The Trouble with Sub Project Update

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Take home messages:

- The survey found that subterranean clovers are poorly nodulated in the pastures of the medium to high rainfall zones of the Riverina region.
- Many of the soils sampled were affected by acidity particularly in the sub soil.
- Few landholders appear to be applying the important (for nitrogen fixation by legume) micro nutrient molybdenum.
- The rhizobia present in the soil are by large of an effective strain and are also generally fixing atmospheric nitrogen.

Project background

Subterranean clover and its associated rhizobium, *Rhizobium leguminosarum* bv. *trifolii,* have added greatly to soil fertility and animal production systems in southern Australia. Australian soils are relatively low in fertility and the use of annual legumes in grazing systems increases the production of the system via the extra nitrogen entering the soil that subsequently is available for non-fixing plants. Annual legumes, particularly subterranean clover, improve the quality of the feedbase in terms of digestibility, palatability and protein content, which in turn increases both overall carrying capacity and individual livestock growth rates.

It has been estimated that legume fixed nitrogen contributes approximately $AUS4 billion to the Australian economy. The cost of nitrogen fertiliser is predicted to increase over time, thus resulting in the increasing importance of legume fixed nitrogen to our agricultural system.

From the 1970's there have been increasing reports of general pasture legume decline. Many mechanisms have been suggested for this apparent overall decline. Some of the main factors implicated include increasing soil acidity, use of pesticides toxic to rhizobia, dilution of effective rhizobia types in the soil with ineffective types and the declining use of molybdenum fertilisers.

The relationship between the legume species and rhizobia strain is generally very specific. In the case of subterranean clover, the various strains of the *Rhizobium leguminosarum* bv. *trifolii* are the bacteria to form this symbiosis. It has been noted that low soil pH and high aluminium levels can interfere with the persistence of rhizobia and its ability to infect the host plants.

The *Trouble with Sub* is a survey project that was undertaken in 2015 to determine whether subterranean clover was in decline across the Riverina region. The project was managed by Harden Murrumburrah Landcare Group in partnership with Riverina Local Land Services, other local Landcare and farmer groups, and the CRS at Murdoch University. This project worked closely with other relevant Meat and Livestock Australia (MLA) and Grain Research and Development Corporation (GRDC) funded projects. This project was funded by the Australian Government under the National Landcare Program.

Project aims

The project aims were:

- To determine if there were measurable growth and nodulation issues with pasture legumes in medium/high rainfall areas of the Riverina.
- To undertake a preliminary determination of the factors that may be contributing to this decline (i.e. soil restrictions, inadequate nodulation and/or the lack of effective rhizobia in the soil).

Survey process

Over 80 paddocks containing subterranean clover were surveyed from Wagga, Lockhart, Coolamon, Bland, Temora, Junee, Cootamundra, Gundagai, Young, Harden and Tumut Shires (Figure 1). The paddocks ranged from long to short term pastures in rotation with crops. The date the paddocks had been originally sown ranged from paddocks sown in the 1950's (with clover regenerating each year), to new paddocks last sown down in 2014. Over 50 percent of the paddocks sampled had been sown since 2000, Table 1.

Figure 1. Survey area showing average nodule ratings and pH ranges.

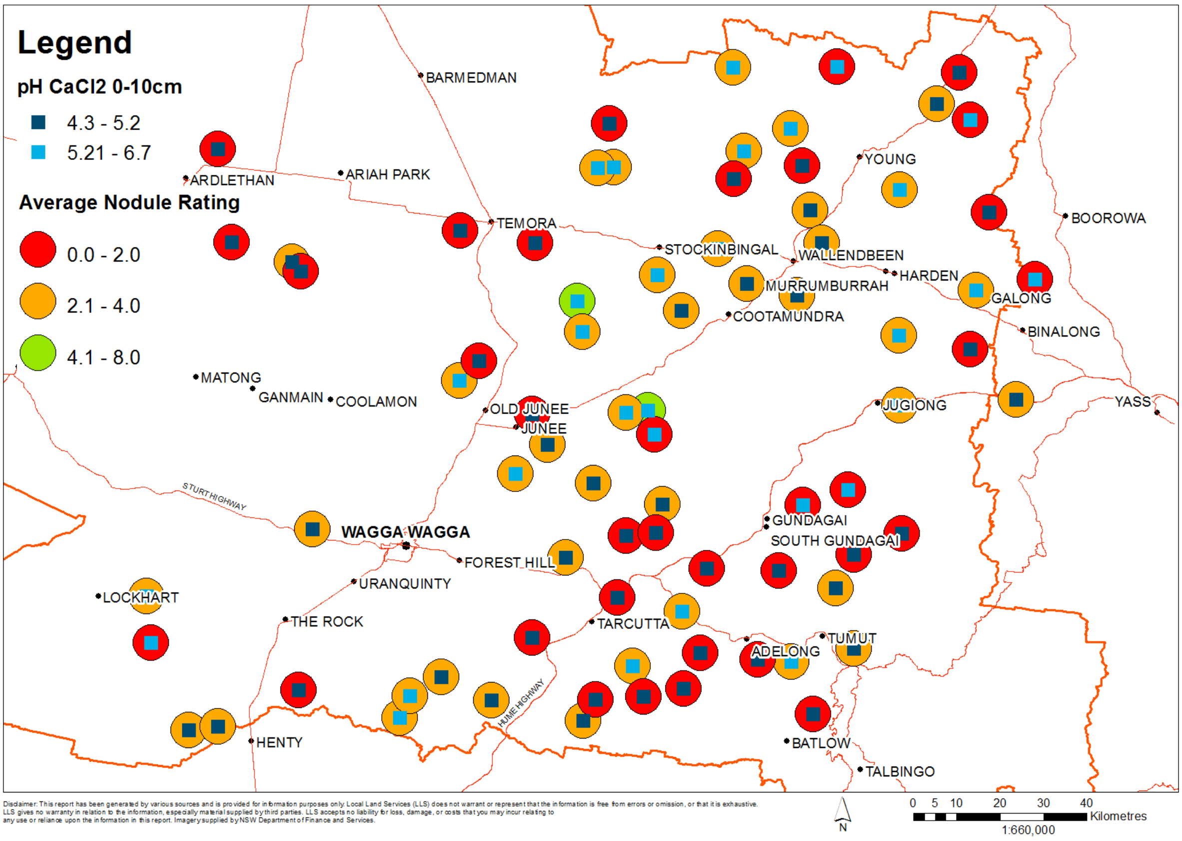


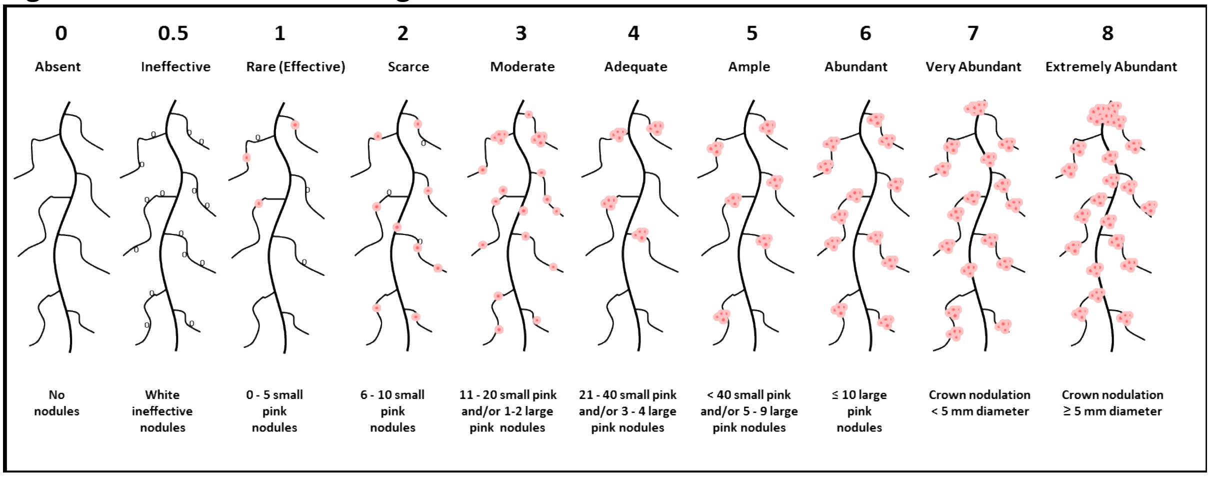
Table 1. Samples and nodule scores.

Year Sown	Sample (n)	Av. Nodule Score
1950s	4	2.31
Unsure*	9	1.8
1990-99	10	1.64
2000-2009	13	2.04
2010 – 2015	30	2.73

***Some paddock histories to be collected**

The first paddock sampling occurred about 8 - 10 weeks after the autumn break, starting in the last week of July 2015. Information collected at this sampling included scoring the root nodules for presence, number, colour and size (Figure 2). Nodules were also collected for strain identification. The collected nodules were placed in desiccating vials and sent to Murdoch University for analysis using the novel MALDI-ID approach. The MALDI technique was compared to DNA based methods and was effective in identifying the rhizobia strains taken from the nodules. The MALDI method provides quick identification and a high laboratory throughput making the technique relatively accurate and cost effective compared to more tradition identification methods.

Figure 2. Score card for scoring root nodules.



A soil sample was also collected for comprehensive soil testing at the 0 - 10 cm depth. A second less comprehensive soil test was used on soil samples collected from the 10 - 20 cm depth. The landholders supplied paddock histories, including information such as fertiliser, chemical usage and cropping history. Pasture composition from the sample sites was also recorded (Table 2).

Table 2. Pasture composition for sample sites.

Pasture Composition	%
Ann Grasses	24
Peren Grass	11
Native Grass	3

Legumes	48
Peren Broadleaf	3
Broadleaf Sps	9
Bare ground	0

The second paddock sampling occurred in mid-October and was timed to occur at approximately the time of peak spring pasture production. The purpose of this visit was to collect above ground leaf tissue samples for nitrogen fixation effectiveness determination, using the N^{15} technique. This required the collection of legume plants, and also an annual non-legume (therefore non-nitrogen fixing) reference plant with a similar root depth as the legume, such as barley grass or annual ryegrass.

Project results

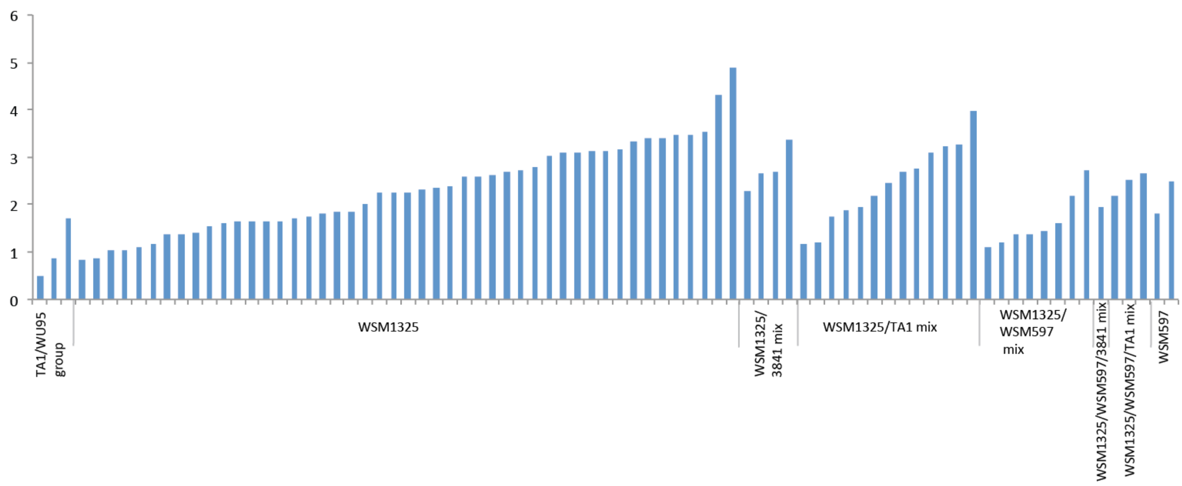
The results from the 81 paddocks sampled indicated that 97 % of the paddocks surveyed (from those sown in the 1950's to 2014) had poor nodule formation on their roots systems, scoring less than a rating of 4 (Figure 2). The average rating of nodulation across all samples was 2.24. Table 3 shows the percentage of paddocks in each nodule score category.

Table 3. Percentage of paddocks in each nodule score category.

Nodule Score	Percentage of Paddocks Sampled (n=81)
0-1	5
1-2	40
2-3	32
3-4	22.5
>4	0.5

The results of the MALDI identification indicated that much of the rhizobia found within the nodules were of the more current inoculant strain WSM1325, either as the sole type found in the sampled paddocks or in combination with older and less effective strains including TA1, WU95 and CC275e and other naturalised strains. Figure 3 shows the dominance of the WSM1325 strain.

Figure 3. Innoculant strains identified in the project samples.



The results of nitrogen fixation from the second paddock visit indicated that 97 % of the samples collected were fixing atmospheric nitrogen at the collection date. This data concurs with the nodule scoring information, which showed that almost all of the 2025 legume plants scored had some (if few) nodules present.

Researchers in the past have explored the link between poor rhizobia nodulation rates and low soil pH. Commercial rhizobia in laboratory cultures have be shown to have suppressed activity at pH (CaCl) <5.5 and be completely inhibited at pH (CaCl) <4.5. High levels of aluminium in the soil affect the ability of rhizobia to form symbiosis with legumes. Aluminium (if naturally high in soils) becomes more plant available in the soil as pH decreases.

Many of the sampled paddocks were affected by soil acidity. Table 4 shows many of the sampled paddocks had acid topsoils (less than pH 5.2 CaCl2 in the 0 - 10cm depth), however many more had acid subsoil (less than pH 5.2 $CaCl_2$ in the 10 - 20cm depth) in a range that is considered to have an impact on rhizobia nodule formation and function.

Table 4. Number of paddocks with acid topsoils and subsoils.

pH CaCl2 % Paddocks Sampled (n=81)	0-10cm	10-20cm
<5.2	42	79
≥5.2	58	21

The soil test results also showed that in association with high levels of soil acidity there were correspondingly high levels of plant available soil aluminium. The results showed that in the soil, 22 % (0 - 10cm depth) and 40 % of paddocks (10 - 20cm depth) had aluminum levels in a range toxic to rhizobia (Al%> 5). Figure 3 demonstrates the relationship between nodulation score and the occurrence of acid soils.

The soil micronutrient molybdenum has an important function in the process of rhizobia extracting nitrogen from the atmosphere. Therefore rhizobia infected legumes have a higher molybdenum requirement than non-fixing plants. Another compounding factor is that as soils become more acid the availability of molybdenum in the soil solution becomes restricted. It is a possibility that low soil levels of molybdenum could be restricting the ability of the nodules to extract atmospheric nitrogen in acid soils. The paddock histories collected indicated that only four of the 55 landholders who returned the paddock history had been using molybdenum fortified fertiliser or pesticides in the past 10 years.

The project survey yielded a large quantity and variety of soil and cultural practice data. There are still relationships between legumes rhizobia levels and these factors currently being analysed. A further project is also being designed to test some of these factors implicated in rhizobia decline due to start in autumn 2017.

Conclusions

Subterranean clover and its accompanying rhizobia, *R. leguminosarum* bv. *trifolii,* have added greatly to Australian agricultural systems, both in terms of increased soil fertility and also in overall production from pasture and livestock systems. The symbiotic relationship between legume and rhizobia form the basis of these advantages. However, this project adds some weight to the idea that subterranean clover is somewhat in decline in southern Australia. Many factors have been attributed to this decline, but the main factors investigated in this project including soil acidity and aluminium levels appeared to be associated with soil rhizobia population decline.

Acknowledgements

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Photos to include if possible.

1. Poorly nodulated subclover plant (Score 1)

2. Well nodulated subclover plant (Score 8)

Collaborators logos:

Funded by: