrill (2018)	4.1	22	97	4.2	35	Upper slope, highly acidic to depth
Wandsworth (2018)	4.4	27	93	4.2	11	Traprock soil, lower slope, level area
Guyra (2020)	4.6	25	66	2.2	8	Granite soil, mid-low slope, level area

ND, not determined.

Table 2. Perennial legume species and cultivars evaluated (●) on high fertility (Series 3) and low fertility soils (Series 4) at experiments sown at Glen Innes (GI), Orange (OE), Paling Yards (PY), Bombala (BA), Dry Plain (DP), Mandurama (MD), Tirrannaville (TA), Middle Arm (MA), Merrill (ML), Wandsworth (WH) and Guyra (GA) in 2018, 2019 or 2020 with subscripts of 18, 19 and 20 (Source: Hayes et al. 2022).

ID	Species	Common name	Cultivar	Series 3							Series 4				
				GI$_{18}$	OE$_{18}$	PY$_{18}$	BA$_{19}$	DP$_{19}$	MD$_{19}$	TA$_{19}$	BA$_{18}$	MA$_{18}$	ML$_{18}$	WH$_{18}$	GA$_{20}$
				Perennial legumes											
1	*Lotus corniculatus*	Birdsfoot trefoil	(LC07AUYF)	●	●	●		●	●		●	●	●	●	●
2	*Medicago sativa*	Lucerne	SARDI Grazer	●	●	●	●	●	●	●	●	●	●	●	●
3	*Medicago sativa*	Lucerne	Titan 9	●	●	●	●		●	●	●	●	●	●	●
4	*Onobrychis viciifolia*	Sainfoin	Othello						●						
5	*Trifolium ambiguum*	Caucasian clover	Kuratas	●	●	●	●	●	●	●	●	●	●	●	●
6	*T. fragiferum*	Strawberry clover	Palestine	●	●	●	●	●	●	●	●	●	●	●	●
7	*T. ochroleucum*	Sulfur clover	(Tas 0433)					●	●						
8	*T. pratense*	Red clover	Astred				●			●					
9	*T. pratense*	Red clover	Relish	●	●	●			●		●	●	●	●	●
10	*T. pratense*	Red clover	Rubitas	●	●	●	●	●	●	●			●	●	●
11	*T. repens*	White clover	Haifa	●	●	●	●	●	●	●	●	●	●	●	●
12	*T. repens*	White clover	Nomad	●	●	●	●		●	●	●	●	●	●	●
13	*T. repens*	White clover	Storm	●	●	●			●		●	●	●	●	
14	*T. repens*	White clover	Tribute	●	●	●		●	●		●	●	●	●	●
15	*T. repens*	White clover	Trophy	●	●	●	●	●	●	●	●	●	●	●	●
16	*T. tumens*	Talish clover	Permatas	●	●	●	●	●	●	●	●	●	●	●	●
17	*T. ambiguum* × *T. repens*	-	Aberlasting	●	●	●	●	●	●	●	●	●	●	●	●
				Annual legume controls											
18	*Ornithopus compressus*	Yellow serradella	Avila						●						●
19	*O. compressus*	Yellow serradella	Santorini												●
20	*O. compressus*	Yellow serradella	Yellotas						●						●
21	*O. sativus*	French serradella	Erica												●
22	*O. sativus*	French serradella	Margurita						●						●
23	*T. subterraneum*	Subterranean clover	Leura	●	●	●	●	●	●	●	●	●	●	●	●
24	*T. subterraneum*	Subterranean clover	SeatonPark												●

Estimates of total herbage and the percentage of sown species in each plot was assessed visually at each site in late spring in year 1 in five quadrats placed randomly per plot and calibrating visual assessments with 10 quadrat cuts. Beyond year 1, measures of aboveground biomass were generally taken at ~ 12-week intervals with sites either grazed by livestock following sampling or mown if livestock were not available. A detailed sampling of the Mandurama site in year 1 was used to determine relative differences in N_2 fixation between species and is detailed later in this report (see Section 3.6, Appendix 5).

3.2.4 Statistical analysis

The Series 3 and 4 experiments were analysed independently. Relationships between seedling density, plant frequency and pasture DM were examined using x-y plots (Sanford *et al.*, 2005) with predicted means derived from the analysis of multiple experiments using the method of residual maximum likelihood (REML) in Genstat 20th Edition (VSN international, Hemel Hampstead, UK). Entries ranked in the top 10 are shown on the x-y plots as being to the right of, or above the dotted lines. Therefore, the entries in the upper right quadrant of the graph were the best-performers for both attributes while those in the lower left quadrant were the worst for both attributes. An assessment of final frequency is presented for each site following an analysis of variance (ANOVA) using cultivar/entry as the random term and replicate as the fixed term in the model.

3.3 Pasture survey, Northern NSW

The pasture survey conducted in northern NSW is reported in detail in a manuscript titled ‘Sown pasture persistence during and post drought on the Northern Tablelands of NSW with an emphasis on white clover: A survey’, in preparation for submission to *Grass and Forage Science* (Harris et al. 2022; Appendix 3). Some data collected early in the survey were included as the basis of an Honours dissertation submitted to the University of New England by Mr Andrew Faithfull, titled ‘The key drivers of sown pasture persistence on the Northern Tablelands of NSW: a survey’.

3.3.1 Sites

Fifty-one paddocks across 11 beef and sheep grazing enterprises on the Northern Tablelands of NSW were surveyed over the autumn–winter of 2019. The properties were selected to represent regions along north—south and east—west bisects of the Northern Tablelands. The regions surveyed, from north to south, included Glen Innes (Reedy Creek) Ben Lomond, Guyra, Armidale, Uralla, Walcha, and from west to east, West Guyra, Guyra, Wongwibinda and Ebor (Fig. 1). The project concept was presented at three Local Land Services and Landcare events across the Northern Tablelands and interested producers were asked to present an expression of interest. Selected paddocks were required to have been sown to exotic grasses and white clover (but not precluding other legumes), have a history of fertiliser application and regularly grazed by sheep and/or cattle. The paddocks were also selected to represent the three broad soil types of the Northern Tablelands: basalt, granite and traprock.

For each property and paddock, data were collected from the property owner and/or manager (hereafter referred to as managers) and supplemented with other sources (such as weather data from the Bureau of Meteorology) where applicable. Data collected included; General (location, altitude, aspect, topography, soil type, paddock size), Climate (average annual rainfall, temperature maximum and minimum in January and June, approximate first and last frost dates), Pasture history (sowing date, white clover cultivars, companion species/cultivars & sowing rates, sowing technique), Fertiliser history (soil fertility data from historic soil analysis, fertiliser application including rate, type and timing), Grazing history (stock type, grazing system, stocking density) and Other (e.g. pests, diseases). At each property, 5-6 sown paddocks of varying ages were selected, and if this was not possible, survey areas were selected on the basis of varying aspect or topography within a large paddock.

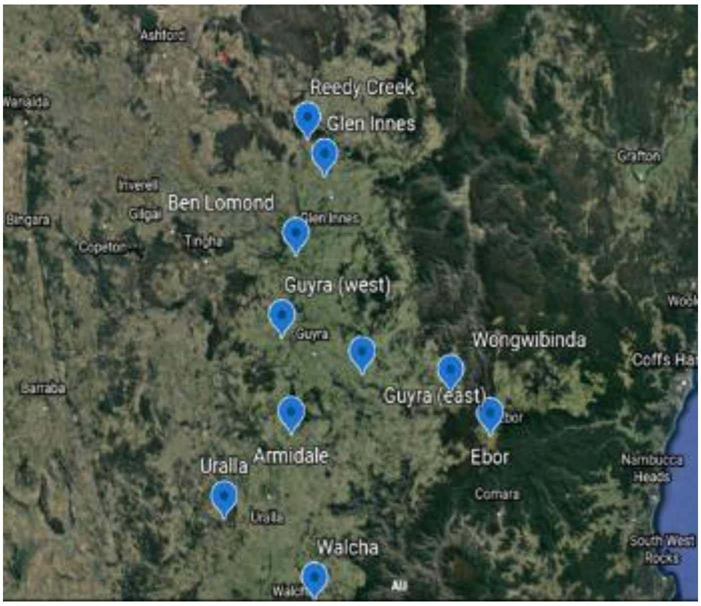

Fig. 1. Site locations of a survey of sown perennial pasture persistence on the Northern Tablelands, NSW, Australia in 2019 and 2020.

3.3.2 Sampling undertaken in 2019

Botanical composition was assessed using the dry-weight rank method (Tothill et al. 1992) along a 60 m transect. The transect area was established in a representative portion (based on aspect, topography and pasture) of the paddock avoiding trees, rocks, stock camps and tracks. The transect was GPS located and mapped using landmarks to assist relocation on subsequent visits. Thirty 0.1 m^2 quadrats were sampled on each transect, approximately 2 m apart, and assessed for the top 3 species present, based on weight of dry matter (DM). A visual estimation of yield, percentage green and groundcover were also assessed within the quadrat. Five to 10 calibration cuts were taken in most paddocks and sorted into green and dead (some paddocks were not calibrated due to lack of green presence and low DM). Calibrations were then dried for 48 hours in a dehydrator at 80^0C and weighed on digital scales. A regression equation was generated to calibrate the visual estimations of yield and % green.

Twenty 0–10cm soil cores were taken at each paddock adjacent to the transect and combined for analysis at a NATA accredited commercial laboratory. Samples were not collected at one site as recent (sampled in same year as survey) laboratory analyses were available for all paddocks. A comprehensive test was conducted on all soil collected and included Soil pH ($CaCl_2$), Colwell P, P buffering index (PBI), Bray P, KCl-40 sulfur (S), Colwell K, Organic carbon and cation exchange capacity (CEC).

Twenty random cores (75 mm in diameter x 50 mm deep) were collected at each paddock bordering the transect area (10 each side of the transect). White clover seed was recovered from the soil samples by means of washing soil through a 0.5-mm mesh with water. Once dry, the bulk of the remaining organic matter was further removed with dry sieving. White clover seed was physically separated from remaining trash and counted. The seeds were stored in paper seed envelopes at room temperature until germination tests were undertaken.

Seeds recovered from paddock were combined to provide a bulk seed sample for each property giving 10 samples. The bulk seed sample for one site was not inadequate (< 80 seeds) for testing compared with the other seed lots (approximately 250 seeds) and was not included. Each of the 10 bulk samples contained 250 seeds except for one sample which contained 200 seeds. The seeds were placed on moistened filter paper in Petri dishes and placed in a germination cabinet at 20^0C for 14 days. Germinated seeds were counted and removed daily over this time. At day 14 the remaining seeds were counted and classified as either soft but non-viable or unswollen (hard seeds). The hard seed samples were air dried, lightly scarified with a fine grade sandpaper. Numbers of seed were low for some sites, but all the hard seed recovered after the first germination test were subjected to further germination testing to confirm viability.

3.3.3 White clover seedling recruitment and frequency

In autumn of 2020 seedling recruitment of white clover was measured at five sites from the original survey in the Glen Innes region. In each paddock white clover seedlings were counted in six 0.5 m^2 quadrats. Three quadrats were located on each side of the sampling transects at 15, 30 and 45 m. Counts were conducted every 4 weeks from late February 2020 to early June 2020. Five seedlings per quadrat at each assessment were marked with coloured tags and monitored for survival. If less than 5 seedlings were observed, all seedlings were tagged. If a seedling died or when it developed 4–5 trifoliate leaves (recorded as established) the tag was removed. Survival/establishment was monitored until early autumn 2021. This process was repeated in autumn 2021 (March) to March 2022 except for one site which was resown by the farmer.

At the same five sites mature white clover frequency was recorded in autumn and spring each year for 2019, 2020, 2021 and autumn 2022. In each paddock plant frequency was recorded in four 1 m^2 quadrats (containing 100 by 0.1 m x 0.1 m cells) placed on both sides of the transect at 20 and 40 m.

3.3.4 DM sampling undertaken in 2021

In spring 2021 the sampling of botanical composition and DM was repeated on twenty of the original sites using the same procedure as described for 2019. Unfortunately, the remainder of the sites were not available for resampling due to sites been sown down to forage crop (12) or new pasture (4) or were inaccessible due to prolonged periods of wet weather.

3.3.5 Manager survey

All 11 managers participated in the in-person survey where they were asked to answer pre-set questions around perceptions of legume (white clover) productivity and persistence. The managers were asked to rate the importance of several influences on legume persistence using a scale of 1–5, where 1=least important and 5=most important. The influences included competition from grasses, diseases, insect pests, grazing management, rainfall (AAR), seasonal rainfall, soil fertility, soil pH, stocking rate, temperature, competition from weeds and other.

3.3.6 Statistical analysis

Descriptive statistics were used to describe the basic features of the survey data to better understand the data set. ‘Sown’ content was plotted against multiple explanatory variables, including parameters such as soil fertility, climate and grazing management to look for correlations.

Statistical analysis was then performed in RStudio using the 'party' package. A conditional inference tree (CIT) model was created with sown grass percentage as the dependent variable and independent variables: pasture age, annual average rainfall, altitude, topography, aspect, groundcover, soil type, pH, Colwell P, Bray P, indexed P fertility, PBI, S, K, organic carbon (OC), CEC, grazing management and stock type. Further CIT models were run to try and explain 'native grasses', 'grass weeds', 'broadleaf weeds' and 'other' presence in the system. A conditional inference tree model was chosen in preference to general linear modelling.

3.4 Glasshouse experiments

Three glasshouse experiments were conducted at the Wagga Wagga Agricultural Institute in the period 2019-2021. The experiments were conducted to explore the survival mechanisms of white clover under moisture stress. The first experiment also included lucerne for comparison while the final experiment included strawberry clover, as both species persisted well at some field sites and were observed to withstand dehydration better than white clover, particularly on higher fertility soils. The first experiment was published in 2021 as part of the previous AGA Symposium special issue in *Crop and Pasture Science* (Norton et al. 2021; Appendix 4).

3.4.1 Dehydration tolerance of white clover and lucerne

Dehydration tolerance and plant survival of white clover (cv. Grasslands Trophy) and lucerne (Stamina GT 6) were compared in a drying cycle experiment under glasshouse conditions. Each pot contained 6.15 kg of an air-dried Red Chromosol soil collected at the Wagga Wagga Agricultural Institute. Owing to the different growth habits and rooting depths of these two species, the lucerne was grown in pots of 0.1 m diameter and 0.6 m depth whereas the white clover pots were of 0.15 m diameter and 0.3 m depth.

Four treatments were arranged in a split-plot design with species (white clover, lucerne) as the main plots and water availability (fully irrigated, drying cycle) as the subplots, replicated four times. Harvest occurred on four occasions. For the two watering treatments, the two species were either: subjected to a drying cycle, which commenced on the same date; or maintained at 90% of field capacity by watering to weight three times per week for the duration of the experiment. Sixteen additional pots (rehydration pots) of each species subjected to the drying cycle were maintained, four until each harvest, at which time these pots were rehydrated for the purpose of determining the proportion of plants still alive. The experiment was blocked by species to ensure that the taller lucerne pots did not shade the white clover. Glasshouse location effects were minimised by relocating pots in replicates within the glasshouse every 2 weeks.

Pots were sown with germinated seeds on 29 May. White clover was inoculated with group B (TA1) and lucerne with group AL rhizobia. Emerged seedlings were thinned to three robust plants per pot on 17 June. In the period before application of treatment, all pots were watered up to a weight equivalent to 90% of field capacity three times per week and were grown under non-limiting nutrient availability. The drying cycle treatments were imposed on 8 October, after the roots of plants had reached the bottom of all pots.

Soil gravimetric water content (θg) was determined in the drying cycle treatments by weighing the pots. The lost mass from one weighing to the next was attributed to evapotranspiration. This allowed an ongoing assessment of soil water status and plant water use in the pots. All pots of each species in the drying cycle treatments were weighed three times weekly from 8 October until termination of the experiment.

Measurement of leaf elongation commenced on 9 October, the first day of the drying cycle. The rate of leaf elongation (or stolon elongation in the case of white clover) was measured in both the drying cycle and irrigated pots until elongation ceased in the drying cycle treatments. Measurement involved selecting and marking the highest order leaf or stolon and then measuring the distance between the axil from which it arose and the tip of the organ. This continued until cessation of elongation on that leaf/stolon, after which time measurement was moved to a higher order leaf/stolon, continuing in this manner until elongation ceased across all leaves/stolons in the pot. Measurement occurred three times per week on alternate days and coincided with measurements of pot weight.

The harvests of white clover occurred on 1, 4, 8 and 13 November, being Days 24, 27, 31 and 36 of the drying cycle. Lucerne harvests occurred on 4, 11, 18 and 22 November (i.e. Days 27, 34, 41 and 45 of the drying cycle). At harvest, total aboveground herbage of all four pots of each treatment was cut off at ground level. The herbage of each replicate was then divided into live and dead (senescent) portions. Roots were also harvested after the plant shoots were removed after soaking the pot overnight to facilitate separation of roots from the soil by gently washing over a fine mesh. All portions of shoots and roots were dried in a forced draught oven at 60°C and weighed.

3.4.2 Effect of soil fertility on dehydration tolerance of white clover

Two trials to assess the effects of soil nutrient constraints on dehydration tolerance and survival of white clover under extended drying cycle conditions in a glasshouse pot environment were conducted in 2020. Trial 1 examined the effect of potassium (K) and sulfur (S) deficiencies while trial 2 assessed the effect of addressing P and soil acidity constraints. The white clover cultivar Haifa was used in both trials. Soils with K and S deficiencies were collected from near Goulburn (from near the Middle Arm experiment described in Section 3.2) with deficiencies treated by application of KCl and gypsum. Soils with P deficiency and low pH were collected in the Gunning district (adjacent to the Merrill experiment described in Section 3.2) and treated with sufficient lime to bring the soil to pH_{CaCl2} 5.5 and triple superphosphate targeting a Colwell soil P value of 35 mg/kg.

In each trial, pots of 15 cm diameter and 30 cm height were filled with the same weight of soil and sown with seed inoculated with Group B rhizobium. Plastic beads were placed over the soil surface of all pots to minimise direct evaporation from the soil. All pots were watered to field capacity every week until roots of every treatment began to extrude from the bottom of the pots, indicating that roots were exploring the entire pot volume. Cessation of watering and the commencement of the drying cycle with associated measurements were commenced once all treatment plants had reached this stage. A group of 'well-watered control' pots was also maintained. Measurements taken were as described for the glasshouse experiment conducted the previous year (see previous Section). In addition, osmotic adjustment was estimated by measuring water potential (ψ), osmotic potential (π) and relative water content (ζ). Water potential and osmotic potentials of the same leaves were measured after vapour equilibration in chambers fitted with thermocouple psychrometers with the output being read by a Wescor HR33T dewpoint microvoltmeter (Boyer 1995). Osmotic adjustment was defined as the difference between the measured osmotic potential (π) and the π due to concentration of solutes by water loss, i.e. $\pi_a = \pi - \pi_t \zeta_t / \zeta$ where $_t$ indicates full turgor. Values of osmotic potential (π_t) and relative water content (ζ_t) at full turgor were estimated from the relationships between ψ and both π and ζ using least squares analysis obtained from consecutive measurements of these parameters during the drying cycle.

Harvests were undertaken at 5 time periods: H1- drying cycle commencement; H2- permanent wilting begins; H3- when 25% plants were estimated to be dead; H4- when 50% plants were estimated to be dead; H5- when 75% plants were estimated to be dead. Harvested plants were dried, divided into roots and shoots and weighed.

Herbage taken from the harvest 1 sampling was used for ^{15}N analysis. Following drying, herbage was passed through a coarse plant grinder, then finely ground using a ring and puck mill. Samples were analysed for N concentration and ^{15}N composition via automatic N and carbon analysis (ANCA-SL) interfaced to a 20–20 stable isotope mass spectrometer (Europa Scientific, Crewe, UK). The amount of shoot N accumulated by the different treatments was calculated as:

Shoot N (kgN/ha) = shoot DM (kg/h) x %Ndfa/100

where %Ndfa is the proportion of shoot N derived from atmosphere. %Ndfa was calculated by using the natural ^{15}N abundance method with estimates (B-values) of glasshouse-derived determinations of the ^{15}N composition of the legumes grown with N_2 as the sole source of N (Unkovich et al. 2008):

%Ndfa = 100 x ($\delta^{15}N$ cocksfoot - $\delta^{15}N$ white clover)/($\delta^{15}N$ cocksfoot – B-value)

The cocksfoot used to determine nitrogen fixation was grown in 16 separate pots prepared at the same time as the remaining experiments, two of each soil treatment, and grown in the same glasshouse on a separate Table beside the main experiment. The B-value for white clover was assumed to be -1.48 (Unkovich et al. 2008).

3.4.3 Drought response of strawberry clover and white clover

A key objective of the project is to improve persistence of perennial forage legumes growing in the high rainfall zone where soil and climatic constraints including acidity, low P and periodic dry spells are common. Observations in this project during the intense, prolonged drought of 2019 indicated that strawberry clover survived better than white clover in some field experiments. It was therefore, considered worthwhile to examine, in the glasshouse environment, which traits may have contributed to this superior drought survival of strawberry clover.

The white clover cv. Haifa and the strawberry clover cv. Palestine were used in the trial. The soil selected was collected in the Gunning district, the same source that was used in the 2020 Glasshouse research described in the previous Section. This 'Control' soil had a pH_{CaCl2} of 3.78, a Colwell P of 15 mg/kg and exchangeable Al of 56%. Lime was mixed thoroughly throughout the soil of lime treatment pots and added at rates equivalent to 1.5, 3 and 6 t/ha.

The trial was a randomised complete block design with 2 species and 4 levels of lime which were either subjected to a drying cycle or maintained in a well-watered state approximating 90% of field capacity. Each treatment had 4 replications. Pots of 15 cm diameter and 30 cm height were filled with the same weight of soil and sown with seed inoculated with Group B rhizobium. All pots were watered to field capacity every week until roots of every treatment began to extrude from the bottom of the pots, indicating that roots were exploring the entire pot volume. Cessation of watering and the commencement of the drying cycle with associated measurements were commenced once all treatment plants had reached this stage. Harvests were undertaken at 4 time periods: H1- drying cycle commencement; H2- permanent wilting begins; H3- 25% plants estimated to be dead; H4- 50% plants estimated to be dead. Harvested plants were dried, divided into roots and shoots and weighed. Measurements undertaken included soil water content, stolon elongation rate, dry matter production, plant water status and osmotic adjustment as described for the previous glasshouse experiment.

3.5 Soil nutrition experiments

A series of field experiments was established to examine the response of white clover to elevated soil pH as well as P, K and S fertility when grown in mixtures with the perennial grass, phalaris. Seven field sites were sown, co-located with perennial legume persistence experiments (see Section 3.2) at Dry Plain (sown 26 Mar 19), Merrill (9 Apr 19), Paling Yards (8 Apr 19), Orange (17 Sep 18), Mandurama (13 Sep 18), Glen Innes (30 May 19) and Guyra (13 May 20). Soil treatments were applied prior to sowing by applying 5 t/ha lime to ameliorate soil acidity, gypsum as a source of sulfur fertiliser, at a rate equivalent to 50 kg S/ha, triple superphosphate as a source of P fertiliser, at a rate equivalent to 50 kg P/ha and muriate of potash as a source of K fertiliser, at a rate equivalent to 75 kg K/ha. Plots were sown to a mixture of phalaris (cv. Landmaster) at 4 kg/ha and white clover cv. Tribute also at 4 kg/ha. The white clover seed was not pre-coated and was inoculated with Group B inoculum prior to sowing. A small number of plots at each site were sown just to phalaris or white clover (Table 4). Plots were 4.0 x 7.5 m, except at Paling Yards and Dry Plain where they were 2.0 x 7.5 m, and all treatments were replicated three times at each site.

Sites were not grazed in the year of sowing. From the end of the first summer, assessments of biomass and botanical composition were taken using calibrated visual assessment at 10 random locations along a transect of each plot, at approximately 3-monthly intervals. The site was mown immediately following herbage assessment and material removed, except at Paling Yards which was grazed by sheep following most biomass samplings. The site at Dry Plain was occasionally grazed by cattle. Livestock were completely excluded at the other sites for the duration of the experimental period, minimising the amount of nutrient transfer between plots.

Grab samples were taken in spring at each site for herbage quality and mineral composition analysis. Separate samples were taken for phalaris and for white clover by removing herbage from 15-20 plants per plot, taking care to avoid contamination from other species in the sample. Where there was insufficient white clover available for sampling, a background legume that was present in all plots was sampled instead. Subterranean clover was sampled from Gunning and Mandurama in 2020, black spotted medic was sampled from Paling Yards in 2020 and knotted clover was sampled from Dry Plain, also in 2020. Samples were dried at 60 °C for 48 hours and ground to pass through a 1 mm sieve. Subsamples were sent to two different laboratories for analysis; either to the Nutrient Advantage Laboratory Services, Werribee, Victoria to determine N, P, S, Cu, Zn, Mn, K, Na, Mg, Ca, B, Fe, Cl and nitrate contents, through nitric acid digestion, or to the NSW DPI Industries Feed Chemistry Laboratory, Wagga Wagga, NSW (ISO 17025 (NATA)) to determine feed quality including acid and neutral detergent fiber (ADF and NDF), crude protein (CP), dry matter digestibility (DMD), ash content, metabolisable energy (ME) and water soluble carbohydrate (WSC) via near infrared (NIR) spectroscopy. The latter analysis was conducted in conjunction with the LPP NIR project, P.PSH.1202 ‘New generation NIRS calibrations to improve diet evaluation and animal growth predictions’. Subsamples of the legumes from each site were sent to a third laboratory, at CSIRO Black Mountain (ACT), for determination of N_2 fixation using the natural abundance technique as described in Section 3.4.2.

Table 4. Soil and pasture treatments included (●) at Dry Plain (DP), Merrill (ML), Guyra (GA), Mandurama (MA), Paling Yards (PY), Orange (OE) and Glen Innes (GI).

Treatment abbreviation	Species sown		Soil treatment				Site						
	Phalaris	White clover	Lime	P-fertiliser	K-fertiliser	S-fertiliser	DP	ML	GA	MA	PY	OE	GI
WC_L	Nil	Yes	Yes	Nil	Nil	Nil	●	●	●	●	-	●	●
WC_L_P_K_S	Nil	Yes	Yes	Yes	Yes	Yes	●	●	●	●	●	●	●
WC_U	Nil	Yes	Nil	Nil	Nil	Nil	●	-	-	-	-	-	-
WC_U_P_K_S	Nil	Yes	Yes	Yes	Yes	Yes	●	●	●	●	●	●	●
Ph_L	Yes	Nil	Yes	Nil	Nil	Nil	●	●	●	●	-	●	●
Ph_L_P_K_S	Yes	Nil	Yes	Yes	Yes	Yes	●	●	●	●	●	●	●
Ph_U	Yes	Nil	Nil	Nil	Nil	Nil	●	-	-	-	-	-	-
Ph_U_P_K_S	Yes	Nil	Nil	Yes	Yes	Yes	●	●	●	●	●	●	●
Ph_WC_L_K	Yes	Yes	Yes	Nil	Yes	Nil	●	●	●	●	●	●	●
Ph_WC_L_K_S	Yes	Yes	Yes	Nil	Yes	Yes	●	●	●	●	●	●	●
Ph_WC_L	Yes	Yes	Yes	Nil	Nil	Nil	●	●	●	●	●	●	●
Ph_WC_L_S	Yes	Yes	Yes	Nil	Nil	Yes	●	●	●	●	●	●	●
Ph_WC_L_P_K	Yes	Yes	Yes	Yes	Yes	Nil	●	●	●	●	●	●	●
Ph_WC_L_P_K_S	Yes	Yes	Yes	Yes	Yes	Yes	●	●	●	●	●	●	●
Ph_WC_L_P	Yes	Yes	Yes	Yes	Nil	Nil	●	●	●	●	●	●	●
Ph_WC_L_P_S	Yes	Yes	Yes	Yes	Nil	Yes	●	●	●	●	●	●	●
Ph_WC_U_K	Yes	Yes	Nil	Nil	Yes	Nil	●	●	●	●	●	●	●
Ph_WC_U_K_S	Yes	Yes	Nil	Nil	Yes	Yes	●	●	●	●	●	●	●
Ph_WC_U	Yes	Yes	Nil	Nil	Nil	Nil	●	●	●	●	●	●	●
Ph_WC_U_S	Yes	Yes	Nil	Nil	Nil	Yes	●	●	●	●	●	●	●
Ph_WC_U_P_K	Yes	Yes	Nil	Yes	Yes	Nil	●	●	●	●	●	●	●
Ph_WC_U_P_K_S	Yes	Yes	Nil	Yes	Yes	Yes	●	●	●	●	●	●	●
Ph_WC_U_P	Yes	Yes	Nil	Yes	Nil	Nil	●	●	●	●	●	●	●
Ph_WC_U_P_S	Yes	Yes	Nil	Yes	Nil	Yes	●	●	●	●	●	●	●

Mineral indices were determined, according to the following calculations. The K:(Na+Mg) ratio was calculated from the percentage of mineral in the dry matter, using the formula (K/0.039)/[(Na/0.023)+(Mg/0.012)]. The tetany index was calculated as (K/0.039)/[(Mg/0.012)+(Ca/0.02)] and the dietary cation anion difference (DCAD; meq/100 g) was calculated using the formula (Na/0.023 + K/0.039)-(Cl/0.0355 +S/0.016). The Ca:P and the K:Na ratios were calculated from the percentage of each mineral in the forage.

At the time of writing, analysis of only one sampling date at three sites was completed for the feed quality analysis, with other samples still with the laboratory. Moreover, visual observations of some field sites as late as September 2022 indicated that visual differences in 'greenness' were starting to become apparent (Plate 1). Researchers believe that these visual differences will correspond to even greater differences in forage quality than are reported for the earlier samplings and have taken samples, despite the fact that field trials should now be concluded. These are important observations that are worth following, meaning that the analysis of the effects of soil treatment on forage quality remain ongoing. The observations also highlight the fact that it takes time for some of these differences to manifest in pastures.

Plate 1. Photograph taken at the Merrill nutrition experiment on 2 Sep 2022, showing obvious visual differences between a nil lime/nil fertiliser plot (⟶) compared to neighbouring treatments. Visual differences have been less obvious in the earlier years of these experiments, prompting researchers to continue monitoring experiments in the short term.

3.6 Nitrogen fixation in perennial legumes

Emphasis in this project was placed on assessing nitrogen fixation of legumes because the value to agriculture is maximised when legumes are functional.

3.6.1 Cross host compatibility

Cross-host compatibility relates to the specificity of legume species to form functional symbiosis with multiple strains of rhizobia. It is considered important in legume species that are commonly grown in soil environments that contain large background populations of rhizobia. In south-eastern

Australia, it is common for background rhizobial populations to resemble the strain commonly used on subterranean clover, sold commercially as Group C inoculum or strain WSM1325. This study examined the cross-host compatibility of a range of perennial legumes on offer to farmers in south-eastern Australia. Rigg et al. (2021) described this study in detail in the most recent Symposium of the Australian Grasslands Association, which was subsequently published in Crop and Pasture Science (Appendix 5). The study included a controlled environment experiment as well as a detailed sampling of a field experiment. Both are briefly described below.

The controlled environment experiment included eighteen cultivars of nine legume species, inoculated with five commercial strains of rhizobia including WSM1325 (annual clovers), TA1 (perennial clovers), RRI128 (lucerne), SU343 (*lotus* species) and CC283b (Caucasian clover) following sterilisation of seed. Seeds were then transferred to a sterile 0.8% water agar plate for germination. Once the radicle was fully emerged, the seedlings were aseptically transferred to growth assemblies and inoculated. Plant growth assemblies consisted of glass test tubes containing Jensen’s N-free agar slants adjusted to pH 6.8. Seven treatment groups were assessed for each cultivar: five inoculant treatments, inoculated with 1 mL of the respective rhizobial strain and two control treatments, uninoculated negative (nil N) and positive (+ N as 0.75 g KNO3/L, equivalent to 105 mg N/L). Each treatment was replicated five times. Inoculant effectiveness was measured by comparing the percentage increase in shoot DM of the inoculated plant relative to the negative controls. The number of nodules per plant was also recorded.

Nodule occupancy was assessed in in July 2019, 4 months after sowing at the perennial legume evaluation experiment sown at Mandurama, described in Section 3.2.1. The legumes sown in this experiment are listed in Table 2. For assessment of nodule occupancy, one replicate of the trial was sampled to maximise the number of plants per plot evaluated and reduce error associated with spatial heterogeneity. Ten plants, including their intact root systems to a depth of 60–100 mm, were carefully exhumed from each plot (five samples, 1 m from each end of a plot, with plants taken from every second drill row), giving a total of 200 plants (i.e. 10 plants of each of 20 legume cultivars). Additionally, eight composite soil samples were collected to a depth of 100 mm directly adjacent to the trial (two composites per side) to assess background rhizobial strains present at the site. Upon return to the laboratory, a sample of rhizosphere soil (soil adhering to the root system) was collected, dried at 40°C, and used to determine pH using a 1:5 soil:0.01 M $CaCl_2$ solution. The roots of each plant were gently washed until all soil was removed. Nodule number, location on the roots (e.g. crown or lateral roots), colour and morphology were recorded to determine nodule score. Four nodules were collected from each plant for microbiological culturing, primarily from the crown of the root system. Where there were no nodules at the crown, nodules were sampled from the lateral roots. Each nodule was surface sterilised to minimise presence of contaminating microorganisms on the surface of the nodule. From 736 nodules plated, 1840 subcultures were recovered. Subcultures that were considered by morphological characterisation as likely to be rhizobia were analysed using matrix-assisted laser desorption/ ionisation–time of flight (MALDI-TOF) mass spectrometry (MS) technology. The commercial strains used to inoculate the field trial (CC283b, SU343, TA1, RRI128, WSM1325, WSM471) were used as controls or ‘type’ cultures to ensure that the MALDI-TOF MS method could positively identify and distinguish between strains.

3.6.2 Sainfoin rhizobia

The perennial legume, sainfoin (*Onobrychis viciifolia*), was identified during the project as a species of interest by the Southern Advisory Committee, as it was one of the very few non-bloating perennial legumes potentially available to Australian livestock producers. Consequently, it was included in a small number of experiments that were sown later in the project. However, seed of this species was difficult to acquire as it has not been used commercially in Australia for many years, and it was discovered that rhizobial inoculant was no longer commercially available. (This explains why sainfoin was the only legume in the Mandurama field experiment that was not inoculated prior to sowing, see previous Section). Staff at the Australian Inoculants Research Group searched the historic collection to re-establish a commercial source of sainfoin inoculum. In doing so they uncovered ambiguity as to the true identity of the inoculum, with several different versions of the same numbered strain existing. A study was initiated to clarify the taxonomy of the commercial strain used for this species.

Previously unpublished data was sourced from earlier work at the South Australian Research and Development Institute (SARDI). In 2001 the Cooperative Research Centre for Plant-Based Management of Dryland Salinity (CRC Salinity), sponsored a major initiative to identify alternative perennial pasture plants for use in a range of agricultural environments (Dear and Ewing 2008). Several networks of perennial legume experiments were established which tested many niche or previously untested species and lines against more familiar cultivars. Seed was distributed by the Genetic Resource Centres based either in Adelaide (SA) or Perth (WA) and was accompanied by an appropriate rhizobial inoculant supplied by one of several collaborating agencies. As many of the legume species were previously untested, little was known of their compatibility with rhizobia. In the lead-up to field experiments being established, a large effort was dedicated to screening species to identify appropriate rhizobial strains that could be distributed with the seed. Due to time constraints and the large number of lines to be tested, the screening undertaken was often not comprehensive. Sainfoin was included in many of those experiments and the rhizobia, strain CC1099G, was supplied by SARDI following the initial screening described below (R. A. Ballard pers. comms).

The nitrogen fixation capacity of eight strains of rhizobia (CC401, CC1046, CC1061, CC1099G, CC1107, CC1108, CC1109, CB2000) was determined in combination with five species of *Onobrychis*, including the cultivar *O. viciifolia* cv. Othello, *O. armena*, *O. arenaria*, *O. gracillis*, *O. tenaitica,* replicated five times. Seed was surface sterilised and stored at 4 °C on water agar plates for 2 days and then placed in an incubator set at 25 °C for 16 hours. Germinated seedlings were sown into a mix of 50:50 coarse sand and vermiculite, in 130 mm diameter pots, which had been pasteurised by autoclaving. Each pot was planted with a pre-germinated seedling of each *Onobrychis* line and then watered with 300 mL of a nitrogen-free nutrient solution. After sowing, the surface of the potting mix in each pot was covered with polypropylene beads to reduce evaporation and the potential for transfer of rhizobia between pots. Five days after sowing, each pot was subjected to one of 10 treatments. The seedlings were either (i) inoculated with one of the eight rhizobia strains (1 mL of culture that had been washed off a yeast mannitol agar and suspended in 145 mmol/L NaCl applied to the base of each seedling), (ii) not inoculated or (iii) not inoculated but had 50 ml of 15 mmol/L NH_4NO_3/pot added at 7, 14, 21, 28 and 35 d after sowing. Plants were grown in a temperature-controlled greenhouse (May/June, approximate 22/15°C mean day/night temperature). Shoots were harvested 43 days after inoculation and dried at 60°C for 72 h and shoot dry weight used as a measure of nitrogen fixation. Sand and vermiculite was carefully washed from the roots and nodulation on each plant given a score based on nodule number, position on the root system and nodule appearance.

A further analysis of candidate strains was undertaken by the AIRG. The database of the AIRCS (precursor to AIRG) indicated several accessions of the CC1099 strain. Two of these, CC1099 (NA806-1) and CC1099 (NA808), were able to be revived. Freeze-dried cultures were aseptically revived from glass ampoules by resuspending in RO water, transferring to YMA media and incubating at 26˚C until colonies were observed. It was immediately apparent that there was a stark difference in the two cultures. To confirm the identities of these strains, a 16S rRNA PCR was performed and sanger sequencing was carried out on the products (Ramaciotti Centre for Genomics, UNSW). The 16S rRNA sequence results were analysed using Geneious Prime software. The Basic Local Alignment Search Tool (BLAST, NCBI) was employed to find the most similar organisms. As historical data for the taxonomy or the colony morphology was scarce, it was not possible to determine which would be the effective strain without plant tests. Sainfoin seed was obtained from Matthew Newell (NSW DPI) and was grown in the controlled environment facilities of the AIRG laboratory at the Elizabeth Macarthur Agricultural Institute (Menangle).

Liquid cultures of each strain were grown in yeast mannitol broth (YMB) at 28˚C with shaking until turbid. CC1099 (NA808), being a slow-growing culture, was incubated for 8 days while CC1099 (NA806-1) was incubated for 4 days. Seeds were surface sterilised and soaked in RO water overnight to imbibe, then plated onto sterile water-agar media and incubated at 26˚C to germinate overnight. Germinated seeds were planted into fine vermiculite with liquid Jensen's Nitrate-Free Medium, and the surface of the vermiculite was covered with a layer of alkathene beads (source). Treatments included uninoculated (negative N control), uninoculated control with added nitrate (positive N control), inoculated with CC1099 (NA806-1) and inoculated with CC1099 (NA808). Inoculated treatments received a 1ml inoculum of liquid culture. Plants were maintained in a controlled environment at 24˚C, with a 12h day-night cycle and watered as necessary.

At the time of writing, two small experiments were in progress to validate the effectiveness of the commercial CC1099 in the field. The experiments were located at the NSW DPI Research Stations at Cowra and Wagga Wagga and both included the same four treatments using cv. Othello; nil lime/uninoculated, 5 t/ha lime/uninoculated, nil lime/inoculated with CC1099 and 5 t/ha lime/inoculated with CC1099. Individual plants were sown in pre-cut holes on weed mat spaced 10 cm apart, with 30 spaced plants per plot. Experiments were replicated 4 times. Sampling was scheduled to occur at 10, 16 and 22 weeks post sowing to using destructive sampling to quantify nodules and relative herbage mass. The perennial grass, phalaris, was sown at the end of each plot to enable determination of nitrogen fixation using the natural abundance technique.

3.7 Bioeconomic modelling

Field research indicated that white clover was the perennial legume species offering the greatest potential to improve productivity across the high-rainfall, permanent pasture zone and that P-fertiliser and lime gave the largest improvements in pasture productivity. The aim of this component of work was to define the impacts on whole farm profitability of addressing soil pH deficits with lime and adding phosphorus fertiliser to improve white clover persistence and pasture production. This component of work was subcontracted to Harper-Adams University in the UK and conducted by Dr Karl Behrendt. A detailed report of the economic modelling component is appended to this report (Appendix 7) with key details summarised below.

To define the impacts on whole farm profitability the Sustainable Grasslands Model (Behrendt *et al.* 2020a; Behrendt *et al.* 2020b) was adapted to model the persistence and productivity of white clover in mixed swards at four case study sites in NSW, Australia. The case study sites correspond to some of the key trial sites described elsewhere in this report, located in the Northern Tablelands (Guyra), Central Tablelands (Orange), Southern Tablelands (Gunning; Merrill) and Monaro (Cooma; Dry Plain) regions. The sites represent different agro-ecological zones across a north-south transect, to capture low-high rainfall, seasonal distribution of rainfall and species adaptation. The SGM framework considers the impact of embedded climate and price risk, technology application and management on the state of the soil and grassland resources (including botanical composition) over time, which, in turn, impacts on whole farm economic and the environmental outcomes from different strategies. The SGM operates as a simulation model that is executed for each nominated grazing area (paddock level) on a daily time step and contains biophysical sub-models accounting for soil water, erosion, biomass production, dry matter digestibility, grazing preference, livestock production, herd/flock structure, supplementary feeding and livestock greenhouse gas emissions. The model utilises the biophysical outputs to calculate enterprise income, cash costs and gross margins. The cumulative net flows of cash from enterprises and enterprise assets then interact with whole farm fixed and financial costs, and assets and liabilities, to determine a range of financial key performance indicators that measure profitability, efficiency and viability. Daily cash inflows and outflows are then extracted (including the value of initial capital invested by farmers and its salvage value) to undertake the economic analysis of the farming systems through commonly used investment analysis techniques, such as Net Present Value (NPV).

The modelling focuses on deriving whole farm economic benefits and costs. This is reported through the average Gross Margin (GM) per hectare for the farm, the whole farm annual cash flow, and the NPV of the whole farm investment. A typical 500ha farm with twenty 25ha paddocks is assumed with animals rotationally grazed using biomass-based decision rules. Paddocks are assumed to be homogenous in terms of soil fertility and botanical composition at the start of the simulation period, with input treatments applied to each paddock as and when triggered by decision thresholds, including the establishment/re-sowing of pastures.

A single livestock enterprise type, self-replacing merino flock, is used across all case study sites to minimise the noise that livestock enterprise type may introduce into the analysis. This approach allows the livestock performance (wool and meat production, gross margin) to reflect pasture seasonality (including risk & resilience of growth/quality) based on the imposed soil/legume management treatment, without requiring modelled ‘treatment paddocks’ to be constantly grazed or livestock moved into a run-off zone/drought-lot that doesn’t reflect the treatment.

Capital applications of single superphosphate (SSP) and lime are the amounts required in one year to lift initial Colwell P and soil pH_{Ca} levels up to the target values. For phosphorus, maintenance applications are then determined using the actual change in soil P from the preceding year (which is driven by production levels from each paddock) to ensure soil P levels remain within an acceptable range. Maintenance applications of lime are triggered by a low soil pH_{Ca} threshold and aim to lift the soil pH_{Ca} back up to the initial target pH_{Ca}. It is assumed that any application of lime or phosphorus occurs at the start of a year, and acidification rate and phosphorus utilisation occur during the year to influence the year ending soil pH_{Ca} and soil phosphorus levels, prior to the subsequent input decision.

The modelling has used near past climate data for all simulations (1991 to 2021) and was calibrated using climate data from the case study sites in 2019-2021. The SGM uses Monte Carlo simulation procedures to randomly draw upon uniformly distributed annual sequences of daily climate data (1991-2021) over a 10-year simulation period. Using 250 iterations for each system ensured the model achieved a 95% confidence interval with an error of no more than ±2.5% of the estimated mean NPV.

4 Results

4.1 Review of literature

Key findings of the Review were presented at the Australian Grasslands Association (AGA) Symposium in Launceston, 2019 and subsequently submitted to *Crop and Pasture Science* for inclusion in the AGA special issue. Following peer review, the manuscript was accepted for publication (Hayes et al. 2019; Appendix 1).

The range of viable perennial species available to farmers on many soils remains limited to only one perennial forage legume species, white clover. Despite recent advances in white clover cultivars for increased persistence in dryland environments, the species remains sensitive to drought with its inherently shallow root system and limited capacity to restrict water loss from herbage. With few alternative species likely to become widely available in the foreseeable future, prospects for extending the boundaries of perennial legume adaptation likely rely on a dual approach of improving soil fertility and further genetic improvement in white clover. An opportunity to improve white clover cultivars by focusing on traits to promote more reliable seedling recruitment is identified. Most previous efforts to improve the drought tolerance of this species have instead focused on improved longevity of mature plants.

Addressing soil constraints alleviates periodic moisture stress in legumes by three key mechanisms: (1) increased water availability through improved infiltration and soil hydraulic properties; (2) better maintenance of plant cell structures to foster improved osmotic regulation; and (3) increased root growth to maximise exploration of the soil volume. Inherent in the last dot point includes greater evaporative demand in larger plants growing in high fertility soil, and reduced incidence of stresses, such as mineral toxicities associated with acidic soils, that would inhibit root development. The priority foci for improving soil fertility include overcoming soil acidity and addressing nutrient deficiencies, particularly of phosphorus, potassium, boron and molybdenum, each of which tend to be more widespread in the target region and can be directly linked to plant-water relations.

4.2 Perennial legume evaluation

4.2.1 High fertility soils (Series 3)

Seedling density at establishment was generally lower at Dry Plain (9-77 plants/m^2) and Paling Yards (33-60 plants/m^2) compared to the other sites, but at most sites seedling density was generally considered acceptable and consistent between entries (Fig. 2). Lower seedling densities were noted in birdsfoot trefoil, Haifa white clover and strawberry clover (9, 19 and 20 plants/m^2, respectively) at Dry Plain, strawberry clover at Tirrannaville (18 plants/m^2), sainfoin at Mandurama (25 plants/m^2) and Nomad and Storm white clover at Glen Innes (17 and 22 plants/m^2, respectively), compared to other values that were generally in the range 30-170 plants/m^2. There was no significant correlation (P>0.05) between seedling density and either herbage production or basal frequency (Fig. 2).

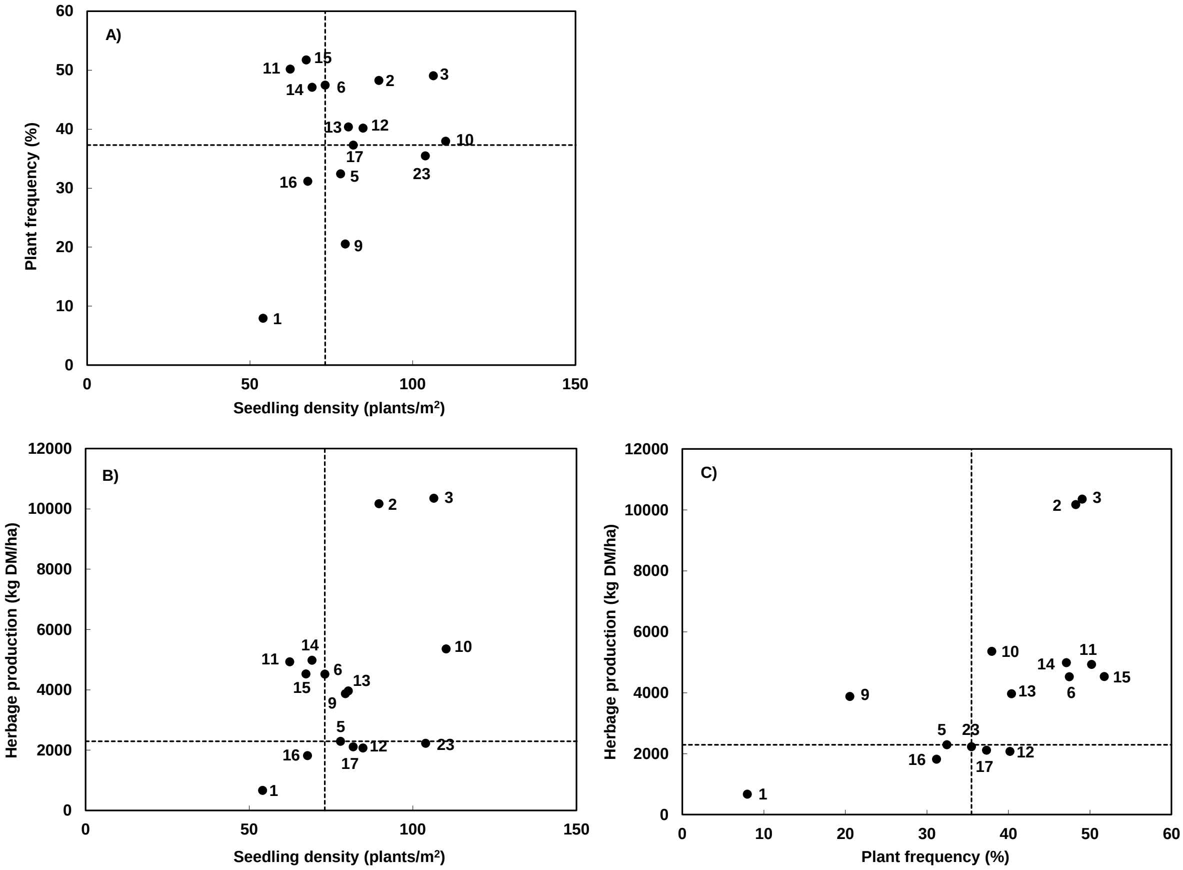

Fig. 2. Scatter plot relationships between a) seedling density and plant frequency (R^2 = 0.058, *P* > 0.05 if a linear relationship is assumed); b) seedling density and herbage yield (R^2 = 0.203, *P* > 0.05); and c) plant frequency and herbage yield (R^2 = 0.378, *P* <0.05) over the life of the Series 3 experiments. Entry ID number for each point corresponds to the species/cultivar ID numbers given in Table 2 (Section 3.2.2). Dotted lines indicate the top 10 entries to the right of the vertical line or above the horizontal line (Source: Hayes et al. 2022).

There was a significant (P<0.01) positive correlation between herbage DM and plant frequency (Fig. 2). The two lucerne cultivars, SARDI Grazer and Titan 9 were substantially more productive than all other species tested, producing roughly double the cumulative biomass of the next most productive species (Fig. 2 B & C). Rubitas red clover, along with strawberry clover and most of the white clover cultivars, were comparable in terms of their DM production and persistence (frequency) throughout the experimental period. Across the white clovers tested, Nomad was the least productive and Haifa the most productive, although very similar to Trophy and Tribute. The alternative perennial legume species, such as birdsfoot trefoil, Talish clover and Caucasian clover, were generally the least productive of the species tested.

At the final assessment of frequency at each site, white clover cultivars Haifa and Trophy were the most consistent entries, both averaging 77% frequency across all sites. Tribute (71%) white clover and Palestine strawberry clover (62%) were also consistent. The lucerne cultivars had an average final frequency of 47% (Fig. 3). Frequency of red clover, Talish clover and Caucasian clover were inconsistent with values comparable to Haifa white clover at some sites, such as Tirrannaville, but had much lower values (<40%) at other sites such as at Orange and Dry Plain.

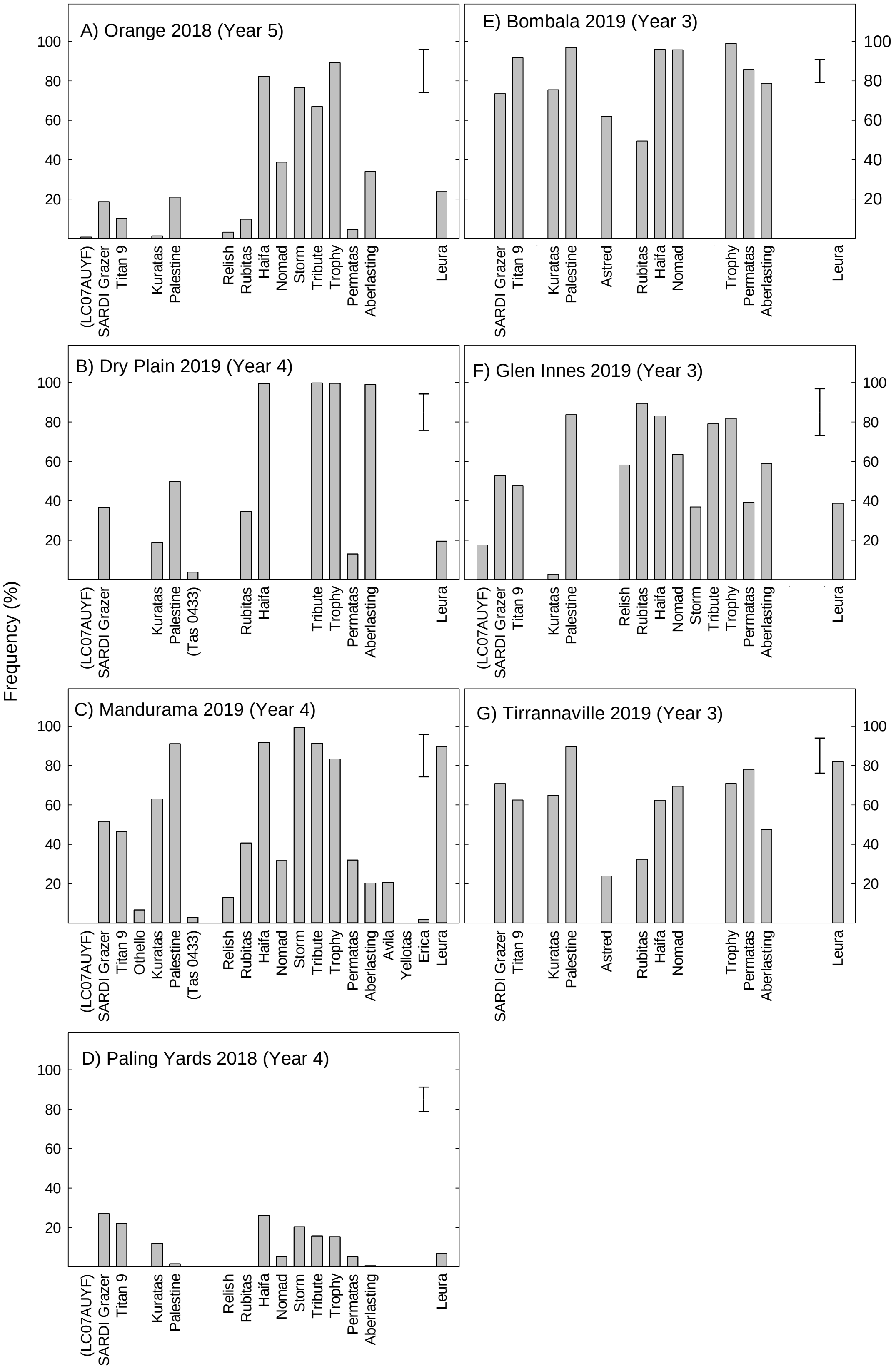

Fig. 3. Final assessment of legume frequency (%) at Series 3 experiments. Error bars indicate l.s.d. at *P*=0.05. (Source: Hayes et al. 2022).

4.2.2 Low fertility soils (Series 4)

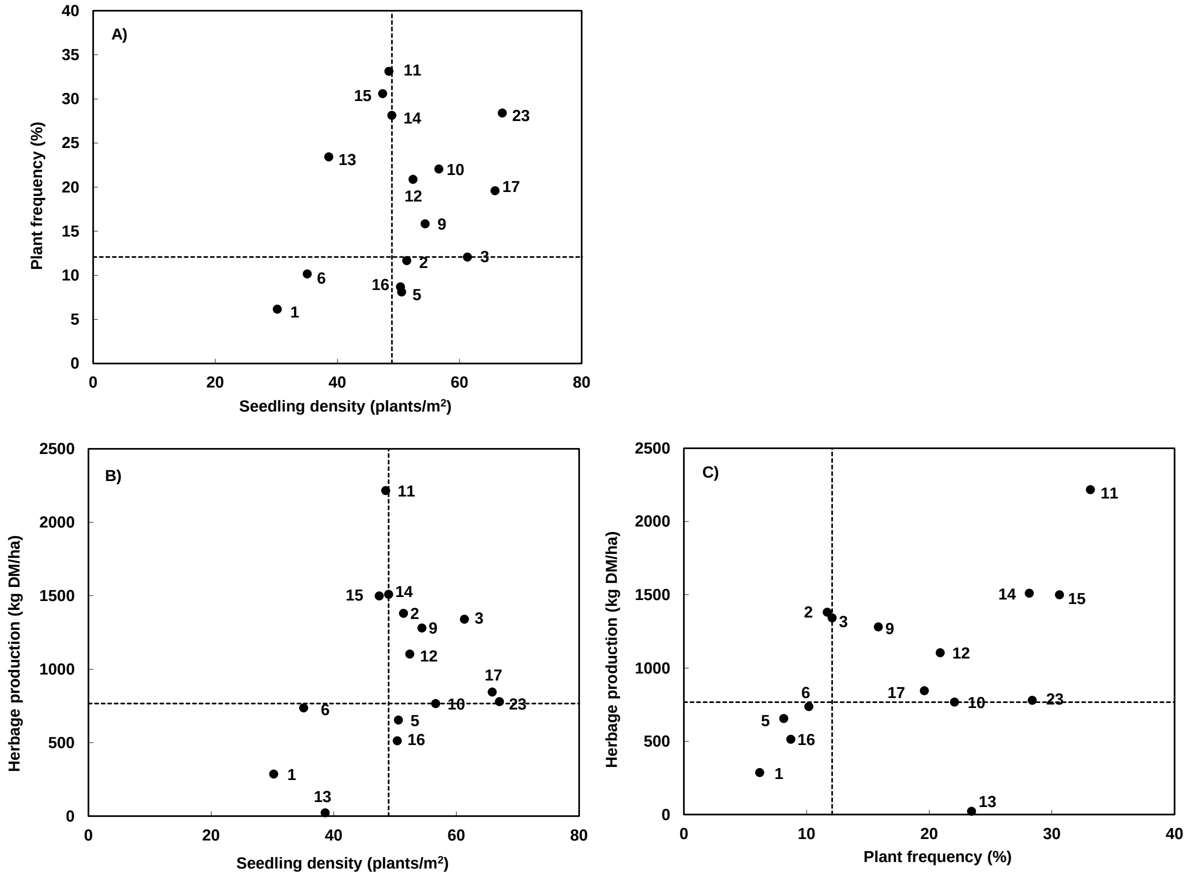

Fig. 4. Scatter plot relationships between a) seedling density and plant frequency (R^2 = 0.078, $P > 0.05$ if a linear relationship is assumed); b) seedling density and herbage yield (R^2 = 0.084, $P > 0.05$); and c) plant frequency and herbage yield (R^2 = 0.249, P = 0.058) over the life of the Series 4 experiments. Entry ID number for each point corresponds to the species/cultivar ID numbers given in Table 2 (Section 3.2.2). Dotted lines indicate the top 10 entries to the right of the vertical line or above the horizontal line (Source: Hayes et al. 2022).

Seedling density at establishment was consistent across species and sites with average density ranging from 48-72 plants/m^2, except at Bombala where the average seedling density was just 25 plants/m^2 (range 11-41 plants/m^2). However, despite the lower average density at that site only five entries had initial densities < 20 plants/m^2 including Haifa white clover (11 plants/m^2), Talish clover (12), Caucasian clover (13), birdsfoot trefoil (17) and Tribute white clover (19). At other sites, only Rubitas red clover (16 plants/m^2) and Erica French serradella (17) at the Guyra site established at densities < 20 plants/m^2. Similar to the Series 3 experiments, there was no relationship (P>0.05) between initial seedling density and either DM production or basal frequency over the experimental period (Fig. 4).

There was a positive correlation between basal frequency and DM production ($P<0.01$, $R^2 = 0.42$). The most productive and persistent entry in this series was Haifa white clover, followed by Trophy and Tribute white clover (Fig. 4). The two lucerne cultivars performed similarly and had an average frequency of around 12% although they were still among the more productive of the entries tested. Birdsfoot trefoil, Talish clover, Caucasian clover and strawberry clover were all observed to have low basal frequency and DM production at this network of sites.

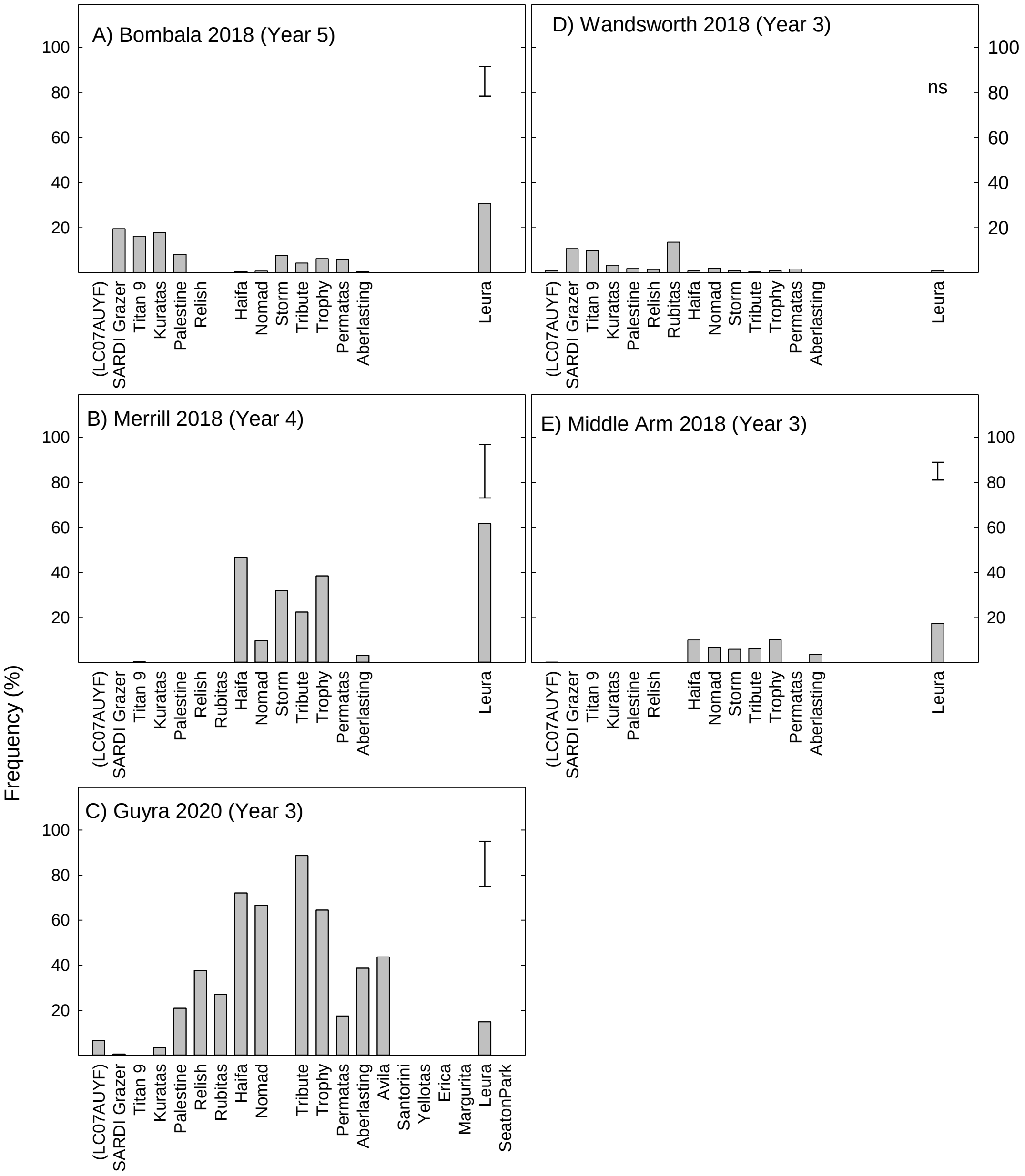

Fig. 5. Final assessment of legume frequency (%) at the five Series 4 experiments. Error bars indicate l.s.d. at *P*=0.05; ns, differences not significant at *P*=0.05. (Source: Hayes et al. 2022)

Final basal frequency across most entries at most sites was low relative to those observed in the Series 3 network. Many of the legumes declined to negligible levels after only 3 years at some sites (Fig. 5). The white clovers were present but had negligible biomass at most of these sites. By the end of the experiment, lucerne had almost completely disappeared at three of the sites, along with strawberry clover and birdsfoot trefoil. At Guyra, where seven annual legume entries were included as controls, only Leura subterranean clover and Avila yellow serradella were observed in year 3.

4.3 Pasture survey

4.3.1 Manager interviews

All managers agreed that legumes are an important component of their pastures, citing nitrogen fixation benefits (100% of respondents), to improve pasture quality, particularly crude protein

(100%), to increase animal production (73%), and to increase seasonal distribution of pasture growth and production (36%). Sixty four percent of managers described a target legume content of 20–30% in their mixed pastures with the remainder (36%) indicating their target legume content is 30–40%. All managers believed that the legume component of their pastures had declined in the previous 3 years largely as a symptom of the prolonged drought. However, over half (55%) of the managers stated that prior to the drought that they struggled to maintain the legume component of their pastures at their target level.

The top three factors identified by the managers as influencing legume persistence were seasonal rainfall (73%), soil fertility (73%) and grazing management (64%). Temperature, particularly heat stress was also rated by 55% of managers. Four managers (36%) nominated that it wasn't one influence alone, but rather a combination of influences that impacted legume persistence, for example, lack of soil moisture and overgrazing. Ninety one percent of the managers do not rest their pastures to aid legume seed set or seedling recruitment. Less than half (45%) were aware that white clover set hard seed. All managers indicated that in favourable (rainfall) springs, bloat was a concern, with 73% having lost stock to bloat in past 5 years despite taking steps to prevent bloat.

4.3.2 Pasture survey

The early stages of the survey coincided with an unprecedented drought period. For all sites, 2019 was the driest on record, with most sites being in the range of 180–280% below average rainfall except for the Uralla site which was ~400% below average. Above average rainfall was recorded for all sites in the 2020-2021 period, meaning that the survey effectively monitored pastures during drought, and in the recovery from drought. A similar study was conducted in the Northern Tablelands following the 1982 drought (Archer and Rochester 1982) providing a useful point of comparison for understanding white clover performance under drought in northern NSW.

Although white clover was the most sown species across all sites, during the first year of sampling (2019) it was recorded in only 21% of the paddocks where it was sown and contributed less than 5% to total pasture biomass. However, with the return to more favourable rainfall and temperature conditions in 2020–2021 there was an increase in white clover by 17% (n=20) and mature white clover plant frequency increased by 45%. For the majority (70%) of the sites, the 'sown' content increased from 2019 to 2021, for 15% of sites 'sown' content was approximately the same and for the remaining 15% of sites 'sown' content declined. On average the 'sown grass' component declined, which was offset by the increase in the 'sown legume' component (predominantly white clover) while the 'sown herb' component remained stable, albeit at a very low level.

The CIT analysis showed that 'Sown' pasture content was significantly higher (P=0.002) at sites where PBI values were greater than 120.9 (n=28) with a median of 88%, while only 16% of pasture DM was contributed by sown species at sites with a PBI ≤120.9. For sites where PBI was >120.9, the data were further split by pH ($CaCl_2$) at 5.1, with sites ≤5.1 (n=21) containing significantly higher 'sown' content than sites with pH greater than 5.1. (Fig 6a). 'Native grass' content (Fig. 6b) was significantly higher at sites with a PBI ≤246.3 PBI (P=0.035) (n=39), while the 'grass weed' content model had insufficient information to produce a decision tree. Broadleaf weeds were significantly higher at sites with a groundcover was ≤82% (P=0.004) (n=15) (Fig. 6c). Of sites >82 per cent groundcover, data were split by stock type, where types 1,2,4 (n=8) were significantly greater (P=0.023) than type 3 (n=28) (stock type 1,2,4 = sheep, sheep-cattle, cattle-sheep, 3 = cattle). Grazing management then split stock type 3, with management types 3,4 (n=14) significantly greater (P=0.004) than type 2 (n=14) (GM 3,4 = Planned & intense rotations, 2 = loose rotation). The

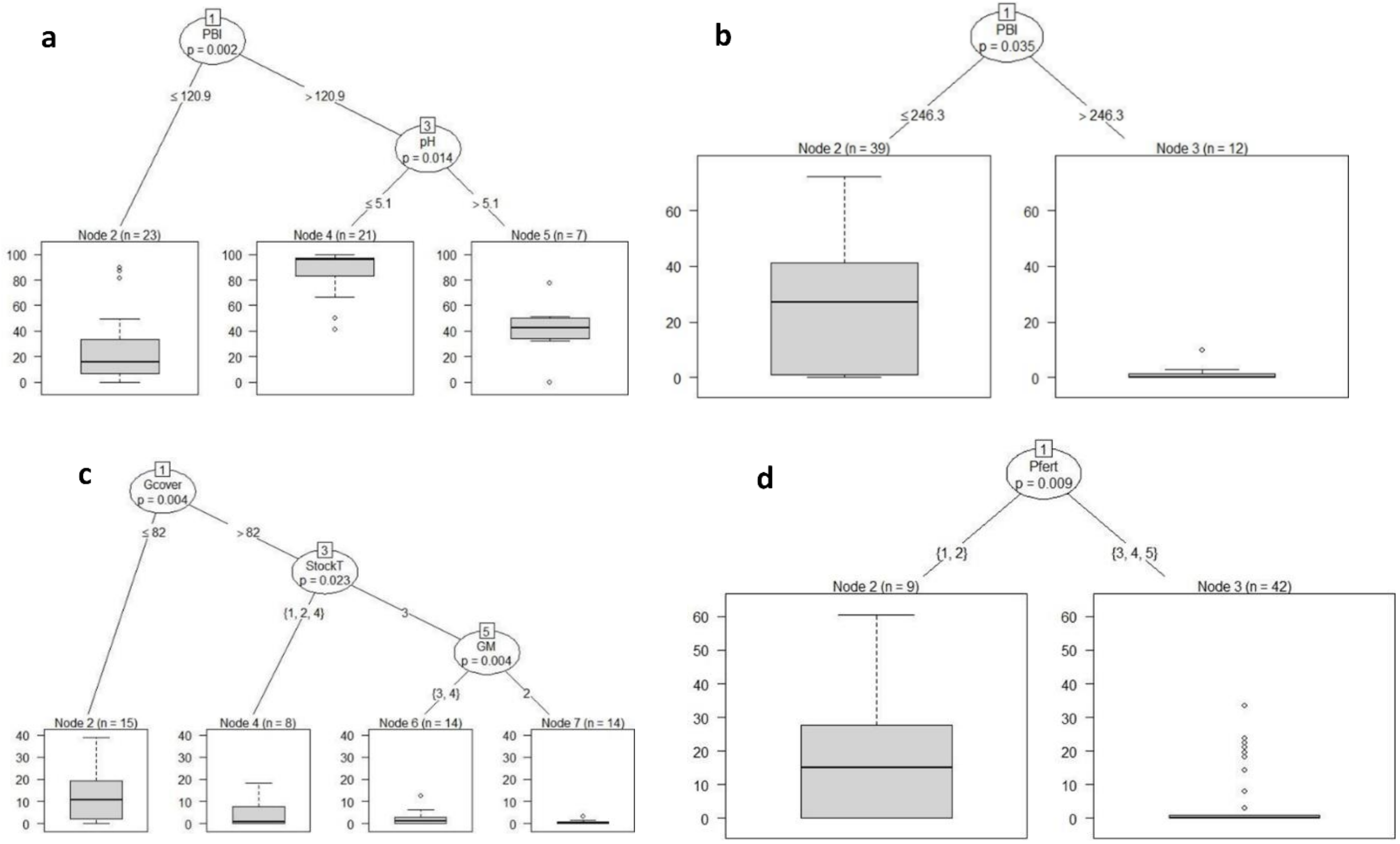

Fig. 6. Conditional Inference Tree examining 'sown' content using soil, climate, stock type and grazing management as explanatory variables. In a) a numerical split of survey sites was made at 120.9 PBI where sites ≤120.9 are graphed in Node 2, while the remaining are split again at pH (≤ or > 5.1) and then graphed for sown content in Nodes 4 & 5. In b) a numerical split of survey sites was made at 246.3 PBI where sites ≤246.3 are graphed in Node 2, while >246.3 then graphed for native content in Node 3. In c) a numerical split of survey sites was made at 82% Gcover (groundcover) where sites ≤82 are graphed in Node 2, while >82 then graphed for broadleaf weed content in Node 4, 6 & 7, with further splits of StockT (stock type) and GM (grazing management). In d) A categorical split of survey sites was made with a Pfert (phosphorus fertility) score of 1 & 2 graphed in Node 2, while 3, 4 & 5 graphed in Node 3. (For box and whisker plot: y axis = is % of botanical composition , n = number of sites, black line = median, grey shade = 25-75 percentile, hats = 10-90 percentile, open circles = outliers).

category ‘Other’ was split by P fertility with index ratings 1, 2 (n=9) significantly higher (P=0.009) than ratings of 3, 4, 5 (n=42) (Fig.6d).

There was a strong correlation (r^2 = 0.98) between ‘sown’ content in 2019 and ‘sown’ content’ in 2021 (Fig. 7) indicating that monitoring ‘sown’ content during a drought could be a useful indicator to predict likely pasture recovery post drought. There were also moderate correlations between 2021 ‘sown’ content and altitude (m) (r^2=0.44) and ‘sown’ content’ and P (Colwell mg/kg) based on 2019 sampling (r^2=0.50).

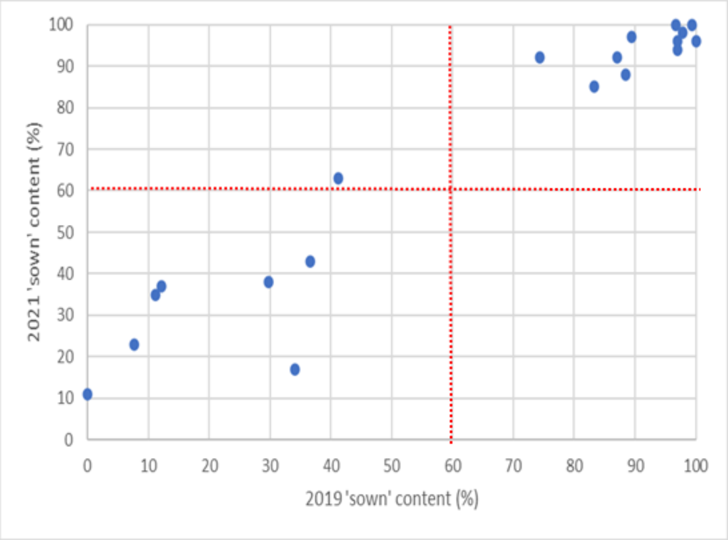

Fig. 7. Correlation between the ‘sown’ content (%) from the 2019 and 2021 botanical composition survey. The dotted red lines indicate the desirable ‘sown’ content of 60% for each survey. Sites in the righthand top quadrat had above the desirable ‘sown’ content in both 2019 and 2021.

4.3.3 White clover seedbank

There was a positive relationship (R^2=0.68) between the number of white clover seeds extracted from cores at surveyed properties and soil P levels (Fig. 8). Although the paddocks with higher levels of seeds per m^2 also had higher levels of S (≥8 mg/kg) and K (≥150 mg/kg Colwell K) the linear relationship between seed extracted and these nutrients was weak (r^2=0.24 and r^2=0.29, respectively). Germination of white clover seed recovered from soil cores averaged 16% and ranged from 9–22% after 14 days. Soft non-viable seed averaged 5% and ranged from 2–14%. Hard seed content of the samples averaged 79% with a range of 76–83%. The relatively low germination rates (16%, with a hard seed content of 79%) of the recovered seeds are comparable to the study conducted in the 1980s (14%, with a hard seed content of 84%) by Archer and Rochester (1982).

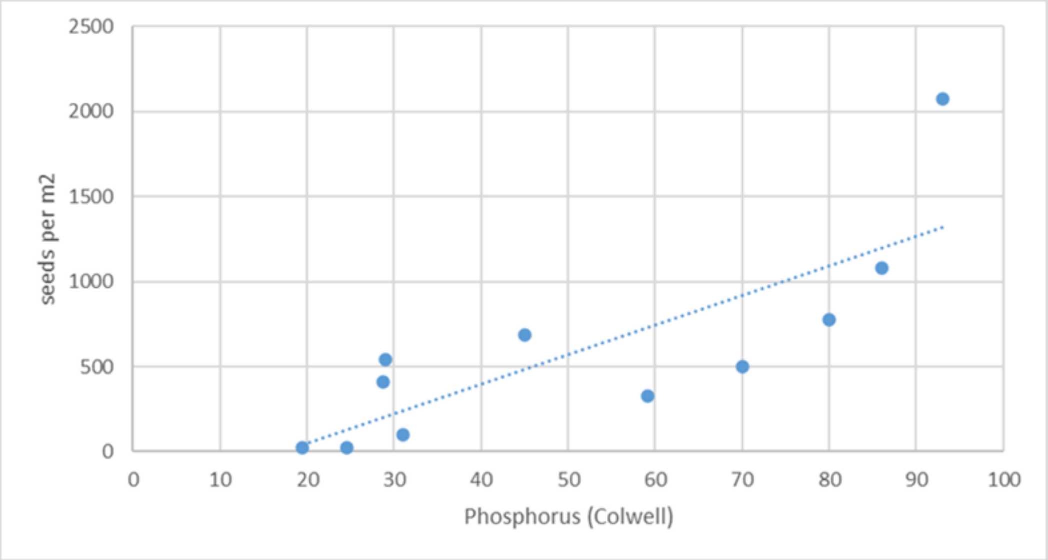

Fig. 8. The relationship between Phosphorus (Colwell mg/kg) and white clover soil seed levels (seeds/m^2) from 11 locations on the Northern Tablelands of NSW as part of a paddock survey to determine white clover prevalence.

4.3.4 White clover seedling recruitment post-drought

Post-drought germination of white clover occurred in all five sites where recruitment was monitored from late autumn to mid-winter (Table 3). In 2020 there were, on average, 60 white clover seedlings/m^2 (ranging from 19–158 seedlings/m^2), 19% of which were recorded as established by the following spring (ranging from 9–31%). In 2021 there were, on average, 126 seedlings/m^2 (ranging from 88–184 seedlings/m^2) with 45% recorded as established by autumn 2022 (ranging from 29–61%). The rate of seedling development was slow in both years with no plants deemed to have established until mid–late spring in each year. Seedlings which emerged in late autumn–winter were likely to have higher survival rates than seedlings that emerged in spring, and there was very little evidence of seedling emergence in early autumn. The main difference between the sites other than the of number of seeds in the seedbank was soil P. Sites 4 and 5 that had more seedlings and established white clover plants in both years had a P Colwell (mg/kg) above 50.

Table 3. The total number of white clover seedlings (seedlings/m^2) that emerged, failed to survive and established at five sites near Glen Innes over 2020 and 2021.

Site	2020–2021			2021–2022		
	Seedlings per m^2	No. seedling deaths per m^2	Established plants per m^2	Seedlings per m^2	No. seedling deaths per m^2	Established plants per m^2
1	44	56	4	88	41	47
2	16	22	5	-	-	-
3	19	31	3	101	72	29
4	158	146	32	184	72	112
5	62	72	10	129	79	50
Mean	*60*	*65*	*11*	*126*	*66*	*60*
SD	*25.96*	*22.01*	*5.4*	*21.29*	*8.50*	*18.01*

4.4 Glasshouse experiments

4.4.1 Dehydration tolerance of white clover and lucerne

A more comprehensive description of results is given by Norton et al. (2021). Essentially, this experiment was established to contrast response of the two species, lucerne and white clover, over a drying cycle. A generic drying cycle, which distinguishes three phases of a terminal drought, is depicted in Fig. 9. In this study, we were primarily concerned with the rate of drying in leaf stage (LS) II, and the length of the tale in LS III as this informs the ability of each species to control water loss to keep vital organs hydrated and the length of time these conditions can be tolerated before plant death.

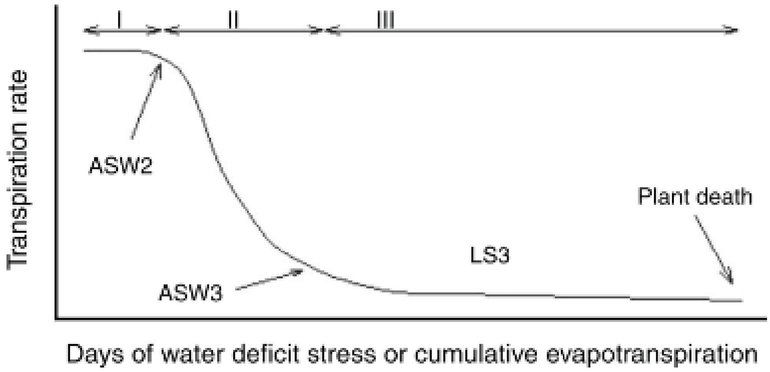

Fig. 9. The three phases of transpiration over a terminal drying cycle. ASW, available soil water, LS3, leaf stage 3. (Source: Norton et al. 2021).

The response of white clover to a terminal drought contrasted that observed in lucerne (Fig. 10). Using gravimetric soil water content (θg) as an indicator over time, we see that white clover dried the soil more quickly and to a lower level than lucerne, resulting in plant mortality sooner. This was despite leaf area being trimmed to similar levels in both species before the drying cycle commenced, the higher root:shoot ratio observed in lucerne, and the nature of the pot environment which precluded lucerne from establishing a deep tap root which is usually required for its survival during drought (Hoffman et al. 2003). White clover dried the soil at a rate of 0.47% θg/day compared to 0.30% θg/day in lucerne and achieved a greater level of drying with soil at the end of the experiment containing only 4.7% θg compared to 8.3% θg in lucerne pots.

Leaf/stolon elongation is one of the first plant processes to cease as water deficit increases; however, elongation was greater in white clover than lucerne at the beginning of the drying cycle, and this trend continued until lower soil water contents were reached. Conversely, leaf senescence generally commenced at quite high levels of water stress and progressed more rapidly to complete senescence in white clover than in lucerne. Lucerne retained tissue relative water content at a higher level than white clover, with final minimum values of 25% and 13.6%, respectively. In lucerne, 50% mortality was observed at θg of 9%, compared with 6% in white clover, albeit with greater variability. In conclusion, lucerne maintained a higher relative water content than white clover even though it endured the drying cycle for longer and without access to water at depth, evidence of its superior dehydration avoidance and better adaptation to dry conditions. However, white clover was more able to extract water from the soil volume in the pot.

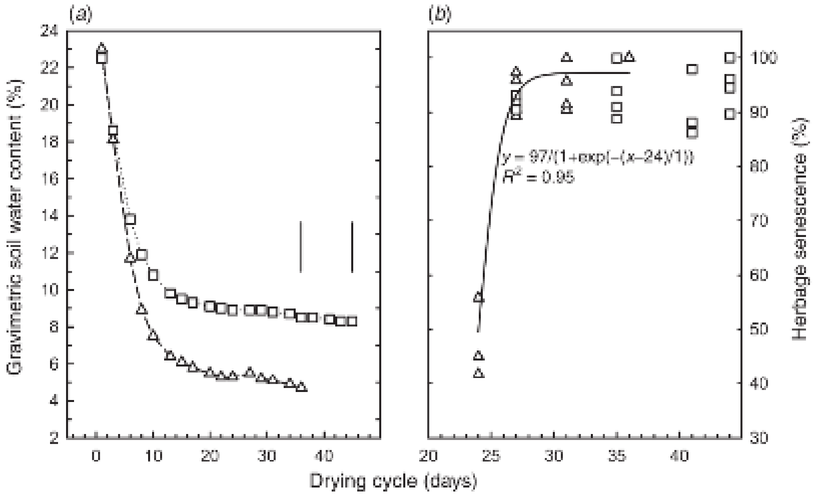

Fig. 10. (a) Time course of soil drying, and (b) percentage of total herbage that was senescent for white clover (Δ) and lucerne (□) as influenced by time from cessation of irrigation. Vertical bars in (a) represent significant differences (P =0.05) between white clover and lucerne in soil gravimetric water content (θg) on Day 36 in white clover and lucerne on Day 45. The relationship between herbage senescence and drying cycle time was significant in white clover but not in lucerne. (Source: Norton et al. 2021).

4.4.2 Effect of soil fertility on dehydration avoidance of white clover

In the K and S trial, neither K nor S treatments caused any differences in water use and stolon elongation rate over the first 5 days of the drying cycle. Moreover, there were no differences in dry weights of either shoots or roots due to the different K and S treatments in either the well-watered control or those treatments that had begun to wilt after having been subjected to the drying cycle. The percentage of herbage mass that was still alive at wilting also did not differ between the treatments. Measurements of water potential, osmotic potential, and relative water content of plants were used to assess the level of expression of osmotic adjustment but it was not possible to demonstrate any association between soil K and/or S levels and the expression of this trait.

In the P and lime trial, neither P nor lime treatments caused any differences in water use and stolon elongation rate over the first 5 days of the drying cycle, except for day 3 of the water use parameter where the P0 treatment had greater water use than the others. Greater shoot and root dry matter were observed in the high P treatment at the first harvest of the drying cycle and the well-watered control demonstrating the commonly observed positive response of legumes to improved P nutrition. Similar to the K and S trial, it was not possible to demonstrate any association between soil P and/or pH levels and the expression of osmotic adjustment. In the P and Lime trial, differences between the treatments at which 50% of plants survived were not consistent so that just as with the K and S trial there were no apparent effects of improving plant survival by soil amelioration treatments.

Calculations of N-fixation showed no difference ($P>0.05$) in the amount of N fixed per pot, regardless of soil treatment in either experiment.

4.4.3 Drought response of strawberry clover and white clover

Lime application greatly changed the soil pH and exchangeable aluminium (Al_{ex}) characteristics of this soil and reduced the concentration of Mn in the herbage of both species (Table 5). Average dry matter weights of shoots from all the harvests are presented in Fig. 11. The greatly reduced dry matter production of both shoots (and roots) of strawberry clover when growing in the soil that had not received any lime (L0) is a most striking result. These L0 strawberry clover plants were so stunted that they were not included in subsequent comparative water status measurements with white clover because with such miniature, stunted plants it was not considered to be a valid comparison. Strawberry clover continued to respond to lime application up until the equivalent of 3t/ha had been applied, whereas there was no response from white clover above 1.5t/ha (Fig. 11).

Table 5. The effect of lime rate on soil pH_{CaCl2} and exchangeable Al (expressed as the percentage of effective cation exchange capacity, % Al_{ex}), and on manganese (Mn) concentration (mg/kg) of strawberry clover (SC) and white clover (WC) shoots.

Lime (t/ha)	Soil pH_{Ca}	Soil % Al_{ex}	Mn in SC (mg/kg)	Mn in WC (mg/kg)
Nil (L0)	3.93	43.1	440	343
1.5 t/ha (L1)	4.34	20.8	158	131
3.0 t/ha (L2)	4.75	8.4	53	46
6.0 t/ha (L3)	5.58	1.7	35	33

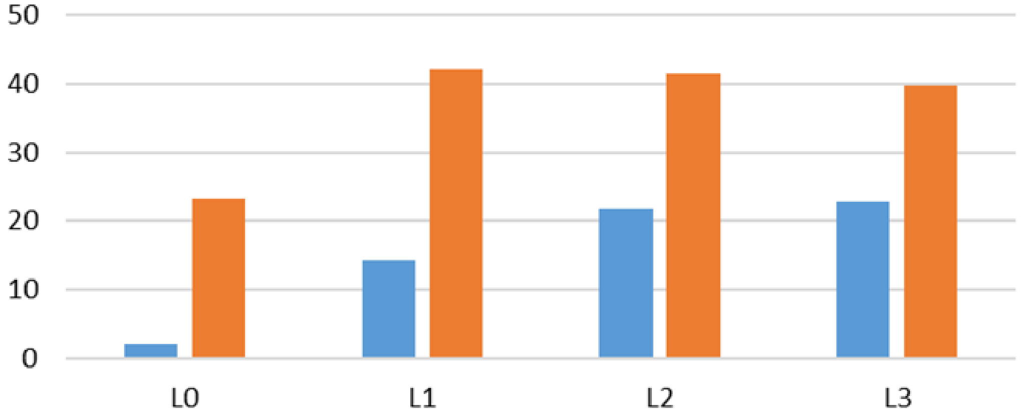

Fig. 11. Average dry matter weights (g/pot) of shoots from strawberry clover (blue bars) and white clover (orange bars) at nil (L0) and increasing applications of lime.

Shoot dry matter production was more severely affected than root production although it is also important to note that even at the higher levels of lime application shoot growth of strawberry clover was rarely more than 50% of that of white clover. This supports the general assertion that strawberry clover is intrinsically less productive than white clover (Kemp *et al.* 2002; Gerrard et al. 2022).

Except for strawberry clover in the absence of lime, the white clover treatment growing in the soil without lime addition had the lowest rates of soil drying, particularly at the beginning of the drying cycle when different rates of water use are always easiest to distinguish (Fig. 12). These differences between the white clover treatments were associated with the different amounts of biomass produced by the different soil environments.

Strawberry clover treatments generally expressed markedly higher levels of osmotic adjustment (average -0.90 MPa) as plant water deficit intensified than the white clover treatments (-0.60 MPa). This observation is consistent with those of Hofmann et al (2007), whose measurements while not expressly determining this trait, pointed to the probability that it would be expressed to a greater degree than in white clover.

We have highlighted that strawberry clover is less tolerant of soil acidity and high levels of Al_{ex} and Mn than white clover. Insofar as we have not been able to show that strawberry clover is able to survive at lower levels of θ g than white clover, we have not been able to demonstrate differences in dehydration tolerance leading to likely differences in survival of severe drought. However, we have shown differences in osmotic adjustment and this may translate into growth differences when water deficit is less severe. Further, we have demonstrated a substantially lower level of biomass production in strawberry clover compared to white clover and illustrated the lower levels of soil drying associated with lower production. This 'conserved water' may contribute to prolonged survival of strawberry clover in the field under drying conditions.

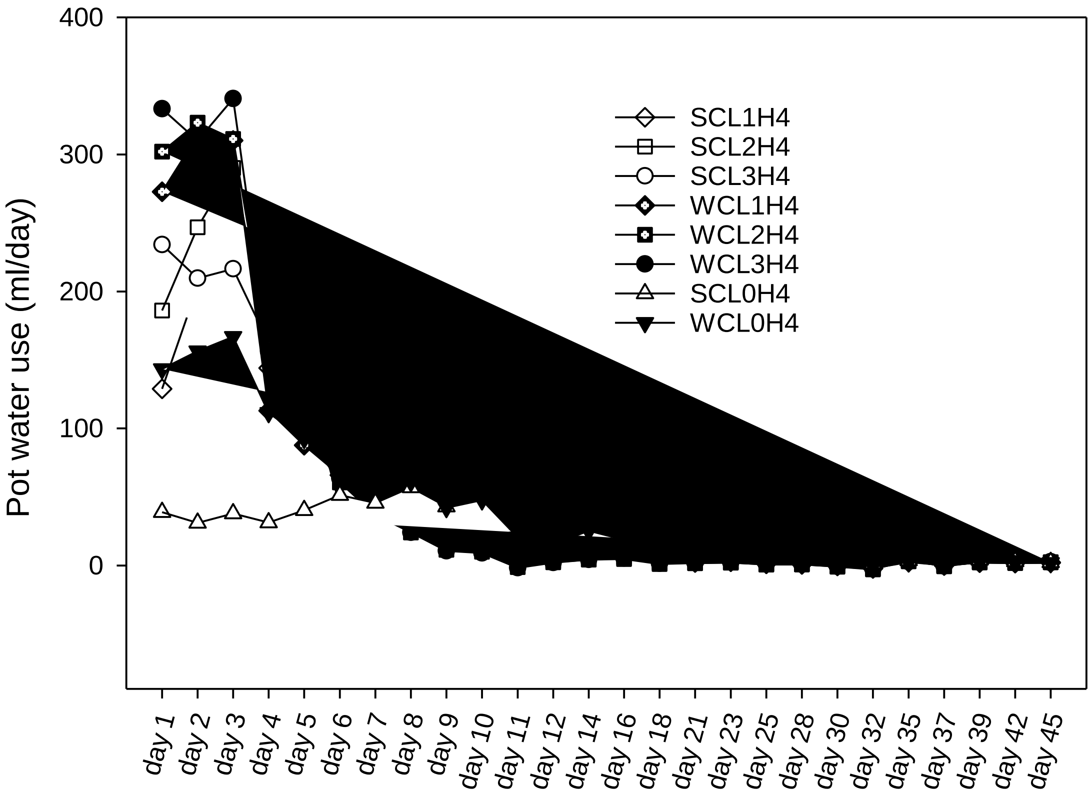

Fig. 12. The effect of different lime application amounts (L0, L1, L2, L3) on rate of water use of four treatments of strawberry clover (SC) and four treatments of white clover (WC) at the fourth harvest (H4) of the glasshouse experiment.

4.5 Soil nutrition experiments

Soil values following the application of lime and fertiliser are presented in Table 6 for each of the sites. The Merrill site had the most acidic surface soil, as indicated by its low pH and relatively high exchangeable Al in plots that did not receive lime. Mandurama and Orange were both relatively low in P and S in the absence of fertiliser. The lowest values of available K were observed at the Guyra, Mandurama and Glen Innes sites. The sites with the highest cation exchange capacity were Glen Innes and Paling Yards, the latter recording the highest values for available P and K, while the former recorded the highest values of available S in the surface depth. Guyra was the site with the lowest CEC (Table 6).

Table 6. Average soil test values for three contrasting soil treatments taken at the 0-10 cm depth at the end of year 1, describing pH (in $CaCl_2$), Aluminium as a percentage of the effective cation exchange capacity (Al%$_{ex}$), available phosphorus (Colwell P; mg/kg), available potassium (Colwell K; mg/kg), available sulfur (KCl-40 S; mg/kg) and cation exchange capacity (CEC; cmol(+)/kg)

Treatment	pH$_{Ca}$	Al%$_{ex}$	Colwell P	Colwell K	KCl-40 S	CEC
			Dry Plain			
Phalaris_Lime	5.7	1	38	147	18	9.3
Phalaris_Lime_P_K_S	5.9	1	59	193	42	10.5
Phalaris_Unlimed_P_K_S	4.4	6	74	163	35	5.3
			Merrill			
Phalaris_Lime	5.4	2	30	270	20	7.5
Phalaris_Lime_P_K_S	5.7	1	58	367	26	9.7
Phalaris_Unlimed_P_K_S	4.1	24	71	267	19	4.1
			Guyra			
Phalaris_Lime	5.6	1	31	98	16	4.4
Phalaris_Lime_P_K_S	5.5	1	57	105	18	5.1
Phalaris_Unlimed_P_K_S	4.5	6	90	137	20	3.0
			Mandurama			
Phalaris_Lime	6.1	1	18	98	7	8.7
Phalaris_Lime_P_K_S	5.7	1	31	116	14	8.6
Phalaris_Unlimed_P_K_S	4.7	2	34	139	13	5.3
			Paling Yards			
Phalaris_WhiteClover_Lime	5.6	1	130	813	19	20.2
Phalaris_Lime_P_K_S	5.6	1	167	900	25	21.0
Phalaris_Unlimed_P_K_S	5.0	1	140	813	28	17.6
			Orange			
Phalaris_Lime	6.4	1	20	143	5	9.6
Phalaris_Lime_P_K_S	6.4	1	36	163	8	9.7
Phalaris_Unlimed_P_K_S	5.3	1	36	163	8	9.7
			Glen Innes			
Phalaris_Lime	5.5	1	45	99	32	20.1
Phalaris_Lime_P_K_S	5.5	1	65	99	42	19.2
Phalaris_Unlimed_P_K_S	5.1	1	44	113	32	16.8

4.5.1 Effects of lime

Averaged across all fertiliser treatments, a positive response to lime was observed in total biomass at all sites, but responses varied from year to year (Fig. 13). The exception was Merrill where no effect of lime was observed. This result was not anticipated given the highly acidic nature of the soil (see Tables 5 and 6) but is likely explained by the strong and consistent response to P-fertiliser at this site (see next section). This result suggests that at Merrill, soil available P was the most limiting factor on plant growth, obscuring any positive response to lime. Further analysis is required to examine if a cumulative response of P and lime existed at this site. Across the sites, lime did not have a consistent effect on legume composition. There were instances where a large positive response was observed in legume biomass, such as the 49% increase observed at Mandurama in year 4, but in other instances there were large negative responses to lime, such as the 40% reduction in legume biomass with lime at Dry Plain in year 2. Across all sites and years, the negative responses in legume biomass to lime cancelled out all of the positive responses observed. There was a consistent positive response to lime in phalaris biomass at Dry Plain, Guyra and Mandurama but results at other sites were more variable.

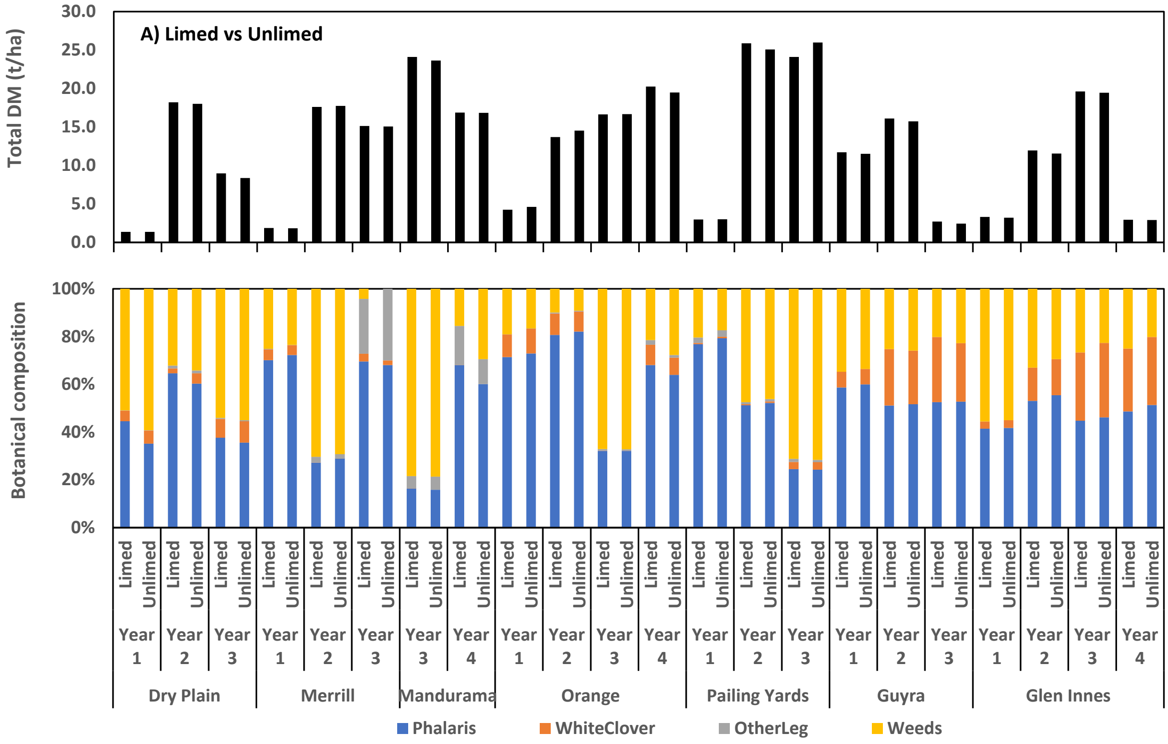

Fig. 13. Response to lime in biomass (t/ha) and botanical composition on an annual basis at Dry Plain, Merrill, Mandurama, Orange, Paling Yards, Guyra and Glen Innes.

Averaged across the three sites for which analysis has been completed (Merrill, Mandurama and Orange) lime increased (*P*<0.05) crude protein (from 25.5 to 25.9%), ADF (from 17.3 to 17.7%) and NDF (*P*=0.052; from 37.0 to 37.7%) but reduced WSC (from 23.2% to 22.2 %). There was no effect of lime on other quality parameters. The herbage mineral data averaged across all sites indicated that lime increased P, Ca and Mo concentrations of herbage, as well as the Ca:P ratio (from 1.31 to 1.39) and reduced the Mn, Zn, nitrate N and ammonium -N of herbage, as well as the tetany index (from 1.74 to 1.68) but had no effect on the K:Na, K:(Na+Mg) ratios or the DCAD in phalaris herbage (P>0.05). There was a significant site by lime interaction in the Ca:P ratio (Table 7), with differences due to lime more apparent as Ca:P values increased.

Table 7. Significant site x lime interaction in the Ca:P ratio of phalaris herbage

	Glen Innes	Dry Plain	Merrill	Mandurama	Orange	Paling Yards
Unlimed	2.37	1.89	3.12	6.37	4.32	1.59
Limed	2.39	2.00	3.59	6.72	4.61	1.6

4.5.2 Effects of P fertiliser

There was a consistent positive response in legume biomass to P fertiliser at the Orange, Merrill and Guyra sites, with responses at other sites more variable (Fig. 14). Surprisingly, there was a ~20% increase in legume biomass at the Paling Yards site due to P-fertiliser, despite very high soil test P values at that site, even in the absence of P fertiliser (Table 6). At Paling Yards, there was a corresponding (15-20 %) decrease in phalaris biomass attributable to P-fertiliser.

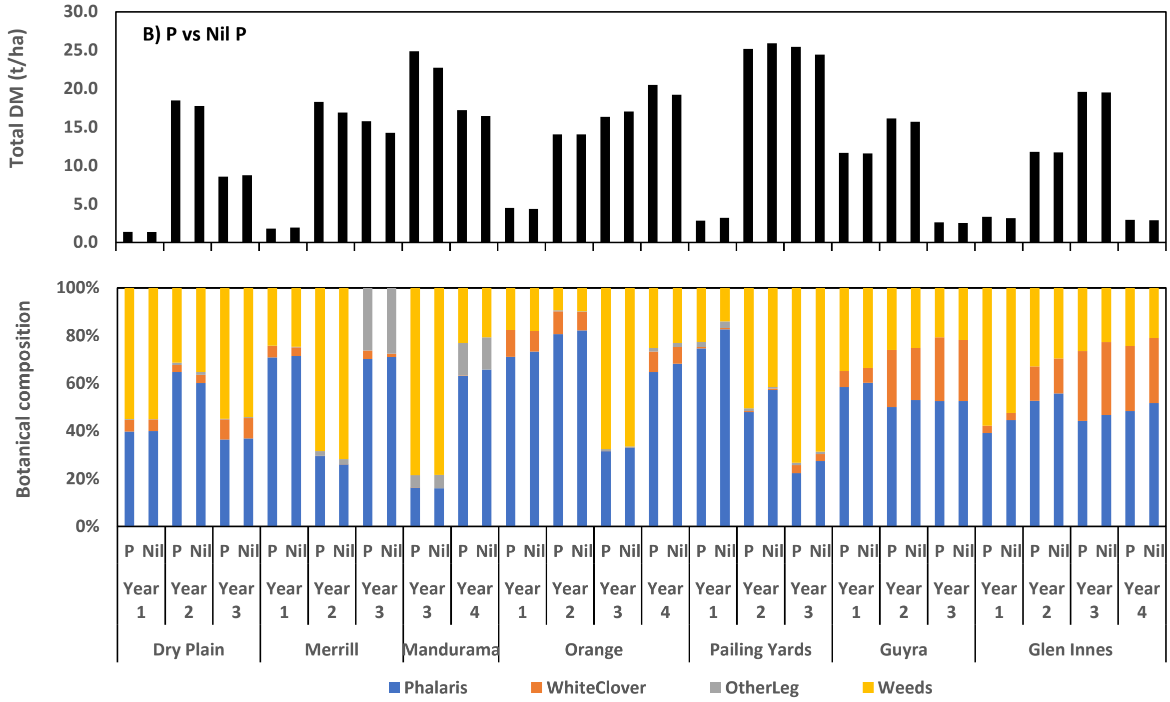

Fig. 14. Response to P-fertiliser in biomass (t/ha) and botanical composition on an annual basis at Dry Plain, Merrill, Mandurama, Orange, Paling Yards, Guyra and Glen Innes.

There was a significant (P<0.05) site x P fertiliser interaction on some forage quality parameters (Table 8), but there was no difference in ADF, OM, WSC or ash content attributable to P fertiliser. Phalaris crude protein and DMD increased with P fertiliser at the Mandurama site only while NDF increased at all sites with additional P, albeit only marginally at the Orange site. Metabolisable energy was slightly lower in the +P plots, but only at the Merrill site (Table 8).

Table 8. Significant site x P-fertiliser effect on dry matter digestibility (DMD), neutral detergent fibre (NDF), crude protein (CP) and metabolizable energy (ME) of phalaris grown with (+P) or without (Nil P) phosphorus fertiliser in spring year 1 at Merrill, Mandurama and Orange.

	Merrill		**Mandurama**		**Orange**	
	Nil P	+P	Nil P	+P	Nil P	+P
DMD (%)	90.1	89.1	94.6	95.1	93.9	93.8
NDF (%)	38.7	40.5	33.4	34.7	38.1	38.4
CP (%)	24.0	23.9	24.8	25.7	28.0	28.0
ME (MJ/kg)	13.9	13.7	14.7	14.7	14.5	14.5

The addition of P-fertiliser increased (P<0.05) the average concentration of P, Na, K, Mg, Cl, S, Mo, Mn and ammonium N of phalaris herbage, and reduced the concentration of Cu. There was a significant site x P-fertiliser interaction on all dietary ratios as well as the tetany index but fertiliser P had no impact (P>0.05) on DCAD. The Ca:P ratio generally decreased, except at the Glen Innes and Paling Yards sites (Table 9), which happened also to be the sites which both had a high soil CEC (Table 6). The K:Na and K:(Na+Mg) ratios, as well as the tetany index, increased with P fertiliser at the Glen Innes and Dry Plain sites, but generally declined at the other sites. There was a large site effect in the K:Na ratio with a ~20-fold difference observed between the sites with the lowest values

(Mandurama) and the site with the highest values (Merrill). This site effect is discussed further in the next section.

Table 9. Significant site x P-fertiliser interactions in the Ca:P, K:Na and K:(Na+Mg) ratios and tetany index of phalaris herbage.

	Glen Innes	Dry Plain	Merrill	Mandurama	Orange	Paling Yards
			Ca:P			
Nil P	1.15	0.99	1.20	2.52	1.59	0.80
+ P	1.24	0.95	1.06	1.89	1.40	0.80
			K:Na			
Nil P	2.27	2.23	22.96	1.53	12.50	17.59
+ P	2.74	2.62	20.92	1.31	10.09	16.70
			K:(Na+Mg)			
Nil P	0.56	0.71	3.49	0.56	2.14	3.09
+ P	0.63	0.77	3.36	0.49	1.94	2.98
			Tetany index			
Nil P	0.69	1.05	3.00	0.97	1.74	2.69
+ P	0.74	1.09	2.98	0.86	1.65	2.62

4.5.3 Effects of K fertiliser

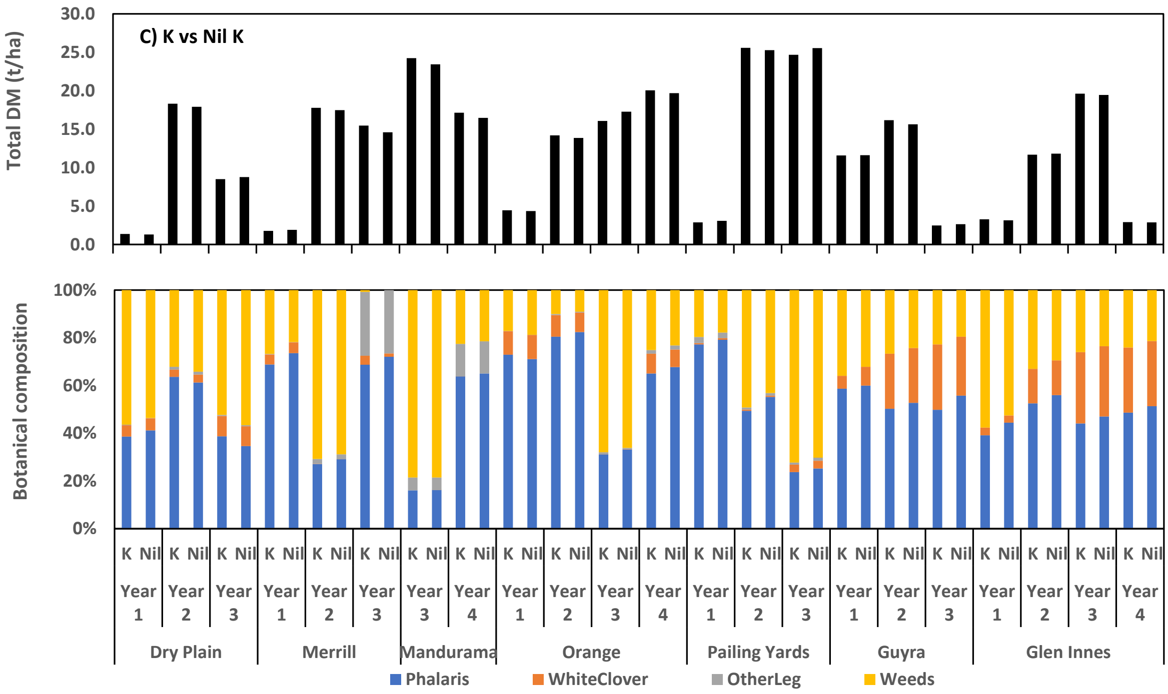

Fig. 15. Response to K-fertiliser in biomass (t/ha) and botanical composition on an annual basis at Dry Plain, Merrill, Mandurama, Orange, Paling Yards, Guyra and Glen Innes.

There was often a positive response to K fertiliser in terms of total biomass, especially in years where high biomass yields were achieved, although there were instances where a nil or negative response was observed (Fig. 15). Potassium fertiliser generally increased legume biomass at the Gunning, Mandurama and Orange sites but had the reverse effect at the Dry Plain and Paling Yards sites (Fig. 15). The response in legume biomass was variable at Guyra and Glen Innes. There were few instances where Phalaris biomass increased with K fertiliser.

No feed quality parameters were affected by K fertiliser, except for ADF and NDF, which increased with the addition of K from 17.3 to 17.8% and 37.0 to 37.7%, respectively. Averaged across all sites and years, K-fertiliser increased ($P<0.05$) the K, P and Cl concentrations of phalaris herbage and reduced the S concentration. As with P -fertiliser, there was a significant site x K-fertiliser interaction on all dietary ratios as well as the tetany index, but fertiliser K had no impact ($P>0.05$) on DCAD (Table 10). The addition of K-fertiliser increased Ca:P at Glen Innes and Paling Yards but had the opposite effect at the other sites. The K:Na, K:(Na+Mg) and tetany index all increased with K-fertiliser at Orange, Mandurama, Dry Plain and Glen Innes, but decreased at Paling Yards and Merrill. Interestingly, it was only at the Merrill and Paling Yards sites where the tetany index of phalaris was above the nominal threshold of 2.2, regardless of fertiliser treatment, meaning that there was no instance in our experiments where the addition of K-fertiliser pushed the tetany index of phalaris herbage above the nominal risk threshold for livestock health.

Table 10. Significant site x K-fertiliser interactions in the Ca:P, K:Na and K:(Na+Mg) ratios and tetany index of phalaris herbage. Nominal thresholds to avoid risk of metabolic diseases in livestock are italicised in brackets.

	Glen Innes	Dry Plain	Merrill	Mandurama	Orange	Paling Yards
			Ca:P (1-2)			
Nil K	1.21	0.98	1.15	2.26	1.56	0.76
+ K	1.31	0.97	1.10	2.12	1.43	0.82
			K:Na (5.6-7.1)			
Nil K	1.81	2.19	22.29	1.15	10.10	18.36
+ K	3.15	2.66	21.52	1.66	12.25	16.08
			K:(Na+Mg) (<6.0)			
Nil K	0.49	0.70	3.47	0.44	1.88	3.16
+ K	0.69	0.78	3.37	0.60	2.17	2.92
			Tetany index (<2.2)			
Nil K	0.64	1.03	3.01	0.81	1.60	2.71
+ K	0.79	1.11	2.97	1.00	1.78	2.60

4.5.4 Effects of S fertiliser

There were few instances where total biomass was increased with the addition of S-fertiliser (Fig. 16). Legume biomass generally increased with the addition of S-fertiliser at the Merrill and Mandurama sites, with more variable responses observed at the other sites. There was a marginal increase in phalaris biomass with S-fertiliser at the Dry Plain site, but generally a negative response in phalaris biomass to S-fertiliser at Paling Yards, Guyra and Glen Innes (Fig. 16).

The ash content of phalaris herbage was reduced with sulfur fertiliser, from 10.6% to 10.4%, averaged across all sites. There were significant ($P<0.05$) site x S-fertiliser interactions for ME, ADF, NDF, DMD and WSC (Table 11). The addition of S-fertiliser had variable effects on herbage quality parameters across the sites. For example, S-fertiliser increased DMD at Mandurama but had the opposite effect at Orange and little effect at Merrill. Both ADF and NDF increased at Orange with S-fertiliser but decreased at the other sites. By contrast, WSC decreased at Orange with S-fertiliser, but increased at the other sites. Variable responses in ME were also observed across the sites (Table 11).

Averaged across all sites and years, the addition of S-fertiliser increased ($P<0.05$) the S, P, Mg, Mn and Mo concentrations in phalaris herbage. There was no effect of S-fertiliser on any mineral ratio or the tetany index but there was a significant site x S-fertiliser interaction on DCAD (Table 12). The addition of S-fertiliser reduced the DCAD across all sites, except for Merrill where there was a small increase. The DCAD was higher at Merrill than any other site, regardless of S-fertiliser treatment. Only at Glen Innes was the DCAD below the nominal threshold (<12 meq/100 g), again regardless of S-fertiliser treatment.

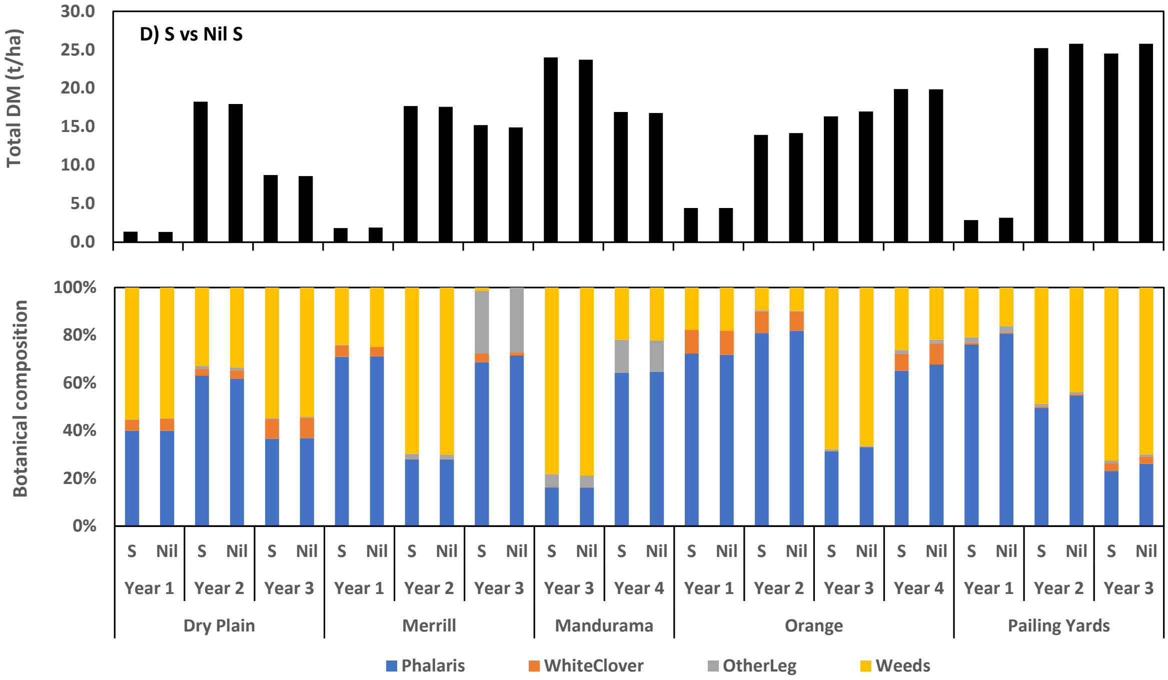

Fig. 16. Response to S-fertiliser in biomass (t/ha) and botanical composition on an annual basis at Dry Plain, Merrill, Mandurama, Orange, Paling Yards, Guyra and Glen Innes.

Table 11. Significant site x S-fertiliser effect on dry matter digestibility (DMD), neutral detergent fibre (NDF), crude protein (CP) and metabolizable energy (ME) of phalaris grown with (+P) or without (Nil P) phosphorus fertiliser in spring year 1 at Merrill, Mandurama and Orange.

	Merrill		**Mandurama**		**Orange**	
	Nil S	+S	Nil S	+S	Nil S	+S
DMD (%)	89.5	89.6	94.5	95.2	94.3	93.4
ADF (%)	20.4	20.1	14.8	14.3	17.2	18.5
NDF (%)	39.8	39.5	34.3	33.9	37.6	38.8
WSC (%)	22.4	22.6	24.9	26.0	20.3	19.9
ME (MJ/kg)	13.8	13.8	14.6	14.8	14.6	14.5

Table 12. Significant site x S-fertiliser interaction in the DCAD of phalaris herbage

	Glen Innes	Dry Plain	Merrill	Mandurama	Orange	Paling Yards
Nil S	10.2	27.5	34.4	26.9	31.7	29.4
+S	8.9	25.1	35.9	25.0	29.1	28.0

4.5.5 Nitrogen fixation in nutrition experiments

There was little effect of soil treatment on the N-fixation efficiency of legumes in the nutrition experiment, largely because legumes were such a small percentage of total biomass in the early years of most experiments. Only at Merrill in 2020 was there a significant treatment effect ($P<0.05$), with the amount of subterranean clover shoot Ndfa increasing from 7.4 kg N/ha to 11.3 kg N/ha with the addition of lime. There was no effect of P, K or S-fertiliser on shoot Ndfa. Although those values at Merrill were low, values at all other sites were lower due to low legume biomass.

4.6 Nitrogen fixation in perennial legumes

4.6.1 Cross-host compatibility; controlled environment experiment

There were highly significant ($P < 0.001$) differences in the number of nodules and shoot biomass production of legumes among rhizobial strains. Certain strains were more effective at increasing the amount of shoot biomass produced relative to the negative control (Fig. 17). For example, WSM1325 and CC283b formed nodules on some white clover cultivars (cvv. Tribute and Nomad), white clover x Caucasian clover hybrid (cv. Aberlasting) and red clover (cv. Rubitas). However, shoot biomass production was poor for these cultivars and TA1 remained the most effective strain. Other white clover cultivars (cvv. Trophy, Haifa and Storm), strawberry clover (T. fragiferum cv. Palestine) and Talish clover (T. tumens cv. Permatas) formed effective nodules with both TA1 and WSM1325. Some legume species (strawberry and Talish clovers) and some cultivars within species (e.g. white clover cv. Haifa) formed ineffective nodules with CC283b (Fig. 17). Only commercially recommended strains resulted in root nodulation for lucerne (cvv. Titan 9 and SARDI-Grazer with RRI128), birdsfoot trefoil (with SU343) and Caucasian clover (cv. Kuratas with CC283b).

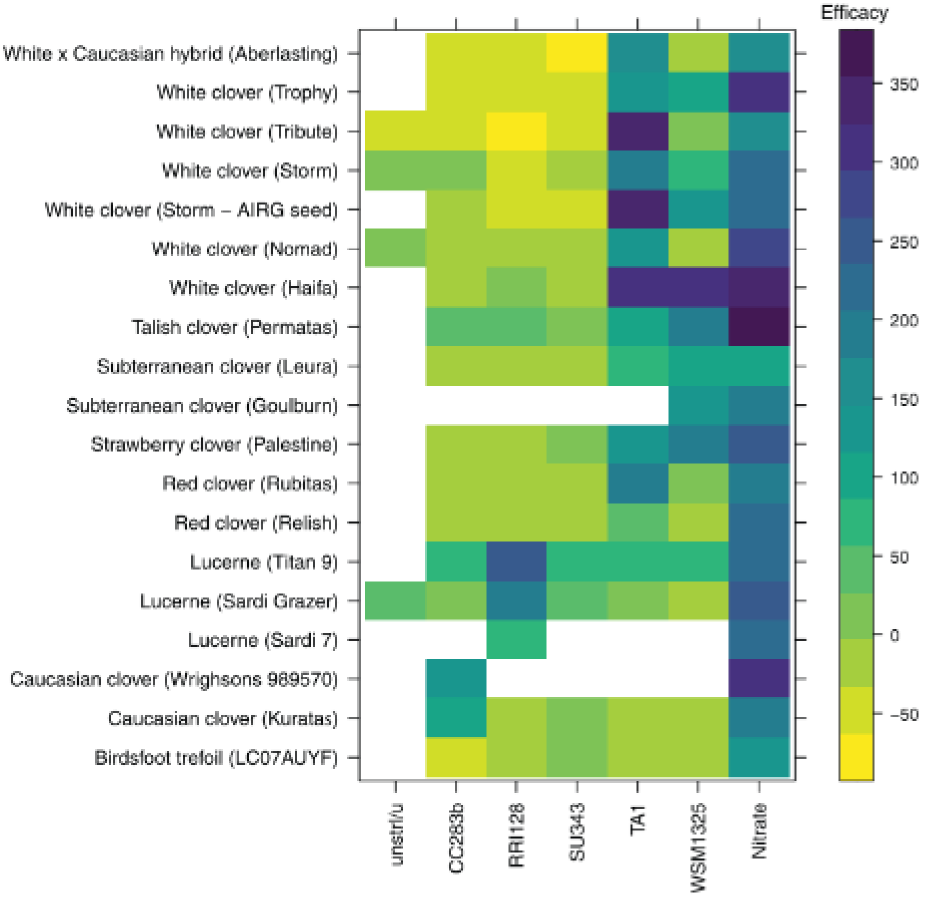

Fig. 17. Heatmap of efficacy (% dry weight increase relative to negative control) for legume–rhizobial strain combinations, as well as multiple control treatments: (-)cntrl, 'negative' uninoculated control; unstrl/u, unsterilised and uninoculated control for pre-coated seeds; Nitrate, nitrate-fed 'positive' uninoculated control. (Source: Rigg et al. 2021).

4.6.2 Nodule occupancy and background strains

Background rhizobial strains present at Mandurama were detected in trap plant assays. Almost all subterranean clover trap plants survived the duration of the assay (30/32 plants). WSM1325-like strains were positively detected in 16 subterranean clover plants and TA1-like strains were detected in two plants. Over half of the white clover trap plants (21/32) survived the assay. Of the 17 surviving and nodulated plants, eight had nodules that were too small (<1 mm) and white to warrant harvesting for nodule occupant assessment. Of the nine white clover plants that were adequately nodulated, WSM1325-like strains were isolated from nodules of five plants, and TA1-like strains from the remaining four plants. The survival of lucerne trap plants was poor (8/32), with most plants yellowing before dying. None of the surviving lucerne trap plants had formed nodules.

Nodule scores were generally low across all sampled legumes in the field, with scores of <4 for all species except white clover (Fig. 18). The legume cultivars that were the most well nodulated included French serradella cv. Margurita, and white clover cvv. Haifa, Storm, Tribute and Trophy (Fig. 18). The French serradella variety generally had better nodulation than yellow serradella cv. Avila. There was large variation in nodulation for some legume cultivars, for example, yellow serradella cv. Avila, white clover cvv. Haifa and Nomad, and red clover cv. Relish (Fig. 18). Some cultivars, Talish clover cv. Permatas, red clover cv. Rubitas and sulfur clover breeding line Tas0433, were very poorly nodulated in the field, suggesting that their rhizobial requirements were not met, and Sainfoin cv. Othello was not nodulated (Fig. 18).

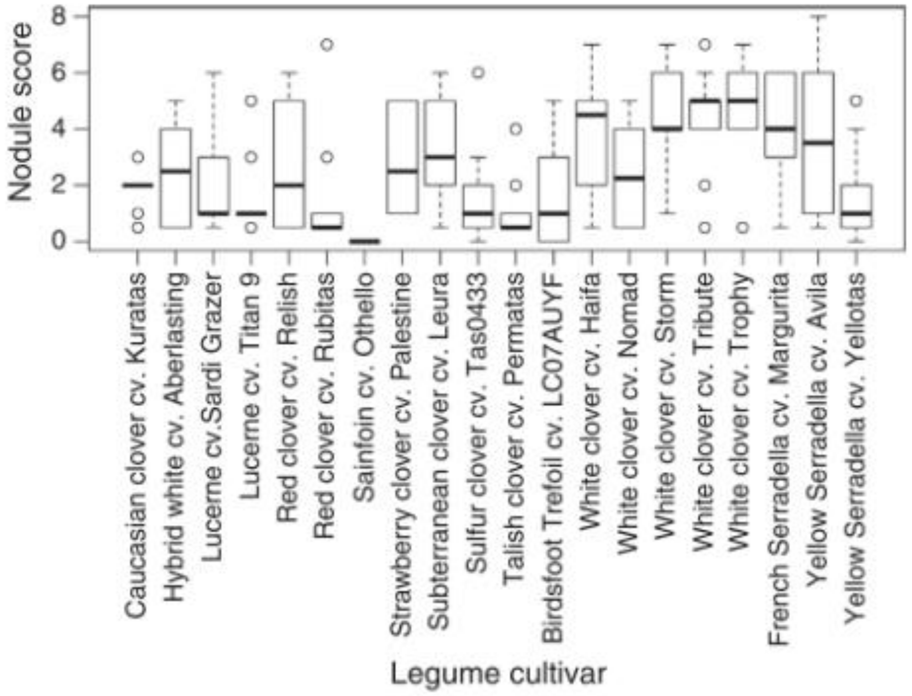

Fig. 18. Box-and-whisker plots of nodule score for each legume cultivar in the field experiment at Mandurama. (Source: Rigg et al. 2021).

Of the 200 plants assessed, 16 were not nodulated, including 10 sainfoin cv. Othello, two sulfur clover cv. Tas0433 and four birdsfoot trefoil cv. LC07AUYF plants. Most Talish clover (8/10) and some red clover cv. Rubitas plants evaluated had small white nodules <1 mm in diameter and therefore rhizobial cultures could not be isolated. The recommended strain used for inoculation at sowing was the occupant most often recovered from nodules of lucerne, serradella, subterranean clover and birdsfoot trefoil. Most nodule occupants cultured from Caucasian clover cv. Kuratas remained unidentified and did not match commercial rhizobial strains. Many other perennial clovers had nodules that contained a WSM1325-like strain instead of TA1, with which they were inoculated. For most cultivars, a small percentage of cultures isolated remained unidentified by the methods used.

Yellow serradella cv. Yellotas produced the greatest biomass during the first year at the field site and serradella cultivars showed the highest amount of shoot N fixed per hectare (Fig. 19). Caucasian clover cv. Kuratas, sulfur clover cv. Tas0433 and Talish clover cv. Permatas had poor biomass production in the establishment year.

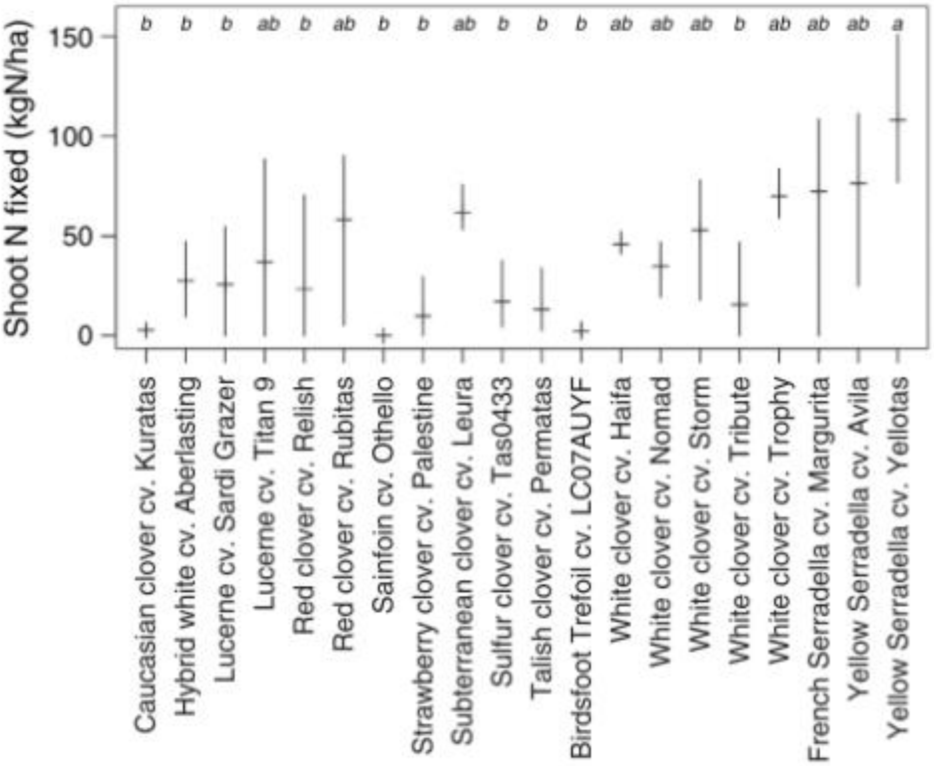

Fig. 19. Shoot N fixed at Mandurama for each legume cultivar. Horizontal lines indicate the mean value of replicates, while vertical lines show the range. Cultivars with the same letter are not significantly different at P = 0.05. (Source: Rigg et al. 2021).

From approximately 800 nodules, 2500 bacterial cultures were generated. Of the potential 2500 isolates recovered, a large proportion (776) have been confidently identified to strain or Genus level using MALDI-TOF MS.

This culture collection is an important representation of bacterial diversity in pasture soils under over 20 legume hosts. The collection includes bacteria such as *Rhizobium sp., Pseudomonas sp., Burkholdaria sp., Sinorhizobium sp*., and some that are yet to be identified. The value of these cultures may yet to be realized. For example, one organism isolated was *Pseudomonas brassicasearum*. Other strains of this species have been used commercially for plant-growth promotion in tomatoes and as a biocontrol agent in potatoes.

Some other examples of use could include but are not limited to, performing experiments investigating the function of non-rhizobial nodule occupants in nitrogen fixation (an emerging topic), to try to identify commercial rhizobial partners for the newly developed pasture legumes from Tasmania or to investigate the plant-growth promotion properties of these cultures.

In 2020, 65 cultures of interest were selected and stored on ProtectBeads, a microorganism preservation system, and stored at -20 °C. Details of each culture accession including the host, experimental treatment, collection date and location of collection (lat/long co-ordinates) have been complied and will be uploaded to the EMu database for future reference.

4.6.3 Sainfoin rhizobia

From the unpublished screening conducted in 2001, the positive (plus N) control treatment produced substantially more biomass than all other treatments, indicating that N was a large limitation to plant growth in this experiment. Two of the strains from the Brockwell collection (CC401 from crown vetch and CC1061) did not form nodules with cv. Othello. Nodulation by a third strain (CB2000) was negligible. Strains CC1099, CC1046, CC1107, CC1108 and CC1109G all formed nodules. However, only CC1109G showed any improvement in growth, compared to the nil control, but only increased biomass by 14% when adjusted for the nil control (Fig.20).

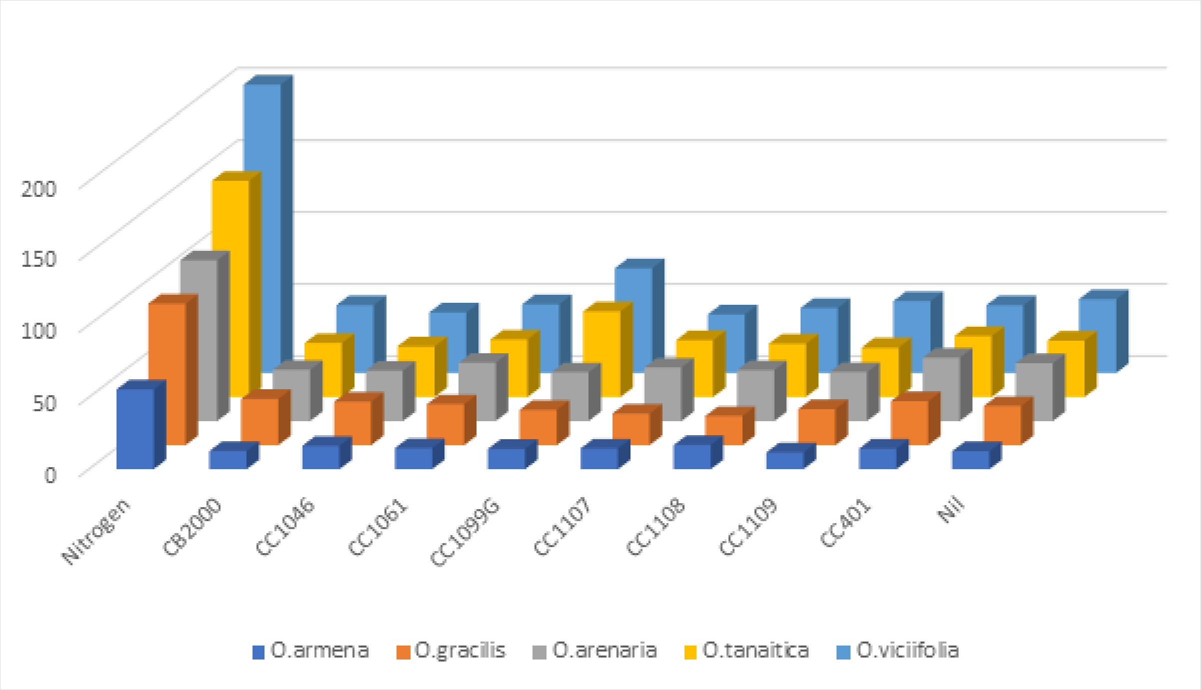

Fig. 20. Shoot DM (g/plant) of five species from the *Onobrychis* genus inoculated with eight different strains of rhizobia compared to negative (nil) and positive N (Nitrogen) controls. (Source: R. A. Ballard unpublished)

Sequencing and analysis of the 16S rRNA gene indicated that CC1099 (NA806-1) belongs to the genus *Rhizobium* and CC1099 (NA808) belongs to the genus *Bradyrhizobium*. Sainfoin seeds showed a very low survival rate past a few days, with 2/3 of plants failing to thrive – leaving too few for statistical analysis.

There was also no indication of effective N-fixation observed within the standard 6-8 week testing period. The plants were grown on until a difference could be observed. It was observed that the +N control plants that survived performed well and produced the most biomass while the plants that received no N, and the plants that were inoculated with NA808 were poorly, with just a few yellowing leaves. The plants inoculated with NA806-1, while also looking poorly at 6 weeks, showed a marked improvement by 12 weeks with more leaves that were very dark green.

At 12 weeks, the surviving plants were harvested, and nodulation assessed. Only the plants inoculated with NA806-1 had formed nodules. The nodules on the root systems of these plants were found throughout, with large, multi-pronged formations on the tap roots. All other treatments showed no nodule formation indicating that the NA806-1 accession of CC1099 is somewhat effective although, similar to the 2001 study, yield increases due to inoculation were modest indicating potential for further improvement. Nevertheless, as of 2022, CC1099 is once again commercially available in Australia enabling industry to continue experimentation and development of sainfoin under local conditions (Appendix 6).

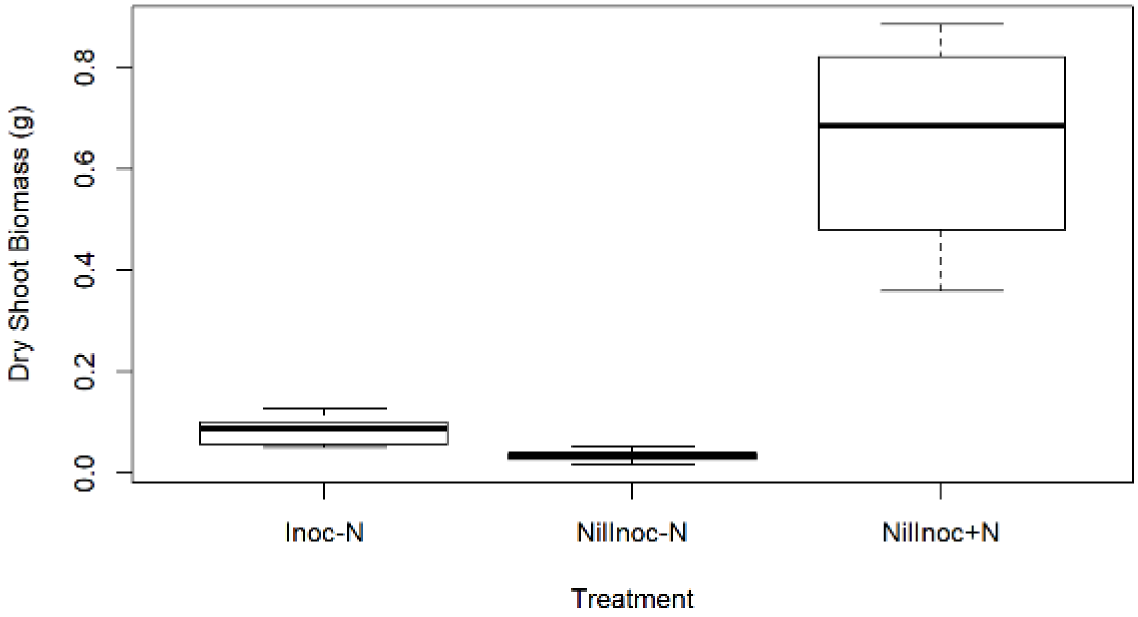

Fig. 21. Dry shoot biomass (g) of Sainfoin when inoculated with CC1099 (NA806-1 recovered in 2021, Inoc -N) compared with an un-inoculated control (NilInoc-N) and plus nitrogen control (NilInoc+N).

At the time of writing, the validation experiments presently being undertaken in the field were still in progress (Plate 2). The first harvest has been undertaken at both sites, 10 weeks after sowing. Consistent with the above experiment conducted in the laboratory, few nodules were evident on any plants at that time and there was no difference in seedling biomass between the inoculated and uninoculated treatments, indicating that more time is required to complete that evaluation. Early indications at the Cowra site suggested a positive response to lime in sainfoin, irrespective of inoculation treatment, but at the Wagga Wagga site there has been little response to lime in seedling sainfoin. The experiments continue to be monitored.

Plate 2. The sainfoin nodulation validation experiment presently underway at the Wagga Wagga Agricultural Institute (photo taken September 2022).

4.7 Bioeconomic modelling

The Guyra and Orange sites had a higher potential carrying capacity than Merrill and Dry Plain, but the carrying capacity increased at all sites with increased lime and P-fertiliser inputs (Table 13). The model simulates the initial flock adjusting to the identified level over the simulation horizon. Full adjustment takes around 4 years for flock size reductions and up to 6 years for doubling the flock size.

Table 13: Sustainable flock sizes identified for each system and site as a proportion of the initial starting flock size of 3017 Merino ewes or 6 ewes ha^{-1}.

Site	Nil Input	Low Input	High Input
Guyra	0.6 (0.7)	1.1 (1.2)	1.95 (2.0)
Orange	0.6 (0.7)	1.1 (1.2)	1.95 (2.0)
Merrill	0.5 (0.6)	1.0 (1.1)	1.65 (1.7)
Dry Plain	0.5 (0.6)	0.9 (1.0)	1.55 (1.6)

Note: proportion in parentheses indicates direction of a potentially more economically optimal flock size with trade-offs on factors such as soil erosion, white clover persistence, biomass and risk (production & economic).

The proportion of area occupied by white clover varied over the 10-year simulation horizon (Fig. 22). The proportion of white clover responded to both soil fertility and grazing. Under excessively high stocking rates the proportion of white clover declined to minimal levels and only recovered when re-sowing was triggered under the low and high input systems at each site. However, under the identified sustainable flock sizes, the proportion of white clover was somewhat maintained (with significant variation around the mean), especially once re-sown. As Orange and Guyra had a higher starting proportion of white clover (based on field experiment data), less paddocks were re-sown early in the simulation period (the reverse applied to Merrill and Dry Plain), and much higher proportions were maintained in the high input system compared to the low input system (partly a soil fertility effect). The curvilinear response at the Guyra and Orange sites for the no input system reflects the effect of rapidly reducing stocking rates as the flock size adjusts early in the simulation period. In the long-term, the proportion of white clover declined to lower levels than the low and high input systems due to declining soil fertility and selective grazing.

At Merrill and Dry Plain, the proportion of white clover had a more linear decline under the nil system, which reflects the limited impact of reducing stocking rates on white clover persistence, and which is much more related to climatic variability and available soil water. Under the low and high input systems, significant areas of the farm are re-sown from years 3-5, which is indicated by the rapid increase in the mean proportion of white clover at Merrill and Dry Plain. The triggering of re-sowing is a result of the lower staring proportion of white clover and the effects of a more variable climate.

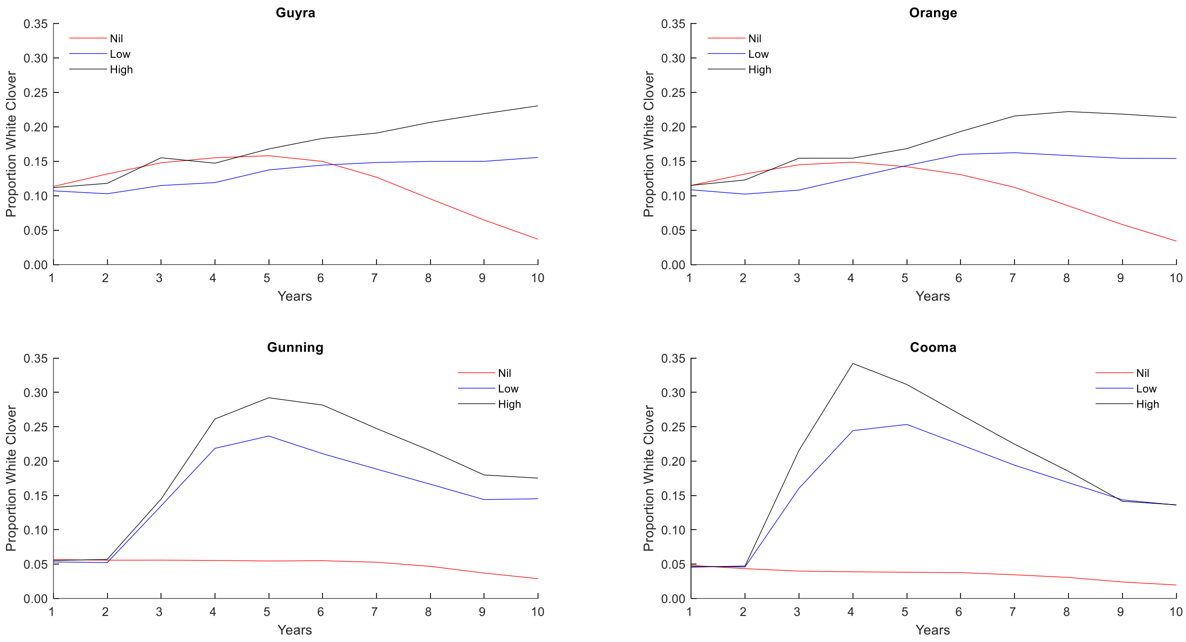

Fig. 22. Trajectories of mean proportion of white clover under nil, low and high input systems for the Guyra, Orange, Merrill (Gunning) and Dry Plain (Cooma) sites.

The trajectories of mean pasture biomass over the simulation period are presented in Fig. 23. For all sites and systems there is large variation around the mean which reflects the biomass-based grazing management rules, occurrence of re-sowing, and the selected sustainable flock sizes. For Guyra and Orange, the overall means for all systems is maintained at a higher level than for Merrill and Dry Plain. Under all systems the nil system initially maintains a higher mean biomass although this rapidly declines during the second half of the simulation period due to declining soil fertility constraining pasture growth. The substantial dip in mean biomass for the low and high input systems during years 2-5 of the simulation at Merrill and Dry Plain indicate the effect of significant areas of the farm being re-sown. This is primarily due to white clover not persisting as well in those environments and the initial proportion of white clover being much lower.

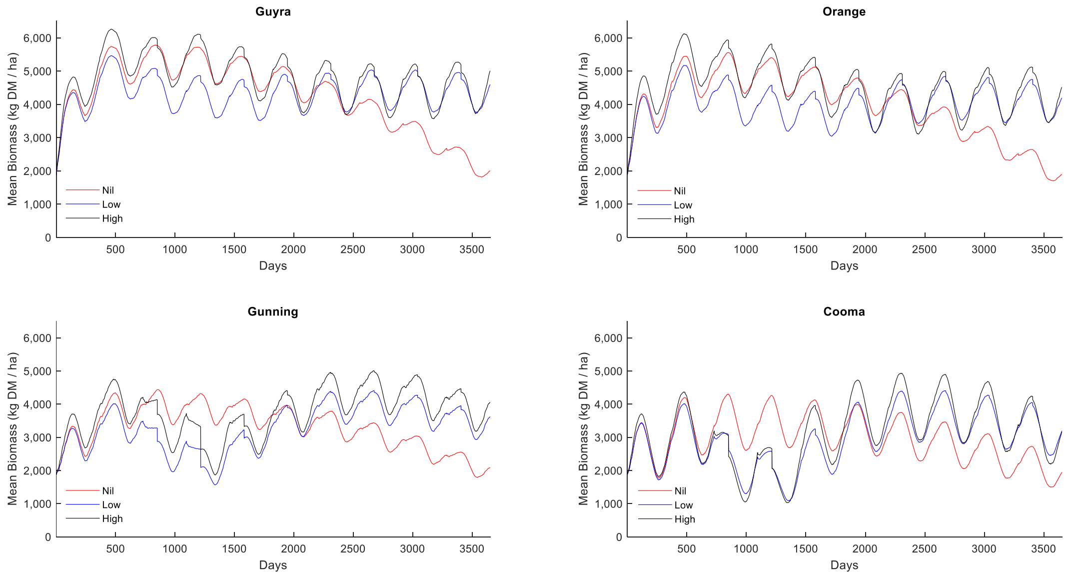

Fig. 23. Trajectories of mean pasture biomass (kg DM/ha) under nil, low and high input systems for the Guyra, Orange, Merrill (Gunning) and Dry Plain (Cooma) sites.

Livestock productivity, in terms of both sheep meat (Fig. 24) and wool production (not shown), varied as expected in response to any changes in flock size. With the biomass-based grazing management and supplementary feeding rules used, and the sustainable stocking rates chosen to model the systems, there were little differences between systems in terms of production per head. Although, during the recursive modelling to identify the sustainable flock size there were substantial differences within systems in response to stocking rates, including significant changes in production risk. The results indicate that both sheep meat and wool production in the high input system was 3-4 times higher than the nil, and the low input system was nearly 2 times higher than the nil.

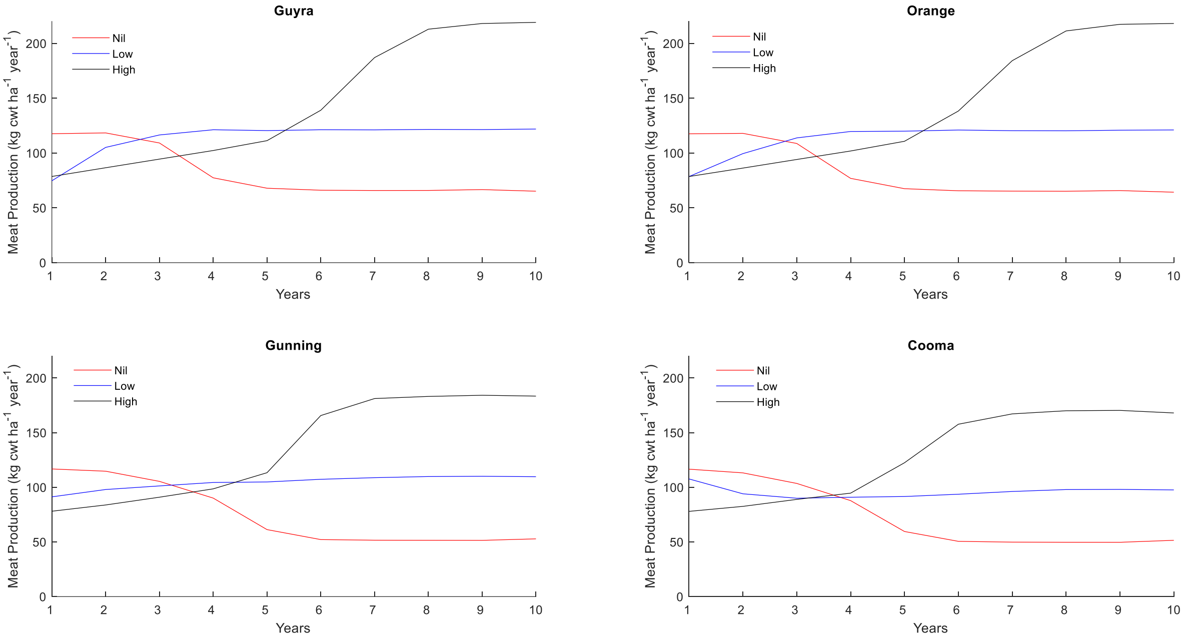

Fig. 24. Trajectories of mean sheep production (kg carcass weight/ha/year) under nil, low and high input systems for the Guyra, Orange, Merrill (Gunning) and Dry Plain (Cooma) sites.

Across all sites and simulated years, the high input systems had the highest GM, followed by the low input, with the nil having the lowest GM (Fig. 25). The high input systems also had significantly higher levels of variability, especially downside variability. This downside variability is partly explained by the large investments into fertiliser and lime at each site, especially where they coincide with drought years.

Between the sites, Orange was the only location to maintain positive GM for all systems, with Dry Plain having the lowest GM. As price risk was not considered in this analysis, the variability in GM shown are the result of capital investments in fertiliser and lime (especially under low and high input systems), the interactions between climate, pasture productivity and quality, and the effects of soil fertility. Across all simulated years, the median marginal gain from adopting a high input system above that of the nil varied from $765/ha at Guyra to $720/ha at Dry Plain. The marginal gain from adopting the high input system above that of the low input system varied from $461/ha at Dry Plain to $410/ha at Orange. The nil input systems across all sites consistently produced positive, albeit, low GM with minimal upside or downside risk.

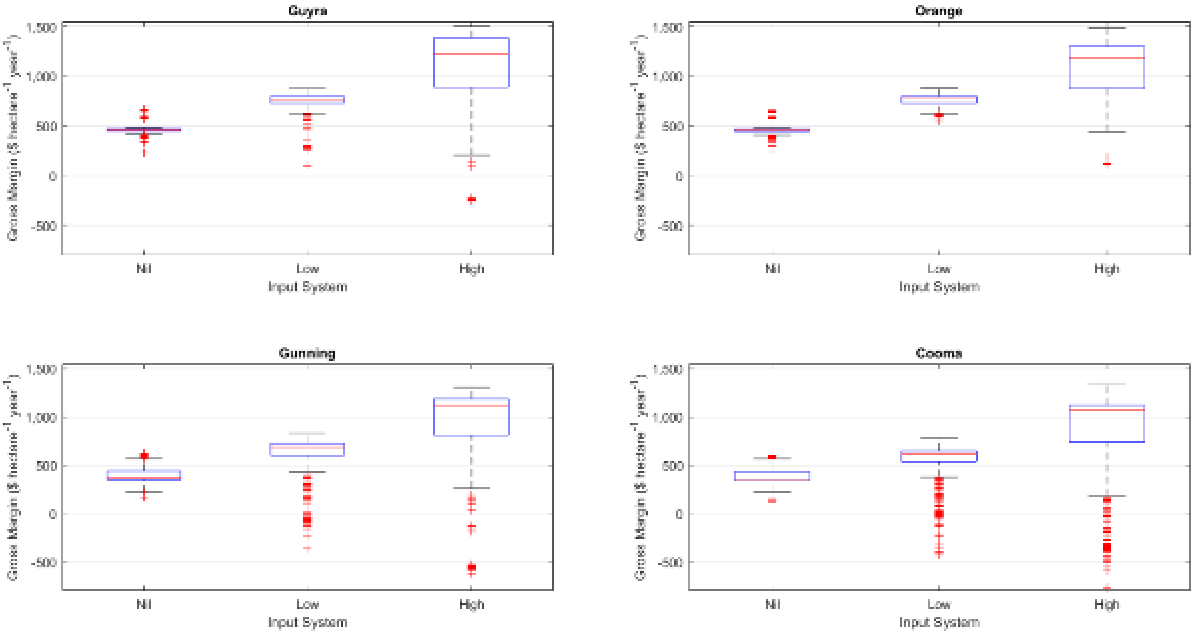

Fig. 25. Box plots of Gross Margin per hectare ($ ha/year inclusive of fertiliser, lime and pasture re-sowing costs) under nil, low and high input systems for the Guyra, Orange, Merrill (Gunning) and Dry Plain (Cooma) sites.

As GM does not indicate the whole farm implications of different input systems and changes in flock sizes in response to changing soil fertility, trajectories of mean whole farm annual cash flow are shown in Fig. 26. These clearly indicate the short-term gain from reducing the flock size under nil systems at each site during the first 4-5 years, but with a resulting lower long-run annual cash flow. The high input systems have the lowest (i.e. negative) cash flows in the first year due to substantial capital investments in fertiliser and lime, as well as reduced income from the retention of breeding females (especially for high input systems). Despite these initial cash flow deficits, equity levels tend to remain above 80% across the low and high input systems with a negligible risk of insolvency (data not shown). In most instances, the high input system achieves the highest cash flow by years 5 (Merrill, Dry Plain) to 6 (Guyra, Orange). The delayed increase in annual cash flows at Merrill and Dry Plain is partly due to the cost of substantial re-sowing of pastures in a larger proportion of years, as well as the costs of supplementary feeding if low rainfall years coincide with substantial re-sowing.

In the long-run, the nil systems are expected to generate annual cash flows of around 15-20% of the level expected to be achieved under the high input systems, and the low input systems will generate around 50% of what is expected to be achieved under the high input system. The highest long-run cash flows are generated at Orange and Guyra ($525-565k p.a.), followed by Merrill (~$465k p.a.), with Dry Plain having the lowest ($420k p.a.). This indicates the difference between the agro-ecological zones in terms of exposure to climatic variation and its interaction with pasture productivity, persistence and quality, and subsequent profitability. The results correspond well to each regions climate, as Dry Plain (Cooma) has the lowest long-term average rainfall in addition to being the most variable climatic zone, whereas Orange and Guyra have the highest rainfall and with less variability, based on historical rainfall patterns.

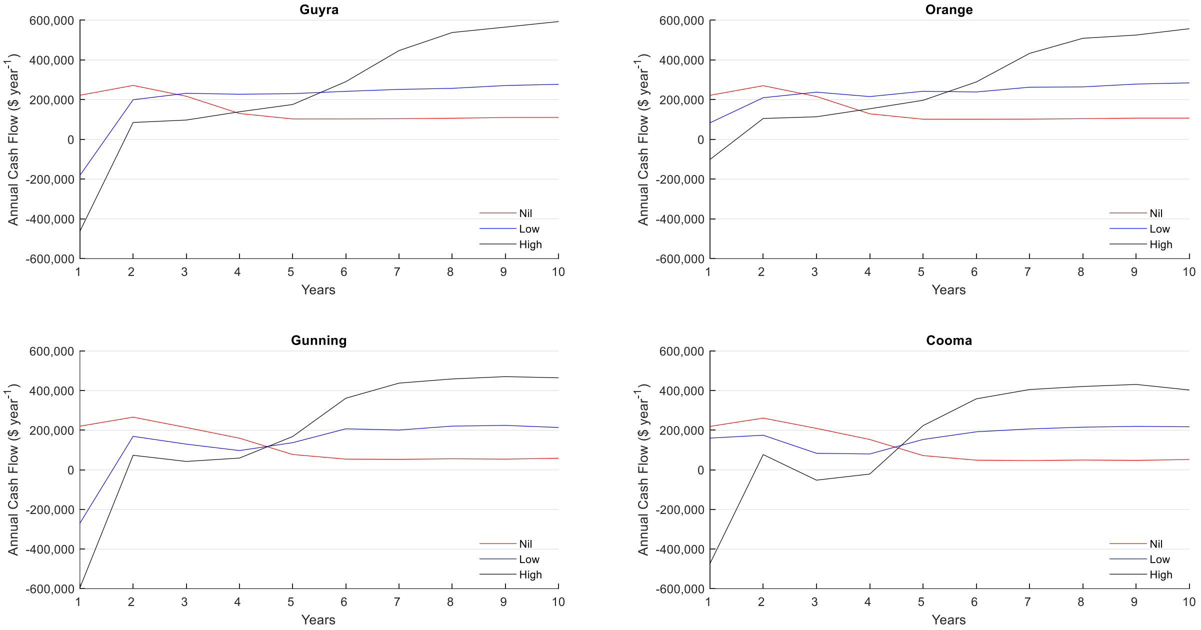

Fig. 26. Trajectories of mean whole farm annual cash flows ($/year) under nil, low and high input systems for the Guyra, Orange, Merrill (Gunning) and Dry Plain (Cooma) sites.

5 Conclusion

5.1 Key findings

5.1.1 Legume choice

The Project highlighted the importance of white clover in permanent pasture regions of south-eastern Australia. Few viable alternative perennial legume species exist, particularly on shallow, acidic and low fertility soils. Even white clover presently lacks the drought-tolerance necessary in many environments in the target region. The Project demonstrated the importance of seedling recruitment for the long-term persistence of white clover, which in the survey of farms in northern NSW, was shown to be improved with higher levels of soil P fertility. The review of literature conducted at the commencement of the Project identified that white clover seedling recruitment has had little attention paid to it in previous cultivar improvement programs (Hayes et al. 2019). Yet, the series of glasshouse experiments demonstrated that white clover had little capacity to conserve water under periods of moisture stress, even at higher soil fertility. Consequently, the persistence of white clover in the face of periodic drought must be reliant upon adequate seed reserves in soil as plant mortality is inevitable in this species. Concerningly, over 90% of surveyed farmers on the Northern Tablelands were unaware of the importance of seedling recruitment for the persistence of white clover and never consciously rest their pastures in spring to maximise seed-set. We believe a large opportunity exists to improve white clover performance and long-term persistence by raising awareness among growers of the importance of seed production and seedling recruitment in this species. There also exists opportunity to substantially expand the zone of adaptation of this important species by focusing on traits essential for successful seedling regeneration in the target environment. In the evaluation of legume persistence in grasslands of south-eastern Australia (Hayes et al. 2022) we propose that subterranean clover presents as a model ideotype to define seed characteristics, such as hard seed levels and breakdown patterns, that may be applied to white clover in much the same way it is being applied to serradella species. Further white clover cultivar development should also pay attention to the compatibility with different strains of rhizobia. Our study demonstrated that cultivars such as Haifa and Trophy, as well as cultivars of some other species, such as Palestine strawberry clover, formed effective symbiosis with the strain used for annual clovers that is commonly naturalised in many soil environments. Farmers may be well advised to select cultivars with these attributes in future pasture renovation programs.

5.1.2 Forage quality

A key objective of the project was to examine the effect of soil fertility on herbage quality. A key motivation for this component of research was the concern that increasing soil fertility may have adverse effects on the anti-nutritional characteristics of the forage. For example, high levels of K in forage is implicated with elevated risks of metabolic disorders such as grass tetany or milk fever. If the use of K-fertiliser increased the K concentration of herbage, what would that do to the risk profile of the forage for vulnerable livestock?

Analysis of a large number of samples from multiple sites over the project period showed that K-fertiliser did indeed increase the K concentration of phalaris herbage. However, there was no instance where soil treatment increased values beyond nominal risk thresholds, based on standard indices and mineral ratios commonly used to assess risk. Risk factors were influenced more by site effects. Similarly, the addition of S-fertiliser had little effect of the dietary cation-anion difference compared to the large impact of site on the DCAD of forage. These findings help allay concerns that the use of these fertilisers will likely increase the risk profile of phalaris pastures but we note that Holland et al. (2018) observed the forage of grazing wheat marginally exceeded the tetany index threshold in late winter at the highest K-fertiliser rate at one site in one year, so acknowledge there may be significant variation in response on different crops, at different sites, and even, from year to year, of which managers should be mindful.

Beyond the antinutritional characteristics, improved soil fertility was shown to increase the mineral density of the forage. Not only did the concentration of the specific mineral in the fertiliser increase in the forage compared to where no fertiliser was applied, but it was common for the concentration of other essential nutrients to also increase. For example, the application of P fertiliser also led to increases in forage Na, K, Mg, Cl, S, Mo and Mn concentrations. The application of S fertiliser led to corresponding increases in the P, Mg, Mn and Mo concentrations. The implications of these changes, in terms of livestock productivity and meat quality, deserve further study but the results highlight that mineral nutrition is a complex field of research where many factors can be impacted by the addition of a single nutrient. This likely explains why we observed no elevated risk of metabolic disorder in livestock with fertiliser application, based on established thresholds, as was initially anticipated. Positive effects of other forage quality characteristics were observed to be associated with soil treatment but these tended to be very site-specific, making broad generalisations difficult.

5.1.3 Nitrogen fixation

There was little evidence in this project of improved soil nutrition leading to greater levels of N fixation in legumes, except for the one example at the Merrill site where a positive response to lime was observed. There were also few treatment effects observed in N_2 fixation of white clover in the glasshouse experiment. Low levels of legume biomass in the field experiments during the early years of the project likely masked treatment effects on N-fixation. Previous work has shown that differences in N_2 fixation result from variations in the proportion of the legume-N derived from atmospheric N_2 (%Ndfa) and/or the amount of legume-N accumulated during growth (Peoples et al. 2012). The accumulation of legume-N relates primarily to the legume content and net productivity of the pasture. Insofar as improved soil fertility improved legume persistence across several project activities, we conclude there was a positive relationship between soil fertility and N_2 fixation but we found little difference in %Ndfa.

5.1.4 Economic analysis

The economic analysis undertaken in this project provides evidence of the financial benefits in investing in better soil fertility, specifically lime and superphosphate. Farms operating at higher levels of soil fertility were more profitable and up to 4 times more productive compared to farms adopting a nil input approach. However, the analysis highlighted the difference in financial outcomes across the 4 different environments examined in our study. Financial benefits of investing in soil fertility was greater in higher rainfall environments compared to drier environments. The lower potential returns achieved in the alpine Dry Plain environment, near Cooma, helps to crystalise the particular challenges that the Monaro environment poses. Its lower rainfall and cold winters lead to highly variable pasture production. Experience with field experiments in that region affirms the greater challenges in establishing pastures in that environment and a need for strategies to help assure livestock production in the face of such variability. This is one environment where the broader adoption of white clover holds great promise, to better utilise temperature and rainfall conditions conducive to pasture growth over the summer months compared to winter-growing annual legumes. However, the success of white clover in the Monaro region will still be dependent upon improving white clover seedling recruitment, as described previously.

5.1.5 Benefits to industry

The project reinforces the importance for livestock producers in permanent pasture regions of south-eastern Australia to invest in better soil fertility, especially phosphorus and lime. Higher soil fertility increases pasture biomass and potential stocking rate, which our modelling indicated would drive up profitability, compared to where nil or lower levels of lime and single superphosphate are used. The project helps allay concerns that the use of K and S fertilisers may lead to higher risk forages for livestock vulnerable to metabolic disorders, with no evidence of either fertiliser increasing nutrient ratios or indicative health indices beyond established risk thresholds.

The project highlights the lack of viable legume alternatives for use in permanent pasture environments. Lucerne is the most productive legume species, but its zone of adaptation is confined to deep, fertile soil not constrained by toxins associated with soil acidity. Red clover and strawberry clover may also be suited to some of these higher-fertility environments. In poorer soil types, white clover is the best perennial legume option available, but it lacks persistence, particularly under dry seasonal conditions.

White clover persistence was improved with higher levels of soil fertility, which we demonstrated was associated with improved soil seed reserves and seedling recruitment. Where white clover is already used in permanent pastures, such as higher or summer-dominant rainfall environments, its persistence will likely be improved by management strategies to increase seed production, such as rotational grazing, strategic rest periods and improved soil fertility. The farmer survey highlighted a lack of awareness among experienced growers of the importance of seed production in the long-term persistence of white clover. Beyond the existing boundaries of white clover adaptation, the potential of white clover will only be realised with further cultivar development that focuses on traits to improve seedling recruitment.

There was no clear advantage in persistence of ‘elite’ white clover cultivars that were developed for improved ‘drought tolerance’, likely because drought tolerance of this species is reliant upon seedling recruitment rather than plant longevity, which is what those elite cultivars were selected for. Rather, the best performing cultivar across a dozen sites, Haifa, was demonstrated to form effective symbiosis with background rhizobia. The importance of this trait is highlighted by the field study that showed only a minority of the rhizobia infecting perennial legume nodules in the field were the strain with which the seed was inoculated. This experience is a reminder of the importance of good inoculation practices immediately prior to sowing.

One clear benefit to industry of this project was the re-establishment of a commercial source of sainfoin inoculant in Australia. Without inoculant, any future trialling of this species was destined to fail. The future role of this species in Australia is uncertain, but it is actively being researched for its potential in low-methane pastures, as a novel forage for honey-bees and as a potential perennial crop.

6 Future research and recommendations

6.1 Research and Development

Three clear priorities emerge from this project for further R&D:

1. Cultivar development of white clover, with an emphasis on traits to improve seedling recruitment in the target environment, such as seed production, hard seed content and pattern of hard seed breakdown. It is noted that whilst we now have a reasonable understanding of the traits required for successful regeneration in the Southern Tablelands of NSW (see Appendix 2), those traits may not be transferable to other permanent pasture environments. We highlight the Monaro region as a priority target environment due to the highly challenging conditions for pasture growth, the paucity of legume options available and the relative importance of pasture growth over summer to drive production. Any future cultivar development initiative should also be mindful of the importance of compatibility with background rhizobia to enhance white clover production over a range of environments.
2. Further exploration of the consequences to livestock production of more nutrient-dense forages, associated with increased fertiliser use. This topic has been identified previously by other research groups (for example, Masters et al. 2019) and was identified as a priority in the present project due to the broad impact that a particular fertiliser may have on a wide range of essential nutrients. It is also not known whether a more mineral-dense forage would lead to more mineral-dense meat, or whether other health-claimable benefits may exist by producing livestock from better-fertilised pastures. A coordinated, multidisciplinary approach encompassing agronomy, livestock production and meat science is warranted, to develop a comprehensive understanding of mineral balance as it relates to forages, livestock and the end product.
3. Investigation of the function of non-rhizobial nodule occupants in nitrogen fixation of legumes. The culture collection amassed from the sampling of the Mandurama site provides

a start on this emerging topic. A better understanding of the microbial diversity within legume nodules may assist in fostering more reliable N_2 fixation in legumes, or to improve symbiotic competence in legumes in which poor symbiosis is suspected as a reason for non-performance, such as Caucasian clover or sainfoin, perhaps.

6.2 Extension and adoption activities

This project informs adoption on several fronts:

1. Lime and fertiliser use. The information generated in this project helps assure producers of the benefits of ongoing use of lime and fertiliser. Improved pasture biomass and legume composition lead to higher potential stocking rates and greater financial returns. Improved mineral density of forage is one more reason why growers might want to apply fertiliser, although the benefits are yet to be fully understood in livestock production. Messaging around fertiliser use is important as growers are increasingly faced with alternative fashionable mantra, badged under ‘regenerative’ or other guises, that sometimes serve to discourage use of fertiliser in livestock production systems.
2. White clover persistence. As a result of this project, we have a sound understanding of the mechanisms driving white clover persistence under drought. The project highlighted a lack of awareness among experienced producers of the importance of white clover seed production and seedling recruitment for persistence.
3. Legume choice. This project highlights the paucity of viable legume options suited to permanent pasture environments, informing species choice for producers undertaking pasture renovation. Included in this is cultivar choice for species such as white clover where options with demonstrated compatibility with background rhizobia exist.
4. Legume inoculation. The project reminds us of the importance of good seed inoculation prior to sowing legumes, particularly when sowing alternative species (such as perennial legumes) in environments where there is a high background population of rhizobia.
5. Farmer case studies. In conjunction with the farmer survey conducted in northern NSW, opportunity exists to develop farmer case studies to highlight how these principles apply on commercial farms, showing other farmers how it can all work in practice.

References

Archer KA, Rochester IJ. (1982). Numbers and germination characteristics of white clover seed recovered from soils on the northern tablelands of New South Wales after drought. *Journal of the Australian Institute of Agricultural Science,* **48**, 99-101.

Behrendt K, Cacho O, Scott JM, Jones R. (2013). Optimising pasture and grazing management decisions on the Cicerone Project farmlets over variable time horizons. *Animal Production Science,* **53**, 796-805. doi:http://dx.doi.org/10.1071/AN11174

Behrendt, K, Liu, H, Kemp, DR, Takahashi, T (2020a) Sustainability modelling of grassland systems. In 'Sustainable Chinese Grasslands.' (Ed. DR Kemp.) Vol. MN210 pp. 97-124. (Australian Centre for International Agricultural Research: Canberra, ACT)

Behrendt, K, Liu, H, Takahashi, T, Kemp, DR (2020b) 'StageTHREE Sustainable Grassland Model (v1.08) – Model description and users guide.' (Australian Centre for International Agricultural Research: Orange, Australia)

Behrendt K, Takahashi T, Kemp DR, Han G, Li Z, Wang Z, Badgery W, Liu H. (2020c). Modelling Chinese grassland systems to improve herder livelihoods and grassland sustainability. *The Rangeland Journal,* **42**, 329-338.

Boyer, JS (1995) 'Measuring the Water Status of Plants and Soils.' (Academic Press: San Diego, Ca.).

Cocks PS (1980). Limitations imposed by nitrogen deficiency on the productivity of subterranean clover-based annual pasture in southern Australia. *Australian Journal of Agricultural Research* **31**: 95-107.

Dear BS, Ewing MA. (2008). The search for new pasture plants to achieve more sustainable production systems in southern Australia. *Australian Journal of Experimental Agriculture,* **48**, 387-396.

Donald G. (2012). *Analysis of feed-base audit. Project B.PAS.0297 Final Report*. Meat and Livestock Australia Ltd, North Sydney.

Gerard PJ, Aalders LT, Hardwick S and Wilson DJ (2022). Investigation into the contrasting production of eight perennial clover cultivars in the first two years at field sites in in Waikato and Canterbury. *New Zealand Journal of Agricultural Research* **65** (4-5): 271-289.

Hayes RC, Ara I, Badgery WB, Culvenor RA, Haling RE, Harris CA, Li GD, Norton MR, Orgill SE, Penrose B, Smith RW. (2019). Prospects for improving perennial legume persistence in mixed grazed pastures of south-eastern Australia, with particular reference to white clover. *Crop and Pasture Science,* **70**, 1141-1162. doi:https://doi.org/10.1071/CP19063

Hayes RC, Newell MT, Li GD, Haling RE, Harris CA, Culvenor RA, Badgery WB, Munday N, Price A, Stutz RS, Simpson RJ (2022) Legume persistence for grasslands in south-eastern Australia. *Crop & Pasture Science* **in review.**

Hayes RC, Li GD, Conyers MK, Virgona JM and Dear BS (2016). Lime increases productivity and the capacity of lucerne (*Medicago sativa* L.) and phalaris (*Phalaris aquatica* L.) to utilise stored soil water on an acidic soil in south-eastern Australia. *Plant and Soil* **400** (1): 29-43.

Hoffmann JD, Eberbach PL, Virgona JM, Katupitiya A. (2003). *Conservative water use by lucerne.* Paper presented at the 11th Australian Agronomy Conference, "Solutions for a new environment", 2-6 Feb, Geelong.

Hofmann, RW, Lin, W, Stilwell, SA, Lucas, RJ (2007) 'Comparison of drought resistance in strawberry clover and white clover, Proceedings of the New Zealand Grassland Association.'

Holland JE, Hayes RC, Refshauge G, Poile GJ, Newell MT, Conyers MK. (2018). Biomass, feed quality, mineral concentration and grain yield responses to potassium fertiliser of dual-purpose crops. *New Zealand Journal of Agricultural Research,* **62**, 476-494. doi:10.1080/00288233.2018.1512505

Kemp DR, Michalk DL and Goodacre M (2002). Productivity of pasture legumes and chicory in central New South Wales. *Australian Journal of Experimental Agriculture* **42** (1): 15-25.

Lattimore MA, McCormick L. (2012). Pasture varieties used in New South Wales 2012-13. In. New South Wales: Published jointly by NSW Department of Primary Industries and the Grassland Society of NSW Inc.

Masters DG, Norman HC, Thomas DT. (2019). Minerals in pastures—are we meeting the needs of livestock? *Crop and Pasture Science,* **70**, 1184-1195. doi:https://doi.org/10.1071/CP18546

Norton MR, Li GD, Xu B, Price A, Tyndall P, Hayes RC. (2021). Differences in dehydration tolerance affect survival of white clover (*Trifolium repens*) and lucerne (*Medicago sativa*) during a drying cycle. *Crop and Pasture Science,* **72**, 723-730.

Peoples MB, Brockwell J, Hunt JR, Swan AD, Watson L, Hayes RC, Li GD, Hackney B, Nuttall JG, Davies SL, Fillery IRP. (2012). Factors affecting the potential contributions of N_2 fixation by legumes in Australian pasture systems. *Crop and Pasture Science,* **63**, 759-786. doi:http://dx.doi.org/10.1071/CP12123

Rigg JL, Webster AT, Harvey DM, Orgill SE, Galea F, Dando AG, Collins DP, Harris CA, Newell MT, Badgery WB, Hayes RC. (2021). Cross-host compatibility of commercial rhizobial strains for new and existing pasture legume cultivars in south-eastern Australia. *Crop and Pasture Science,* **72**, 652-665.

Tothill JC, Hargreaves JN, Jones RM, McDonald CK (1992) BOTANAL—a comprehensive sampling and computing procedure for estimating pasture yield and composition. 1. Field sampling. Tropical Agronomy Technical Memorandum. **78**(24).

Unkovich M, Herridge DF, Peoples M, Cadisch G, Boddey R, Giller K, Alves B, Chalk P. (2008). *Measuring plant-associated nitrogen fixation in agricultural systems*: Australian Centre for International Agricultural Research (ACIAR) GPO Box 1571, Canberra ACT 2601, Australia.

Appendix 1. Review of literature

The copyright of this manuscript is owned by *Crop and Pasture Science* and cannot be published as part of this report. However, the full publication can be downloaded from the *Crop and Pasture Science* website, provided below.

Hayes RC, Ara I, Badgery WB, Culvenor RA, Haling RE, Harris CA, Li GD, Norton MR, Orgill SE, Penrose B, Smith RW. (2019). Prospects for improving perennial legume persistence in mixed grazed pastures of south-eastern Australia, with particular reference to white clover. *Crop and Pasture Science,* **70**, 1141-1162. doi: https://doi.org/10.1071/CP19063

Appendix 2. Legume persistence

The following manuscript has been submitted to Crop and Pasture Science and, at the time of writing, was still in review. It cannot be published as part of this report to avoid jeopardising publication in the Journal, which is necessary to enable data to be more broadly discoverable and available into the future. In the meantime, it is appended to this report for information only. Citation details can be published to aid readers in locating the article when it becomes available. The paper is also scheduled to be presented at the 2023 Australian Grasslands Association Symposium.

Hayes RC, Newell MT, Li GD, Haling RE, Harris CA, Culvenor RA, Badgery WB, Munday N, Price A, Stutz RS, Simpson RJ (2022) Legume persistence for grasslands in south-eastern Australia. *Crop & Pasture Science* **in review.**

Appendix 3. Pasture survey in northern NSW

The following manuscript has been prepared for submission to *Grass and Forage Science*. It cannot be published as part of this report to avoid jeopardising publication in the Journal, which is necessary to enable data to be more broadly discoverable and available into the future. In the meantime, it is appended to this report for information only. Citation details can be published to aid readers in locating the article when it becomes available.

Harris CA, Faithful A, Hayes RC (2022). Sown pasture persistence during and post drought on the Northern Tablelands of NSW with an emphasis on white clover: A survey. *Grass and Forage Science* **In preparation**.

Appendix 4. First glasshouse experiment, 2019

The copyright of this manuscript is owned by *Crop and Pasture Science* and cannot be published as part of this report. However, the full publication can be downloaded from the *Crop and Pasture Science* website, provided below.

Norton MR, Li GD, Xu B, Price A, Tyndall P, Hayes RC. (2021). Differences in dehydration tolerance affect survival of white clover (*Trifolium repens*) and lucerne (*Medicago sativa*) during a drying cycle. *Crop and Pasture Science,* **72**, 723-730. https://doi.org/10.1071/CP20300

Appendix 5. Cross-host compatibility study

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Rigg JL, Webster AT, Harvey DM, Orgill SE, Galea F, Dando AG, Collins DP, Harris CA, Newell MT, Badgery WB, Hayes RC. (2021). Cross-host compatibility of commercial rhizobial strains for new and existing pasture legume cultivars in south-eastern Australia. *Crop and Pasture Science,* **72**, 652-665. https://doi.org/10.1071/CP20234

Appendix 6. CC1099 Certificate of release, 2022

The certificate details the strain of rhizobia now commercially available in Australia, in part a result of efforts undertaken in this project.

Appendix 7. Bioeconomic analysis

The following report is the basis for a scientific journal article currently being prepared for submission to *Crop and Pasture Science*. It cannot be published as part of this report to avoid jeopardising publication in the Journal, which is necessary to enable data to be more broadly discoverable and available into the future. In the meantime, it is appended to this report for information only. Readers can contact the Project Leader (richard.hayes@dpi.nsw.gov.au) to request further details if required.