Local Land Services South East

Knowing Your Nodules – Results from the 2016 Monaro Legume Survey

In spring 2016 South East Local Land Services and Monaro Farming Systems surveyed 54 paddocks across the Monaro looking into the health and nodulation status of pasture legumes.

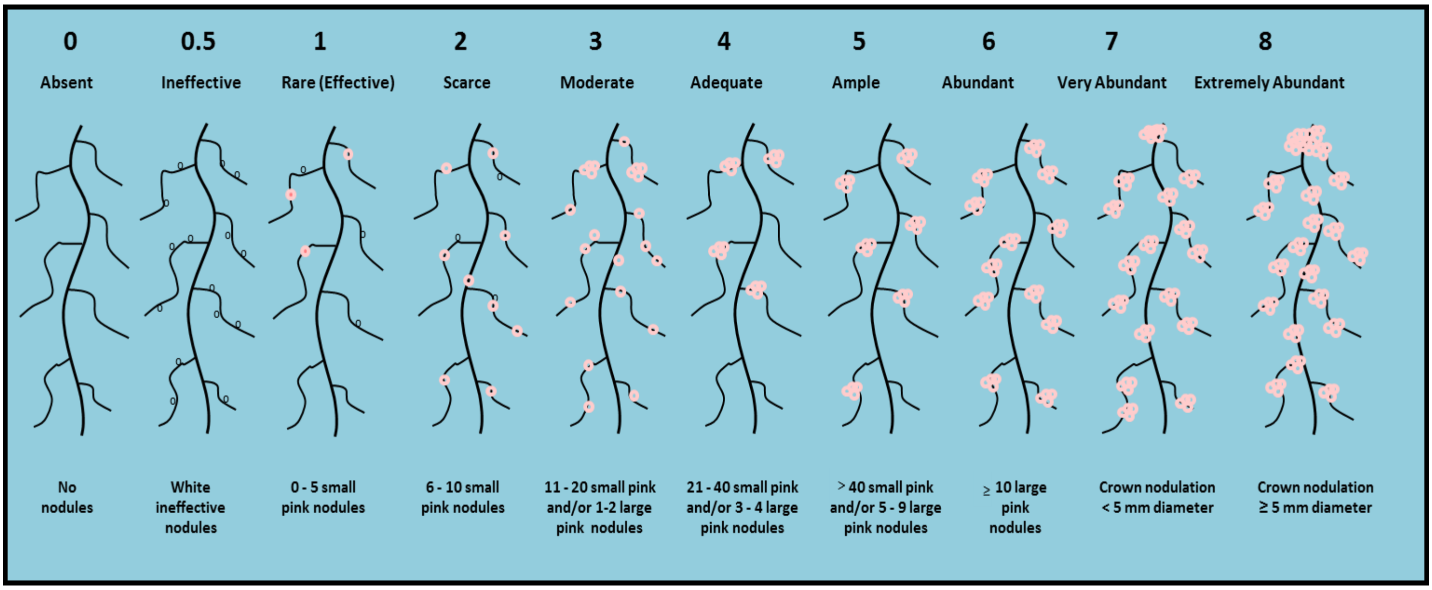
We wanted to find out:

- If healthy looking legume plants (above-ground) were actually fixing nitrogen?
- Did we have the most current rhizobia strains?
- What were the soil characteristics that may be influencing nodulation and N fixation?
- Can we manipulate nodulation & nitrogen fixation in existing pastures?
- What were the limiting factors affecting legume production (including nodulation) and how could we address them?

Sampling at each survey site included soil sampling, pasture composition and nodulation assessment and identification of rhizobia (using MALDI ID).

Nodulation Scoring System

This system was designed by Dr Ron Yates, Department of Agriculture and Food W.A. A nodule score of 4 is considered to be adequate. A mass of large pink nodules located close to the crown or in the higher sections of the root mass of the plant are ideal for maximum nitrogen fixation.



Source: Yates, R.J., Abaidoo, R., and Howieson, J. 2016. Field experiments with rhizobia. Pages 145-166 in: Working with rhizobia, J. Howieson and M. Dilworth, eds. Australian Centre for International Agricultural Research, Canberra.

The 2016 Monaro survey involved the nodule scoring of a total of 828 legume plants including 704 subterranean clover, 71 white clover, 15 suckling clover, 12 Caucasian clover, 6 haresfoot clover, 3 strawberry clover, 3 red clover, 3 arrowleaf clover and 11 lucerne plants.

Monaro Nodulation 2016 Survey Results

Legume	Samples	Monaro average nodule score
Subterranean clover	704	2.6
White clover	71	2.4
Suckling clover	15	3.2
Lucerne (1st year)	11	3.0

Note. Data included only for legumes species that were sampled at multiple locations.

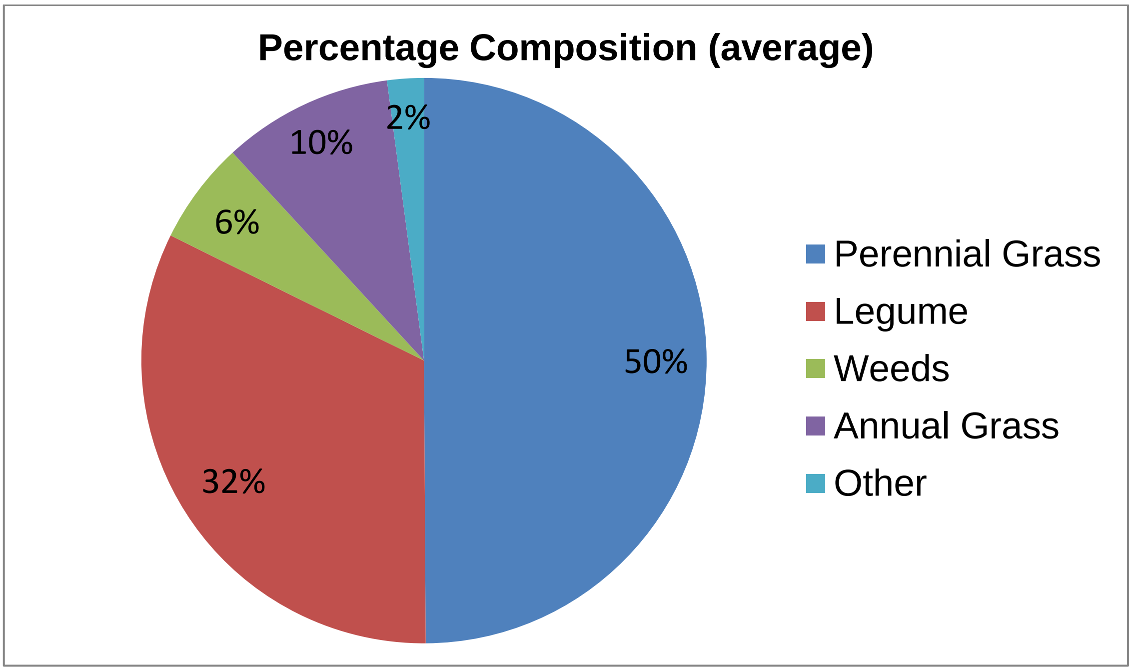
The average paddock nodule score across all legume plants sampled was 2.6 (median 2.5; range 1.1 to 5.1). Only 1 paddock of the 54 sampled had better than adequate nodulation with an average nodule score of 5.1. Only 3 paddocks came close to having adequate nodulation with average paddock nodule scores of 3.8 and 3.9 (2 paddocks).

Legume nodulation surveys in other Local Land Services regions carried out in spring 2015 and 2016 recorded similar sub-adequate levels of legume nodulation:

Local Land Services Region	Number of paddocks sampled	Average Nodulation Score
Monaro	54	2.6
Central Tablelands	30	2.3
Riverina	81	2.2
Central West	60	1.8

Pasture Composition

The pasture composition at each site was intensely surveyed to identify not only the presence of and species of legumes but also the other pasture plants growing alongside them. On average, perennial grasses made up half of the composition of all paddocks surveyed. Pasture legumes made up between 5-87% of the plant species in the surveyed paddocks, averaging 32% across the Monaro.



Rhizobia Identification

Two of the fifteen legume plants that were nodule scored from each survey site were sent to the MALDI ID laboratory at Murdoch University for rhizobia identification. Analysis identified that the most common strain of rhizobia in subterranean clover was the current strain WSM1325 (group C inoculum). Several older strains of group C inoculums were also found at some sites and on several occasions the current group B inoculant TA1 was also found to have caused nodulation on subterranean clovers.

2016 Monaro Survey MALDI ID Results	
73.4%	Current strain of rhizobia present in nodules
12.0%	Old rhizobia strain(s) present in nodules
7.1%	Mix of current and old rhizobia strains present in nodules
6.5%	No rhizobia found in nodules
1.0%	Exotic strain of rhizobia identified in nodules

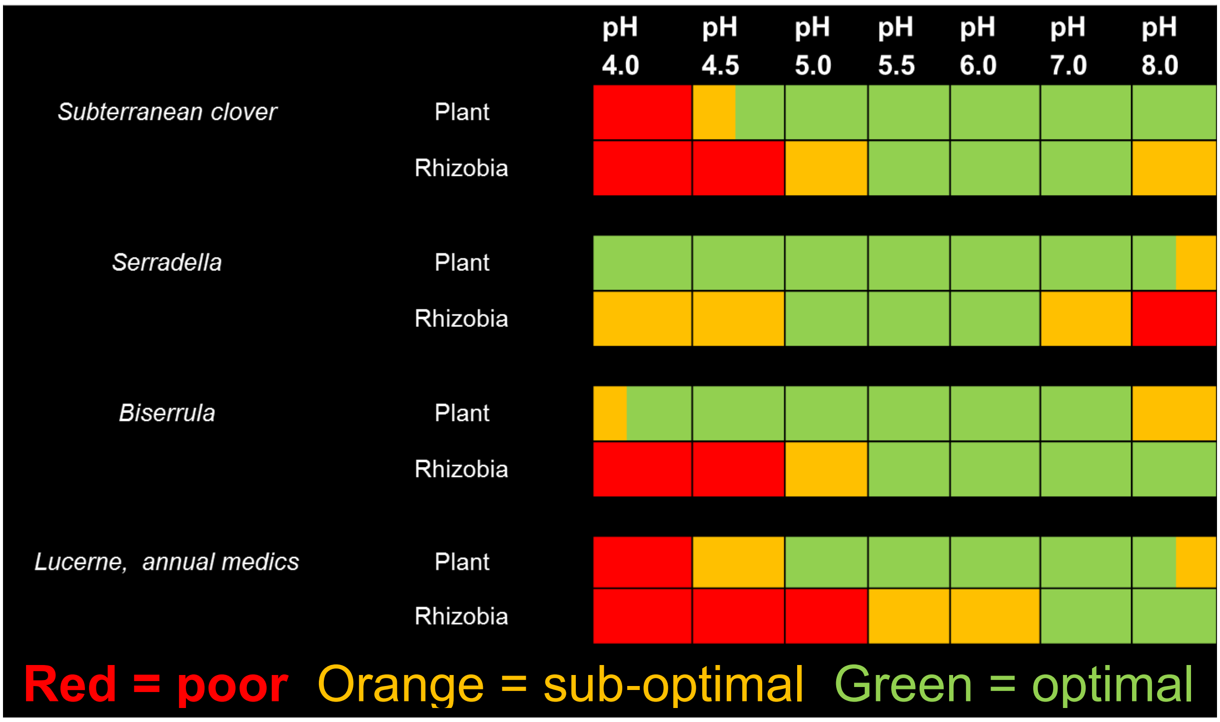
Most of the white clovers tested were hosting the current group B rhizobia. However, some white clover samples were hosting the current group C inoculum (WSM1325). The exotic rhizobia strain WSM597 was also found within nodules at one site. This South American strain of rhizobia is generally ineffective at nitrogen fixation with clovers of mediterranean origin (such as subterranean clover).

Factors Affecting Nodulation and N-Fixation

There are many factors that can potentially affect nodulation & N-fixation including, rhizobia specificity, soil texture, soil pH, soil fertility, certain herbicides, waterlogging in paddocks, fungicide and insecticide use, time since last inoculation, desiccation, time since sowing, time of sowing and seasonal timing and drought.

Soil pH

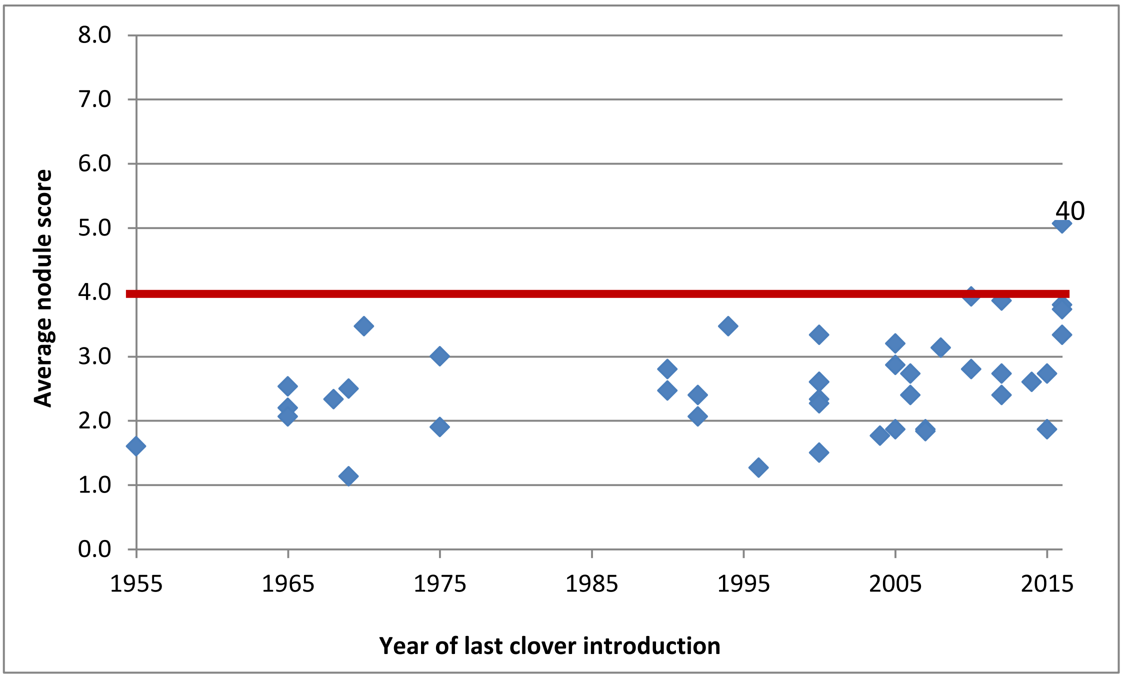
The tolerance of legume rhizobia to low soil pH is not the same as that of their host plants. For example, subterranean clover prefers soils with a pH ($CaCl_2$) above 4.5 however, the associated rhizobia prefer a soil pH above 5.5 ($CaCl_2$). Serradella rhizobia show the most tolerance of low soil pH with lucerne and annual medics the most sensitive.



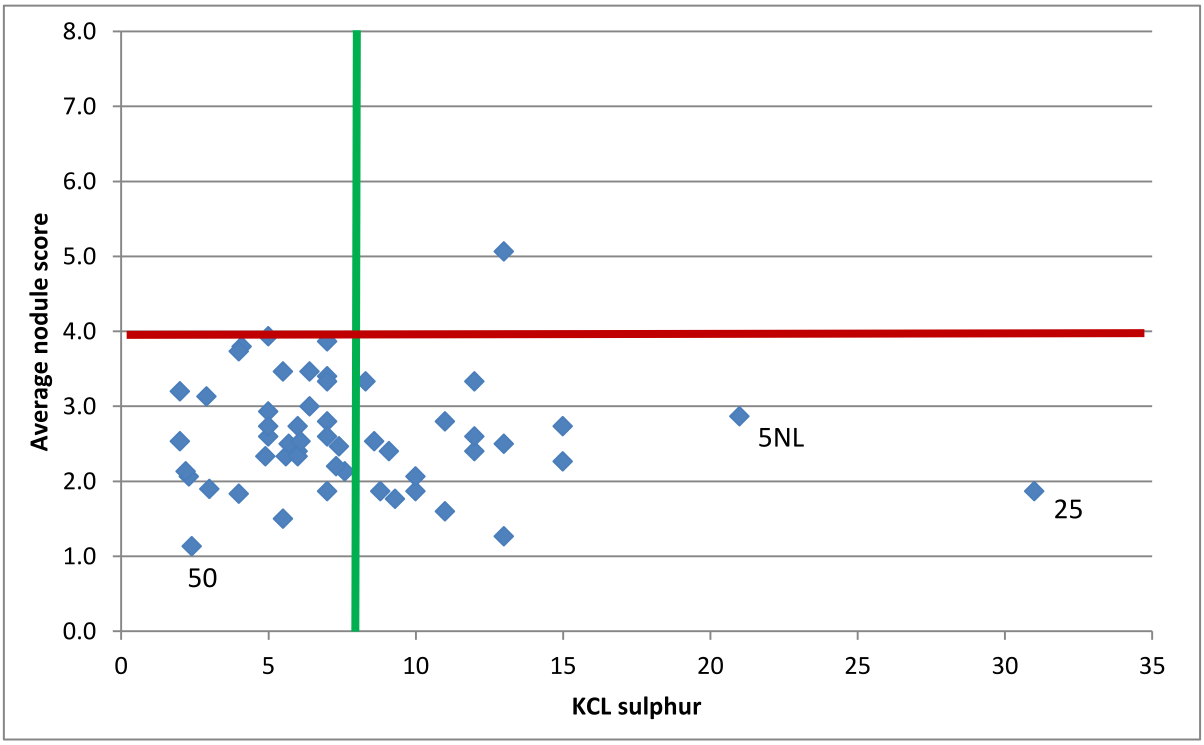
Nodulation Scores and Factors Studied

Paddock information including time since legume introduction, fertiliser history, any recent herbicide, fungicide or insecticide use were gathered for each site. Soil samples were also collected from each site and tested at a NATA accredited soil testing laboratory.

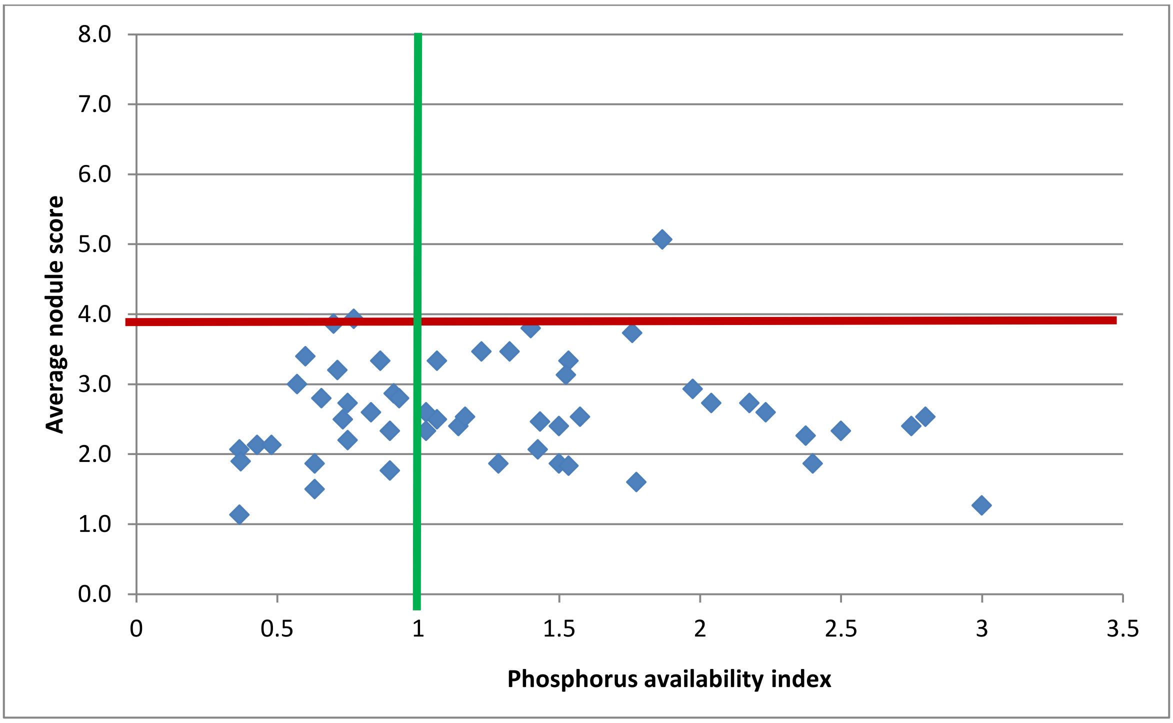
A nodule score of 4.0 is considered to be adequate; this is reflected as the red horizontal line in all the following charts.



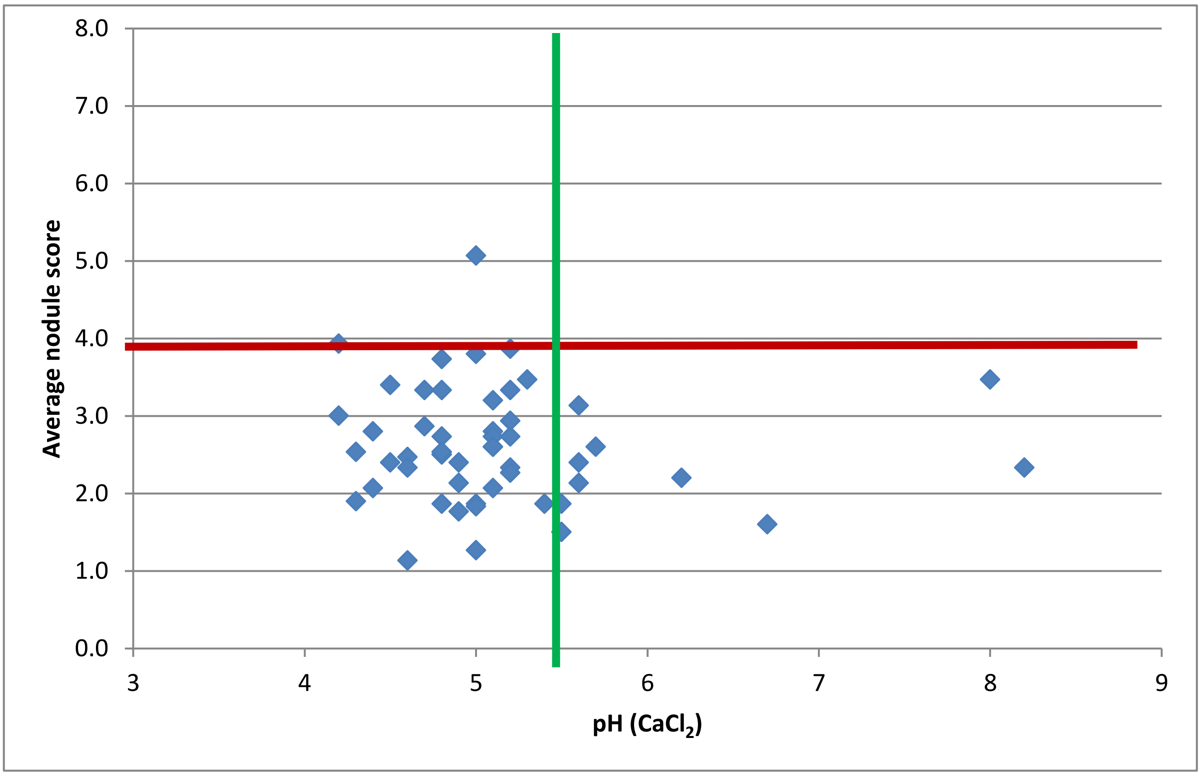
The 2016 survey included sites where clover had been introduced into paddocks from 1955 through to 2016. The year of clover introduction often determines the presence of certain rhizobia strains. For example, older sown paddocks will most likely have older strains of rhizobia.



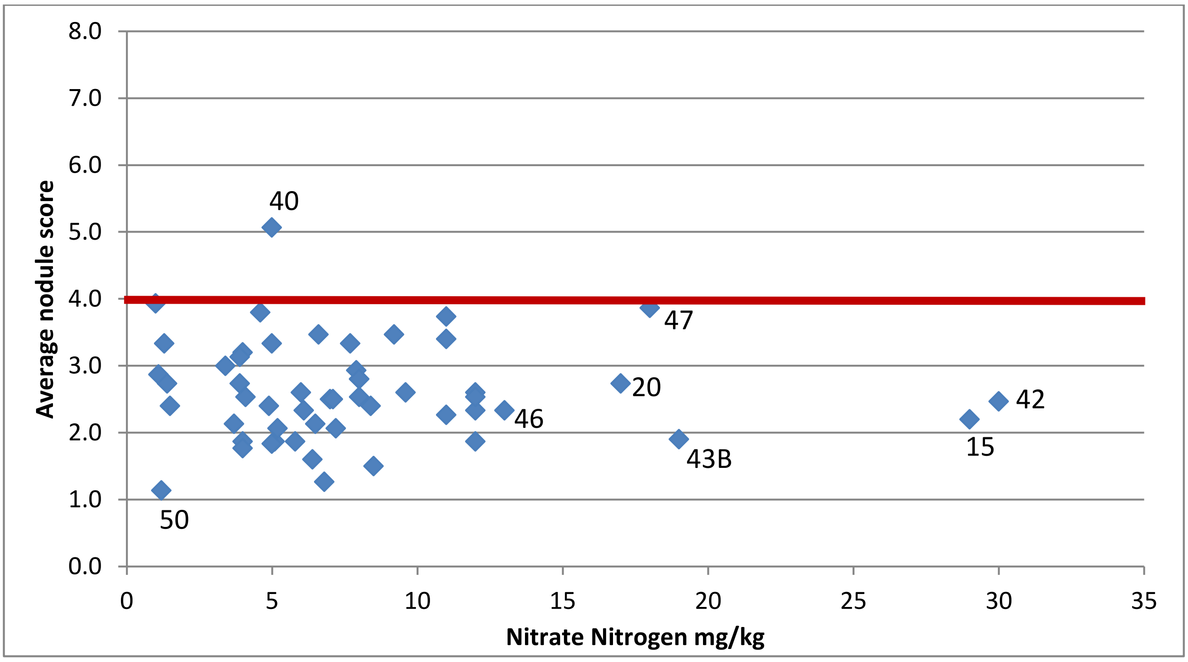
A soil KCL sulphur reading of 8 is considered to be adequate for pastures (green vertical line). Sulphur is critical to the forming of nitrogenase, and the subsequent fixing of soil nitrogen by legume rhizobia. 63% of paddocks tested had less than sufficient levels of sulphur.



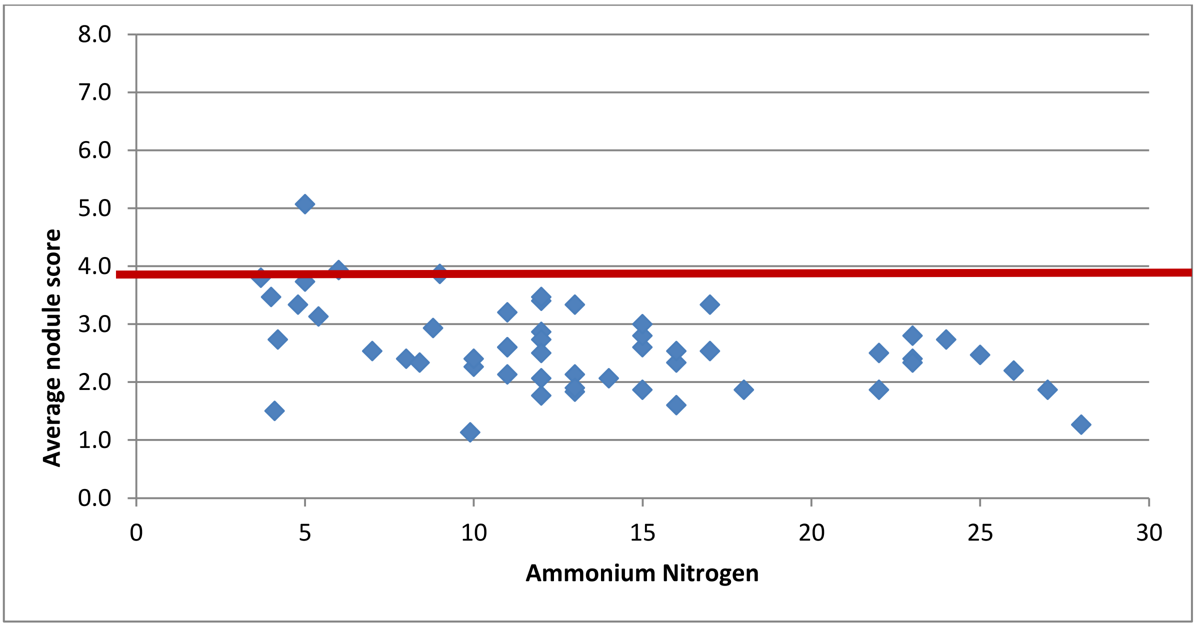
The phosphorus availability index (PAI) is calculated by comparing the soil Colwell phosphorus (Colwell P) reading against the target phosphorus value for each specific phosphorus buffering index (PBI). A PAI value of 1 indicates that the Colwell P is at the target level for that soil type (green line). A PAI above one indicates the soil has more that the adequate level of phosphorus available for plant growth. A PAI of less than one indicates that phosphorus is below target levels. 58% of Monaro sample sites had soil phosphorus levels at or above target P levels.



Whilst many legume species can grow adequately in low soil pH, their associated rhizobia can be less tolerant of such conditions. The rhizobia associated with subterranean clover (group C inoculants) are able to fix nitrogen optimally between pH ($CaCl_2$) 5.5 and 8.0. Only 19% of the sites surveyed (10 paddocks) had a soil pH within the optimal range (to the right of the green vertical line).



Nitrate nitrogen is a measure of the nitrogen currently available for plant use whereas ammonium nitrogen still needs to go through a chemical process in order to become available for plant use. Both measures of nitrogen varied widely across the surveyed area with many factors such as potential waterlogging, recent cropping activities (soil disturbance), nodulation status, soil fertility and presence of appropriate rhizobia all influencing nitrogen in the soil.



Herbicides

There is a growing awareness of the impact of broadleaf residual herbicides (particularly Mode of Action groups B and I) and their impact on legume root development and subsequent nodulation (DAFWA Crop Pasture Group). Two sites in the 2016 Monaro survey had been treated with broadleaf residual herbicides prior to sowing with both showing negative root development and nodulation impacts.

In the images below, note the absence of lateral root hairs and crown nodulation on the herbicide treated plant in comparison to the un-sprayed plant which shows good root development and more than a dozen small nodules on the top 2cm of the plant root.

Subterranean clover plant from a site that had been treated with 5g/ha Metsulfuron-methyl (plants on the right of each picture) and from an unsprayed area within the same paddock (plants on the left of each picture). Photos: Jo Powells

Plant-back or re-cropping interval periods must be observed particularly when using residual broadleaf herbicides. Metsulfuron-methyl has a re-crop interval of between 12 and 20 weeks (depending on rate applied) in soil with a pH below 5.5 and a minimum rainfall of 150mm for pasture legumes. This makes the use of this herbicide in a late summer fallow a risk when planning to sow annual legumes in autumn.

Antas subterranean clover from a Fluoxypyr treated paddock. Photo: Jo Powells

This pasture was sown 16 days after the use of Fluroxypyr (Group I) herbicide for pre-sowing (fallow) broadleaf weed control. A plant-back period of 7-14 days is recommended prior to the sowing of crops for this herbicide. No information on pasture legume plant-back periods was available on label. Like the metsulfuron-methyl affected legumes, lateral root development and crown nodulation was poor in the upper root region of all legumes in this paddock.

Questions and Findings

Were healthy looking legume plants (above-ground) actually fixing nitrogen?

We found that visual assessment of leaf and shoot characteristics of the legumes sampled (prior to root washing and nodule processing) were not effective in indicating the level of nodulation, presence or absence of appropriate rhizobia and any associated level of nitrogen fixation. Examination of legume nodules, the use of MALDI ID and soil testing are the only way to identify legume nodulation status, rhizobia strain identification and N-fixation activity.

Did we have the most current rhizobia strains?

The most current strains of legume rhizobia were found in just over 80% of the legumes plants tested using MALDI ID. Older strains of rhizobia were found co-existing with new strains in 7% of samples and exclusively old strains in 12% of samples. Given several paddocks tested had been sown in the mid-1950s to 1970s and had not been re-seeded with the current strains this result is not a surprising result. Re-inoculation is an option to "top-up" these paddocks with more efficient and productive rhizobia and this practice is worth considering if it will result in more effective N-fixation.

What were the soil characteristics that may be influencing nodulation and N fixation?

The results highlighted that many different characteristics were influencing the nodulation and N-fixation of the legume plants studied. These characteristics varied with each paddock and included available soil nutrients such and phosphorus and sulphur, soil pH, waterlogging and previous herbicide use.

Unfortunately there was not one sole soil factor found to be influencing the nodulation status of all paddocks tested in the 2016 Monaro survey. There was a large variability in paddocks tested with the results influenced by local geology (influencing inherent soil fertility and texture), paddock and soil fertility management post-sowing, seasonal conditions etc.

Can we manipulate nodulation & nitrogen fixation in existing pastures?

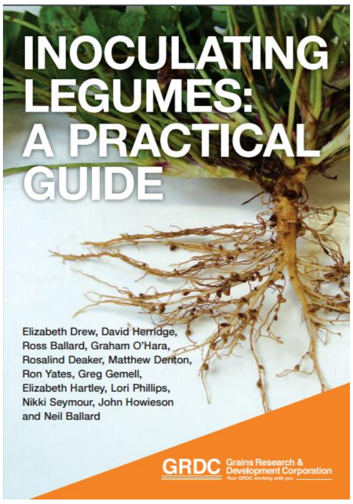
Further investigation is warranted into several aspects highlighted in the 2016 survey in relation to this question including the re-inoculation of old pasture paddocks, correction of key soil constraints and further investigation of residual herbicide impacts. Studies in other regions suggest that nodulation and N-fixation levels can be manipulated however the specific method adopted will need to consider the relevant soil factors in each paddock and the economic value of such actions evaluated.

What were the limiting factors affecting legume production (including nodulation) and how can we address them?

The 2016 Monaro survey highlighted several potential limiting factors affecting legume production and N-fixation as discussed. Additional factors such as seasonal conditions (e.g. drought), presence of root diseases and other pathogenic factors, the presence of available molybdenum and the effectiveness of inoculation methods may also need to be considered.

South East LLS will be continuing to investigate some of these factors in coming years to provide further information to producers on the best ways to maximise legume production and N-fixation.

Further Information



For more information on legumes and inoculation, "*Inoculating Legumes: A Practical Guide*" is essential reading:

https://grdc.com.au/Resources/Bookshop/2015/07/Inoculating-Legumes

Acknowledgements

South East LLS would like to thank all the producers who volunteered their paddocks for sampling and provided management history and relevant information to this survey.

This study would not have been possible without the support of Monaro Farming Systems and the Meat and Livestock Australia funded project B.FDP.0035, "Establishing persistent and productive new legumes".

Air is made up of ~78% Nitrogen….. How much are we fixing??