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## Pasture Soil Biology Molecular Assays – Pilot Delivery

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# Abstract

The purpose of this project is to start delivering DNA assays to pasture scientists to help them study impacts of biological constraints in different pasture management strategies and identify and address any potential sustainability issues. This included assays being developed by SHP.005 for a range of soilborne pathogens, beneficial organisms and key plant species. The latter to measure root growth. Also included were complementary DNA assays for biological function and community structure being developed by CSIRO Division of Sustainable Ecosystems. For these developments to deliver benefits to industry, pasture researchers need to be familiar with the assays and plan to incorporate them into experimental programmes.

A prototype training course and manual was developed to introduce the tests and increase awareness of the role of soil biology.

The assays were used to assess treatment effects at EverGraze sites at Albany WA and Wagga Wagga, NSW, and long term MASTER trial also near Wagga Wagga and P X Grazing trial at Hall, ACT. Significant treatments differences were detected at each site. This stimulated a positive response from local research groups. The use of DNA assays to assess root growth was of particular interest; assessing root growth in mixed swards is currently very difficult.

The potential of the technology to support development of better grazing systems was recognised and the efforts to facilitate adoption of the technology needs to continue.

# Executive summary

This project was developed to support delivery of new DNA-based assays to pasture research groups for use as research tools to study soil biological constraints to production and sustainability.

SHP.005 project has been developing molecular assays for a range of soil organisms including pathogens and beneficial organisms and key pasture plant species. The latter are being developed to provide a DNA option to assess root growth in soil. Concurrently, Dr Steve Wakelin, CSIRO Division of Sustainable Ecosystems, has developed DNA assays for soil biological function and community structure. A number of assays from both groups have reached a stage of development where they can be evaluated in field trials, and help determine if they need further refinement.

The combination of these assays is expected to provide powerful new tools to study biological constraints to pasture production.

To engage pasture researchers and gain valuable field experience, four pasture trials were selected to evaluate the most advanced assays. These included recently established EverGraze sites at Wagga Wagga, NSW and Albany, WA, and two long established experiments including the MASTER site run by NSW DPI, Wagga Wagga and a grazing by phosphorus experiment run by CSIRO Plant Industry, Hall, ACT.

The results revealed significant treatments affects at each site. At the MASTER site a significant impact of lime was detected on levels of arbuscular mycorrhizal fungi (AMF) and several important plant pathogens including Take-all. Levels of some other pathogens such as Pythium and *Rhizoctonia solani* AG2.2 were not affected by liming. This was correlated with sub clover soil DNA levels, which were also not affected by lime. Sub clover is a host of both pathogens. In the P X Grazing trial, the DNA assays detected differences in sub clover, *Pythium* and *Phytophthora clandestina* levels between treatments. Treatments differences reflecting the different pasture compositions were also evident in the EverGraze sites.

Use of molecular assays to assess plant DNA levels in soil, is a novel approach to monitor root growth. Combining assays for different plants, pathogens and beneficial organisms should provide a better insight to biological constraints impacting on productivity and water use efficiency, and ultimately facilitate development of more efficient and robust pasture systems.

The CSIRO assays for key functional genes and community analysis also detected significant changes in abundance of functional genes and species composition between the different treatments, which included liming, grazing rates, P application and pasture composition.

A pilot training course and draft manual was developed to support introduction of the DNA assays. Workshops were conducted at Wagga Wagga NSW and Albany WA. These were attended by 30 scientists from State agricultural research agencies and universities. Their backgrounds included senior pasture researchers, visiting scientists, research students and some senior technical staff. The material was well received and stimulated considerable discussion on how this technology could be incorporated in the research programs. The feedback from participants was constructive and will contribute to refinement of future training activities, and priorities for development of future assays.

Engagement with pasture scientists through this project has further highlighted the value of the DNA approach to provide a practical method to study soil biological constraints as part of development of robust pasture management systems. There is strong interest in seeing the technology grow and applied and particular enthusiasm for using the plant assays to monitor root growth in soil.

Further development and promotion of DNA assays as research tools is warranted.

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# 1 Background

This project developed and delivered a pilot program to help educate pasture scientists on biological constraints to pasture production and demonstrate the potential value of the DNA assays being developed by SHP.005 for use as research tools, and DNA assays for biological function and community structure being developed by CSIRO Division of Sustainable Ecosystems.

SHP.005 is developing molecular assays for organisms in the soil including pathogens, beneficials and pasture plants (as roots in soil). A number of these assays are reaching a stage of development that warrants evaluation under field conditions. It is also appropriate for researchers to begin to use them and provide feedback on the need for further refinement.

The combination of pathogen, beneficial and plant assays is expected to be a powerful research tool to study biological constraints to pasture production.

The outcomes and lessons learnt from this pilot delivery process during 2006/07 will underpin the formulation of a more comprehensive delivery program for the future.

## 1.1 Pasture soil biology molecular assays

### 1.1.1 Soil organisms

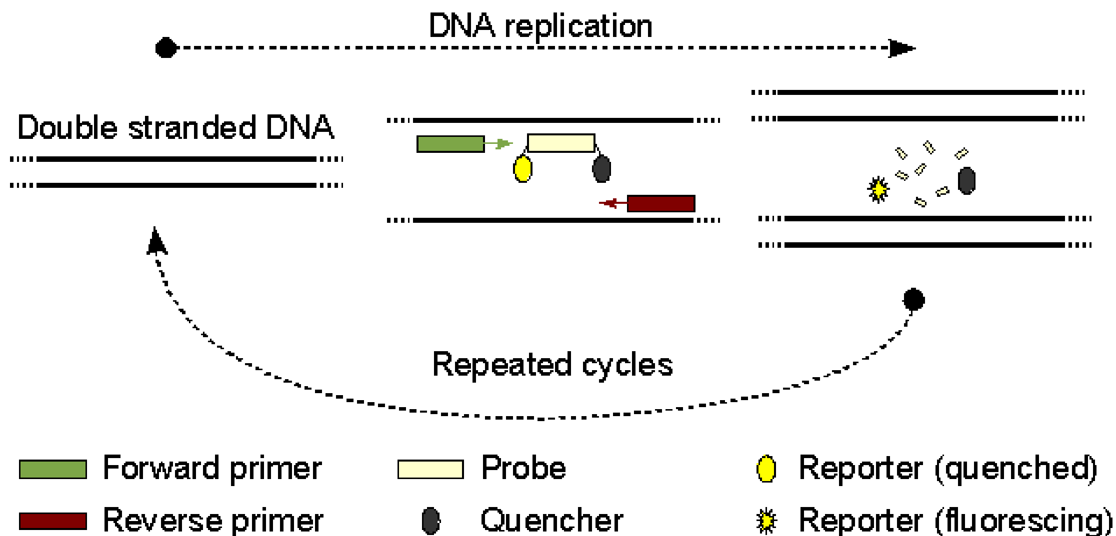
Soil provides plants with water, nutrients and anchorage, functions that are only made possible by the community of organisms that live therein. Plants provide photosynthates through live and dead roots and surface residues that feed the soil community. Understanding, and potentially manipulation of soil organisms, is important for achieving and maintaining optimal pasture production. With the development by SARDI of efficient means of extracting DNA - which all organisms contain - from meaningful volumes it is now possible to quantitatively monitor a range of soil organisms by a single method.

DNA extracted from soil, amplified and probed for a DNA sequence specific to the target of interest, which can be a single organism or group of related organisms (clade), can be quantitatively assayed. In the past, to estimate numbers of different organisms, a plethora of resource intensive extraction, isolation and bioassay methods were required, which meant investigations had to be narrow and conducted and interpreted by experienced specialists.

The development of a new test must proceed though a series of stages; a) collection of DNA sequences of target and related organisms, b) phylogenic analysis, c) collection of organisms for test validation, d) selection of a diagnostic region, e) design of primers and probes, f) evaluation of the specificity and sensitivity of the assay again the target as pure DNA, pure cultures, and in soil, and finally g) validation and calibration in the field. This procedure may not be linear, in that some steps might need to be repeated to achieve the robust and sensitive test desired.

For this work, a proprietary DNA assay system, TaqMan® has been adopted, because it has many advantages including linearity of a wider range of target DNA concentrations.

DNA from the soil is replicated using target-specific primers and a fluorescently labelled probe. Through each DNA replication cycle the fluorescent reporter is released and can be measure using laser technology. The diagram below illustrates this process.




The quantity of the target DNA is estimated by the number of replication cycles taken for the fluorescence to reach a certain threshold and comparing this to standards of known concentration. The value is then converted to picograms of DNA per gram of soil (pg DNA/g soil) and then to units such as nematode eggs per gram of soil, where such relationships have been determined.

The final step of the development of a new test is usually undertaken in collaboration with other researchers. It is not possible or desirable to fully deliver tests in isolation, as it is only by application to field experiments can tests be validated and sufficient data generated to develop meaningful interpretation.

New molecular tests have been developed to quantify key pasture plants and associated pathogens (fungi, nematodes and oomycetes) and beneficial organisms (mycorrhizae) in pasture soil. These add to existing test developed for cropping systems that are also directly relevant to pasture or indirectly through the impact of pastures on rotational crops.

The new tests were selected to cover a range of key organisms relevant to major pasture systems in winter rainfall areas of southern Australia and demonstrate suitability to the technology to assess the different types of organisms that would be desirable to quantify. For these reasons, the list of tests does not comprehensively cover all pasture types or the even all organisms relevant to the major pasture systems. This is the first application of this technology to pastures and will clearly grow as its merits are demonstrated.

The DNA tests for plants allow the quantification of live roots of the target species in soil even in mixed swards. It is expected to these tests will be received as major advancements in the study of roots in soil and their responsiveness to the impact of genotype, soil environment, management, pathogens and beneficials organisms.

Quantification of plant DNA appears to provide responsive measure that might be more closely linked to root activity than simple biomass, likely to provide new insights to root biology. In pastures, with variable botanical composition and distribution, the precision in the study of microorganisms (pathogenic and beneficial) in soil is likely to be greatly enhanced by the parallel assessment of plant roots.

### 1.1.2 Soil community function and structural analysis

Soil biological processes underpin many terrestrial ecosystem functions and thereby directly affect productivity and the health of both farming systems and the landscapes in which they sit.

Examples are numerous, and include biogeochemical cycling of most major elements central to plant health and ecosystem fertility (e.g. C, N, Fe, S, P), plant pathogenic organisms and their competitors, beneficial organisms such as mycorrhizae and rhizobia, decomposition of animal, plant, and chemicals. The combined action of these processes underpins soil formation, structure, stability and function.

Understanding the interactions and links between soil microorganisms, the processes they drive and resultant functions (effects) in the environment has many benefits (Figure 1). Direct benefits include understanding and managing microbial communities and processes for (1) enhanced productivity of plants and livestock, and (2) environmental impacts or services such as reduced nitrate leaching to groundwater or greater C sequestration in agricultural soils. Indirect benefits include understanding how sustainable these processes are. For example, the ability of an ecosystem to resist change or stress (ecosystem resistance) or the ability to recover after disturbance such as drought (ecosystem resilience).

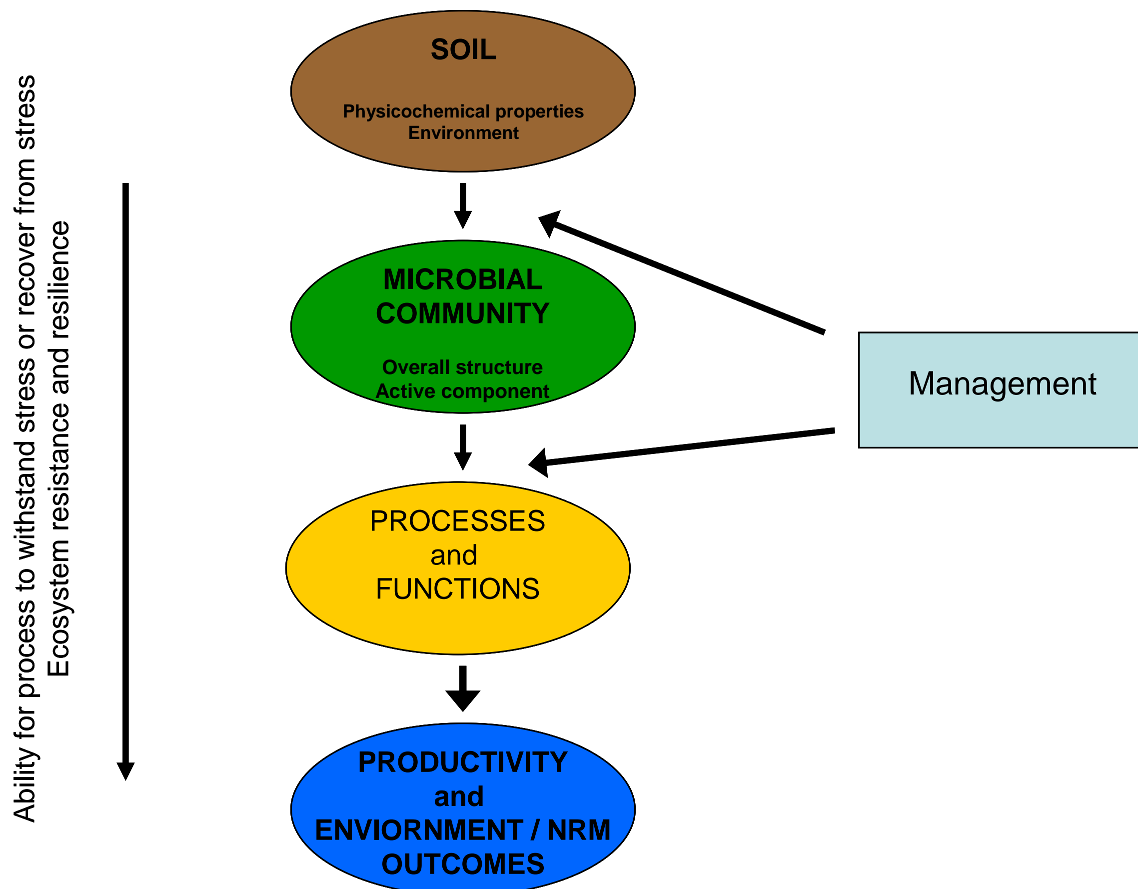



**Figure 1.** Links from soil and environment, through biology and functions in ecosystems. Very little is known about the microbial community and its interaction with the environment. As such, directed farm management towards desired production or environmental outcomes is poorly understood is progressing only incrementally.

To date, our understanding and ability to harness potential gains from soil biology research to benefit production and environmental endpoints has been limited. Principally, this has been due to technological constraints. These constraints have necessitated that most research occurs at the single organism or single processes level. Compounding this has been a strong reliance on cultural methods, which severely limit research to investigating microorganisms that grow in laboratory culture - a very different environment to soil. These culturable organisms represent an extremely small component of the total soil microbial diversity and function. Despite these constraints, soil microbiology research has resulted in significant advances in the productivity and sustainability of production systems; one only needs to consider the benefits gained from our

understanding the management of soil pathogens, beneficial mycorrhizae and the consider the benefit of rhizobia to soil fertility in Australia.

Although much benefit has arisen from single organism/single process work, it is now appreciated that community-level approaches are needed to understand soil microbiology at ecosystem levels (in this context, an agricultural or production based ecosystem (Figure 1). Confounding this understanding of soil microbial communities are complex interactions between the diverse range of organisms present, the driving effects of soil and environmental properties on the microbial community, and the sum of these effects in terms of production or environmental impacts. As such, not only must dominant organisms and processes be described and management-driven controllers understood, but the knowledge must be understood in terms of the system and boundaries in which it operates.

Recent technological advances in molecular microbiology are providing entirely new ways of investigating soil microbiology and opening windows to the processes therein. Key to harnessing these advances is the ability to investigate soil microbiology at a community level at both phylogenetic and functional levels - i.e. not only describing what is there but also what functions are present. These techniques can be used to 'fingerprint' microbial communities, understand how the dominant community shifts in composition and function with management, stress, time, space etc. They can also be used to identify primary drivers of biotic community composition and functions in agri-ecosystems. Most of the data generated are highly dimensional and new approaches in multivariate statistical analysis are therefore being applied to community level soil microbiology to understand the systems in which they operate. The methods are able to relate specific changes in biological community structure to management practices and soil physicochemical properties and, for the first time, provide a way to closely describe pasture soils according to their biology.

Soil microbiology in pasture-based agro-ecosystems in Australia is poorly understood. To date, most research has focused on single issues – single organism/process interactions. Despite this, the benefits to understanding and managing microbial activity and processes are clearly well defined both in terms of productivity and sustainability (examples of which are given above). The reasons for not exploring these potential benefits may be due to a number of factors including (1) technological limitations – "it can't be done", (2) funding opportunities – "money better spent elsewhere", (3) lack of industry 'drive' to lift sustainable production in such a manner, (4) lack of scientific resources to conduct the work necessary, or (5) perception of limited short term benefits – "it will take 5-10 years for gains to be made".

## 1.2 Pilot education package

The study of soil borne organisms and their function with traditional methods has been difficult and time consuming in the past, and impossible in complex mixed pasture systems.

New molecular (DNA based) assays are now being developed that are able to identify and quantify specific organisms and functions in soil environments and offer the opportunity to accelerate our learning in soil biology dynamics under pastures.

The training conducted sought to introduce some of these new molecular tools for pasture soil biology research to encourage their utilisation in current and future research projects.

The goal is to see these tools used to better understand the impacts of management and environment on different pasture based systems with a view to understanding how best to manipulate such systems for soil biological health and optimum pasture productivity.

These recent advances in molecular technologies offer opportunities to study soil biological function and impacts of specific soil borne organisms in a range of complex agricultural systems.

The training workshops sought to introduce the use of these DNA based assays to study critical aspects of soil biology in pasture systems.

While the technology requires further development and refinement, the assays that have been developed so far are suitable for researchers and advisors to evaluate in experiments and use to monitor changes in soil biological components over time, season and in relation to imposed pasture management treatments.

The training aimed to:

- Explain the advantages and limitations of these assays
- Provide examples of where they have been used
- Encourage researchers and advisors to begin to use these assays as research and monitoring tools
- Help begin to create a collaborative network of researchers using these assays to accelerate development of knowledge in pasture soil biology.
- Provide a basic background in soil biology and soil borne organisms to provide context for use of the assays
- Obtain feedback on the workshop content and the technology to help shape future improvements.

## 1.3 Researcher engagement

To engage researchers in the use of the DNA technology four existing pasture research activities were selected, two EverGraze, the MASTER (Managing Acid Soils Through Effective Rotations) project (NSW DPI) and a CSIRO long-term phosphorus by grazing experiment.

The collaborators were

- Dr Richard Simpson – CSIRO, PI, Site Leader P x Grazing Trial, Hall ACT
- Dr Guangdi Li – NSW DPI, MASTER Site Manager
- Dr Michael Friend – CSU, EverGraze Site Leader, Wagga Wagga
- Dr Paul Sanford – DPI WA, EverGraze Site Leader, Albany

### 1.3.1 EverGraze – Albany

EverGraze is an initiative of MLA and the Cooperative Research Centre for Plant-based Management of Dryland Salinity. The primary goal is investigate perennial-based pastures to reduce groundwater recharge and increase productivity in high rainfall areas. EverGraze is investigating new grazing systems within the context of the whole farm system of pastures, animals and management and with potential relevance across the whole of the high rainfall zone of southern Australia. The EverGraze goal is to increase profitability and reducing groundwater recharge, both by 50%. Given the whole systems approach the ability to quantitative DNA assays to monitor changes in soil organisms is potentially highly relevant to the EverGraze program.

The EverGraze site at Albany is comparing perennial pastures based on lucerne, kikuyu, tall fescue plus lucerne, tall fescue and chicory with annual pasture. The treatments were established in spring 2005.

### 1.3.2 EverGraze – Wagga Wagga

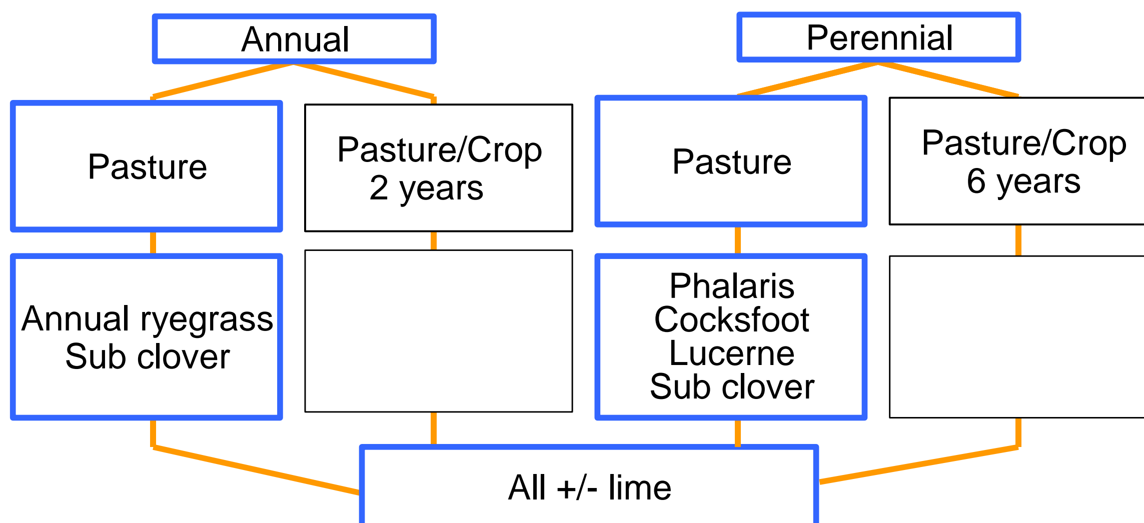
The Wagga Wagga site is another in the EverGraze program that is described above.

The EverGraze site at Wagga Wagga is comparing perennial pastures, lucerne, phalaris and tall fescue.

### 1.3.3 NSW DPI – MASTER

MASTER is a long-term agronomic experiment that commenced in 1992 to demonstrate approaches to effectively manage highly acid soils in the high rainfall areas of south-eastern Australia (see Li *et al*. 2006). The experimental site is located on a farm 40 km SE from of Wagga Wagga with a rainfall of about 600 mm pa. The initial pH was about 4.1 in the top 100 mm, with subsoil being highly acidic. The goals include the evaluation for perennial vs. annuals pastures and liming on the production system and its economic viability.

The experiment included 80 plots treated as described by Li *et al.* (2001) and summarised by the simplified schematic below (only the permanent pasture treatments are highlighted, as only these were assessed in this study).




Lime was initially applied in limed plots at 3.7 t/ha, incorporated into top 100 mm of soil, followed by maintenance applications of 1.6–1.8 t/ha top-dressed every 6 years.
Measures included chemical soil properties, crop and pasture performance (including botanical composition) and animal production data. Effects on soil biology were not part of the initial design.

### 1.3.4 CSIRO – Phosphorous by grazing trial

This experiment was established in 1994 by the CSIRO at Hall, ACT to examine the long term benefits/effect of phosphorus application under two grazing intensities. The site has been continually grazed since establishment except for periods of de-stocking during dry periods in 2002-05.

The experiment included two phosphorus regimes, P0 – no added P (Cowell P 8-12 µg P/s soil in top 100 mm) and P1 – fertilised annually (Colwell P 20-25 µg P/g soil in top 100 mm). Two stocking rates were applied, SR9 – 9 DSE (dry sheep equivalents) to both P0 and P1 and SR18 – 18 DSE to P1 only.

# 2 Project objectives

To develop a pilot education package on soil biology and the emerging molecular assays for soil borne organisms, biological function and microbial community analysis for delivery to researchers. The aim is to encourage the use and evaluation of these research tools in their research underpinned with some basic understanding of the importance of soil biology in pasture and grazing systems research.

To engage with existing field research projects to collaborate in the evaluation of the soil based molecular assays.

# 3 Methodology

## 3.1 Pilot education package

A training manual "Pasture Soil Biology: New tools for research" and with parallel lesson plan was prepared.

Two training workshops were conducted, the first at Charles Sturt University, Wagga Wagga, NSW on 17 April 2007 and the second at Department of Agriculture and Food, Albany, WA on 23 April.

Dr Bob Hannam convened the workshops and invited pastures researchers and other interested in potential application of the technology.

Dr Hannam chaired the workshop and presented the introductory session. Drs McKay and Riley presented other sessions.

All participants were provided with provided with feedback forms at the beginning of the workshop and were asked to make comments during the sessions.

## 3.2 Researcher engagement

### 3.2.1 EverGraze – Albany

Twenty soil samples (composites of 30 cores, 10 x 100 mm, about 500 g total) were collected from treated plots from 14 to 28 August 2006. Four replicate plots for lucerne, fescue+lucerne, fescue and chicory were sampled and two for kikuyu and annuals.

The samples were transported at field moisture content to Adelaide by road freight in insulated containers and dried on arrival in a dehumidifying oven.

#### 3.2.1.1 Soil organisms

DNA was extracted by the proprietary procedures used by the SARDI Root Disease Testing Service and assayed by real-time PCR for the following organisms.

| | |
|---|---|
| Plants | *Lolium/Festuca* spp., *Trifolium subterraneum* and *Phalaris aquatica* |
| Fungal pathogens | *Bipolaris sorokinana* (Common root rot), *Gaeumannomyces graminis* var. *tritici* and var *avenae* (Take-all), *Fusarium culmorum/graminearum*, *Fusarium pseudograminearum* (two types), *Mycospaerella pinodes*/*Phoma medicaginis* var. *pinodes* (black spot complex), *Phoma* sp (associated with Black Spot) and *Rhizoctonia solani* (AG2.1, AG2.2, AG4 and AG8) |
| Oomycete pathogens | *Phytophthora clandestina* and *Pythium* Clade F (after Levesque and de Cock, 2004) |
| Nematode parasites | *Ditylenchus dipsaci, Heterodera avenae*, *Meloidogyne javanica/incognita/arenaria*, *Pratylenchus neglectus, P. penetrans, P. thornei* |
| Arbuscular mycorrhizal fungi | AMF groups a, b, c, d and e |

The test results are converted to picogram of target DNA per gram of oven-dried soil, except for the nematodes, where the concentration is estimated in numbers of nematode per unit weight of soil.

At the time of analysis, standards had not been developed for the *Phalaris* and AMF assays. The number of PCR cycles taken to detect the target was converted to DNA concentrations for *Phalaris* based on the *Trifolium* test. The AMF assays were converted using a common standard curve to allow comparison of levels between treatments for the same group, however this does not allow comparisons to be made between AMF groups or other tests.

The ANOVAR (GenStat, release 8.2) was applied to the DNA data using with logarithmic transformation, if needed. Botanical composition and feed-on-offer (FOO) data was available and relationship with DNA data examined by correlation and linear regression analyses as with GenStat.

### 3.2.1.2 Soil community function and structural analysis

Extracts of DNA were supplied by SARDI to CSIRO Land and Water for analysis using a Picogreen based dsDNA quantification procedure. All DNA samples were diluted to a standard quantity of 5 ng DNA/µL buffer.

The copy numbers of 3 genes involved in key steps of the geochemical cycling of N (Figure 2) were quantified in the DNA extracts. The genes, the enzymes they encode and the functions of these enzymes are given in Table 1.

**Table 1**: Functional genes measured in this work and their enzymes/functions.

| Gene | Enzyme | Function |
|---|---|---|
| *nif*H | dinitrogenase reductase | $N_2$ fixation |
| *amo*A | ammonia monooxygenase | Nitrification/N conversion |
| *nar*G | nitrate reductase | Denitrification |

Functional genes quantification was based on real-time PCR amplification against appropriate standard curves containing known copy numbers of each gene. PCR chemistry was based on SYBR detection; PCR products were also separated electrophoretically to ensure amplification of a single fragment of the expected size.

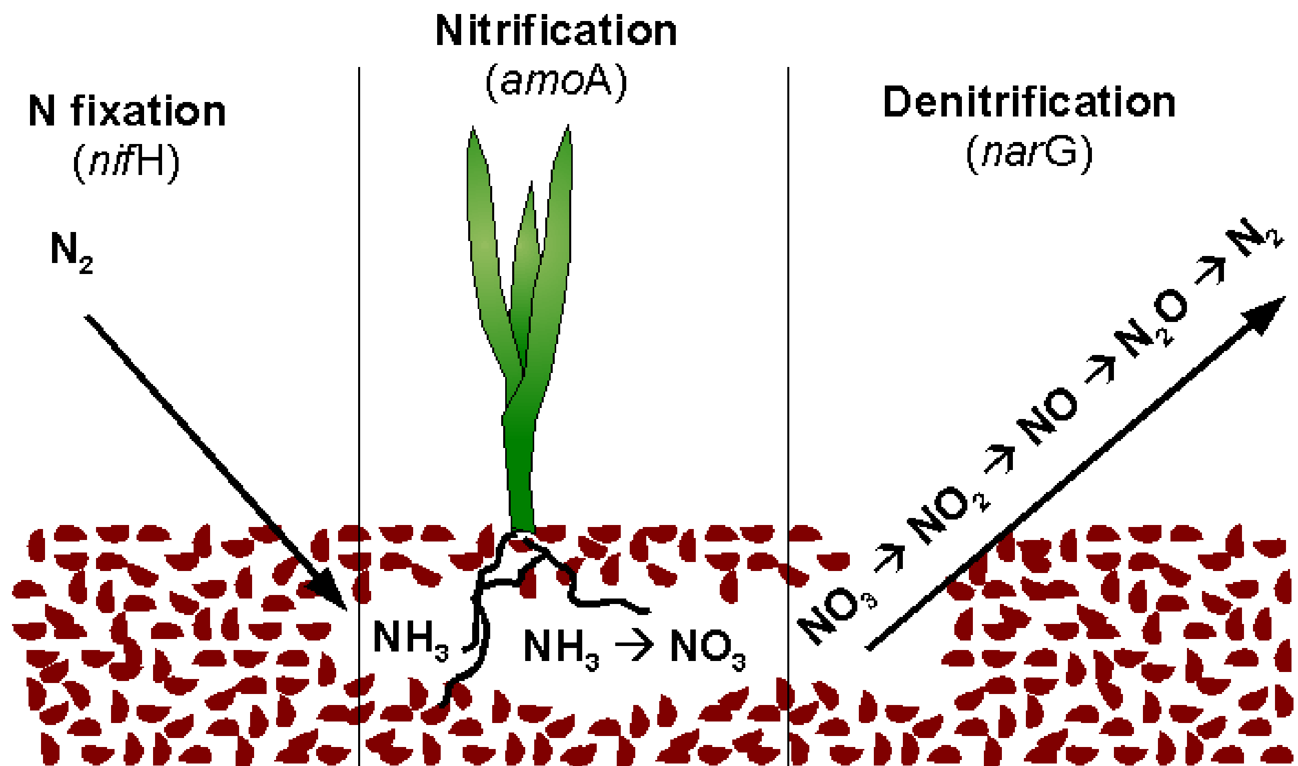



**Figure 2.** The role of *nif*H, *amo*A, and *nar*G in nitrogen fixation, nitrification and denitrification. Nitrogen fixation adds to soil and ecosystem fertility, nitrification is involved in the cycling and reprocessing of different N-based molecules in soils, denitrification results in the loss of N (fertility and profits) from soil and can lead to leaching of $NO_3$ to waters and emissions of $N_2O$, a potent greenhouse gas.

The fungal community structure was assessed using DGGE of PCR-amplified ITS ribosomal DNA. DGGE banding patterns were digitally captured and processed using image analysis software.

Bacterial community structures were assessed using tRFLP of PCR-amplified 16S ribosomal DNA. Terminal restriction fragment sizes were measured on a capillary sequencer (AGRF) against known oligo-size fragments (ABI). Peak locations and heights were captured using 'genemapper' software and imported to other software for community assessment.

Similarities between community structures were determined using Bray-Curtis algorithm on 4th root transformed abundance data. Cluster plots (using the group average method) were generated and the significance of grouping tested (SimProf routine). Non-metric multidimensional scaling (MDS) was used to present (scale down to 2 dimensions) the distance (similarity or resemblance) of community types.

The effects of specific management treatments on community structure were tested by 1 or 2-way analysis of molecular similarities (ANOSIM). ANOVA was used to test the significance of management effects on copy numbers of functional genes in DNA taken from the sites.

### 3.2.2 EverGraze – Wagga Wagga

Ten soil samples (composites of 30 cores, 10 x 100 mm, about 500 g total) were collected from treated plots on 10 July 2006. Three replicate plots for lucerne, fescue, and phalaris were sampled, along with one from a nearby roadside.

The soil was transported to Adelaide at field moisture content and dried in a dehumidifying drier commencing 12 July.

The DNA assays and data processing applied were the same as those described for EverGraze (Albany).

### 3.2.3 NSW DPI – MASTER

Sixteen soil samples (composites of 30 cores, 10 x 100 mm, about 500 g total) were collected from pasture plots on 10 July 2006. Four plots for each combination of permanent perennial and annual pasture, with and without lime were sampled.

The soil was transported to Adelaide at field moisture content and dried in a dehumidifying drier commencing 12 July.

The DNA assays and data processing applied were the same as those described for EverGraze (Albany).

### 3.2.4 CSIRO – Phosphorous by grazing trial

Soil samples (composites of 8 cores, 25 x 100 mm, about 500 g total) were collected from three replicate plots of each treatment in the first week of each month from April to November 2006. The soil was air dried before transport to by road freight to Adelaide. The samples were oven dried in dehumidifying drier on arrival.

The DNA assays and data processing applied were the same as those described for EverGraze (Albany), with the exception that Repeated Measures ANOVAR (GenStat, release 8.2) was used for the analysis of the organisms quantified by real-time PCR.

# 4 Results and discussion

## 4.1 Pilot education package

Nineteen people attend the workshop in Wagga Wagga, representing NSW DPI (from Wagga and Orange) and Charles Sturt University. In Albany, eleven people attended, representing WA DAF and the University of Western Australia. The participants included researchers with long standing in pasture agronomy, early career scientists, technical officers and research students.

The participants largely gave positive feedback and made helpful suggestions for improving the training when delivered again.

The presentations stimulated considerable interest and future collaboration is being discussed with several parties. In most cases, current funding levels do not permit adoption of the technology, so funding will be sought in new projects. Given that most of the participants did not have strong plant pathology or soil biology backgrounds, the greatest interest was generated by the prospect of being able to study root biology using DNA.

## 4.2 Researcher engagement

### 4.2.1 EverGraze – Albany

#### 4.2.1.1 Soil organisms

Figure 3 shows the log-transformed data across the spectrum of tests. Sub clover occurred in all samples and it is likely that some of this represents seedbank. No *Phalaris* was detected as was expected. The kikuyu and chicory plots had low *Lolium*/Fescue compared to the other treatments.

*Pythium* Clade F was the pathogen in highest concentration, followed by Phoma (black spot complex), which might reflect the level of sub clover in the site. The only pathogens to show statistical significance at this stage were *Pythium* and take-all.

The AMF assays are not quantitatively comparable between groups at this stage as the efficiency of the individual test can only be determined when suitable standards are developed. Nevertheless, there are within group differences that are significantly related to the pasture composition.

The possible combinations of 26 DNA assay results and 21 botanical components (% and biomass estimates) included many measures that had insufficient data to allow valid analysis. So the analysis undertaken concentrated on *Pythium* and take-all (Ggt) as these showed significant responses to the treatments.

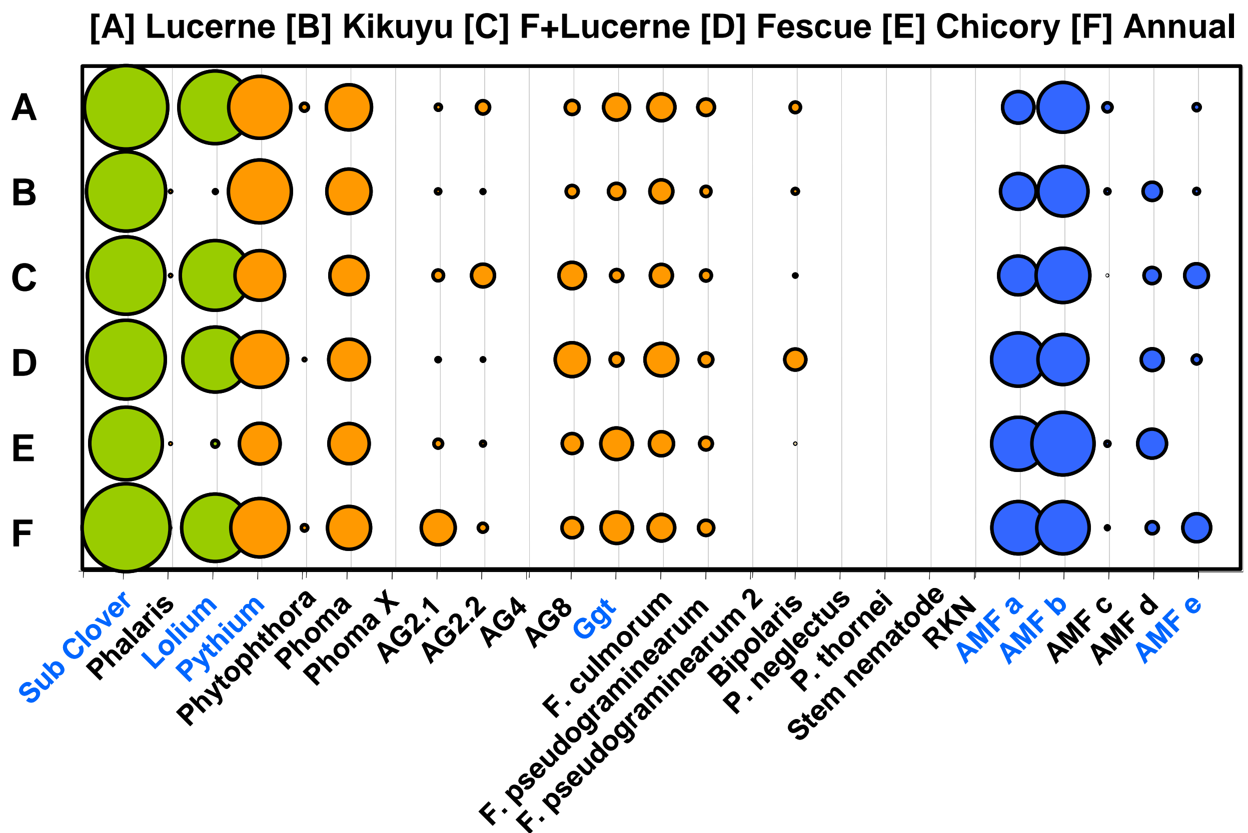



**Figure 3.** DNA soil profiles for EverGraze site at Albany (width of circle proportional to log(pg DNA/g soil) or log(nematodes/g soil)). Organisms highlighted in blue text responded significantly to the pasture treatments.

*Pythium* DNA had a significant positive relationship with the sub clover estimates, both proportion and biomass, explaining 22 and 40% of the variation respectively (P =0.02 and 0.002). The relationship with FOO was stronger, explaining 50% of the variation (P<0.001). It was not correlated with the biomass of grasses but showed positive relationship with the biomass of broadleaf species (4% variation explained, P=0.001).

Take-all DNA was negatively correlated with tall fescue (proportion and biomass, P=0.02) explaining 21% of the variation, with a similar relationship with total grasses.

These data are of a base-line nature as the site was only established a year ago. The DNA is stored and can be tested further if additional tests are developed.

The results show that even in the first year the treatments have significantly changed the spectrum of microorganisms in the system, so it is anticipated that stronger difference will emerge in time if the botanical differences are maintained.

Although the relationships with biomass were not examined exhaustively at this stage, some relationships were evident. *Pythium* increased with sub clover and total plant biomass as would be expected. However, the factors contributing to the take-all concentration need further exploration; it is a pathogen of grasses, however, host susceptibility varies between species. Nevertheless, this demonstrates the potential to examine the relationships between soil microbes and other factors in a way that was not previously possible.

### 4.2.1.2 Fungal community structure

The figure below (Figure 4) is an example of a section of a DGGE gel of the fungal community in the soil.

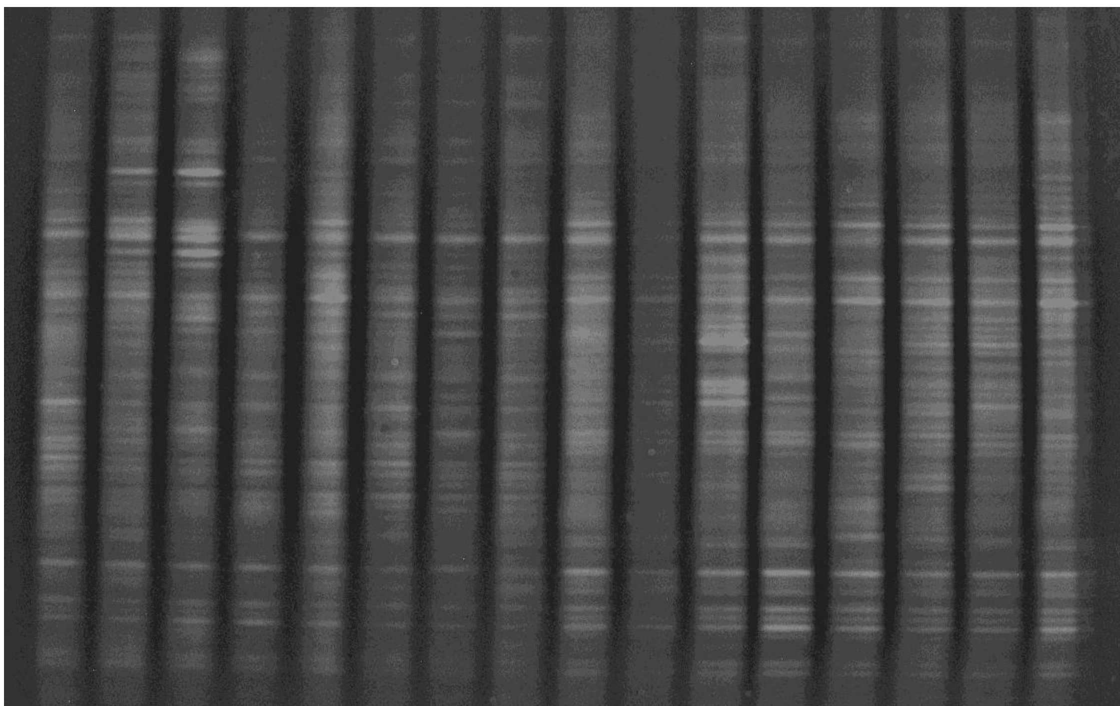


**Figure 4.** DGGE gel showing community structure of fungi in soil. Each lane (vertical) is from a treatment replicate. Each of the bands in the lanes represent an individual fungal species.

The fungal community structure was significantly different in the soil from under the different pasture species types (overall R=0.894; P = 0.001). The fungi from under fescue and chicory

had very distinct community types (Figure 5), whereas there was more variation between replicates in the lucerne, kikuyu and fescue+lucerne treatments. The fungal communities under kikuyu were not significantly different to those under lucerne, chicory or lucerne+fescue.

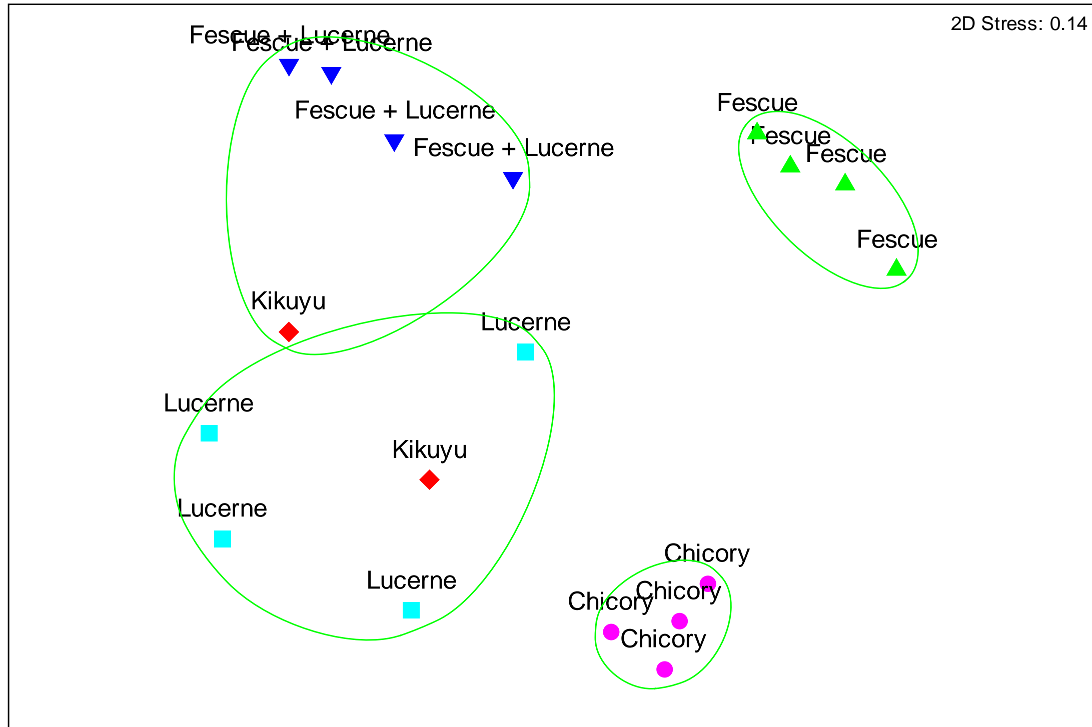



**Figure 5.** MDS plot of fungal community structure from soil under different pasture crop plants at the Albany field site. Circled symbols represent significant groupings at 70% similarity.

#### 4.2.1.3 Bacterial community structure

The species assemblage of bacteria in soil was evaluated using terminal restriction fragment polymorphism (tRFLP) of 16S rDNA. An example of a bacterial tRFLP plot is given in Figure 6.

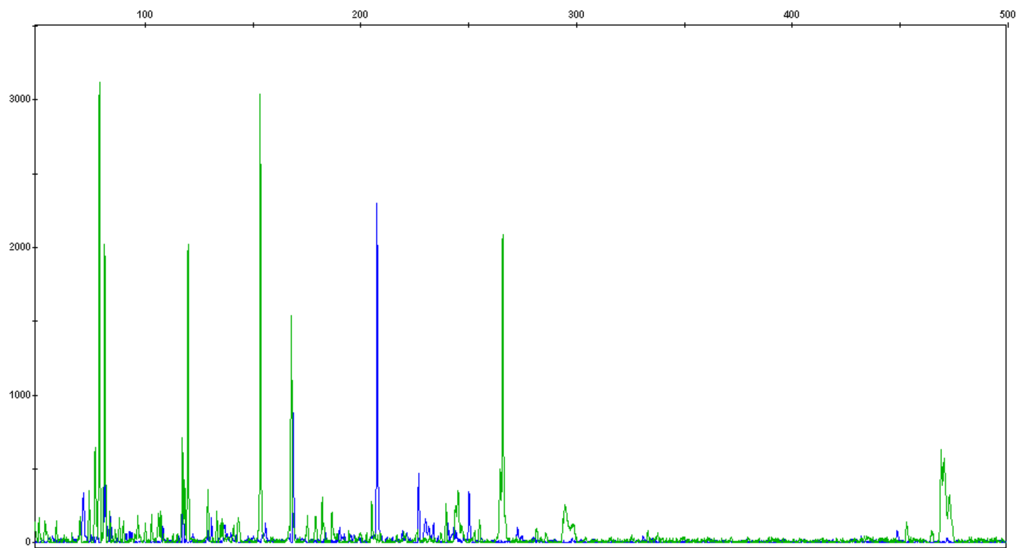



**Figure 6.** Terminal restriction fragment length polymorphism (tRFLP) trace analysis as separated by capillary sequencing. Each peak on the trace resembles a specific bacterial type. The location and size of peaks is used to generate a picture of the bacterial species present in the samples.

Pasture treatment had a significant overall effect on bacterial species present (R=0.848 P=0.001; Figure 7). However, bacteria under lucerne and kikuyu were grouped together, as did bacteria under fescue and fescue+lucerne. As such, lucerne can be considered to be not as strong as selecting bacterial species as fescue or kikuyu (i.e. bacterial communities did not group to lucerne). Bacterial communities under chicory were distinct from the other pasture species.

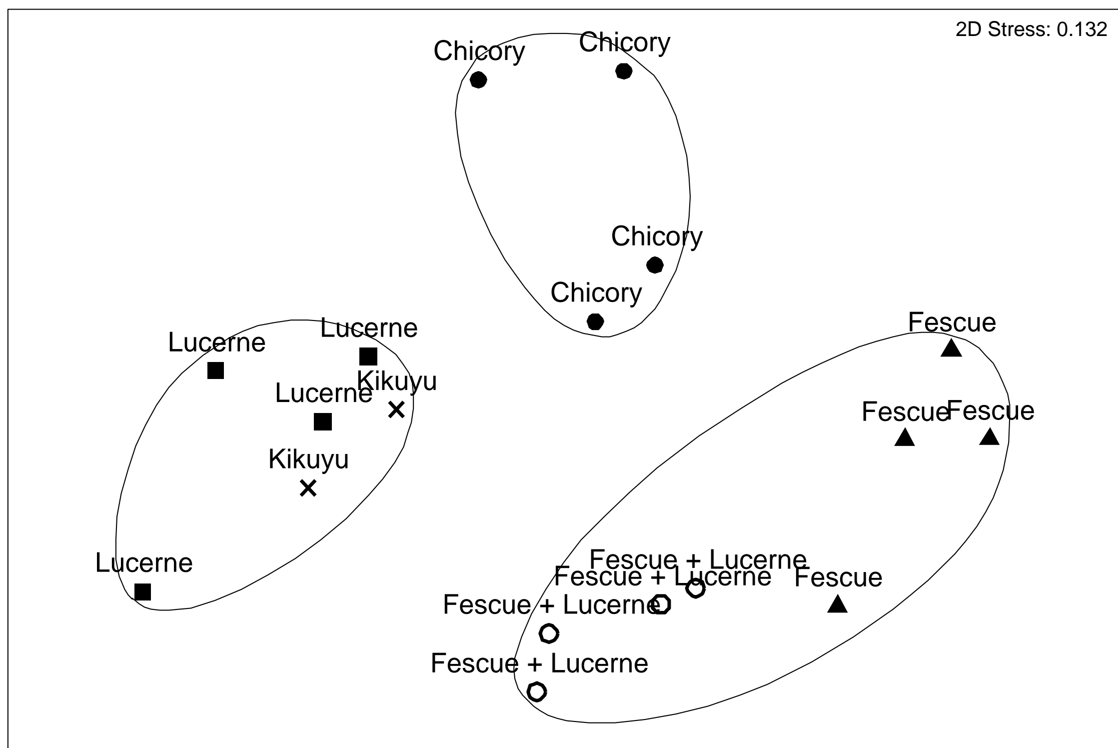



**Figure 7.** Multi-dimensional scaling ordination plot showing similarity of soil bacterial communities under different pasture crops from the Albany site. Circled samples are statistically significant and are grouped at 75% community similarity.

#### 4.2.1.4 Functional groups

The numbers of genes responsible for nitrogen fixation, nitrification and denitrification were measured in the DNA extracted from soil from under the 4 pasture species. The abundance of each gene/ng of soil-extracted DNA are presented in Figure 8.

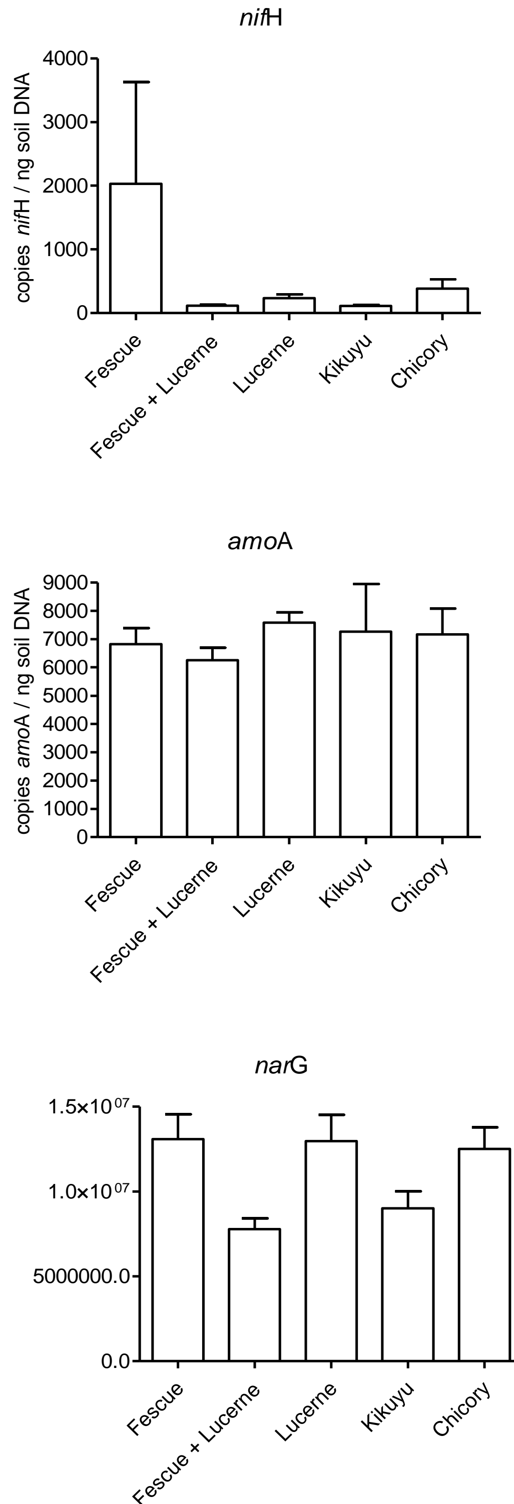



**Figure 8.** Effect of pasture species on the abundance of genes involved in N transformations in soils at the Albany site.

The abundance of the N-fixation gene nifH, was significantly greater in the plots plated to fescue compared with the plots planted to the other pasture species. At this site, the biological potential for nitrification (*amo*A) did not vary between plant species ($P>0.05$). Significantly lower potential for denitrification ($P<0.05$) occurred in plots sown to kikuyu and fescue+lucerne.

### 4.2.2 EverGraze – Wagga Wagga

#### 4.2.2.1 Soil organisms

Figure 9 shows the log-transformed data across the spectrum of tests. Sub clover occurred in all samples, although the population in the roadside was much less than in the experimental plots. *Phalaris* was only detected in the plots in which it had been established. *Lolium*/fescue was present in all treatments but was lower in the lucerne plots and along the roadside.

The pathogen abundance and diversity was much less than in Albany, and there were no treatment effects on pathogen levels evident at this stage. *Pratylenchus neglectus* was found at low numbers at the Wagga Wagga site, whereas none of the plant pathogenic nematodes subject to assay where found at Albany site.

The pattern of AMF occurrence did show treatment significant treatment effects.

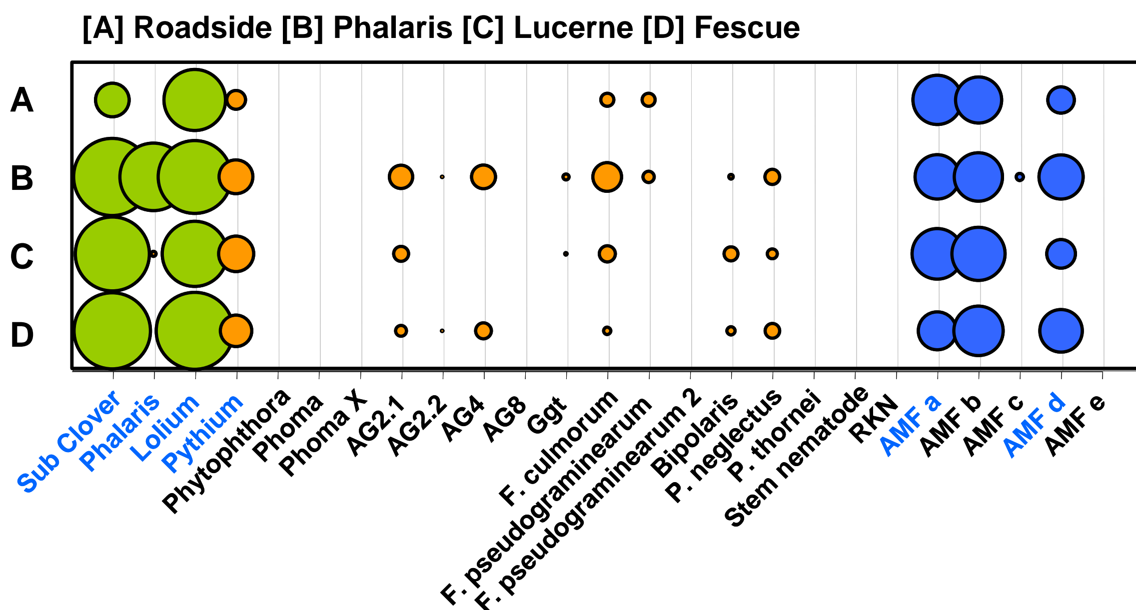



**Figure 9.** DNA soil profiles for EverGraze site at Wagga Wagga (width of circle proportional to log(pg DNA/g soil) or log(nematodes/g soil)). Organisms highlighted in blue text responded significantly to the pasture treatments.

As with the EverGraze site in Albany, the data from Wagga Wagga are to establish base-line levels and the stored DNA can be retested when additional tests become available.

The pathogen densities and diversity were low and, at this early stage, no treatment effects were statistically significant. It would be expected, if conditions were conducive, that future sampling will reveal differences that develop over the course of the experiment.

#### 4.2.2.2 Fungal community structure

Pasture species type was a significant driver of fungal species present (R=0.844; *P*=0.04). This is reflected in Figure 10, showing grouping of fungal communities according to the pasture species under which they were taken.

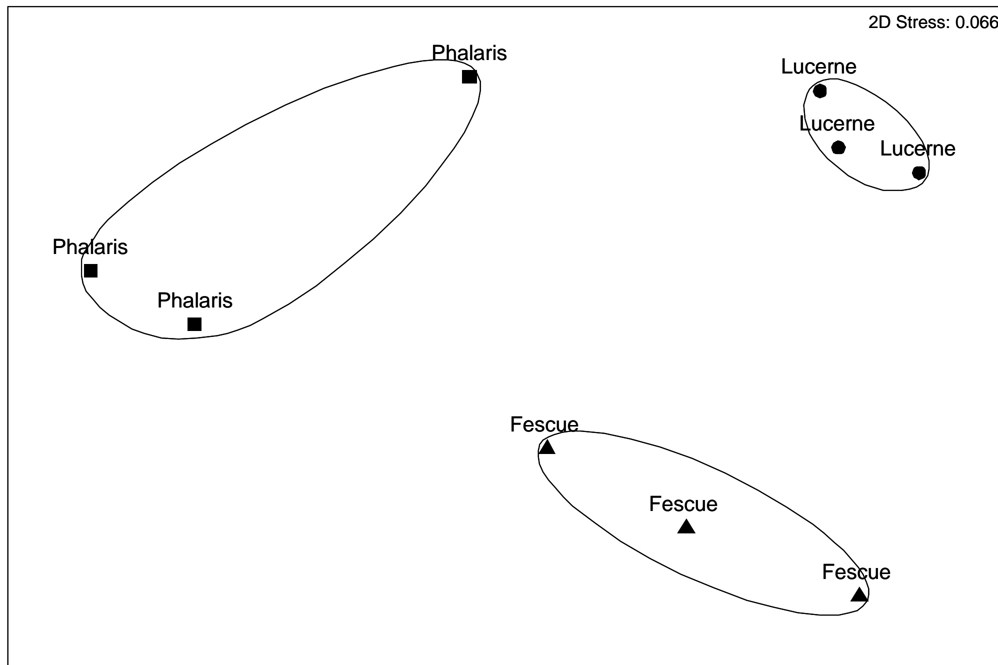



**Figure 10.** MDS plot showing similarity of fungal species in soil according to the pasture species present. The line circling the samples represents 81% similarly (statistically significant) between samples.

#### 4.2.2.3 Bacterial community structure

The bacteria community structure in the soil from under the 3 pasture species was compared (Figure 11). Soil bacterial communities were most similar according to pasture species type; i.e. the pasture type had a direct and significant effect on the bacterial species present; R=0.638; *P*=0.04).

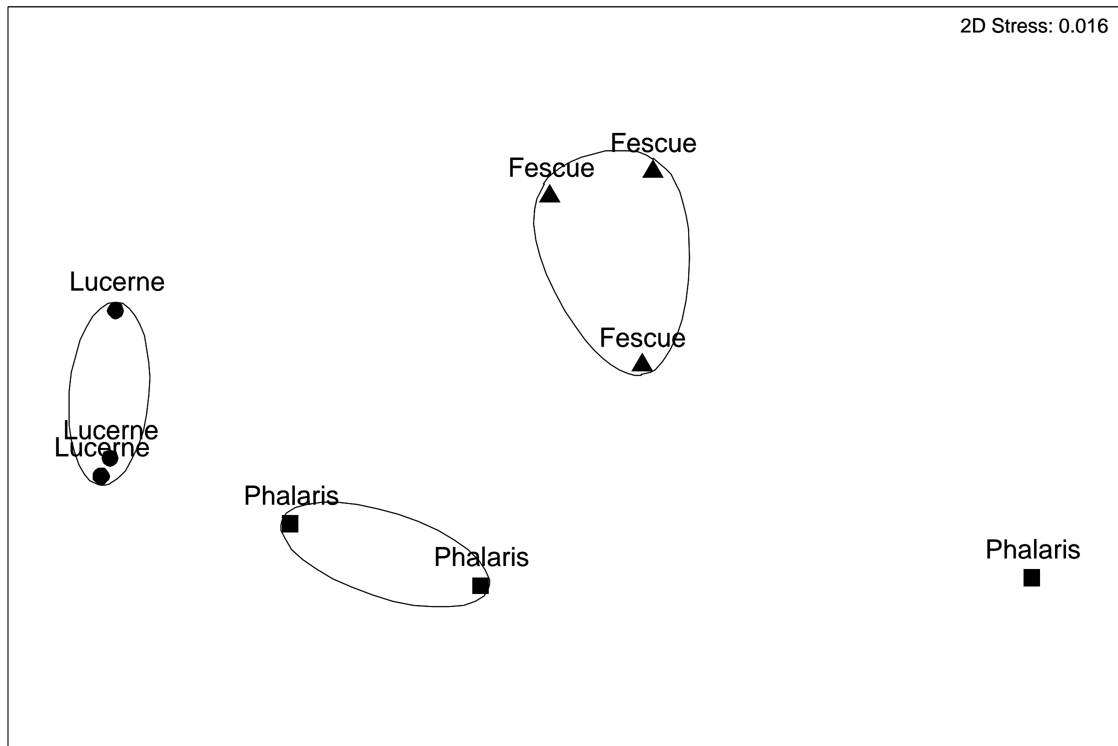



**Figure 11.** MDS plot showing similarity of bacterial species in soil according to the pasture species present. Line encircling samples represents 80% similarly (statistically significant).

### 4.2.2.4 Functional groups

The numbers of genes responsible for nitrogen fixation, nitrification and denitrification were measured in the DNA extracted from soil from under the 3 pasture species. The abundance of each gene/ng of soil-extracted DNA are presented in Figure 12.

Soil planted to lucerne had significantly greater levels of *nif*H and *amo*A genes ($P<0.05$) than soil planted to fescue or phalaris. These genes are responsible for nitrogen fixation and soil N re-cycling respectively. The abundance of *nar*G, responsible for denitrification, did not significantly vary between pasture species.

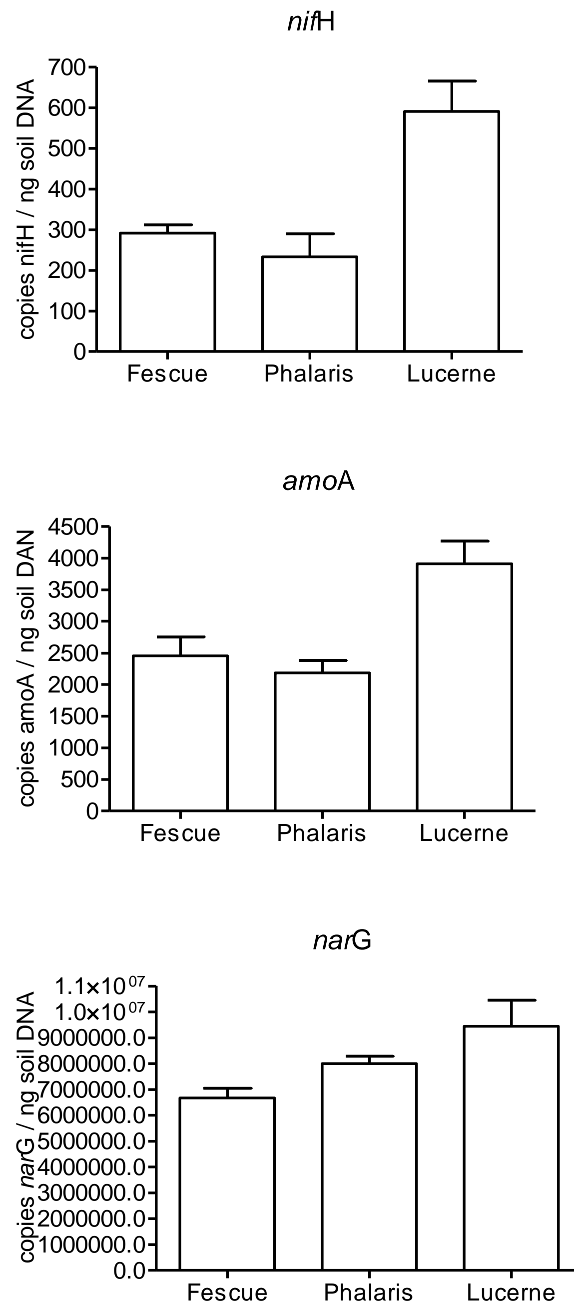



**Figure 12.** Effect of pasture species on the abundance of genes involved in N transformations in soils.

### 4.2.3 NSW DPI – MASTER

#### 4.2.3.1 Soil organisms

Figure 13 shows the log-transformed data across the spectrum of tests. Sub clover was present in all treatments, phalaris only in the perennial plots and *Lolium*/fescue significantly more abundant in the annual plots.

*Rhizoctonia* AG 2.2 was the most abundant pathogen across the experiment but was not affected by the treatments. However, the density of two of the pathogens, take-all (Ggt) and

*Bipolaris* were influenced by lime application. In both cases the liming had increased their density. Note the main hosts of both pathogens were probably barley grass and silver grass for which we do not yet have DNA assays.

Liming had also had a strong effect on AMF abundance and diversity. A small effect of perennial vs. annual pastures was also evident, but not to the magnitude of the lime effect.

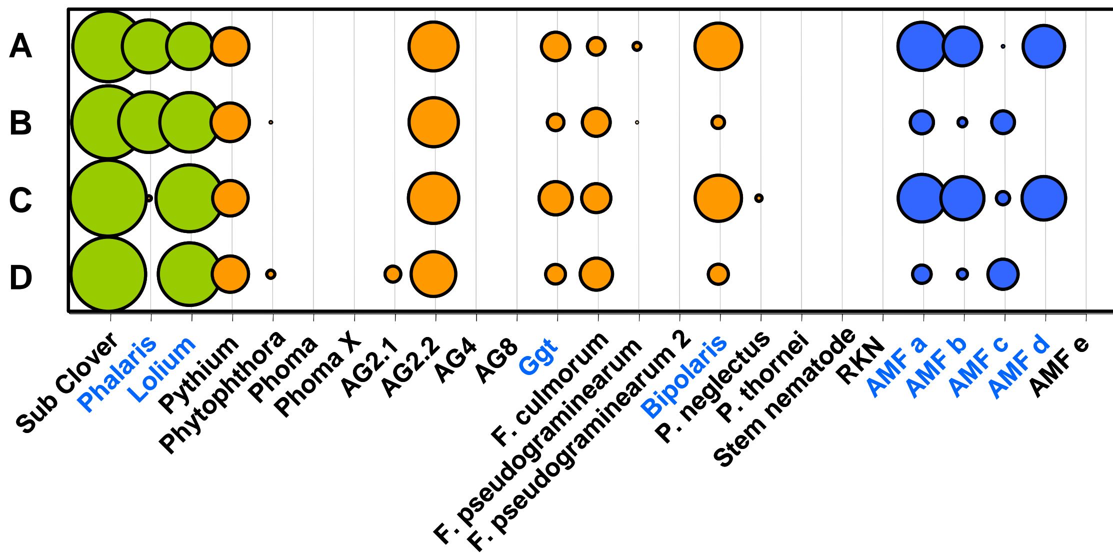



**Figure 13.** DNA soil profiles for MASTER site at Wagga Wagga (width of circle proportional to log(pg DNA/g soil) or log(nematodes/g soil)). Organisms highlighted in blue text responded significantly to the treatments.

The application of the DNA assays was to plots that had been managed differently for nearly 14 years, so it would be expected that equilibrium soil biology would have established in that period. Interestingly liming appeared to drive pathogen abundance more than botanical composition. Although only a limited assessment of botanical composition was undertaken in this work and it is possible that the botanical components (eg sub clover) that persisted across all treatments were the main hosts for *Pythium* Clade F and *Rhizoctonia* AG 2.2.

The clear benefit of liming to AMF populations could be very important. Not only will uncontrolled acidification affect the chemical environment in which plants derive their mineral nutrition, it also affects microbial factors.

#### 4.2.3.2 Fungal community structure

Non-metric multidimensional scaling (MDS) of fungal community structure (Figure 14) revealed two statistically significant groupings of fungal communities. The two distinct communities were affected by addition of lime as the primary driving factor (ANOSIM ρ=0.953; P=0.001). The type of pasture – annual or perennial – had no significant affect on fungal community type (ANOSIM ρ=0.188; P=0.082)

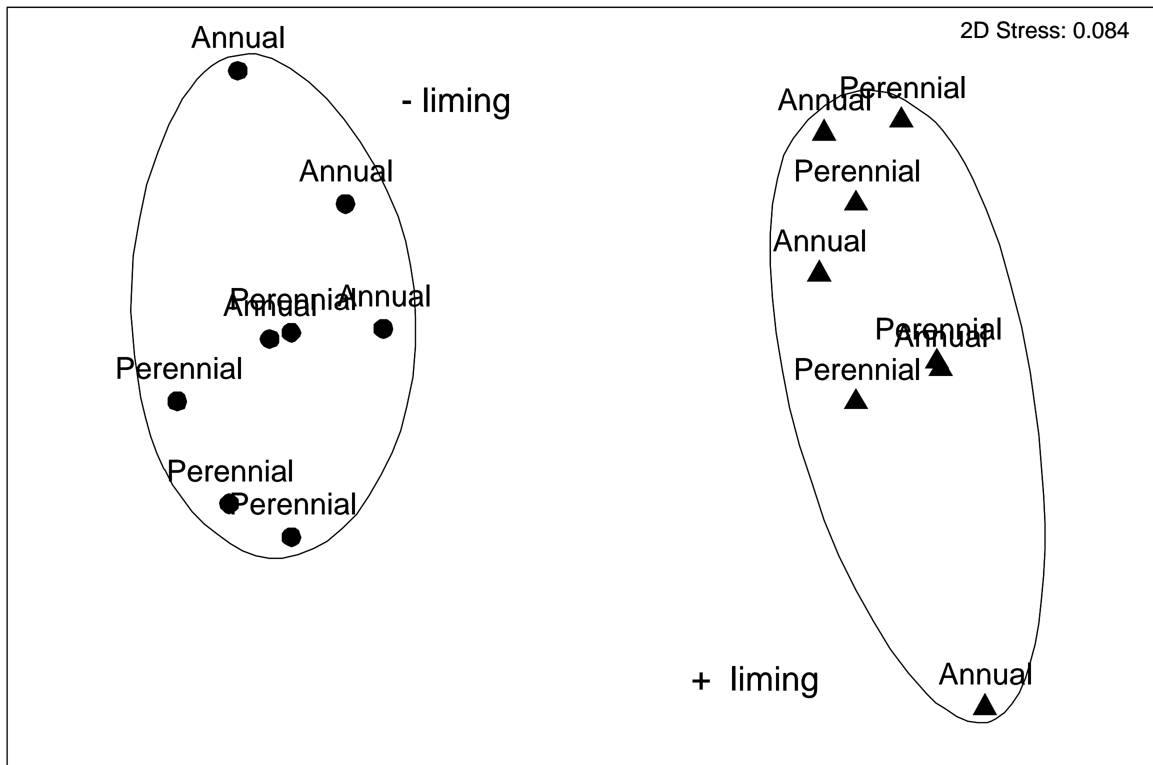



**Figure 14.** Non-metric multidimensional scaling plot representing similarity of fungal communities between treatments. Increasing distance between points on the plot represent increasing level of change in fungal species present.

#### 4.2.3.3 Bacterial community structure

The similarity between bacterial communities according to management at the site is given in Figure 15.

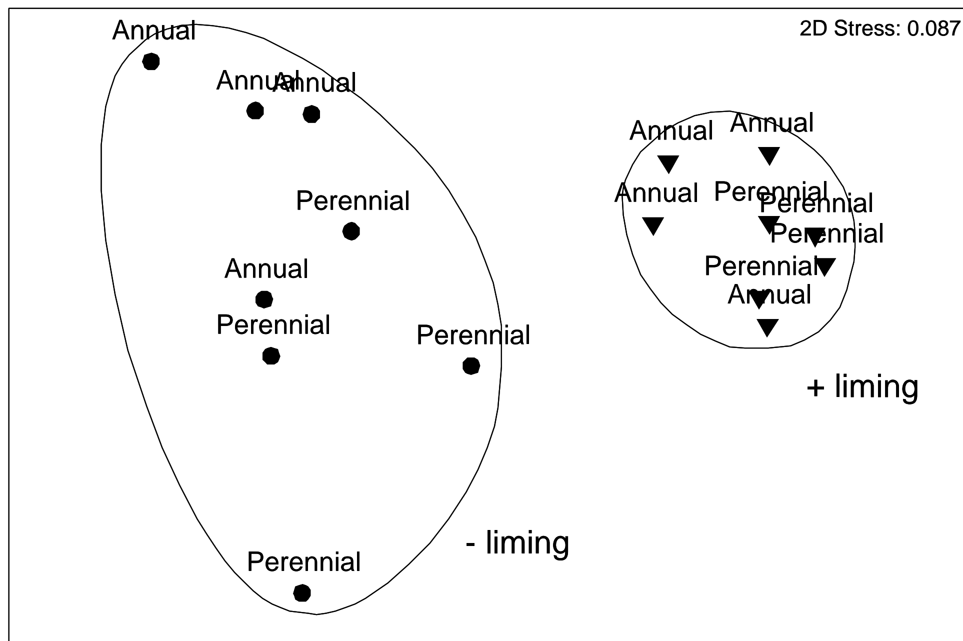



**Figure 15.** Non-metric multidimensional scaling plot similarity of bacterial communities between treatment. Increasing distance between points on the plot represent increasing level of change in bacterial species present.

As with the fungal community structure, the types of bacterial species present in the soils from the Master site were significantly different between the 2 liming treatments (ANOSIM R=0.969; P=0.001). However, there was also a secondary effect of pasture species type within each of the liming treatments (Figure 15; (ANOSIM R=0.292; P=0.021). Clearly, however, the effect of lime addition is the major driver of biological community type at the Master site and pasture species is secondary to this (Figure 15).

#### 4.2.3.4 Functional groups

The numbers of genes responsible for nitrogen fixation, nitrification and denitrification were measured in the DNA extracted from soil from the Master site. The abundance of each gene/ng of soil-extracted DNA are presented in Figure 16. The results showed that the addition of lime significantly increased the biological potential for nitrification (cycling of various forms of N in soil) regardless of pasture type. The biological potential for N fixation (Figure 16) increased following the addition of lime in annual pastures but had less effect in perennial based permanent pastures. There was little no significant difference ($P$>0.05) in the levels of biological denitrification potential in soil due to either liming or pasture type.

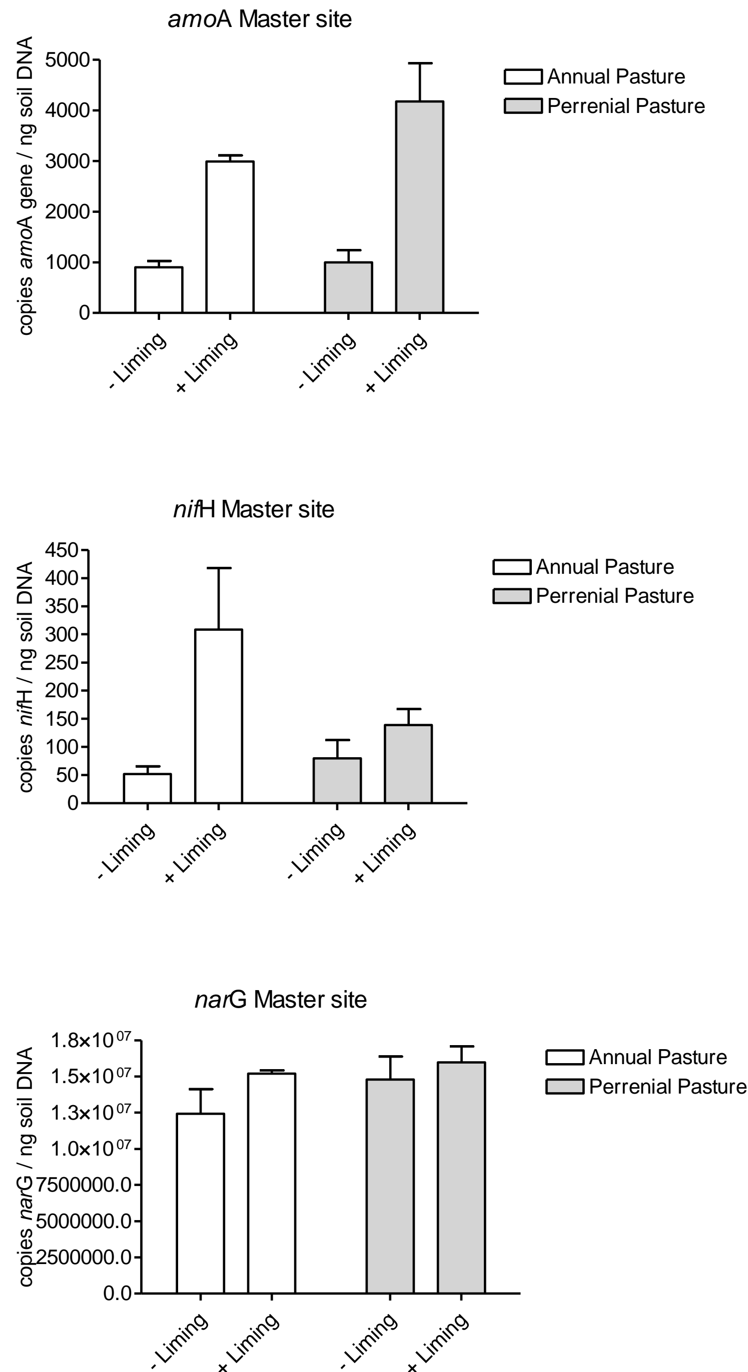



**Figure 16.** Abundance of genes (mean + SEM) for N fixation (*nif*H), nitrification (*amo*A) and denitrification (*nar*G) in DNA extracted from soil at the Master site.

### 4.2.4 CSIRO – Phosphorous by grazing trial

#### 4.2.4.1 Soil organisms

Statistically significant effects were found in *Trifolium*, *Phalaris*, *Lolium*, *Pythium* and Take-all tests (these are shown in Figures 17 to 22). In addition, the black spot fungal complex, *Rhizoctonia* AG2.2 and AG4 (some plots) were at moderate to high densities at the site with no obvious treatment effects.

Sub clover was highest with P application and the higher stocking rate (Figure 17). There was no treatment effect on *Phalaris* but it did rise and fall during the season (Figure 18). *Lolium* tended to only occur were P was applied but not tightly grazed (Figure 19).

*Pythium* increased during the year and occurs at highest densities under P1 SR18, probably because this treatment favoured sub clover. *Phytophthora* only occurred at any significant density in the unfertilised treatment (P0) and rose rapidly during winter to decline again in late spring. Take-all did not change though the year and it was lowest in P1 SR18, again probably due to this treatment combination favouring sub clover and suppressing grasses.

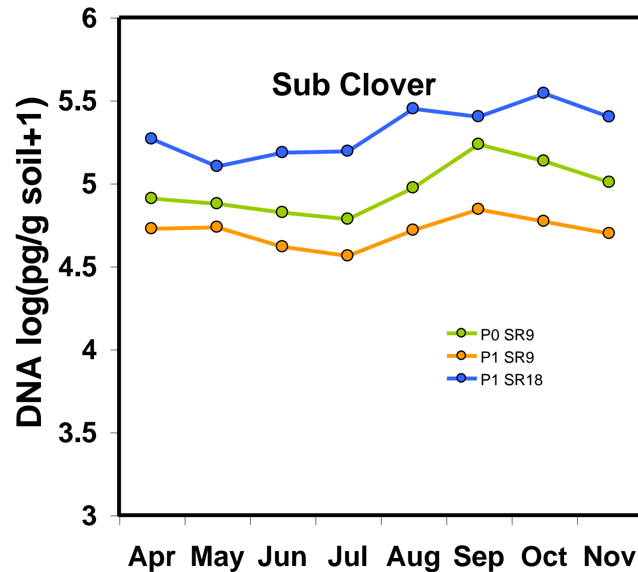



**Figure 17.** Effect of phosphorus application (P0, no fertiliser; P1, P applied annually) and grazing regime (SR9, 9 DSE; SR18, 18 DSE) on sub clover (*Trifolium subterraneum*) DNA concentration at monthly intervals in 2006 at Hall, ACT (CSIRO experiment)

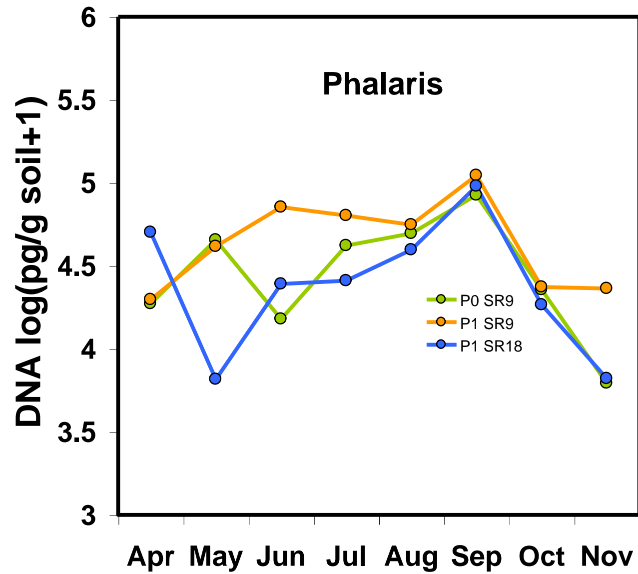



**Figure 18.** Effect of phosphorus application (P0, no fertiliser; P1, P applied annually) and grazing regime (SR9, 9 DSE; SR18, 18 DSE) on *Phalaris* DNA concentration at monthly intervals in 2006 at Hall, ACT (CSIRO experiment)

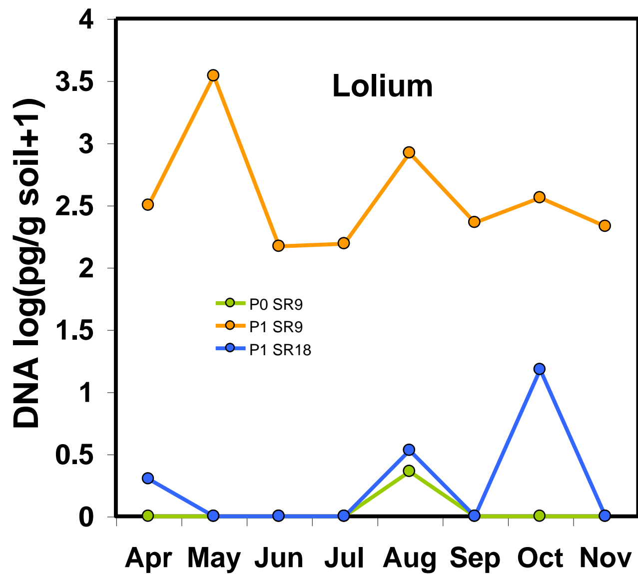



**Figure 19.** Effect of phosphorus application (P0, no fertiliser; P1, P applied annually) and grazing regime (SR9, 9 DSE; SR18, 18 DSE) on *Lolium*/fescue DNA concentration at monthly intervals in 2006 at Hall, ACT (CSIRO experiment)

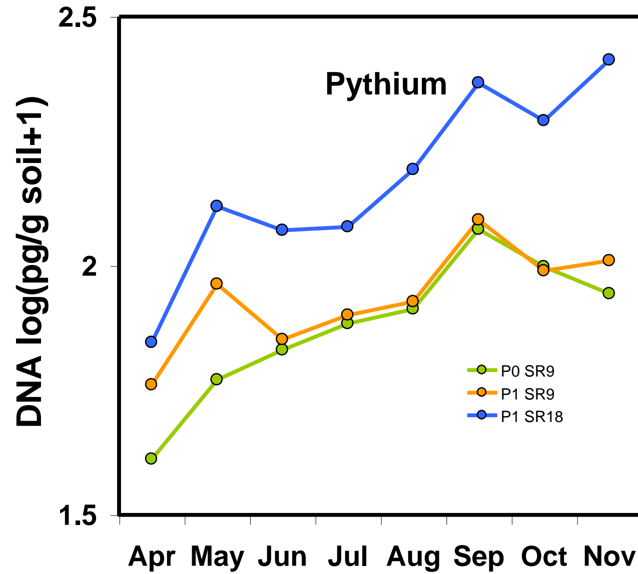



**Figure 20.** Effect of phosphorus application (P0, no fertiliser; P1, P applied annually) and grazing regime (SR9, 9 DSE; SR18, 18 DSE) on *Pythium* Clade F DNA concentration at monthly intervals in 2006 at Hall, ACT (CSIRO experiment)

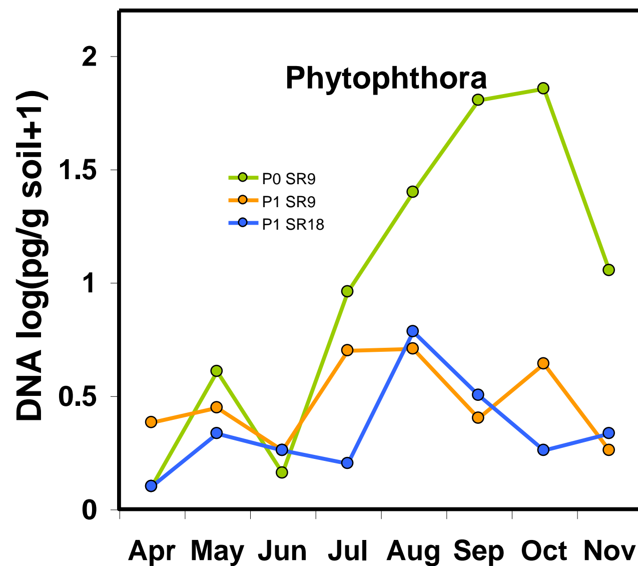



**Figure 21.** Effect of phosphorus application (P0, no fertiliser; P1, P applied annually) and grazing regime (SR9, 9 DSE; SR18, 18 DSE) on *Phytophthora clandestina* DNA concentration at monthly intervals in 2006 at Hall, ACT (CSIRO experiment)

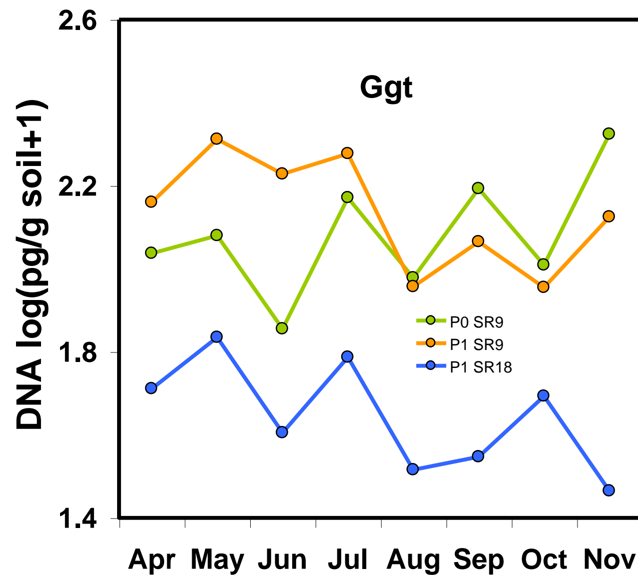



**Figure 22.** Effect of phosphorus application (P0, no fertiliser; P1, P applied annually) and grazing regime (SR9, 9 DSE; SR18, 18 DSE) on take-all (*Gaeumannomyces graminis* var. *tritici*) DNA concentration at monthly intervals in 2006 at Hall, ACT (CSIRO experiment)

The soil DNA analysis was again able to reveal effects of the treatments on soil biology and proved a useful tool for examining seasonal changes. *Pythium*, *Phytophthora* and *Gaeumannomyces* (take-all) showed significant responses to the treatments and relationships to changes botanically composition.

Changes in botanical composition as a result of the treatments appeared to be the main determinant of the changes in pathogen densities. The lack of effect on AMFs is unexpected given the results from the other sites studied. Also, given that 2006 was an unusually dry year, it would be expected that the patterns of response would be quite different and potentially more informative in a normal season.

### 4.2.4.2 Fungal community structure

Changes in fungal community structure under different P and stocking regimes were tested over time.

Phosphorus was found to be a significant driver of the species of fungi in the soil R=0.833, *P*=0.01;) and was a stronger driver than time (Figure 23). Time was found to be a significant driver (R=0.528; *P*=0.001), with the major differences being between a grouping between September/August and other months. It should be noted that soil samples were taken during drought conditions.

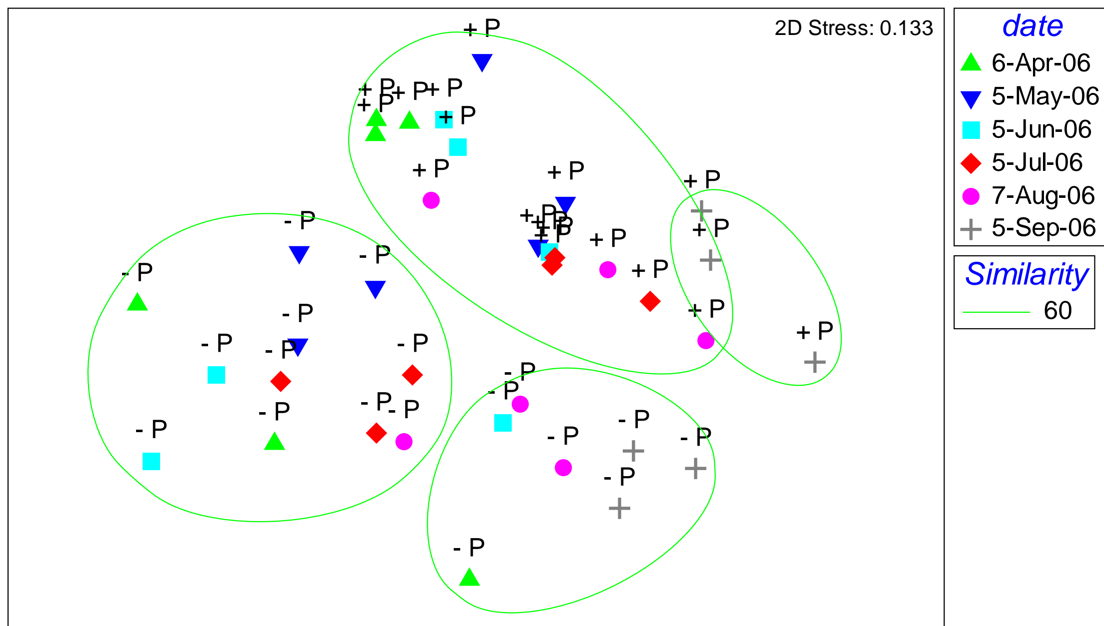



**Figure 23.** MDS of fungal community structure under different levels of P addition (all at stocking rate of 9 livestock units) over time. The major groupings are according to +/- addition of P.

Similarly, the effect of stocking was investigated over time. The fungal communities grouped strongly according to stocking rate (R=0.79; *P*=0.001; Figure 24). However, 2 major clusters also occurred at approximately 55% similarity (Figure 24). The top grouping incorporated samples taken from July to September, while the lower grouping included samples from April to June.

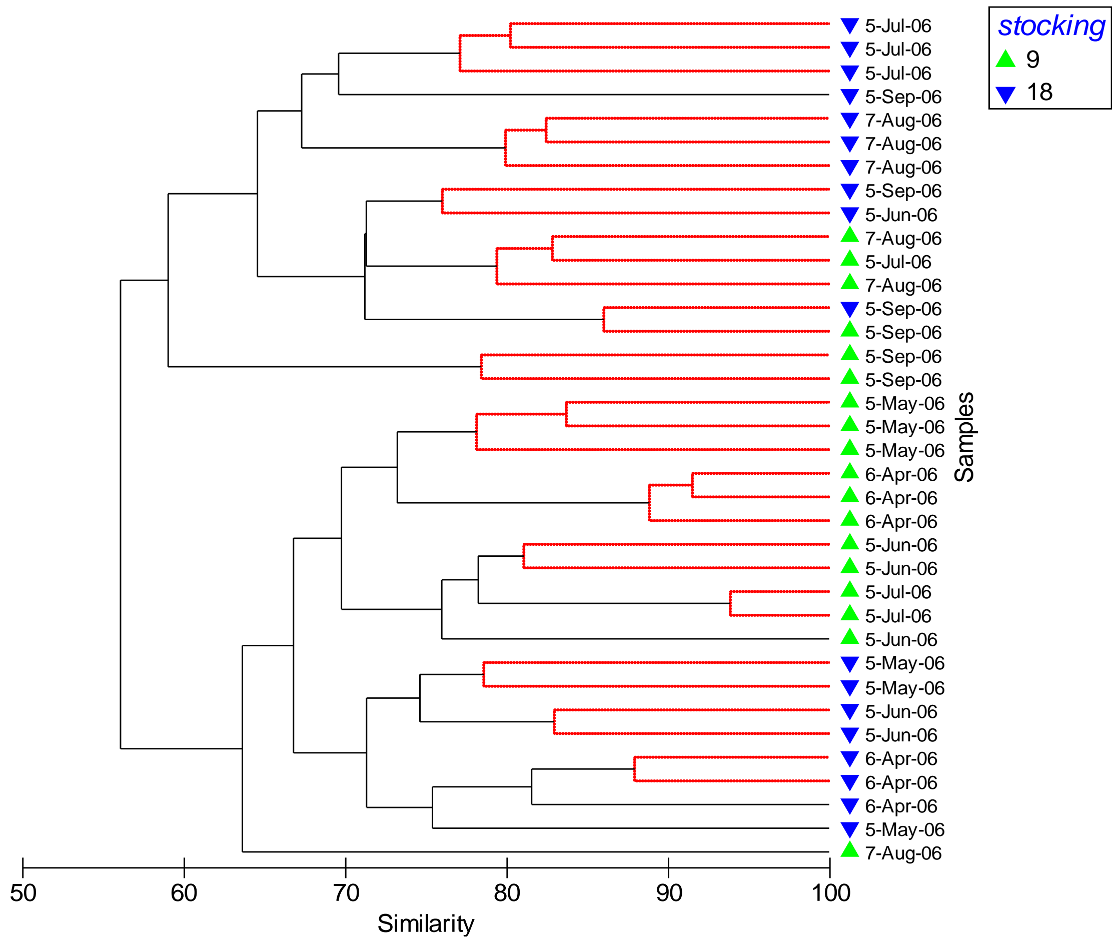



**Figure 24.** Cluster plot of fungal communities structure under alternate levels of stocking (9 and 18) and over time.

MDS ordination was used to determine the effects of time on fungal species assemblage (Figure 25). A noticeable shift in community composition occurred over time (X-axis) whereas the Y-axis described differences in stocking rate. When analysed by ANOSIM, time was found to be a significant driver of species assemblage (R=0.762; *P*=0.001)

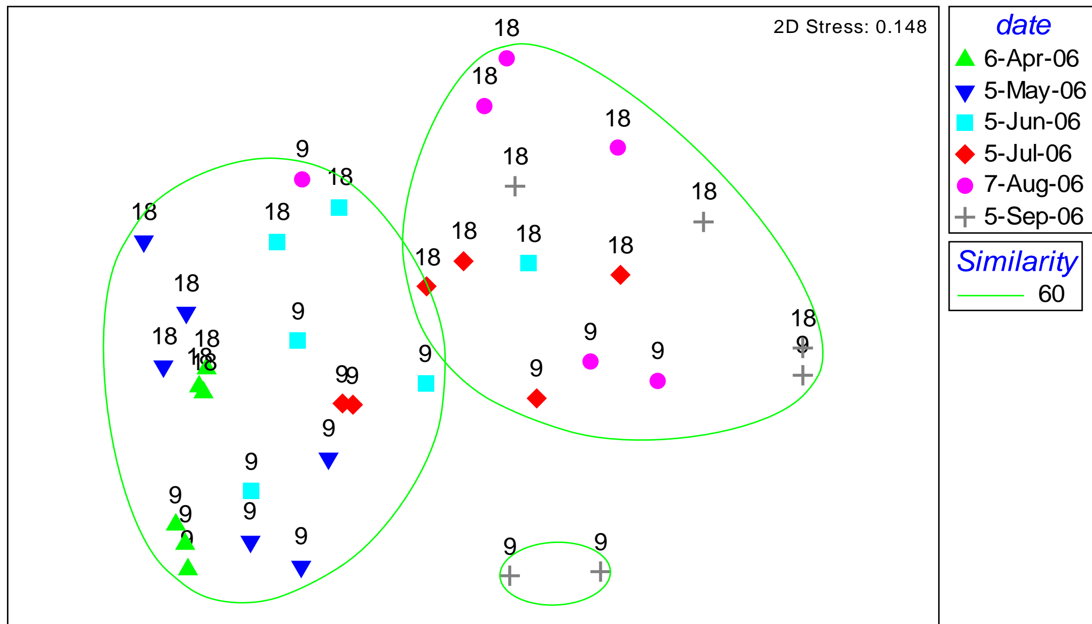



**Figure 25.** Multi-dimensional scaling plot of fungal community composition for the stocking treatments over time. The X-axis represents a shift in time from April (left) to September (right).

### 4.2.4.3 Bacterial community structure

Changes in bacterial community structure under different P and stocking regimes were tested over time.

Both P and time had small but significant effects on the species of bacteria present (for P, R=0.247, *P*=0.018; for time, R=0.236, *P*=0.03). The small magnitude of these effects are represented in Figure 26.

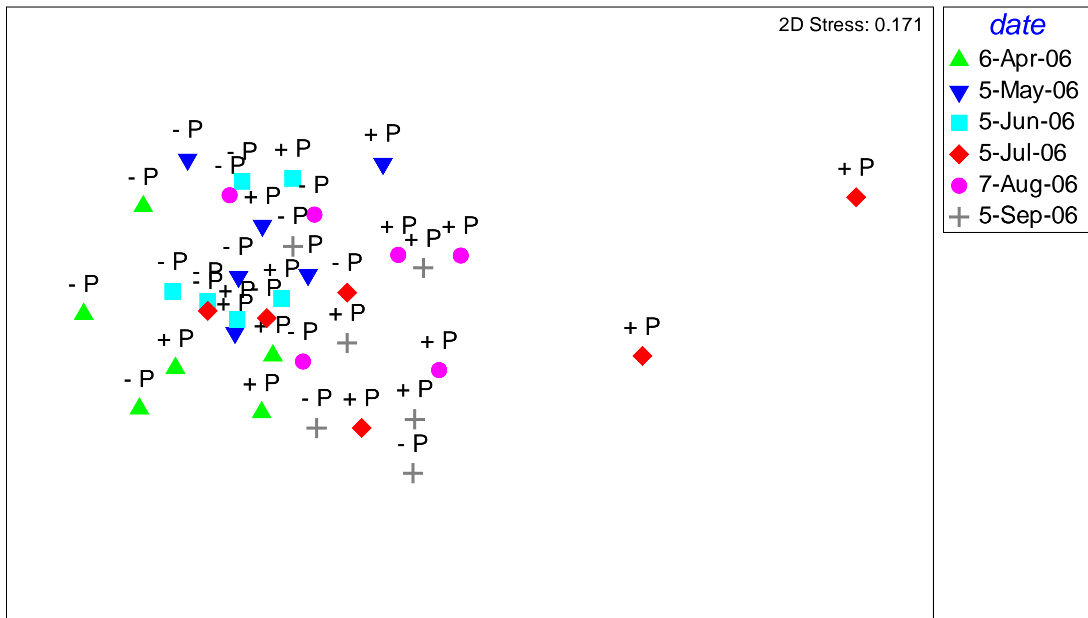



**Figure 26.** Multi-dimensional scaling plot showing similarity between bacterial community structures as affected by phosphorus and time (at stocking rate of 9 only).

Stocking rate did not significantly change the community of bacterial species present in the soil (R=0.049; *P*=0.3; Figure 18) at the P rate of 1. However, a successional change in bacterial species over time occurred (Figure 18) and this temporal change was highly significant (R=0.383; *P*=0.01).

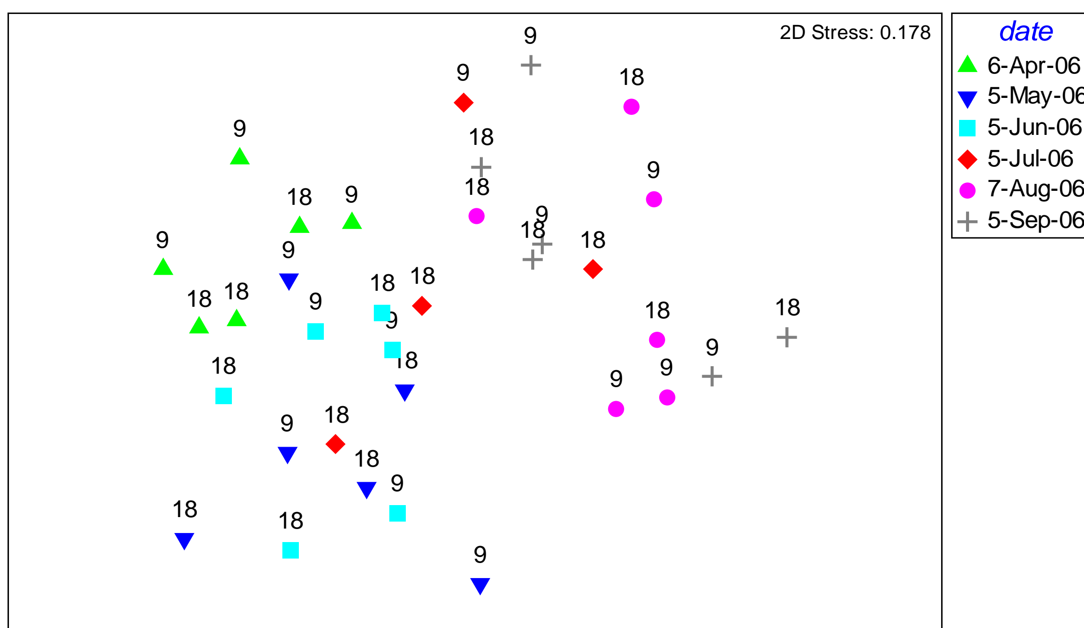



**Figure 27.** Multi dimensional scaling plot of bacterial community structure as affected by stocking rate and time. The effect of time is evident on the X-axis (April/May on left progressing towards August and September on the right).

### 4.2.4.4 Functional groups

The numbers of genes responsible for nitrogen fixation, nitrification and denitrification were measured in the DNA extracted from soil from under the different rates of P, stocking and over time.

The biological potential for $N_2$ fixation (*nif*H) was significantly affected by stocking rate but not P addition (Figure 28). At the higher stocking rate, copy numbers of *nif*H significantly increased ($P$<0.05). Over time, the abundance of *nif*H copies within the pool of soil DNA did not significantly vary. However, there was a dip in *nif*H in July (Figure 28).

The biological potential for nitrification (*amo*A copy numbers) was significantly affected by all treatments (Figure 29). Copy numbers of the *amo*A gene increased with stocking rate and P (Figure 29). The numbers of the *amo*A gene in soil varied significantly over time ($P$=0.013), dipping markedly in July and peaking in October.

Similarly, the biological potential for denitrification (*nar*G copy number) was also significantly affected by all treatments (Figure 30). As for *amo*A, *nar*G increased with both stocking and P. However, both increased stocking and P addition had similar magnitudes of effect on the gene numbers (Figure 30). Copies of narG in soil varied over time (P<0.05) and dipping in July and peaking in November.

# *nif*H

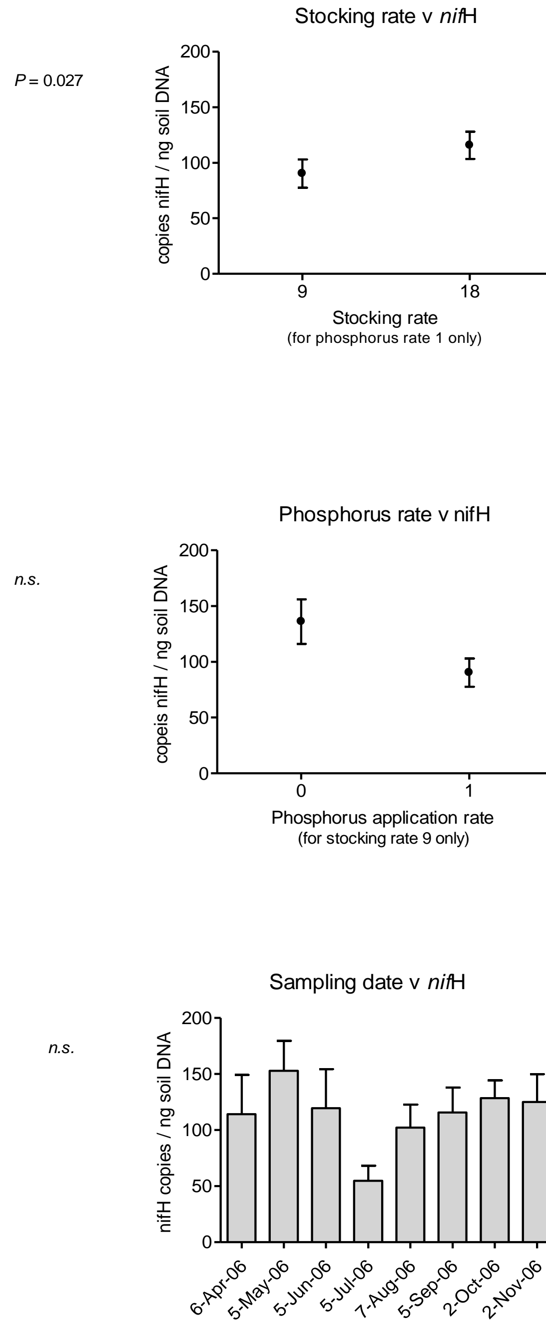



**Figure 28.** Biological potential for $N_2$ fixation in soil DNA from alternate P, stocking management and over time.

## ***amo*A**

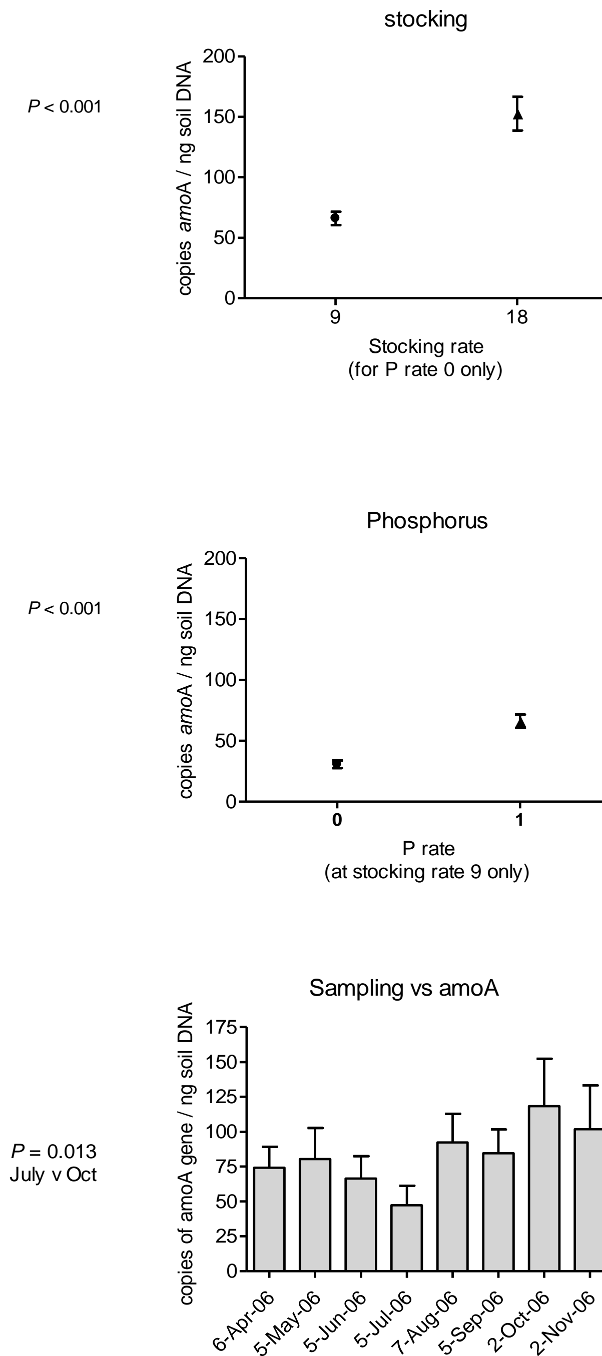



**Figure 29.** Biological potential for nitrification in soil DNA from alternate P, stocking management and over time.

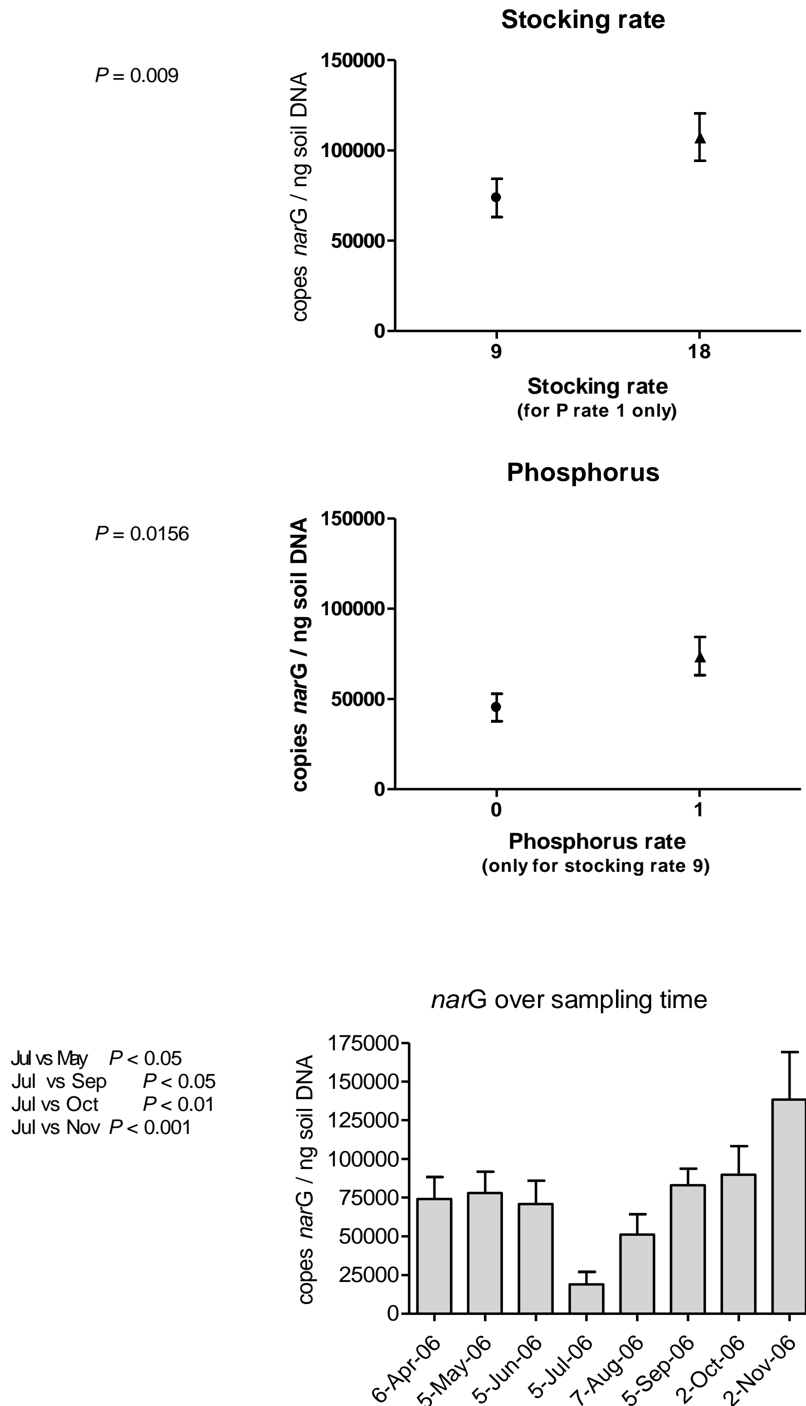



**Figure 30.** Biological potential for denitrification in soil DNA from alternate P, stocking management and over time.

### 4.2.4.5 Between Site Comparisons

The analysis proved useful for exploring treatment effects within the experimental sites and for establishing base-line data, however the between site comparisons also appear to be potentially useful.

Figure 31 shows the DNA profiles for the four study sites. In the case of most organisms tested differences between the sites are evident. The botanical composition varied, the pathogens showed obvious examples of site effects and the AMF patterns also changed markedly between sites.

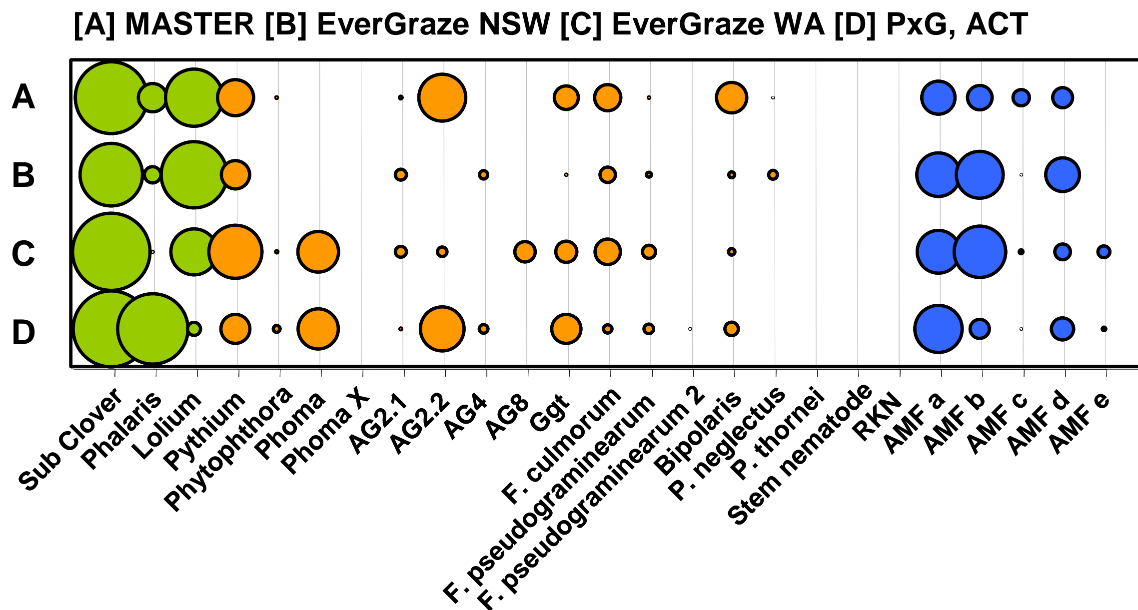



**Figure 31.** DNA soil profiles for the MASTER, EverGraze (Wagga Wagga, NSW and Albany, WA) and P by grazing experiments (width of circle proportional to log(pg DNA/g soil) or log(nematodes/g soil)), means of all plots sampled).

It is clear that the DNA tests provide tools not only for monitoring treatment effects and changes with experiments but also the effects of large-scale factors such as industry-level shifts in management practices, soil types and variations in climates and seasonal conditions.

# 5 Success in Achieving Objectives

The objectives of the project were met successfully.

The pilot education package on soil biology and the emerging molecular assays for soil borne organisms, biological function and microbial community analysis was developed and delivered to researchers. Many of those participated were enthusiastic about the new opportunities the technology provides and some have entered in to active negotiation use and evaluated the DAN assays in their research.

The project engaged with four field research projects and the results generated showed that pasture composition and management activities affect soil biology and that his can be measure at a single organism, though to community structure and function scale.

# 6 Impact on meat and livestock industry – now and in five years time

The project was not designed to have direct impact for the meat and livestock industry, rather it was intended to begin developing knowledge and confidence of pasture scientists to use and interpret DNA capability and results to assess impacts of biological constraints in pasture trials to ultimately develop more efficient and robust grazing systems.

It is anticipated that within the next year the technology will be further applied in existing experiments and by five years researchers will be planning their experimental programmes with DNA assessments as an integral part. We encourage the funding organisations to accept and endorse the use of this technology in future pasture based research programmes.

The interest generated by the new approach to studying root distribution and function and its application is likely to provide a vehicle for strengthening an interest in and assessment of plant interactions with the soil microbial community.

It is possible that within five years, results of the research aided by the soil DNA technology will become available to industry. However, this will depend on continued commitment to the extension and refinement of the methods, so that the data generated can be meaningfully interpreted and applied in development of practical solutions.

# 7 Conclusions and recommendations

The engagement with researchers through this project has further highlighted the value of the DNA approach in that it can provide data and insights not available by any other means. There is strong interest in seeing the technology grow and applied. There was particular enthusiasm about the potential of monitoring root development and activity in soil with the plant assays.

Therefore it is recommended that further development and promotion of DNA assays for tools to understand and assist in the favourable manipulate of soil ecosystems be supported.

## 7.1 Pilot education package

The project successfully introduced the new approach and opportunities for study of pasture soil biology to about 30 scientists from WA and NSW. The pilot delivery of the training course has identified strengths and weakness and obtained valuable feedback that will be incorporated as the course is modified for future delivery.

Interaction with a broader range of potential users also provided useful feedback that will help in setting priority for future test development and refinement.

Summary of feedback from workshop attendees

Highlights

*Technology*

- New tools and potential they have for research.
- Update on tests under development
- New technology for root disease and root research.
- Taqman technology for high throughput assessment of soilborne pathogens.
- Benefits of the technology to investigate soil biology.
- Interesting technology.
- Plant assays
- Good overview of range of tests available.

*Manual & Presentation*

- Manual great- easy to follow with slides and handy notes pages. Well presented.
- Work book excellent, plenty of time for Q&A
- Manual and presentation good

- Manual very good.
- Manual is a great resource.
- Good workshop most informative.
- Good PowerPoint slides, comprehensive manual, OK for scientists.
- Good presentations well presented.
- Presentation good, very useful manual.
- Excellent presentation, manual well put together.
- Interesting concepts – manual good.
- Manual great, good in colour – information presented in presentation- structure of discussion.
- Presentations very good not to long
- Manual excellent and relates well to people with limited backgrounds.
- Speakers made a lot of sense.
- Multidisciplinary presentation.

*Soil biology*

- Relationships between pathogens soil plant and their DNA – application to pasture systems – appreciated case studies.
- Background / update on pathogens and soil organisms.
- Good coverage of soil pathogens association in soil with plants.
- The number of cases for the technology is very surprising. Manual is very good.
- Handy to go through all important pathogens, hosts conditions for suppression etc, and available tests.
- Discussion on pathogens.
- Beneficial organisms – mycorrhiza
- Several practical applications
- Relevant to my work on cover crops.
- Relevant to current pasture sites, could refocus future work.
- Having a soil biology workshop in Wagga.

Lowlights

*Manual & Presentation*

- Need to explain whole soil environment and principle of assays much better. Less detail of individual pathogens. A brief explanation of functional groups would be better precursor to the application of techniques.
- Some sessions running up to an hour without discussion.
- Too much detail in talks
- Introduction a bit slow.
- Pathogen talk a bit stale – could be improved with more photographs of diseases and pathogens.
- May have bogged down in the section illustrating technology through site specific examples.
- Brushed over beneficial microbes.
- No details about DNA techniques - assumptions of molecular skills of audience may be over rated.
- Assumption of knowledge of DNA application – though appreciate this requires separate workshop.
- Detached, e.g. nematode biology without context.
- Results presented weren't explained clearly in terms of site history – hard to interpret applications of technology.

- Results section needed, but the applications are not all clearly shown by the results. Other ways to show how the technology could be used would be good.
- Presentation of ”baseline” data was not very beneficial, hopefully when next lot of sampling is done presentation can be more focussed – key outcomes with examples shown from the data.

What would you like more of?

*Soil biology*

- More understanding of why soil biology responds in a particular way to management – will come with more work.
- More emphasis on soil ecology
- Bio-indicators to assess soil health.
- More depth on beneficial microbes and promoting these in soils
- More simple explanation at start of presentations on what the technology is and how it works.
- More information on link between DNA results and root mass to complement conventional root research.
- Information on interaction between pathogens – application to manage improve pasture.
- More examples of pathogen associations.
- More time course information.

*Technology*

- Expansion into perennials.
- Broader range of pathogen and plant assays.
- More annexes about calibration and other technical issues.
- Basics on DNA technologies.
- More detail on what is an assay.
- Application to crop pasture interface – conservation cropping systems – impact on microbial communities.
- Details of current use and effectiveness of fungicides etc on suppressing / eliminating populations and effect on beneficial microbes.
- Better explanation of figures.
- Tools and measurements for beneficial soil biota – DNA of important organisms to measure and compare with known data eg microbial biomass.
- More on beneficial biota – more on applications – obviously come with time.

What’s missing

- Role of beneficial plant growth rhizobacteria in trials.
- Presentations and manual very informative. Presentation would be better next time after the discussion questions.
- Brief outline of the types of organisms and their roles in soil function.
- Quick explanation on how to interpret cluster analysis diagrams are created and what they mean.
- Outline of traditional methods of looking at soil biology eg biomass, CO2 evolution, plate cultures etc
- Relationship between plant DNA and beneficial soil biota.

## 7.2 Researcher engagement

### 7.2.1 Soil organisms

This pilot project has demonstrated the potential value of combining a range of assays to monitor soil DNA levels of key plants, pathogens and beneficial organisms to evaluate management strategies in field trials.

DNA tests detected significant levels of a range of plant pathogens and AMFs in all sites, and significant treatment differences were detected in the long-term experiments. Treatments differences were also becoming evident in EverGraze sites, which had only recently been established.

The use of DNA assays to monitor plant DNA levels is soil is a novel approach to monitor root growth. The results from the field trials reflected the botanical composition and warrant further evaluation as a method to monitor root growth.

Combining assays for plants, pathogens and beneficial organisms should become a powerful research tool to assist scientists to identify biological constraints impacting on productivity and water use efficiency, and facilitate development of more efficient and robust pasture systems.

### 7.2.2 Soil community function and structural analysis

This work has shown that agri-management practices in pasture based farming systems significantly drive the composition of the microbiological communities in soils. Using molecular microbial community fingerprinting methods, we demonstrated that liming, pasture type, P and stocking rates significantly alter the composition of the bacteria and fungi present. Soil bacteria and fungi are directly responsible for many of the ecosystem services and functions occurring in soils. These include decomposition of organic matter, soil formation and structure (affecting porosity, water holding capacity etc), plant disease and disease suppression, removal of chemical pesticides, cycling of major and minor elements – C, N, P, S, Fe etc, production or catabolism of greenhouse gasses. As such, altering the structure of the biological community by farm management practices can potentially have a significant effect on plant productivity and ecosystem health and function.

In addition to significantly affecting the types of bacteria and fungi present in the soils, the management practices also affected key groups involved in defined functions. This was quantified through measuring the abundance of genes known to encode key rate-limiting enzymes in the N-cycle: *nif*H, responsible for nitrogen fixation; *amo*A, ammonia oxidation and N cycling and recycling in soil; and *nar*G, the first step in the denitrification pathway. The abundance of these genes, which can be viewed as the biological potential for the processes to occur, varied according to liming, stocking rate, P addition, time and pasture crop type. The genes amoA and nifH were particularly sensitive to management practices. The processes that these two genes control are especially important for the supply of N to crops throughout the growing season and will be strongly related to production and sustainability of the cropping system.

The next step is to couple these new molecular microbiology techniques with basic measurements and understandings of soil process and functions. This information can then be used to generate information relating to the effects of management decisions on biotic communities and processes therein. To capture these gains in biologically mediated processes for enhanced pasture production, NRM outcomes and more sustainable agro-ecosystems (i.e. more resilient, resistant and with less off-site impact on natural ecosystems), it is necessary to be able to measure, understand and interpret both the biotic and functional components and the links between these components. This knowledge then needs to be tested and understood in terms of management practices and ecological outcomes (production or environment). Finally,

these need to be put into context of the overall agri-ecosystem; a sum of production × profit × sustainability × environment.

# 8 Bibliography

Li, G. D., Helyar, K. R., Conyers, M. K., Cullis, B. R., Cregan, P. D., Fisher, R. P., Castleman, L. J. C., Poile,G. J., Evans, C. M., Braysher, B. (2001) Crop responses to lime in long-term pasture-crop rotations in a high rainfall area in south-eastern Australia. *Australian Journal of Agricultural Research* **52**, 329–341.

Li, G. D., Helyar, K. R., Evans, C. M., WilsonM. C., Castleman, L. J. C., Fisher, R. P., Cullis B. R., Conyers, M. K. (2003) Effects of lime on the botanical composition of pasture over nine years in a field experiment on the south-western slopes of New South Wales. *Australian Journal of Experimental Agriculture* **43**, 61–69.

Li, G. D., Helyar, K. R., Conyers, M. K., Cullis, B.R., Poile, G.J., Knight, P.G. (2004) *Phalaris* persistence under rotational grazing on a highly acidic soil on the south-west slopes of New South Wales. *Australian Journal of Experimental Agriculture* **44**, 771–778.

Li, G. D., Conyers, M., Cullis, B. (2006), MASTER - Experimental design. *Primefact* 31, NSW Department of Primary Industries, pp. 2.

# 9 Appendices

## 9.1 Appendix – Collaborators and contributors

- Dr Bob Hannam – RJ Hannam & Co., collaborator liaison, design of training course and manual, convenor of the training workshops
- Dr Steven Wakelin, CSIRO Land and Water, assessments and review of chapter on microbial community structure & functional genes.
- Dr Richard Simpson – CSIRO, PI, Site Leader P x Grazing Trial, Hall ACT
- Dr Guangdi Li – NSW DPI, MASTER Site Manager
- Dr Michael Friend – CSU, EverGraze Site Leader, Wagga Wagga
- Dr Paul Sanford – DPI WA, EverGraze site Leader, Albany
- Dr Martin Barbetti – UWA, Literature Review, technical input
- Dr K Sivasithamparam - UWA, Literature Review, technical input
- Dr Kathy Ophel Keller – SARDI, advice on content and presentation of training manual and workshops
- Di Hartley, CSIRO Entomology for designing the assays
- Dr Suzanne McKay – SARDI Lucerne Bioassay sites
- Dr Sue Wiebkin and Dr Herdina – SARDI, for developing the tests
- Ina Dumitrescu, Danuta Szot, Irena Dadej, Russell Burns and Aidan Thomson for processing the samples
- PIRSA Publishing Services for training manual cover design and assisting with formatting.

## 9.2 Appendix – Draft Press Release

**New research tools for pasture soil biology**

Dr Alan McKay and his team from the South Australian Research and Development Institute (SARDI) in Adelaide have commenced pilot delivery of new DNA assays as research tools for scientists to study biological constraints in pasture trials.

This project also delivered DNA assays being developed by Dr Steven Wakelin CSIRO Division of Sustainable Ecosystems to assess levels of selected functional genes and monitor changes in community structure in soil.

To maximise pasture production and demonstrate livestock industries are good custodians of soil resources and the environment, it is important that researchers can assess the detrimental and beneficial impacts on soil microbial communities.

The new methods involve extraction of DNA from all of the organisms in a single soil sample. The DNA is then tested using a range of assays to quantify specific organisms, community structure and frequency of key genes that drive important biochemical processes.

Dr McKay says "the information we can now get from DNA extracted from a single soil sample would have taken a huge effort and the skills of many specialists using the old methods. So this development can be seen as moving pasture soil research into a new era."

The SARDI team has been funded under a Pasture Soil Biology Initiative lead by Meat and Livestock Australia in partnership with Australian Wool Innovation Ltd and Grains Research and Development Corporation.

To engage pasture research agronomists and help validate the assays in the field, SARDI has used the tests to assess field experiments run by EverGraze (WA, NSW), NSW Department of Primary Industries and the CSIRO (ACT).

To support delivery and interpretation of the tests, a training package was developed and delivered in two pilot workshops in Wagga Wagga, NSW and Albany, WA.

Thirty pasture scientists attended the training sessions and showed considerable interest in the potential of the new technology.

Dr McKay said the feedback from the training highlight strong interest in the capability the tests. In particular the use of DNA assays to study root development and distribution in soils created a lot of interest. Assessing root growth in mixed swards using existing techniques is very difficult.

Dr McKay said it now seems the use of DNA assays to monitor root growth could drive the use of the technology in pastures. Any serious study root growth will need to consider the biological factors affecting root health, and the DNA technology can deliver this information.

For further information contact Dr Alan McKay on 08 8303 9375.