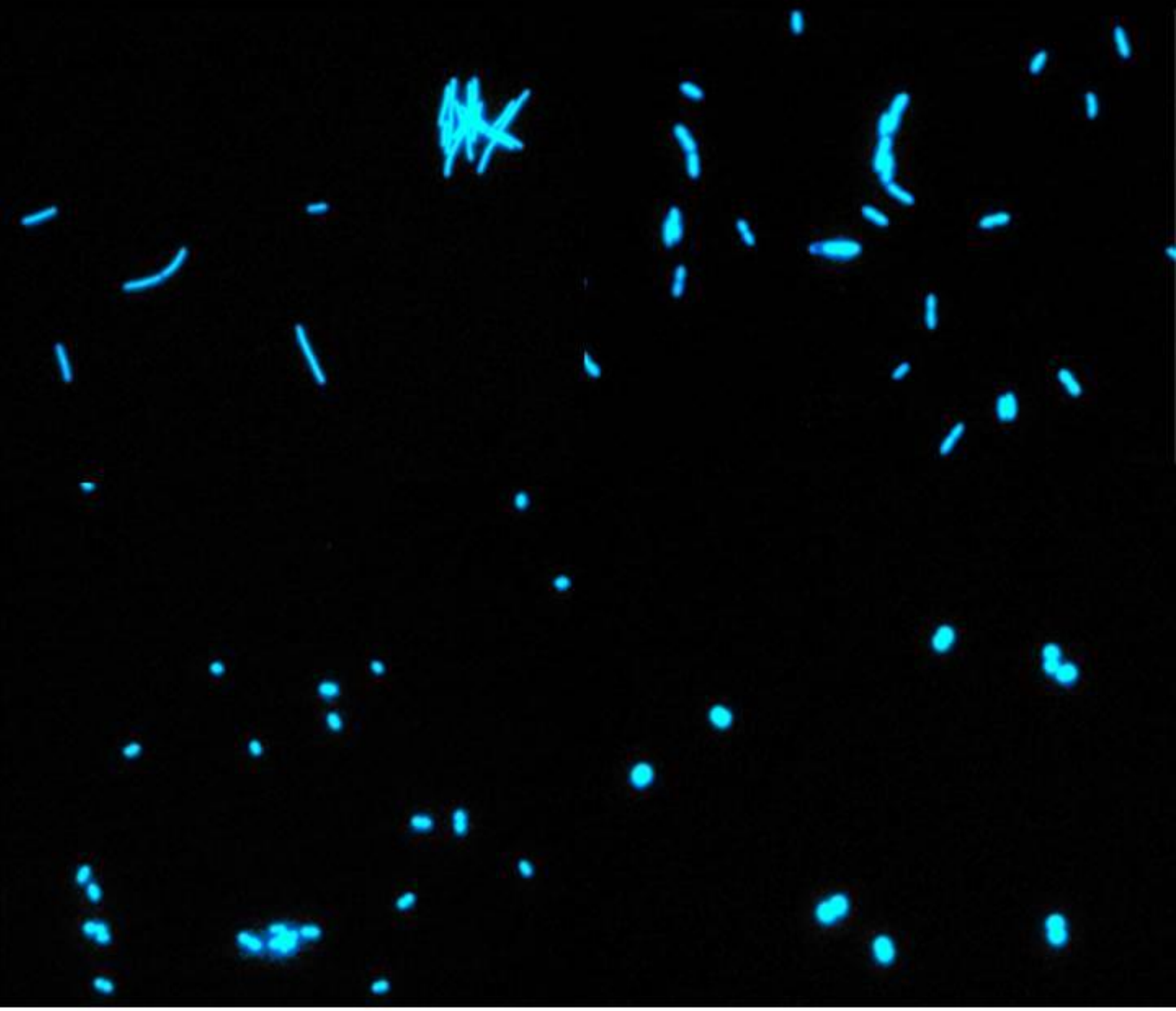
Annual Report 2014

NEW ZEALAND
AGRICULTURAL GREENHOUSE GAS
Research Centre



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Cover image: Four different species of New Zealand rumen methanogens

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PROGRESS TOWARDS SOLUTIONS

Identifying mitigation solutions is a key component of the New Zealand Agricultural Greenhouse Gas Research Centre's (NZAGRC) Vision and Mission. The complexity of the problem means that identifying solutions is a long term goal. Successfully reducing greenhouse gas (GHG) emissions below a 1990 baseline within the New Zealand context of an expanding agricultural sector will require progress in both direct and indirect mitigation options. Direct mitigations are those solutions that reduce absolute emissions per unit of substrate (e.g. feed, nitrogen). Indirect mitigations are those that arise as a result of general improvements in the efficiency of production (for example by improved animal genetics and feeding practices which will reduce emissions per unit of product but not necessarily reduce absolute emissions).

Practical direct mitigation options for methane emissions from grazing animals are now being demonstrated at the field scale. The NZAGRC's Methane Research Programme, which is jointly funded in association with the Pastoral Greenhouse Gas Research Consortium (PGgRc) and the Ministry for Primary Industries (MPI) investment through the Sustainable Land Management and Adaptation to Climate Change (SLMACC) programme, has now shown in field trials that diets based on 100% forage brassicas can reduce emissions per unit feed from sheep by as much as 30%. Indoor trials have also shown that the reductions in emissions are directly proportional to the proportion of brassica in the diet. This will make it easier for the effect of brassicas to be incorporated into the national inventory, although the impact on national emissions is limited due to brassicas being a minor component of sheep and cattle diets in the New Zealand pastoral farming system. In addition, the impacts on nitrous oxide emissions under practical field conditions need to be assessed before any moves are made to incorporate the impacts of brassica feeding into the national greenhouse gas inventory.

Developing an animal genetic solution to methane production has made substantial progress this year. In addition to obtaining more data demonstrating that emissions per unit intake is a heritable and repeatable trait, more evidence has been gathered to support the view that the low emitting trait is not negatively correlated with production traits. Divergent lines of high and low methane emitting sheep continue to be studied and the finding that repeated short duration measurements (1 hour) taken in portable 'accumulation chambers' shows promise as an alternative to measurements in respiration chambers could well reduce the cost of identifying high and low emitting animals. Volatile fatty acid (VFA) levels in rumen and blood have been found to be genetically correlated with methane yield emissions and have some potential as indirect predictors of methane emissions in sheep. The metagenomic and metatranscriptomic work on rumen microbial populations has shown that the abundance of particular rumen microbial genes and transcripts could eventually be used as a proxy to predict CH_4/kg DMI.

The search for compounds that can safely and cost effectively inhibit methane production continues to identify targets that can inhibit methane in pure methanogen cultures, but only one compound has been identified as suitable for progression to an animal trial; this will take place in Spring 2014. The vaccine programme made a significant breakthrough when a prototype vaccine successfully inhibited its target methanogen population.

A new mathematical model of the rumen of a sheep, which has an improved predictive ability over existing models, has been developed and tested against experimental data; more validation of the model is needed before we are confident that it can be used as a tool to 'screen' potential low emitting feeds.

No new technologies have emerged for reducing nitrous oxide emissions to replace the well-proven DCD which was removed from the market in 2013 due to concerns over residues in milk. DMPP has similar efficacy to DCD, but is currently only available in combination with fertiliser so has less potential than DCD to reduce emissions. Fundamental work on exploring whether it is possible to produce ryegrass plants combining high yield with low nitrogen requirements has

identified a gene that has appears to have a major role in determining dry matter production in ryegrass; further work is needed to validate the role of this gene.

Since such large quantities of carbon are stored in soils (150-200 t/ha), even small changes in the rate at which it accumulates can substantially offset emissions of GHGs. Four years of monitoring carbon inputs and outputs at a grassland site in the Waikato suggest that the net gain in carbon is 0.6 t/ha/yr. Complementary modelling studies suggest that intensification via increased nitrogen inputs and increased stocking rates, a feature of New Zealand dairying over the last 20 years in many areas, will result in sustained greater losses of nitrogen but any positive impacts on rates of soil carbon storage are likely to be transitory. The effects of earthworm introduction on long term carbon stocks in the field is contradictory and, although under controlled conditions it has been demonstrated that biochar addition does not affect the stability of existing soil carbon, there are major doubts as to the practical applicability of biochar addition as a method of increasing carbon stocks in New Zealand's pastoral soils.

It is important that the new knowledge developed in NZAGRC funded/co-funded research programmes is utilised in order to have a practical impact, wherever possible, on the greenhouse gas emissions resulting from New Zealand agriculture. The table below highlights key outputs from 2013/14 and their envisaged impacts.

NZAGRC output	*Expected impact*
The breeding work has shown: • VFA levels in rumen and blood are genetically correlated with methane yield emissions and have some potential as indirect predictors. • Brief (1 hr) measurements are as useful for genetic selection as 24 hr measurements, if sufficient measurements are recorded. • Selection for low methane yield does not negatively affect key production traits.	This work opens up the opportunity for more cost-effective identification and testing of large numbers of animals needed for the successful breeding of low emitting phenotypes. The significant, positive combined economic value for the low emitting selection line vs the high emitters will play a key role in encouraging on-farm adoption of this research.
Preliminary results suggest that methane yield and rumen pH decreased almost linearly with increasing proportion of forage rape in the diet.	This will make it far easier to incorporate the effect into the national inventory as the reduction can be based on an assessment of the proportion of the national diet that is brassicas and the reduction when brassicas make up 100% of the diet.
Results from animal trials that show prototype vaccinations can produce sufficient levels of antibody in the saliva and rumen to have an effect on ruminal methanogens.	These results are approaching proof of concept for the vaccination research strategy. Further animal trials are planned for 2014/15 which will include methane measurement of the vaccinated animals.
Lead inhibitor compound able to reduce methane production *in vitro* by >80%.	Lead inhibitor compound to proceed to animal testing and *in vitro* testing of derivatives to begin in Spring 2014.
Study of metagenomic and metatranscriptomic datasets have shown that the abundance of particular rumen microbial genes and transcripts may be used as a proxy to predict CH_4/kg DMI.	Further detailed examination of the data will be undertaken to identify the minimum gene and transcript set that can give good correlations with methane yield.
A new mathematical model of the rumen of a sheep, which has an improved predictive ability over existing models, has been developed and tested against experimental data that included fresh temperate forages offered at a wide range of sheep feeding levels.	After further validation the model will be used to guide the search for low methane emitting feeds and to provide better prediction of the quantity of methane emitted by animals consuming particular feeds.

Preliminary evidence for an association between genetic variation of the DELLA gene and dry matter yield in ryegrass.	Further investigation required in order to validate the role of DELLA as a key candidate gene. Eventually this could lead to development of molecular tools that would allow breeding of higher yielding ryegrass cultivars that require less N.
Field trial results suggesting that liquid formulations of both DMPP and DCD have the potential to be used as nitrification inhibitors to reduce N_2O emissions in grazed pasture soils, but there is no apparent advantage of DMPP over DCD.	Given current costs and formulations it appears unlikely that DMPP will make a significant impact on nitrous oxide emissions from grazed pastures in New Zealand.
Four year of monitoring carbon inputs and outputs on a Waikato dairy farm suggest that the net carbon gain is 0.6 t/ha/yr. A global compilation of 54 site years of carbon balances made over grazed pastures suggests that many of these sites were net carbon sinks when accounting for all imports and exports	Positive message for NZ farmers around the potential of grassland soils to store carbon, although care must be taken not to generalise this result to all farms, managements and environments. In soil carbon terms 4 years is not a long term study.
Inconclusive results regarding whether earthworms have an effect on soil carbon accumulation.	Available results suggest it is not possible to say with any certainty that the addition of earthworms will significantly impact levels of soil carbon in the short and long term.
Findings that addition of biochar to soil does not promote the decomposition of natural organic matter, at least when incubated for a period of 510 days.	This finding that the addition of biochar does not affect the decomposition of native soil carbon provides some reassurance that adding biochar results in greater net stable soil carbon inputs.

CHAIR'S REPORT

The emissions intensity of New Zealand agriculture, that is the gases generated per unit of meat or milk produced on farms, has declined on average by about 1% since at least 1990. This is a result of farm businesses becoming more efficient over the past 25 years. Improved animal genetics and management, combined with better grassland management and feeding practices mean that farms are using resources more efficiently to increase their outputs. This has happened across all sectors of the pastoral industry. However, the reduced emissions intensity has been more than offset by the increased overall product generated by the sector. As a result, New Zealand's total agricultural emissions have risen by 15%. Without the efficiency gains on farms, emissions would have grown much more, by more than 30%. So, while New Zealand farmers' efficiency gains are addressing a large portion of the problem, they are not enough to counter the extra GHGs being produced overall.

New Zealand has made international commitments to take action to lower its GHG emissions. Whilst the country is small by global standards, its reputation as a trading nation confers an obligation to contribute fairly towards the global effort to reduce emissions and the risks from climate change.

Farmers are already making a contribution. By continuing to improve on-farm efficiency, there is the opportunity to further reduce the intensity of emissions per unit product. However, this is unlikely to stop the country's total agricultural emissions from rising in the future. Practical and cost-effective mitigation approaches are required to help meet environmental, social and international aspirations and obligations as well as economic growth targets. This is the role of the NZAGRC alongside the jointly industry/government-backed PGgRc. Government, industry and researchers are working together, combining resources to identify and develop additional interventions that will provide effective and practical results by 2020 and beyond.

During 2013/14, the NZAGRC and PGgRc research programmes have formally combined and jointly funded contracts are now in place. The governance bodies of both organisations continue to meet quarterly and there is a strong drive towards engaging commercial partners by mid-2015. A number of key science results in 2013/14 demonstrate that the science teams are getting closer to viable solutions to reduce agricultural GHGs.

Through its national and international roles and responsibilities, particularly through its active involvement in the Global Research Alliance on Agricultural Greenhouse Gases (GRA), the Centre continues to build on its reputation as an important source of clear and unbiased advice on the science behind agricultural greenhouse gases and their mitigation options.

Professor Warren McNabb
Chair of NZAGRC Steering Group
August 2014

NZAGRC DIRECTOR'S REPORT

The 2013/14 financial year has been another eventful one for the Centre. Working alongside MPI and the PGgRc, usable results, outputs and publications continue to emerge from the research. We keep a close eye on ensuring that the outcomes of our funding are able to be easily translated into practical solutions and in some areas results have reached the stage where engaging with potential commercial partners is a priority. The PGgRc will lead the interaction with potential commercial partners with strong support from the NZAGRC.

A key focus this year has been to re-evaluate and update the Centre's investment in nitrous oxide, soil carbon and integrated systems for the period 1 July 2014 to 30 June 2017. This has involved significant input from NZAGRC Principal Investigators, science teams and our wider network of industry and policy representatives who provided review and feedback. I would like to thank everyone that has contributed to this process. I am happy that the new work plans are both scientifically rigorous and highly targeted towards solutions and look forward to the first outputs emerging from these updated programmes.

In addition to our core science programmes, we have contracted a three year project that aims to assist the Māori pastoral sector to improve its collective capacity to increase resource efficiency and farm productivity while lowering emissions. This will allow us to ensure that our research is applicable to all sectors of New Zealand society and also provide tangible knowledge transfer materials that can be used by our Member organisations and industry partners.

Our working relationship with the PGgRc has been further cemented this year. We collaborate in the science, governance, commercialisation and communication areas and continually strive for improved efficiency and better outcomes. In addition, the Centre's role in administering GRA funding on behalf of MPI ensures good coordination for the New Zealand research programme with international efforts.

Highlights for the Centre staff this year include contributions to the latest IPCC report by the Centre Director (Lead Author) and in particular the Deputy Director, Andy Reisinger, who was not only a Coordinating Lead Author of a chapter but also actively participated in preparing the Summary for Policy Makers and acted as a topic facilitator for Topic 4 (adaptation and mitigation options). At an operational level, we have welcomed Emma Arnott to the NZAGRC team as the new Centre Administrator and Heather Went has returned to her role as Centre Operations Manager.

I would like to express my thanks to all of our Advisory Groups, and particularly to the Steering Group, for their dedication to the Centre and the knowledgeable advice that they have provided throughout the last year.

Dr Harry Clark
NZAGRC Director
August 2014

THE NEW ZEALAND AGRICULTURAL GREENHOUSE GAS RESEARCH CENTRE

The NZAGRC is 100% government-funded by the Ministry for Primary Industries through its Primary Growth Partnership Fund. It is a core component of the New Zealand Government's approach for addressing the reduction of greenhouse gas emissions from agriculture. This includes New Zealand becoming: (a) a major investor in agricultural GHG mitigation research; (b) a world leader in finding solutions to agricultural GHG emissions via its domestic investment programme; and (c) a leader in international initiatives to advance the search for mitigation solutions and help ensure international treaties address agricultural GHG emissions in an appropriate manner. The Centre is a science funder, has additional responsibilities for strategic research coordination, capacity building and leads New Zealand science input into international activities and policy processes in the agricultural GHG area.

The NZAGRC is a partnership between the leading New Zealand research providers working in the agricultural GHG area and the PGgRc. About NZ$48.5 million is being invested by the NZAGRC into research and development activities over ten years. The NZAGRC is a "virtual" Centre and the research that it funds is carried out by researchers working in their own organisations and collaborating across organisations.

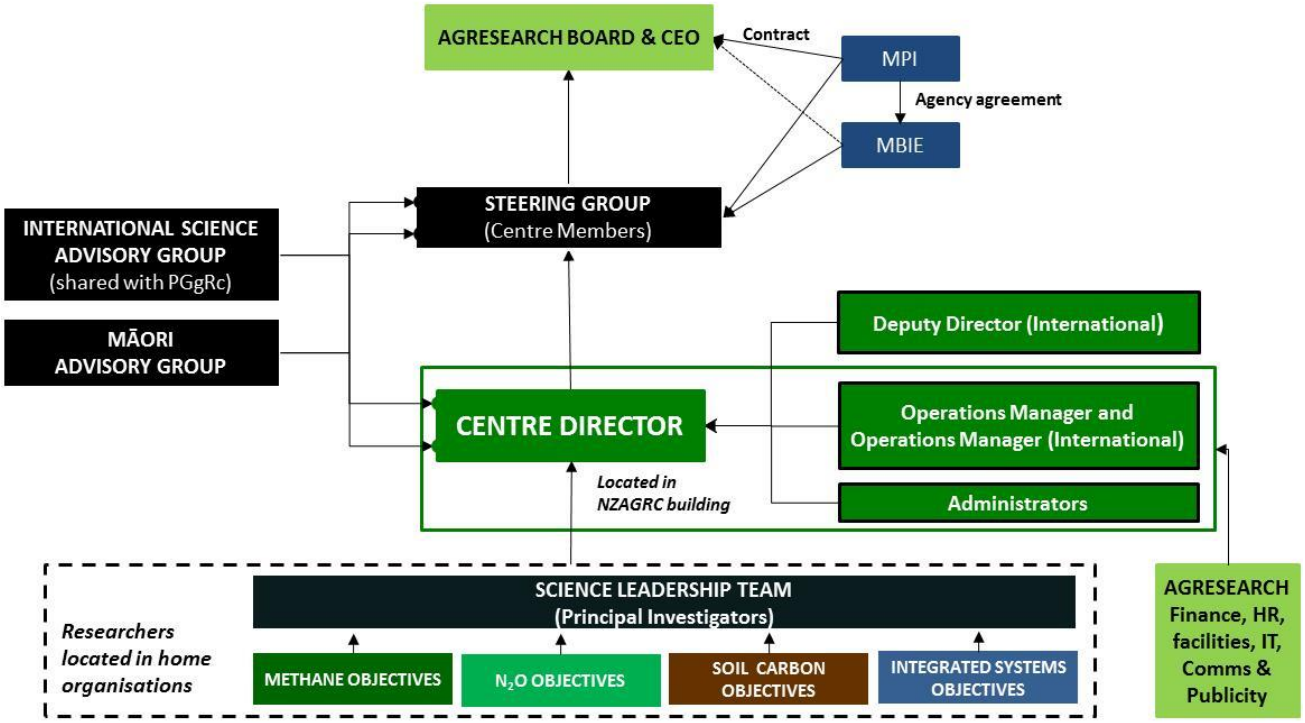
NZAGRC is not the only significant investor into agricultural GHG mitigation research in New Zealand. Much of NZAGRC methane research builds on research investments made by the PGgRc, and since 2013 the NZAGRC and PGgRc investments have been formally aligned. This involves a single research strategy with shared advisory groups and administrative processes. Targeted mitigation research and proof-of-concept trials are also carried out under the Sustainable Land Management and Adaptation to Climate Change (SLMACC) programme coordinated by MPI. In addition, the New Zealand government provides funding for projects that support the goals and objectives of the Global Research Alliance, which build on and extend New Zealand-based research through international collaboration and data sharing. Various investments by industry into on-farm tools and trials and extension complete the picture. Research investment by NZAGRC within this funding landscape is based on an assessment of national needs and priorities, existing knowledge and expertise, and major gaps.

The NZAGRC is physically located on the AgResearch Grasslands Campus in Palmerston North. The Director, Operations Manager, Operations Manager (International), Project Analyst and Administrator are employed by AgResearch on behalf of the NZAGRC and are based in this building. The Deputy Director (International), also employed by AgResearch, is located in Wellington.

NZAGRC GOVERNANCE

As the NZAGRC is set up as a unit operating within AgResearch, the Board and Chief Executive (CEO) of AgResearch have ultimate responsibility for the NZAGRC. However, a Steering Group (SG) comprising a representative of each NZAGRC Member provides advice and recommendations to the AgResearch CEO and Board on the operation of the NZAGRC. The NZAGRC Director reports to the AgResearch CEO and Board via the NZAGRC's SG. The International Science Advisory Group (ISAG) monitors, advises and reports on the NZAGRC's science quality and direction to the SG and NZAGRC Director. The NZAGRC SG meets formally with the PGgRc Board every quarter and this provides guidance in relation to the needs of the industries that are intended to take up its research outcomes. The advisory roles of the ISAG and PGgRc Board are primarily in the areas of science quality, research direction and industry relevance.

A Māori Advisory Group (MAG) was established in 2011/12 to ensure that the research and development undertaken by the NZAGRC is relevant and accessible to all sectors of New Zealand society. The primary role of the MAG in 2013/14 has been to provide input and guidance into the establishment of a research programme focussing on low emission farm systems for the Māori sector which commences in July 2014.



NZAGRC Governance Structure

Role of the Steering Group (SG)

The NZAGRC Director reports to the Steering Group of the NZAGRC Members and via them to the AgResearch CEO and Board on the performance of the NZAGRC, including (with appropriate quantitative measures):

- Relevance of the NZAGRC's R&D to the agriculture sector and New Zealand
- Science quality
- Performance to contracted goals
- Human resource development and constraints
- Financial performance.

The main roles of the SG over the past financial year have been to ensure that the NZAGRC is operating effectively, funding decisions are made in a robust fashion and that there is a good alignment with the PGgRc at a governance level.

During 2013/14 the SG met quarterly in Wellington and also provided comment and feedback on documents via video/teleconference and email as required. Quarterly face-to-face meetings were run in a similar fashion to Board meetings with papers circulated prior to, and detailed minutes signed off after, each meeting.

The compositions of the SG, ISAG and MAG and meeting dates during 2013/14 can be found in appendix 1.

2013/14 SUMMARY OF ACTIVITIES AND ACHIEVEMENTS

The need for research to find cost-effective practices, tools and technologies to reduce agricultural GHG emissions that are consistent with New Zealand's pastoral farming base is as important as ever. Consequently, the Centre's vision and mission (see below) remain highly relevant in the changing context in which it operates.

The Vision

'To be an internationally renowned centre for research and development into agricultural greenhouse gas mitigation solutions'

By 2015, the NZAGRC plans to be (i) a source of practical, cost effective technologies and/or practices that reduce emissions/increase sinks and clearly demonstrate that farm businesses can be both lower emitting and profitable; (ii) a focal point for New Zealand activities in agricultural greenhouse gas mitigation/soil carbon sink solutions; (iii) the key authoritative source of technical advice and support on agricultural greenhouse gas emissions and soil carbon sinks. Additionally, the NZAGRC will lead NZ's science input into the Global Research Alliance.

The Mission

'To provide knowledge, technologies and practices which grow agriculture's ability to create wealth for New Zealand in a carbon-constrained world'

The Goals

The NZAGRC has five major goals for the first five years of its life:

1: Advance knowledge and understanding
2: Enhance awareness among stakeholders
3: Contribute to policy
4: Develop science capability
5: Develop science and commercial partnerships

These have been defined and quantified in order to be consistent, realistic and achievable and detailed targets are included in the NZAGRC Strategic Plan. The Centre has made substantial progress towards achieving its Vision and Mission through its on-going achievements in the five major business goal areas. Each goal is discussed in more detail in the following pages.

Centre progress towards achieving vision and mission

In 2013/14 particular high level achievements include:

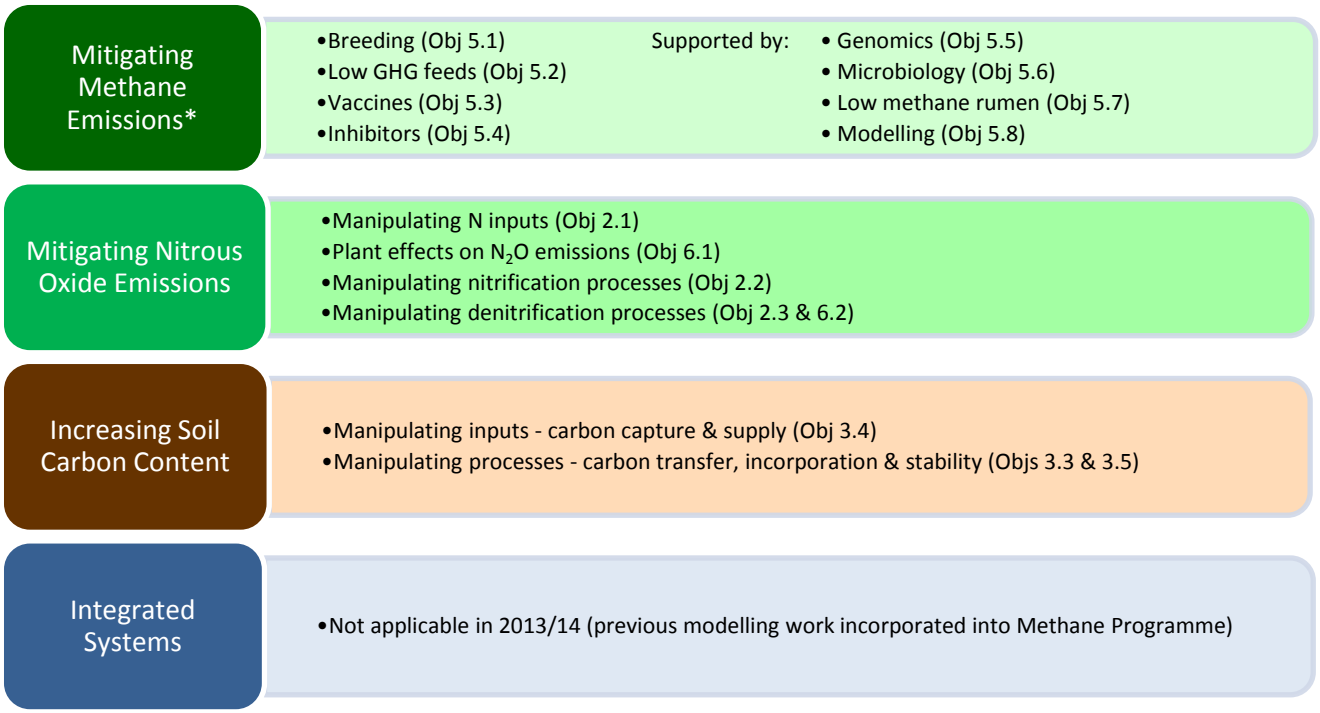
- Continuing to act as a focal point for New Zealand research activities in agricultural GHG mitigation, building on international reputation for the quality of our research and progressing towards solutions.
- Running an efficient organisation with sound governance and financial control.
- Cementing alignment with the PGgRc and, through this relationship, starting to actively engage with commercial entities to establish pathways to market for our technologies.
- Enhanced engagement with Māori, including working with the MAG to commission a new three year Māori-focussed research programme.

- Actively contributing to the success of the Global Research Alliance and coordinating New Zealand's science input to the Alliance.
- Active engagement with policymakers through the Global Research Alliance, the Sustainable Land Management and Climate Change (SLMACC) fund investments, Methanet, Intergovernmental Panel on Climate Change (IPCC) and FACCE-JPI committees.
- Actively contributing to the development and retention of GHG-related scientific capability.

Goal 1: Advance knowledge and understanding

By 2015, the NZAGRC will be the most important and trusted NZ source of scientific knowledge in the field of agricultural GHG emission mitigation.

The NZAGRC supports four Science Programmes in alignment with other agencies and private investors.



*Joint programme with the PGgRc

Formal alignment with the PGgRc has led to a joint science plan and contracting in the Methane programme being implemented from 1 July 2013. These contracts cover the period 1 July 2013 – 30 June 2015 and have an annual review clause in them to ensure that the research remains solution-focussed.

The majority of the remaining contracts initiated in 2010 in the N_2O and Soil Carbon areas were completed at the end of 2013/14. A significant amount of time and effort has been spent formulating new work programmes in these areas and these will commence 1 July 2014. Two new contracts were signed in the N_2O area in 2013/14.

- Work to investigate plant effects on N_2O emissions has been continued and extended (Objective 6.1). Previous work has found differences in nitrification between species, between cultivars and between endophyte-grass combinations. The new programme will test whether differences apparent in these initial experiments are evident in a field situation, and if so, whether the effect is quantitatively important and whether there are trade-offs (e.g. in forage production) that might reduce the desirability of a low emitting species as a mitigation option. A broad screening approach will also complement industry testing of alternative pasture species by providing valuable information on the most suitable material for testing in grazing trials.
- The previous denitrification-focussed NZAGRC Objective 2.3 is being extended for a year to finish off existing work (Objective 6.2). The last contract concentrated on testing and improving the latest microbiological techniques to identify pathways to reducing N_2O production during denitrification and develop mitigation technologies that reduce N_2O emissions by lowering N_2O/N_2 ratio during denitrification, including in areas where denitrification is maximised to reduce nitrate leaching losses (e.g. riparian buffer zones). The mitigation effect of liming in order

to modify denitrifier community structure, accelerating complete denitrification and mitigating N_2O emissions from urine will be evaluated.

The Integrated Systems programme, which was aligned with a MPI funded SLMACC programme, finished formally in June 2013. Support for the rumen modelling work was continued until 30 June 2015 and is reported under the Methane programme.

In 2013/14, key science achievements included:

- Results from animal trials that show prototype vaccinations can produce sufficient levels of antibody in the saliva and rumen to have an effect on ruminal methanogens.
- Identification of a lead inhibitor compound which is able to reduce methane production in vitro by >80%, with animal trials planned for 2014/15.
- Significant progress towards including the low methane trait into a commercial breeding index.
- A new mathematical model of the rumen of a sheep, which has an improved predictive ability over existing models, has been developed and tested against experimental data that included fresh temperate forages offered at a wide range of sheep feeding levels.
- Preliminary evidence for an association between genetic variation of the DELLA gene and dry matter yield in ryegrass.
- Results from laboratory trials that demonstrate that DCD has only a small and transient impact on microbial communities, with impacts being comparable to those seen from urine deposition by ruminants.
- Field trial results suggesting that liquid formulations of both DMPP and DCD have the potential to be used as nitrification inhibitors to reduce N_2O emissions in grazed pasture soils, but there is no apparent advantage of DMPP over DCD.
- Monitoring carbon inputs and outputs on a Waikato dairy farm suggest net carbon gains of 0.6 t/ha/yr.

More detailed information regarding science progress during 2013/14 can be found in appendix 2 which includes the submitted annual reports from all NZAGRC-funded Objectives.

Goal 1 metrics:

Measure	*Progress in 2013/14*
Peer-reviewed scientific journal papers	11
Scientific conference papers	12
Patents relating to agricultural GHG emission mitigation technologies	Patenting decisions are the joint responsibility of MPI and PGgRc (not the Centre directly); new IP protected and managed as commercial (in confidence) IP or shared freely as public-good information.
Practical on-farm mitigation practices and technologies identified and being promoted	Brassica feeding Promotion of improved efficiency as the immediate action farmers can take to help reduce emissions

Goal 2: Enhance awareness among stakeholders

By 2015, the NZAGRC will be the most important and trusted source of information for New Zealand agricultural stakeholders on agricultural GHG emission mitigation.

PGgRc Alignment

From 2002-2012, the PGgRc invested more than $37m in GHG (mainly methane) mitigation research. During 2012/13, PGgRc successfully renewed its Partnership funding with MBIE for a further $37m over seven years. This renewal triggered a move for the Centre to develop a much closer working relationship with the PGgRc.

Close cooperation with the PGgRc is a key pathway for the Centre to interact with industry stakeholders, assist MPI to manage IP and enable knowledge transfer through commercialisation of new tools, technologies and practices. Current industry co-investors within PGgRc are: Fonterra, DairyNZ, Beef+Lamb NZ, Landcorp, Deer Research and PGG Wrightson. AgResearch is the CRI member. Since February 2013, the Centre Steering Group members have been meeting jointly with the PGgRc Board members to monitor progress on joint initiatives and co-funded R&D. The Centre Director is an observer on the PGgRc Board.

Key joint initiatives in 2013/14 with the PGgRc included:

- Establishing and implementing a single contracting, reporting and review process for the jointly-funded Methane Research Programme.
- Development and roll out of a joint brand.
- Agreeing and implementing a joint communications strategy and plan.
- Working together to attract and engage commercialisation partners.

Other Stakeholder Engagement

Although the PGgRc provides a robust pathway for the NZAGRC to link with industry stakeholders, the Centre continues to maintain direct links with a broad range of other stakeholders, including policy makers, end-users, the science community and the wider public.

In its on-going support of knowledge transfer the Centre was involved in key activities in 2013/14 that included:

- Hosting meetings with farmer groups and individual companies and organisations (e.g. Fonterra, Federated Farmers, DSM, DairyNZ) and giving presentations at farmer forums.
- Presenting at conferences where industry is well represented (e.g. New Zealand Society of Animal Production).
- Publishing dedicated publications (e.g. Highlights document and general information brochure) and articles in farming and general press and presenting on television and radio.
- Membership of MPI industry-related advisory groups (e.g. SLMACC, Methanet, Agricultural Inventory Advisory Panel).
- Presenting directly to government officials and hosting science planning workshops with industry, policy and science for the new nitrous oxide, soil carbon and integrated systems science programmes.
- Developing a new communications strategy and action plan in partnership with the PGgRc and MPI.

Māori Engagement

During 2013/14 NZAGRC staff worked closely with the MAG to develop an RfP for a focussed Māori research project. A proposal for a three year programme of work was accepted from a cross-organisation team (AgFirst, AgResearch, Lincoln University and Scion) led by AgFirst, in late 2013/14 and work will begin on 1 July 2014.

Māori-focussed research	•Development of farm systems and farm typologies and selection of case study focus farms •Mitigation modelling and scenario design •Sector adoption and integration of project outcomes and practice change strategies

The "Low emission farm systems for the Māori sector" programme aims to assist the Māori pastoral sector to improve its collective capacity to increase resource efficiency and farm productivity while lowering greenhouse gas emissions. The programme will achieve this by developing a set of Māori farm typologies, which represent the predominant pastoral farming systems, identify key factors that underpin farm productivity, resource and emission efficiency and sustainable profitability, and then identify and test a range of mitigation strategies. Farm typologies are important to avoid the problems of homogenizing a heterogeneous group that range from very small farms to large multi-enterprise corporates. These typologies will be compared against existing databases and help in the selection of in-depth representative case study farms for scenarios of alternative farm system configurations that will evaluate mitigation options.

The programme will improve understanding of the critical characteristics of GHG profiles (both in terms of absolute emissions and emissions intensity) of existing Māori pastoral farming systems and produce a range of mitigation options to modify farm systems to lower absolute emissions and/or emissions intensity. A key contribution to the literature will be an enhanced understanding of the Māori farm typologies with economic, environmental, social and cultural implications of low emission farming systems within the Māori sector, with wider implications across NZ.

Communications and media

In early 2014 a joint communication strategy and action plan (CSAP) was approved by the NZAGRC SG and PGgRc Board. The key goal of the plan is to raise visibility, understanding and relevance of the work undertaken by the Centre and Consortium. The plan will achieve this by including changes in emission intensity in the overall scope of communications, and by engaging more directly with our targeted audiences and building direct links to editors/journalists. This will improve our target audiences' understanding of where we and our work fits in the overall 'NZ Inc' approach to increasing agricultural production within environmental and GHG constraints.

Work to implement the action plan has progressed well, with highlights as follows:

- Production of an information brochure on historical and potential future reductions in emissions intensity arising from productivity improvements.
- Engagement with New Zealand journalists from a key contact list through email and a mailed information package. Familiarisation visits are scheduled for interested journalists.
- Development of a register of target events and fact sheets for production in 2014/15.
- Identification of key science progress areas for development of media/news release potential.

With respect to the media, the Centre and Deputy Directors' involvement in the IPCC AR5 report has provided an excellent opportunity for open and varied discussions with a range of audiences. These include participation in IPCC Outreach workshops, television, radio and newspaper interviews and ongoing briefings to New Zealand local government/councils (Wellington, Auckland, Dunedin, rural/provincial) and interested stakeholders.

Additionally, during 2013/14 the NZAGRC has both hosted and attended a significant number of meetings and presentations with a diverse group of external parties, both in New Zealand and internationally. The NZAGRC has also actively promoted itself and its role in the media and to a scientific audience via conference papers and peer-reviewed publications. These are summarised below and detailed in appendix 3.

Type of interaction/output	# in 2013/14
Meetings and Presentations (New Zealand)	63
Meetings and Presentations (International)	30
International Visitors and Groups	5
Global Research Alliance related interactions	28
Media interactions	20
Conference presentations	19
Journal articles in press	18
Journal articles published	11
Other interactions/publications	18

Goal 2 metrics:

Measure	*Progress in 2013/14*
Page views of Centre's website	37,499
Senior Centre staff presentations to meetings of New Zealand industry and policy stakeholders	18
Centre funded scientist presentations to the farming community and general public	13

Goal 3: Contribute to policy

By 2015, the NZAGRC will be the authoritative source of information for the New Zealand government on agricultural GHG emission mitigation.

Policy Advice

A key aim of the Centre is to be a trusted and independent source of knowledge - particularly to policy agencies – to enable sound, evidence-based policy development. The Centre's relationship with MPI (and other government departments in general) has continued to grow stronger and deeper in 2013/14, particularly as a result of its increased role in the GRA. Likewise, MPI policy staff continue to appreciate the NZAGRC's robust scientific input and encourage and foster a culture of trust and open engagement.

The Centre's on-going inputs into the GRA and IPCC are prime examples of activities that the Centre engaged in during 2013/14 related to this goal.

Other activities by the Centre in 2013/14 include:

- Director and Deputy Director acting as lead and coordinating lead authors, respectively, on IPCC AR5. The Deputy Director also served as a member of the Core Writing Team for the Synthesis Report, with the added responsibility of acting as topic facilitator for Topic 4 (adaptation and mitigation options).
- Director and Deputy Director are members of MPI's Agricultural Inventory Advisory Board.
- Director is Chair of MPI Methanet (science grouping advising MPI on methane inventory development).
- Director is a member of SLMACC science assessment committee and Deputy Director is the NZ representative on the FACCE-JPI Call Steering Committee.
- Director is a member of the FACCE-JPI Science Advisory Board and Chair of the FACCE-JPI GHG Mitigation call International Advisory Committee plus Chair of science assessment committee on EU call on Climate Smart Agriculture.
- Director is the Chair of Manure Management project Advisory Board for the CCAC.
- Operations Manager (International) attending UNFCCC in Bonn.
- NZAGRC hosting international visitors (e.g. Ecuadorian Minister of Agriculture, Irish Minister of Agriculture, Food and Marine, Kenya Agricultural Research Institute, Managing Director of Animal Sciences Group – Wageningen UR, amongst others).

Goal 3 metrics:

Measure	*Progress in 2013/14*
Senior Centre staff presentations to meetings of New Zealand government policy staff	13
Written reports prepared for government policy makers	3
Centre's science contributions directly influence and reflected in government policy.	Range of technical advisory roles

Goal 4: Develop science capability

By 2015, the NZAGRC will be a major source of new capability in the field of agricultural GHG emission mitigation.

Students and Post-doctoral fellows

Increasing the pool of researchers with skills in the agricultural greenhouse gas mitigation area is a major objective for the NZAGRC, due to an aging science population and the need for increased capacity and capability. To achieve this objective the NZAGRC is strategically funding students to build capability for the future. Some of this funding is embedded within the funding of the core science programme, with additional funding being available on a discretionary basis when high quality students or projects are identified.

1. The provision of short term scholarships to promising undergraduate students with the aim of encouraging them to undertake post graduate studies
2. The provision of well-funded PhD stipends to high quality undergraduates
3. Employing high quality post-doctoral fellows and early stage scientists on 2-3 year contracts

In 2013/14 the dedicated undergraduate "pipeline" scholarship scheme continued with Massey and Lincoln Universities and was extended to include Waikato University. Part of Waikato's funding was used to provide partial support to a new PhD student. One of the initial recipients of a NZAGRC PhD studentship funded via the nitrous oxide core science programme submitted her thesis at the end of 2013/14. A number of other PhD students are expected to complete their studies during 2014/15. The new science programmes starting 1 July 2014 incorporate new PhD positions which will run until 2017.

Type of Capability Development	# new in 2013/14	Total funded to date*
Undergraduate - Summer student	2	17
Undergraduate - Honours student	1	4
Masters Project	1	3
Masters	0	1
PhD	1	11
Post-doctoral fellow	0	3
Early career scientist	0	1
	5	40

*Including new 13/14 numbers

The NZAGRC continues to be a major funder of PhD students in agricultural sciences related to nutrition, animal and plant performance and greenhouse gas emissions in New Zealand.

Funding for international students under the LEARN fellowship scheme (under separate contract with MPI; see below under Goal 5) provides an international dimension to NZAGRC's overall capacity building efforts.

Goal 4 metrics:

Measure	*Progress in 2013/14*
PhD students studying and graduated	11
Post-doctoral researchers completed 2-year projects*	3
FTEs of professional researchers working on NZAGRC research programmes	107 researchers (13.01 FTE) contributing to the Centre's research programme

*Includes LEARN Post-doctoral fellows

Goal 5: Develop science and commercial partnerships

By 2015, the NZAGRC will be a key player in many research and commercial partnerships relating to agricultural GHG emission mitigation.

International

The Global Research Alliance on Agricultural Greenhouse Gases, initiated by the New Zealand Government, continues to be a key pillar in New Zealand's international science and policy engagement in climate change and agriculture. Leadership of New Zealand's engagement in the Alliance rests with MPI and the Centre plays key supporting roles by providing science leadership in the Alliance's research groups, monitoring and administering research contracts in support of the Alliance on behalf of MPI, and providing strategic advice to MPI on collaborative funding opportunities, capacity building initiatives and linking of research projects with existing international initiatives.

The core focus of New Zealand's engagement, other than providing the Alliance Secretariat and supporting the development and activities of the Alliance Council through MPI, is the leadership of the Alliance's Livestock Research Group (LRG). The Centre Director co-chairs this group together with his colleague from Wageningen UR (Netherlands), and the Centre Deputy Director acts as New Zealand's representative on the LRG. The Deputy Director and the Operations Manager (International) support the co-chairs in developing and monitoring the LRG's work plan, ensuring appropriate LRG presence at international events, and identifying opportunities for further engagement with existing research programmes, science institutions, international organisations and the private sector.

To increase awareness of LRG activities and engage new members and stakeholders, NZAGRC communicate the work and scope of the LRG to a global audience via quarterly newsletters, a regularly updated website, and presentations at meetings including an international workshop in Jakarta and the GGAA in Dublin during 2013/14, and to visiting international delegations (including from the US, China and the UK).

Key activities for 2013/14 included:

- Ensuring appropriate representation of New Zealand on other Research and Cross-Cutting Groups of the Alliance.
- The Alliance Council met in The Hague, the Netherlands in June 2014 and the NZAGRC coordinated presentations by the LRG to the Alliance Council.
- Facilitating the collaboration between the LRG and the UNEP Climate and Clean Air Coalition initiative, including membership of the Advisory Board of the Manure Management initiative and being invited to lead development of an enteric methane initiative.
- As contracting agent for MPI, NZAGRC oversaw the monitoring and, in some cases, completion of a wide range of research projects in support of the Alliance. Examples include:
    - A completed guidelines document for the SF6 tracer technique which was launched at the AnimalChange conference in Madrid, May 2014.
    - Two new research contracts were signed worth $1.1M from round 2 of the New Zealand Fund for Global Partnership in Livestock Emissions Research (GPLER). These contracts are between MPI and research providers, but NZAGRC manages these contracts on behalf of MPI. NZAGRC also provided expert advice to MPI on the design, operation and scientific merit of expressions of interest for the third round of the GPLER fund. The full proposals are due for submission in late 2014.

- Contracts worth almost $1.0 million were signed for three projects stemming from the FACCE-JPI multi-partner call on agricultural greenhouse gas research with a group of European countries, the US and Canada. While four new contracts worth $0.8M were signed for New Zealand scientists to participate in the 'Filling the Research Gap' (FtRG), a component of the Australian Agriculture Department's Carbon Farming Futures programme supporting research into agriculture GHG mitigation and carbon sequestration.
- NZAGRC continued to manage a suite of LRG priority research projects in the area of methane and nitrous oxide.

- Continued administration of the LEARN/GRASS fellowship scheme, with 10 fellows involved this year.
- International capacity building and engagement efforts:
  - Initiated discussions with AnimalChange and the University of Pretoria to hold a 2 week technician training course on measurement of GHG emissions from livestock in South Africa. The course will be held in September 2014 with participants from 12 countries across Africa. NZAGRC will organise the event funded by MPI, with financial support from the EU funded AnimalChange programme.
  - Organised engagement workshops on the Global Research Alliance in:
    - Hyderabad, India at a side-event of the World Agricultural Forum (October 2013). NZAGRC attended and chaired the event to showcase more of the work of the Alliance and emphasise the opportunities that international collaboration brings, and generate bottom-up as well as top-down momentum for Indian engagement.
    - Mozambique, Africa at a side-event of the RUFORUM conference (July 2014). NZAGRC attended and chaired the event to showcase more of the work of the Alliance and emphasise the opportunities that international collaboration brings, and generate bottom-up as well as top-down momentum for African engagement. The event attracted scientists, African funding agencies, University leaders and representatives from pan-African organisations.

IP and knowledge management

The Centre does not own IP generated from its science investments and patenting and commercialisation decisions are the direct responsibility of MPI and/or PGgRc. The Centre's role is simply advisory and administrative. An on-line Release of Information (ROI) system, established and maintained by the NZAGRC, is used to keep track of the number and type of publications/presentations generated under NZAGRC funding and ensures that new IP is appropriately identified, protected and managed. The system is also used for approval and tracking of PGgRc and GRA outputs. During 2013/14, the possibility of establishing an on-line reporting and progress management system for science contracts has been investigated. A decision has not yet been reached regarding whether to implement this type of system in 2014/15.

Thus far, only the methane mitigation area has identified products (e.g. methanogen inhibitors, anti-methanogen vaccines and low emitting sheep), with clearly identified commercial potential. During 2013/14, the NZAGRC has actively supported the PGgRc in its efforts to engage with industry partners to move these research areas closer to commercial reality.

Goal 5 metrics:

Measure	*Progress in 2013/14*
Leadership of science input into Global Research Alliance and coordination of Livestock Research Group with the Netherlands	Proactive NZAGRC input into Alliance during 2013/14
Visiting fellows from overseas research organisations hosted	3 exchanges funded by LEARN/GRASS Fellowships 2 NZ researchers also went overseas funded by GRASS
Memoranda of understanding covering research collaborations agreed with research centres around the world	Agreements with national and international research centres on-going and productive
Confidentiality agreements with companies to discuss information related to agricultural GHG mitigation technologies	Signing confidentiality agreements with interested companies is the joint responsibility of MPI and PGgRc. The PGgRc are taking a lead role with regards to adoption and commercialisation, on behalf of industry and MPI. The NZAGRC role is one of advice and support.
Licenses to companies to sell agricultural GHG emission mitigation technologies that the NZAGRC or its partners have developed or imported and implemented to suit NZ requirements	Signing licensing arrangements with interested companies is the joint responsibility of MPI and PGgRc. The PGgRc are taking a lead role with regards to adoption and commercialisation, on behalf of industry and MPI. The NZAGRC role is one of advice and support.

SCIENCE FUNDING REPORT

Funding

In accordance with the NZAGRC's Business, Strategy and Science Plans, and with the approval of the SG, $3.36 million was allocated to core Research Programmes in the 2013/14 financial year. The distribution of funding between Programmes is reported in detail later in this section. All figures are exclusive of GST.

Infrastructure Update 2013/14

All spending on infrastructure was completed in the 2010/11 financial year with the New Zealand Ruminant Methane Measurement Centre (at the AgResearch Grasslands campus in Palmerston North) and the New Zealand Nitrous Oxide Measurement Centre (situated at Lincoln University) becoming operational.

No new contracts for infrastructure were executed in 2013/14.

Capability Development Funding 2013/14

The NZAGRC's strategy in this area is outlined under Goal 4 (see previous section). A portion of the Centre funding for this is embedded within the core science programme, another portion is provided via the undergraduate "pipeline" scholarship schemes, with the remaining funding being available on a discretionary basis when high quality students are projects are identified. Additionally, the NZAGRC advises MPI with respect to international capability building efforts and assists with the administration of Alliance funds in this area (see Goal 5).

Research Programmes 2013/14

The current Science Plan consists of 16 Research Objectives which align under three key areas: (i) methane; (ii) nitrous oxide; and (iii) soil carbon. Those programmes marked with a dagger (‡) are co-funded with the PGgRc and/or PGgRc/MPI and those marked with a diamond (◊) are solely funded by the PGgRc.

Area	#	Objective Title	Objective Leader	Objective Leader Organisation	2013/14 Research FTE**	2013/14 $NZ NZAGRC (GST excl)*
Methane	5.1‡	Breed low methane ruminants	J McEwan & C Pinares-Patino	AgResearch	1.0	300,000
	5.2◊	Identifying low GHG feeds	D Pacheco	AgResearch	0	0
	5.3‡	Vaccine	N Wedlock	AgResearch	0.6	150,000
	5.4‡	Identify inhibitors that reduce ruminant methane emissions	R Ronimus	AgResearch	0.7	235,000
	5.5‡	Microbial genomics to underpin methane mitigation	E Altermann & S Leahy	AgResearch	1.1	232,692
	5.6◊	Microbiology to underpin methane mitigation	S Kittelmann & P Janssen	AgResearch	0	0
	5.7‡	Understanding the low methane rumen	M Tavendale & G Henderson	AgResearch	1.0	170,000
	5.8	Modelling rumen methane production	D Pacheco	AgResearch	1.9	280,000
Nitrous Oxide	2.1	Manipulating N inputs	C de Klein	AgResearch	1.2	250,000
	2.2	Manipulating nitrification processes	HJ Di	Lincoln University	4.0	500,000
	2.3	Manipulating denitrification processes	S Saggar	Landcare Research	1.9	250,000
	6.1	Plant Effects on N_2O Emissions	S Bowatte	AgResearch	0.5	100,000
	6.2	Denitrification Processes	S Saggar	Landcare Research	0.5	62,500
Soil Carbon	3.3	Process-based modelling of drivers of soil carbon change	AJ Parsons	Massey University	2.4	200,000
	3.4	Manipulation of carbon inputs, incorporation and retention to protect and enhance soil carbon	D Whitehead	Landcare Research	3.7	470,208
	3.5	Improved soil carbon measurements	F Kelliher	AgResearch	0.4	140,000
				Total	20.80**	$3,340,400

*N.B. 2013/14 funding includes personnel costs, consumables and in certain cases, significant expenditure on travel, items such as SNP chips or services such as DNA sequencing. **NZAGRC PhD students and post-doctoral researchers time is included.

Methane Research Programme Report - 2013/14

Principal Investigators: Dr Graeme Attwood and Dr Peter Janssen

The NZAGRC CH_4 programme is jointly funded with the PGgRc and aligns with existing MPI programmes. It aims to reduce emissions by directly targeting the CH_4-producing methanogens through small molecule inhibitors and vaccines and indirectly through feeding and changes in animal phenotype. The current objectives within the NZAGRC CH_4 programme have made significant progress this year and remain on track to deliver their contracted milestones.

The inhibition of ruminant methane emissions, without compromising the normal digestive functions of the rumen, requires the targeting of methanogen-specific features. Targeted rumen methanogen genome sequencing is being undertaken to confirm current targets for inhibitor and vaccine development and to identify new targets for further investigation. The genomes of the following rumen methanogens have been completed this year – *Methanobacterium formicicum* BRM9, *Methanosarcina barkeri* CM1, Rumen Cluster C sp. ISO4-H5, Rumen Cluster C sp. ISO4-G1 and *Methanobrevibacter* sp. D5. These genomes have been intensively studied and their sequence information has been added to a growing rumen methanogen gene database.

In the inhibitor discovery objective, key methanogen enzyme targets have been cloned, expressed, crystallised and their three dimensional structures solved using data collected at the Australian Synchrotron facility. Four such structures have been used to screen over 400,000 compounds *in silico* and nearly one hundred potential 'hit' compounds have been identified. Additionally, four different enzymes have had their assays optimised and been screened against a large compound library and a number of hits identified. A 60 mL rumen *in vitro* assay system has been used to screen >100 compounds derived from either enzymes or pure culture screenings and several of these have been found to cause inhibition. One of these is scheduled for testing *in vivo* animal trials in spring 2014.

In the vaccine development pipeline, important work continues on the identification and testing of new vaccine targets. Three animal trials using both sheep and cattle have been conducted this year and re-boosting of sheep and cattle with fractions is underway. Subcutaneous vaccination of sheep with two peptides (based on conserved motifs) resulted in significant changes in methanogen populations in the rumen providing further 'proof-of-concept' for an anti-methanogen vaccine. Further trials are planned for 2014/15.

Research in underway to address the question: what happens in the animal's rumen when methane is no longer formed? Specifically does the hydrogen, no longer forming methane, become incorporated into microbial products that may be of benefit to animal production, and/or does the accumulating hydrogen influence the fermentation of feed components such as carbohydrates? Results of a short-term trial confirm methanogen inhibition increases ruminal homoacetogenesis and propionate producing microbes become more abundant. To evaluate long term effects an assessment of the suitability of acetylene for reducing methanogenesis was undertaken by daily administering acetylene for 21 days to sheep. Methanogenesis returned towards the end of the trial confirming acetylene's unsuitability for use in long-term studies.

In the animal variation objective, the high and low emitting sheep selection lines continue to be measured, selected, genotyped and monitored for a wide variety of productive traits. The following results have emerged. It was found that volatile fatty acid (VFA) concentrations in both the rumen in fasting animals and in blood are genetically correlated with methane yield. VFAs are a key rumen derived energy source in sheep and it appears that there has been a shift to propionate production. This offers a potential method for indirect selection of low methane producing animals,

however, these results are observed under extremely defined feeding conditions and the results may differ on pasture diets. Measurements using Portable Accumulation Chambers (PAC) at pasture where the animal is measured for 1 hour in an enclosed chamber have shown these measurements are repeatable and can detect differences in the selection lines when grazing and also are related to estimated breeding values for methane yield. This provides hope that PAC chambers can be used as a low cost method to rank animals and that the differences in respiration chambers will be confirmed under grazing conditions. Finally, results from animals genotyped with up to 600,000 SNPs have shown that genomic selection based on DNA profiles is possible with a prediction accuracy equivalent to an individual being measured for 4 days in a respiration chamber for methane yield (CH_4/kg dry matter intake) and a day for gross methane emission (kg CH_4/day). While some regions appear to explain more variation than others this trait appears to be a consequence of many rather than few loci.

The feeds programme this year has focussed on improving the understanding of the mechanisms behind the reduction in enteric methane emissions of sheep fed forage brassicas, and how those mechanisms relate to feeds such as starchy supplements. Preliminary experimental results suggest that feeding forage rape reduces methane emissions even when mixed with ryegrass. If confirmed, this novel finding means that forage rape does not have to be fed as a sole diet to reduce methane emissions. New insights on digestion processes influencing CH_4 emissions when fresh forages are fed have also been acquired. By understanding these effects, we will be in a better position to understand variation in responses in CH_4 emissions to different feeds.

Underpinning microbiology work which aims at understanding the microbial processes occurring in the rumen during the application of different methane mitigation strategies, thereby guiding the four different research streams within the programme, has continued this year. The analysis of rumen samples stemming from various animal trials conducted by the different research streams has been accompanied by the constant development of sequencing and analysis pipelines to achieve the highest possible resolution of the community structure in a cost-effective way.

Modelling work (previously reported under Integrated Systems) has continued this year and the predictions of methane from the Molly dairy cow model have been significantly improved by developing a sub-model of particle and outflow rate from the rumen. Additionally, the original Molly95 model has been rescaled to represent the rumen of sheep. This model will be useful to support the analysis and interpretation of animal experiments that use sheep as an animal model. The sheep model has also been improved to allow implementation of a model of methanogen growth. This leads to an improved ability to predict the consequences of putative methane mitigation strategies.

Nitrous Oxide Research Programme Report - 2013/14

Principal Investigators: Dr Cecile de Klein and Prof Hong Di

A significant amount of time during 2013/14 has gone into summarising and analysing the results obtained to date in the N_2O programme. During 2013/14 the nitrification, manipulating nitrogen inputs and denitrification programmes started in 2010 were completed and new contracts further investigating plant effects on N_2O emissions and wrapping up the denitrification work were established.

A principal focus of the nitrous oxide mitigation programme has been the optimisation and improved performance of nitrification inhibitors (NI). Work was completed in this area this year. This research has significantly improved our knowledge and understanding of soil moisture effect on N_2O emissions and ammonia oxidiser growth, and the results showed great potential for the use of DCD to reduce N_2O emissions in wet soils such as those under winter forage grazing. The results have also identified ammonia oxidising bacteria (AOB) as the prime target for inhibition in high-N loading urine patch soils and this is important in the development/testing of new nitrification inhibitors. The effectiveness of the new nitrification inhibitor DMPP has been compared with DCD and the results showed that the two NIs were equally effective in reducing N_2O emissions in grazed pasture soils and there was no apparent advantage of DMPP over DCD for use in grazed pastures to reduce N_2O. This research has significantly improved our knowledge on the long-term viability of DCD use, and results showed no adverse effect on other microbial communities or enzyme activities after seven years of DCD use, thus demonstrating the longer-term viability of the NI technology.

With respect to manipulating nitrogen inputs, research has confirmed that the growth rate of forage plants is not at all times growing to the limit of resource supply and revealed that this can be manipulated externally. A set of genes related to gibberellic acid (GA) biosynthesis and degradation have been identified that play a critical role in regulating grass regrowth and carbohydrate metabolism and the molecular mechanisms limiting plant growth under N limitation unravelled. DELLA has been identified as a candidate gene underlying a ryegrass growth quantitative trait locus in a mapping population, supporting the development of molecular tools for breeding higher yielding ryegrass cultivars that require less N.

The denitrification programme has demonstrated that both soil type and specific location were powerful regulators of both emissions and the final N gas being emitted (N_2 vs N_2O). The results of changes in soil water content show that the denitrifier functional genes abundance under saturated soil conditions reflects better the ability of the soil to enhance the reduction of N_2O to N_2 than using field moist soils.

New work has been started this year focussed on measuring plant effects on N_2O emissions in the field. This will provide evidence to determine whether observed differences in potential nitrification rates among different plant species/cultivars under controlled conditions are translated into meaningful reductions in N_2O emissions in the field when urine is applied.

Soil Carbon Research Programme Report - 2013/14

Principal Investigators: Prof Frank Kelliher
and Dr David Whitehead

Increasing the quantity of carbon stored in agricultural soils has the potential to offset emissions of greenhouse gases to the atmosphere. However, realising this potential is technically challenging when soil carbon stocks are already high as they are in New Zealand, potential changes in soil carbon are small and spatial variability is high. The initial NZAGRC's programme (started in 2010) had three distinct components (1) assessing the potential to store carbon across the range of physical and climatic conditions found in New Zealand, (2) devising management practices that can increase the long term soil carbon store and (3) development of tools for verifying that soil carbon stocks have in fact been changed. Parts (2) and (3) have been completed in 2013/14 and a significant amount of time has gone into reflecting on the results obtained to date in the programme and planning the next stages out to 2017.

Modelling soil C and N pools and fluxes using the Hurley Pasture model has provided new insights into how they are influenced by management practices. N fertilizer rates, stocking rate and physiological state (lactating vs dry) affect both C and N cycles with short-term (2-5 years) effects not necessarily being good indicators of longer term (10+ years) impacts. Initial indications are that increased fertilizer use accompanied by increased stocking rates lead to a sustained increase in N losses but only a transitory increase soil carbon storage. Therefore indicators such as emission factors and C and N balances based on field measurements collected during the period of transition (some 2 to 10 years minimum) may be misleading about the drivers and the scale of changes in soil carbon and nitrogen pools and fluxes.

The work with CenW has focused on a detailed comparison between model runs and site observations with eddy covariance (EC) data at a Waikato experimental farm. Analysing grazing systems with EC measurements poses significant challenges as the respiration from grazing animals can result in large short-term CO_2 fluxes. As paddocks are grazed only periodically, eddy covariance observations derive from a mosaic of paddocks with very different exchange rates. This violates one of the key assumptions underlying the use of EC data, and various approaches were trialled to overcome this key methodological challenge. The work ultimately developed a novel approach whereby gas exchange from 26 paddocks around the EC tower was modelled individually based on the detailed grazing history of each paddock. These simulations were then coupled to a footprint analysis which estimates the source are of the gas exchange flux observed at the tower to estimate the net fluxes at the EC tower that provided the appropriate comparison against actual EC observations.

Overall, good agreement was obtained between modelled fluxes and measurements, especially for daily evapotranspiration rates and gross primary production. This work has made it possible to use short-term EC measurements to gain insights into the effect of management changes on changes in productivity and carbon storage. These insights can then be used through the model for longer-term scenario analysis of the effect of different management regimes on long-term carbon storage.

We have continued measurements in the laboratory and at field sites and combined these with the use of models to determine the effects of weather and management variables on changes in soil carbon stocks. Our three areas of focus have been:

a) Increasing carbon inputs to soil by replacing conventional ryegrass/clover grassland with mixed swards
b) Improving the rate of incorporation of carbon from dung into the soil profile by introducing earthworms

c) Determining the impacts of biochar additions on total and 'native' soil carbon and the stability of soil carbon fractions.

(a) Measurements of the exchange of carbon dioxide for a grazed grassland at a dairy farm in the Waikato combined with data on carbon imports from additional feed and exports from meat, milk and methane emissions have continued. Averaged over 4 years, the site was a sink for both carbon dioxide exchange (1.65 t C ha^{-1} y^{-1}), and total carbon including imported and exported carbon (0.60 t C ha^{-1} y^{-1}). A global compilation of 54 site years of carbon balances made over grazed pastures suggested that many of these sites were net carbon sinks when accounting for all imports and exports.

Continuous measurements of carbon dioxide exchange at three sites at a dairy farm in the Waikato with the three treatments commenced: undisturbed ryegrass/clover, ryegrass replaced with ryegrass/clover and ryegrass replaced with a mixture of species to give a diverse sward incorporating plants with deeper root systems. Biomass production for the first year for the re-grassed sites was about 17.2 t DM for the ryegrass/clover and 16.9 t DM for the diverse sward. Both were greater than the old ryegrass/clover mix which produced 15.3 t DM.

(b) The anecic earthworm *Aporrectodea longa* was successfully introduced into three different soils at field sites and reached abundances of 50 individuals/m^2 at all sites despite differences in soil type and climate. There was little evidence that *A. longa* increased the rate of dung incorporation into the soil. At a different site where *A. longa* had been introduced more than 20 years previously and reached abundances of 200 individuals/m^2, more dung was detected in the soil profile compared with a soil containing no anecic earthworms. The implications of this work are that introducing earthworms is likely to increase the rate of incorporation of carbon from dung into the soil but the build-up of soil carbon is slow and unlikely able to be detected in periods less than a decade. Monitoring soil carbon stocks at sites where *A. Longa* had been introduced > 20 years ago gave contrasting results.

(c) The stability of biochar in soils with added plant residues and its effect on carbon stocks was modelled. The carbon lost from both biochar production and decomposition of plant material 'broke even' with that lost from fresh plant residue decomposition by 35 weeks but this was dependent on soil type. These results provide experimental evidence for the potential of biochar to sequester carbon and avoid carbon losses from respiration of plant material while protecting the native soil carbon. However, long-term field studies along with system analysis are needed, prior to deciding whether biochar technology is a viable option for increasing soil carbon in New Zealand's pastoral soils.

Work to address the complex issue of measuring soil carbon stocks has discover that to estimate carbon stocks, soils should be sampled deeply across depth intervals reflecting the vertical distributions of soil mass per unit area and carbon concentration. By measuring the carbon stock and establishing a baseline, change can be determined over time. Although carbon stock change can be inferred by sampling soils on treated and control sites, the results of our dairy farm conversion study suggested soil carbon stock change can depend on the sampling depth, calculation basis, the period between sampling campaigns and suitable control sites.

FINANCIAL SUMMARY

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EXPENDITURE	
Core research spending	
Methane	1,379,797
Nitrous Oxide	1,162,500
Soil Carbon	810,208
Research Total	**3,352,505**
Other research costs	
Additional Fellowships and Studentships	42,000
Planning, engagement & knowledge transfer	77,870
Policy support	42,638
Special IT and communications	36,588
Total	**199,096**
Administration	**555,784**
Total Expenditure (actual)	***4,107,385***
REVENUE*	***5,328,844***
Balance unspent carried over **	***1,221,459***

*Includes $478,844 carried over from 2012/13.
**The unspent funding is primarily due to delays in contracting new integrated systems and DCD-related research during 2013/14. New research programmes have been agreed and this underspend will be contracted out in 2014/15.

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APPENDIX 1 – COMPOSITION OF NZAGRC SG, ISAG and MAG

Compositions of the SG, ISAG and MAG

The tables below set out the compositions of the SG, ISAG and MAG and the dates of governance meetings held during the course of the financial year.

Steering Group

Four Quarterly meetings held in Wellington (21st August 2013, 20th November 2013, 19th February 2014 and 13th May 2014). Plus one teleconference (6th May 2014).

Name	Organisation
Prof. Warren McNabb	AgResearch (Chair)
Dr Rick Pridmore	DairyNZ
Dr Peter Millard	Landcare Research
Dr Stefanie Rixecker	Lincoln University
Prof. Mike Hedley	Massey University
Dr Murray Poulter	NIWA
Mr Warrick Nelson	Plant & Food Research
Mr Mark Aspin	PGgRc
Dr Trevor Stuthridge	Scion
Dr Gerald Rys	MPI (Observer*)
Dr Marc Lubbers	MBIE (Observer*)
Dr Andrea Pickering	MPI (Observer**)

*MPI and MBIE hold Observer (non-voting) positions on the Steering Group.

**Dr Andrea Pickering was invited to attend SG meetings in 2011/12 following recommendation from MPI that an Alliance representative attend SG meetings to ensure coordination.

International Science Advisory Group

No formal meeting in 2013/14. Next meeting planned for early 2015.

Name	Organisation	Rotation end
Dr Richard Eckard	Melbourne University	28 February 2014
Prof Dorian Garrick	Iowa State University	28 February 2015
Prof Keith Goulding	Rothamsted	28 February 2015
Dr Tim McAllister	AgCanada	28 February 2014
Prof Jamie Newbold	Aberystwyth University	28 February 2014
Dr Frank O'Mara	Teagasc	28 February 2015
Prof Pete Smith	Aberdeen University	28 February 2015
Dr Jean-Francois Soussana	INRA	28 February 2014

Membership runs from 1 March to 28 February in each year.

All existing members' commitments will cease at the end of February 2015.

Māori Advisory Group

One meeting held in Palmerston North with some members joining by TC/VC (2nd October 2013).

Name	Organisation
Lorraine Stephenson	Independent
Jamie Tuuta	Māori Trustee
Dr Tanira Kingi	AgResearch
Geoff Taylor	DairyNZ
Marino Tahi	Landcare Research
Prof. Hirini Matunga	Lincoln University
Dr Nick Roskruge	Massey University
Dr Charlotte Severne	NIWA
Alby Marsh	Plant & Food Research
Peter Bennett	Scion
Erica Gregory	MPI

APPENDIX 2 – ANNUAL OBJECTIVE SUMMARY SCIENCE REPORTS (SUBMITTED)

Objective Level Summary – 2013/14

Key:

Objective completed
Objective on track
Objective on track with agreed revisions
Objective on track apart from publications
Current issues with Objective (e.g. behind on experimental work)

Those programmes marked with a dagger (‡) are co-funded with the PGgRc and/or PGgRc/MPI and those marked with a diamond (◊) are solely funded by the PGgRc.

Area	#	Objective Title	Objective Leader	Objective Leader Organisation	2013/14 $NZ NZAGRC (GST excl)	Status End 2013/14
Methane	5.1‡	Breed low methane ruminants	J McEwan & C Pinares-Patino	AgResearch	300,000	On track vs milestones finalised prior to end 13/14
	5.2◊	Identifying low GHG feeds	D Pacheco	AgResearch	0	Minor delays to outputs
	5.3‡	Vaccine	N Wedlock	AgResearch	150,000	Some minor delays in outputs due to consideration of IP and incomplete results
	5.4‡	Identify inhibitors that reduce ruminant methane emissions	R Ronimus	AgResearch	235,000	*In vivo* trials of lead compounds delayed due to lengthened approval process
	5.5‡	Microbial genomics to underpin methane mitigation	E Altermann & S Leahy	AgResearch	232,692	Minor delay to a conference presentation
	5.6◊	Microbiology to underpin methane mitigation	S Kittelmann & P Janssen	AgResearch	0	Delay to report due to issues establishing new sequencing technique & minor delay to paper
	5.7‡	Understanding the low methane rumen	M Tavendale & G Henderson	AgResearch	170,000	Yet to identify a suitable medium term CH_4 inhibitor
	5.8	Modelling rumen methane production	D Pacheco	AgResearch	280,000	Minor delay to paper
Nitrous Oxide	2.1	Manipulating N inputs	C de Klein	AgResearch	250,000	Minor delays re final paper submission
	2.2	Manipulating nitrification processes	HJ Di	Lincoln University	500,000	Completed
	2.3	Manipulating denitrification processes	S Saggar	Landcare Research	250,000	Contract replaced by 6.2 in 13/14
	6.1	Plant Effects on N_2O Emissions	S Bowatte	AgResearch	100,000	On track
	6.2	Denitrification Processes	S Saggar	Landcare Research	62,500	On track

Soil Carbon	3.3	Process-based modelling of drivers of soil carbon change	AJ Parsons	Massey University	200,000	Minor delay in final publication
	3.4	Manipulation of carbon inputs, incorporation and retention to protect and enhance soil carbon	D Whitehead	Landcare Research	470,208	Minor delay to publication and workshop
	3.5	Improved soil carbon measurements	F Kelliher	AgResearch	140,000	Completed

*Minor revisions agreed to Methane 13/14 workplans following Annual Review meetings in May 2014. Methane 14/15 workplans will be finalised and contracts formally varied in early 14/15.

5.1 - Breed low methane ruminants

Jointly supported programme

Objective Leader – Drs John McEwan & Cesar Pinares-Patiño (AgResearch)

The aim of this research is to understand the genetics of host control of ruminant methane emissions. If successful to then develop and make genetic and genomic selection technologies available to reduce methane yield (gCH_4/kgDMI) and methane intensity (gCH_4/kg product) in sheep by June 2016 and in cattle by June 2018. This would be via a beta test format with subsequent full scale industry implementation.

Current research priorities for the next 2 years are:

- determine the genetic correlation of methane emissions with maternal production traits and anatomical changes in the rumen;
- test the stability of the results across a variety of feedstuffs and ages;
- improve genomic predictions and attempt to localise loci with major effects.

In order to undertake these tasks a structured approach has been taken:

- Determine the heritability and repeatability of methane emissions using a wide range of New Zealand maternal sheep;
- Estimate genetic and phenotypic relationships with economically important traits;
- Create divergent selection lines and examine the detailed changes in: anatomy, emissions at different ages, feeding levels and feed stuffs, production traits in young and adult sheep, including carcass and meat quality traits;
- Make the selection lines progeny and samples available for other research e.g. rumen microbial changes, vaccine and forage trials as required.

The second component is to extend these results into industry via:

- Development and validation of low-cost rapid measurement technologies.
- Implementation via genomic selection which is being separately developed in sheep and cattle industries, because it can be applied at effectively zero additional cost to the breeder.
- Development of appropriate selection index weighting based on expected long term methane emission costs.

An important aspect of using genetic change is that it is slow, but permanent and cumulative. As a consequence it is important that an on-going monitoring of genetic changes in other traits is undertaken to detect any unfavourable changes at an early stage. Sheep are being used for initial development as they are markedly cheaper and we expect broad consistency of results across these related species.

After an initial pilot project in 2008, a formal research programme commenced in with 2009 born progeny. To date heritability and repeatability of methane emissions has been estimated and published as have correlations with production traits to one year of age. The measured animals and subsequent selection lines are being monitored for responses at later ages and detailed studies of anatomical changes are being undertaken. Similarly, studies have commenced using rapid measurement techniques and examining responses on pasture under a variety of

physiological ages and seasons. Sufficient genotyping has been done to identify certain host genome regions associated with methane emission and validate that genomic selection can be undertaken.

5.1 – Progress in 2013/14

Genetic and genomic selection offers a potential method to reduce both gross cattle and sheep methane emissions (gCH_4/d), methane yield (gCH_4/kg dry matter intake) and improve methane efficiency (g CH_4/kg end product). Previous work from this project has shown that gross methane emissions and methane yield were heritable and repeatable traits when measured in respiration chambers. Similarly, it found that selection using the existing Sheep Improvement Limited production index was improving methane efficiency (via increases in reproduction and decrease in disease), but gross methane emissions/ewe wintered were increasing and methane yield were unchanged. These results led to the development of selection lines of low and high methane yield animals based on measurement of the trait in respiration chambers. The work undertaken in the current year has focused on continuing this selection to create divergent lines, while at the same time seeking to understand the relationship of methane yield with a variety of physiological and productive traits. In addition work is underway to investigate the value of brief measurements and the potential of genomic selection to assist in breeding for reduced emissions.

The following results have emerged. It was found that volatile fatty acid (VFA) concentrations in both the rumen in fasting animals and in blood are genetically correlated with low methane yield. Specifically the concentration of acetate is reduced. VFAs are a key rumen derived energy source in sheep and it appears that there has been a shift to propionate production perhaps coupled with better rumen absorption of these compounds. This offers a potential method for indirect selection, however, these results are observed under extremely defined feeding conditions and the results may differ on pasture. Separate work, now published, on these lines has shown that the microbial profiles obtained from rumen samples also differ with low methane yield animals either having higher *Quinella* or *Sharpea*-type bacterial communities. The former is thought to be a propionate producer and the latter may be a lactate producer. Both pathways reduce hydrogen production - a substrate of methane production. Consistent changes are also observed in the *Archaea* microbial gene expression pathways which produce the methane end product. Similarly computed tomography and slaughter studies identify that the rumens of the low methane yield are smaller. The animals therefore have a higher dressing percentage (proportion of the live animal that consists of carcass). While not a production trait as such this suggests the digestive anatomy of low methane yield animals has altered. Acetate can only be used by the host as an energy source or to deposit lipid so it could be postulated that some change in fat deposition may also be observed. To date we do have limited evidence that the low methane yield animals are leaner and have more muscle, but further work is required.

Measurements using Portable Accumulation Chambers (PAC) at pasture where the animal is measured for 1 hour in an enclosed chamber have shown these measurements are repeatable and can detect differences in the selection lines when grazing and also are related to estimated breeding values for methane yield. The differences observed in PAC chamber gross emissions adjusted for liveweight are similar or greater to methane yield differences in respiration chambers. This provides hope both that PAC chambers can be used as a low cost method to rank animals and that the differences in respiration chambers will be confirmed under grazing conditions. Similarly, studies using brief measurements obtained in respiration chambers (either 6 minutes, or 1 hour) have been shown to be highly genetically correlated to daily emissions and suggest several brief measurements can be as useful as daily measurements. Finally, results from animals genotyped with up to 600,000 SNPs have shown that genomic selection based on DNA profiles is possible with a prediction accuracy equivalent to an individual being measured for 4 days in a respiration chamber for methane yield and a day for gross methane emission. While some regions appear to explain more variation than others this trait appears to be a consequence of many rather than few loci.

Key achievements for 2013/14:

- Estimated heritabilities and genetic correlations of VFA with methane emissions for value as an indirect predictor
- Estimated genetic correlations, repeatabilities and heritabilities of 6 minute and 1 hour measurements versus 1 day
- Investigated and reported on differences in rumen size and carcass dressing percentage and carcass yield
- PLoS One paper submitted on the accuracy of genomic selection for methane traits and genomic regions where loci affecting the trait may occur in sheep along with a comparison with similar results from a separate project in dairy cattle.
- Continued to measure and select the selection lines and monitor them for a wide variety of productive traits.

5.2 - Identifying low GHG feeds

PGgRc sole funder

Objective Leader – Dr David Pacheco (AgResearch)

We aim to develop feeds and feeding strategies that result in reduced GHG emissions from ruminants. Nutritional strategies will take advantage of desirable characteristics of different forages, feed crops and feed ingredients to design appropriate feeding systems. Such feeds and feeding systems will then direct rumen fermentation towards pathways that are conducive to reduced methane and increased nitrogen utilisation efficiency. The devised nutritional strategies will provide benefits on GHG that are above and beyond any benefits in emission intensity achievable via increased productivity when current feed supplementation practices are used. We will not only identify nutritional strategies that work, but also understand the mechanisms behind the GHG reductions so that low GHG feeding systems can be designed for current and future markets of livestock feed.

Initially, we will use the leads from forage brassica experiments to develop hypothesis of mechanisms underpinning the reduction in GHG emissions. From a mechanistic perspective, the initial lines of enquiry will be the quantification of the contribution of pH and carbohydrate structure as factors affecting fermentation patterns leading to lower methane production in the rumen. The purpose of understanding these rumen processes is to design manipulations of rumen fermentation to reduce GHG emissions, while maintaining or increasing animal productivity. In all animal experiments, nitrogenous aspects of rumen fermentation will be studied to ensure that feeds and feeding strategies will deliver reductions for both methane and nitrous oxide.

The knowledge generated will be used by scientists in the NZAGRC/PGgRc programme. For example, to help define the role of digestive processes as a contributor to the variation in animal to animal methane emissions and also to understand their potential role as modulator of responses to methanogen inhibitors and vaccines.

In the medium term, knowledge generated from this objective will be used by nutrition scientists, applied nutritionists, forage and crop breeders to develop feeds, feeding practices and forages that will result in reduced methane emissions from the process of feed digestion.

5.2 – Progress in 2013/14

The programme this year aimed at improving the understanding of the mechanisms behind the reduction in enteric methane (CH_4) emissions of sheep fed forage brassicas, and how those mechanisms relate to feeds such as starchy supplements. This programme has strong linkages with SLMACC projects evaluating the potential of supplements and forage brassicas as mitigation tools for on-farm application.

Our previous studies have confirmed that feeding forage rape to sheep consistently results in less CH_4 emitted per unit of intake, whether expressed as dry matter, organic matter or digestible organic matter. The proportional difference between brassicas and ryegrass increases if the emission is expressed per unit of organic matter or digestible organic matter, which has positive implications for emissions intensity.

The lower CH_4 emissions from sheep fed forage rape were accompanied by a reduction in ruminal pH. Low pH has been postulated as a mechanism for the lower CH_4 emissions in ruminants fed high amounts of grain. We were able to partially abolish the effect of forage rape on CH_4 emissions from sheep by increasing the rumen pH to values comparable to those measured when ryegrass is fed. We observed that the responses in methanogenesis appeared gradually as the rumen pH in rape-fed sheep was increased and then allowed to return to low values. The pH manipulation was achieved by introduction and subsequent removal of a buffer salt in the diet. Our observation is

compatible with a mechanism in which pH, within physiological values, promotes a gradual selection for a 'low CH_4' rumen, but is yet to be elucidated if the mechanism involves an initial effect on methanogens or in propionate producers. We have characterised the rumen microbial communities in sheep fed brassicas, and found them to be consistent with populations that have fermentation patterns leading to more propionate production, and hence lower hydrogen production and less CH_4 emitted. This has positive implications for animal metabolic performance and productivity.

We have acquired new insights on digestion processes influencing CH_4 emissions when fresh forages are fed. For example, data from sheep fed a variety of fresh forages suggests that water content of the forage has an effect on CH_4 emissions. This could be explained by changes in the dynamics of liquid outflow rate. Additionally, a large proportion of 'dry matter' is present as a soluble fraction or contained in small particles in fresh forages, which could result in a significant proportion of fermentable material leaving the rumen shortly after ingestion. By understanding these effects, we will be in a better position to understand variation in responses in CH_4 emissions to different feeds.

We have investigated how increasing levels of grain in the diet affects CH_4 production, and currently an experiment is under way with increasing levels of forage rape substituting ryegrass pasture. We have confirmed previous observations that a large proportion (>65%) of grain needs to be fed to reduce CH_4 emissions. Our experiments also confirmed that lower proportions (20-50%) of grain can increase CH_4 production. In contrast, our preliminary observations from the inclusion trial with forage rape suggest a linear reduction in CH_4 yield as the proportion of forage rape in the diet increases. If confirmed, this result would mean that feeding of forage brassicas can lead to a reduction in CH_4 emissions at a larger range of inclusion than previously thought. It also mean that reductions in CH_4 observed in brassicas occur through a different mechanism to those elicited by feeding grains. Detailed measurements of the interactions between chemical composition and physical attributes of fresh forages, and the fate of feed entering the rumen, require further research to progress our understanding on ways to reduce CH_4 emissions from ruminants fed fresh forages.

Key achievements for 2013/14:

- We have established and used an *in vivo* model to assess the effect of pH on methane emissions independently of feed type.
- We have preliminary experimental results suggesting that feeding forage rape reduces methane emissions even when mixed with ryegrass. If confirmed, this novel finding means that forage rape does not have to be fed as a sole diet to reduce methane emissions.
- Our summary of work on dietary mitigation practices using fresh forages has been accepted for publication in Animal Production Science and selected as the Stobbs Memorial Lecture of the Australian Society of Animal Production.
- Our contributions have been accepted for presentation at the combined ISRP-ISNH meeting in Canberra. This will increase the international exposure of our research programmes.
- We have completed a review on forage carbohydrates, which identifies key gaps in information as well as the NZ capability required to fill those gaps.

5.3 – Vaccine

Jointly supported programme

Objective Leader – Drs Neil Wedlock & Art Subharat (AgResearch)

The immediate goal of the vaccine programme is to produce a prototype vaccine which has shown efficacy in either sheep or cattle such as a change in methanogen communities in the rumen. Further development of the vaccine (by optimising antigens, adjuvants and delivery) will lead to a vaccine which is targeted at reducing methane emissions in sheep and cattle by at least 20%.

The programme in Y13-14 will seek to provide antigens and adjuvants for a prototype vaccine and 'proof-of-concept' that a vaccine will work, i.e., sufficiently high levels of antibodies can be produced following vaccination of sheep or cattle and there is evidence of vaccine-mediated changes in methanogen populations in the rumen. To achieve this, experimental vaccines formulations, consisting of new antigens selected by bioinformatics analysis of genomes from the most rumen-abundant methanogens, and formulated with current 'best' adjuvants will be administered to sheep and cattle. In each trial, animals will be monitored for their antibody responses to the methanogen antigens, anti-methanogen activity measured in *in vitro* assays and rumen microbial profiling undertaken to determine antibody induced changes in microbial populations in the rumen.

From these vaccine trials we will determine:

1. Which is the best model – sheep or cattle? After two trials, we expect to understand if cattle are more promising, or if sheep are the most suitable species for further research.
2. Do the adjuvants increase salivary IgA, and ruminal IgA (and other classes of antibody) resulting in very high levels of antibody in the rumen?
3. Do the serum antibodies inhibit the target methanogens in pure culture?
4. Do any combinations of adjuvant and antigen change the ruminal methanogen community?

The scheduled work will make efficient use of resources and effectively makes every set of experiments an animal trial, and also a search for further 'proof-of-concept'.

Because of the structure of the process, if both the right antigen and the correct adjuvant are administered, positive results will be gained for points 2 and 3, and possibly 4. If the right adjuvant is combined with an ineffective antigen, increased IgA (or IgG) will be measured in the saliva and rumen (point 2), but there will be no impact on pure cultures (point 3) or on methanogens in the rumen (point 4). If an effective antigen is tested with an ineffective adjuvant, results from points 2 and 4 will be negative, but from point 3 will be positive. In each round of trials, we will formulate the best antigens with the best adjuvants and test those combinations, and also introduce new antigens or adjuvants.

Once we have obtained positive results in point 4, we will have the next 'proof- of-concept' step needed. Depending on the nature/magnitude of the change in the rumen methanogen community, we can then proceed to conduct a larger vaccination trial in either sheep or cattle with quantification of the reduction in methane emissions using respiratory chambers. This will be negotiated with the funders, since it may require reallocation of resources, and changes in milestones.

The prototype vaccine arising from research will be available for scientists within the NZAGRC-PGgRc programme to develop further and along with critical knowledge of vaccination (antigens/adjuvants/route of vaccination and relevant IP will be available for entering negotiation with an Animal Health Company for commercialisation.

5.3 – Progress in 2013/14

Milestone 5.3.1. Antigen identification and testing - dominant/conserved epitopes from dominant methanogens identified by bioinformatics and fractions produced from dominant methanogens. The D5 'variants' of GT2 and SecE have been produced in *E. coli* for future vaccination of sheep and testing antigens. Work has progressed on producing 3F5 variant of SecE and the recombinant protein has been produced. Additional amounts of cytoplasmic fractions were prepared from methanogens D5 and 3F5 for the purpose of vaccination of sheep and cattle in Milestone 5.3.2. Two previously untested targets (SND and GspF1) were produced as recombinant proteins for raising and testing antibodies. A new list of potential vaccine targets has been produced for testing in our pipeline of antigen discovery.

Milestone 5.3.2. Vaccination of sheep and cattle to determine vaccine-induced changes in rumen microbial populations (2013-2014). Three animal trials have been completed with antisera, saliva and rumen contents collected for analysis. Two other trials (re-boosting animals are underway, see below). These trials have used 3 or more animals per target/adjuvant combination to help determine vaccine-induced changes in rumen microbial populations. In each trial, non-vaccinated animals were monitored as controls. Antibody responses to the various antigens and vaccines in these trials have been analysed by ELISA. Testing the antisera produced against the various antigens and fractions for inhibitory activity in *in vitro* methanogen cultures is being conducted. DNA was prepared from the rumen contents samples from trials 1 and 2 for pyrosequencing and microbial profiling.

Trial 1 (18 sheep): Sheep were vaccinated with D5 cytoplasmic fraction, 3F5 cytoplasmic fraction, D5 'variant' of SecE peptide or a mixture of two peptides based on conserved regions within the large extracellular domain of GT2 identified by bioinformatics. Two additional sheep were included; one vaccinated with recombinant SND and another with GspF1. All antigens were formulated with the current 'best' adjuvant Montanide ISA61 and the vaccines administered by the subcutaneous route. Trial 2 (18 cattle): Cattle were vaccinated with D5 cytoplasmic fraction or 3F5 cytoplasmic fractions formulated with Montanide ISA61 (identical vaccines to those used in the sheep trial allowing comparison of antibody responses between sheep and cattle). Trial 3 (23 sheep): Sheep were vaccinated with M1 SecE2-KLH with FIA adjuvant, recombinant M1 GT2 with FIA adjuvant. Sheep were also vaccinated with recombinant SecE (D5) and recombinant GT2 (D5) and AAVLPs, and four other recombinant proteins. Trial 4 (re-boosting, 10 sheep): The sheep vaccinated with the new D5 and 3F5 fraction had produced variable antibody responses while the responses in cattle were low. The sheep were revaccinated with D5 and 3F5 fractions using a 10-fold higher dose of proteins than previously used to boost the antibody titres further. New rumen content samples will be collected from the re-vaccinated animals for further rumen microbial community analysis. Cattle will also be re-vaccinated to boost antibody titres.

Antisera produced against the various targets in Trial 1 have been tested for anti-methanogen activity in *in vitro* methanogen cultures. Preliminary results indicated that antibodies against SND caused extensive cell agglutination. Archaeal community analysis was performed on rumen content samples collected from the animals in trials 1 and 2. The results indicated a significant reduction in methanogens belonging to the *Mbb. gottchalkii* clade and a significant increase in *Mbb. ruminantium* clade methanogens in the animals vaccinated with D5 GT2 peptides compared to non-vaccinated animals. This indicated that antibodies produced by this vaccination strategy were active against methanogens belonging to the *Mbb. gottchalkii* clade, a result consistent with effects produced by a vaccine based on the D5 variant of GT2. The result would indicate that vaccination by the subcutaneous route using Montanide ISA61 has produced sufficient level of anti-GT2 antibodies in the saliva and rumen to have had an effect on ruminal methanogens. This is a significant result and provides further 'proof-of-concept' for a vaccine.

Milestone 5.3.3. Mucosal vaccination strategy for producing high levels of salivary antibodies for 'proof-of-concept' and vaccination studies. Good progress has been made on producing AAVLP that express the methanogen antigen GT2. Production of the modified AAVLP was successful but the yield of purified VLPs was low. Sufficient amount of AAVLPs expressing GT2 in a crude form

was produced to vaccinate sheep parenterally and mucosally (intranasal route). Analysis of immune responses indicated that only minimal GT2-specific responses were induced. Higher levels of AAVLP will need to be produced for further vaccination studies.

Key achievements for 2013/14:

- Three animal trials using both sheep and cattle have been conducted; re-boosting of sheep and cattle with fractions is underway. Subcutaneous vaccination of sheep with the D5 'variant' of GT2 (2 peptides based on conserved motifs) resulted in significant changes in methanogen populations in the rumen providing further 'proof-of-concept' for an anti-methanogen vaccine.
- Two new potential vaccine candidates, SND and GspF1 have been tested in our pipeline of antigen discovery.
- AAVLP displaying GT2 were produced for testing in sheep.
- Improved methods developed to produce large quantities of purified recombinant GT2 and other methanogen proteins for animal trials.
- Immunofluorescence microscopy confirmed binding of target-specific antibodies to the surface of methanogen cells.

5.4 - Identify inhibitors that reduce ruminant methane emissions

Jointly supported programme

Objective Leader – Dr Ron Ronimus (AgResearch)

The aim of this objective is to develop cost-effective inhibitors that reduce methane emissions by at least 20% in sheep and cattle without reducing productivity. The research will use an already established "pipeline" that uses rumen methanogen enzyme assays and enzyme structures to screen for inhibitors in a cost-effective and rapid way. The pipeline has produced a number of "hits" (compounds that inhibit in simple tests). Taking these compounds through to testing in animals has the highest priority. The discovery of novel inhibitors will be also accelerated using the developed enzyme assays to screen large compound libraries for potential inhibitors (5,000 compounds) and by enhancing the efficacy of hit compounds using drug design techniques. The compounds will be tested in animal trials for efficacy and productivity effects. The data from the animal trials will be used to engage a commercial partner (or partners) for the development of appropriate technologies for delivering the inhibitors.

5.4 – Progress in 2013/14

Methane emissions from ruminants are produced by a unique group of archaeal microorganisms termed methanogens and are widely recognised as contributing to climate change. Inhibitors of rumen methanogens are likely to play a significant role as one of a suite of strategies used in methane mitigation. Small molecule inhibitors have been previously shown in a number of *in vivo* studies to markedly reduce methane emissions (average of circa 65%). However, these compounds are unsuitable in modern animal husbandry practices as they contain halogen atoms (e.g. chlorine, bromine, fluorine). These studies reinforce, however, the notion that alternative and effective inhibitors can be discovered using 'standard' drug discovery approaches that have been extensively used by the scientific community to target cancer cells and pathogens. Small molecule inhibitors of rumen methanogens that are cost-effective, non-toxic and environmentally-friendly could be quickly developed and manufactured to rapidly alleviate methane emissions on a global scale.

Methods for developing novel inhibitors of microorganisms generally rely on three strategies. **One strategy** that has been utilised in PGgRc-NZAGRC Objective 5.4 has sought to determine new rumen methanogen enzyme structures. The target key enzymes are indicated to be essential for the survival of the majority of rumen methanogens. Once the newly-derived structures become available they can then be used to screen large virtual compound libraries (>100,000 compounds) via computer-based methods ('*in silico*'). The highest scoring compounds are ordered and then tested either in the respective enzyme assays or against pure cultures of rumen methanogens to reconfirm inhibitory activity. Enzyme structures can be used to guide the development of derivatives with increased potency compared to the original hit by modelling the compound in the active site of the enzyme. Increases in potency using these techniques have been known to exceed 10,000-fold. **A second method** that is also used in the PGgRc-NZAGRC Objective 5.4 involves producing large quantities of a rumen methanogen enzyme and directly screening compound libraries (5,000-20,000 compounds) using enzyme assays. 'Hit' compounds are then tested against pure cultures of rumen methanogens to confirm their ability to inhibit methanogens. **A third approach**, termed phenotypic screening, is used in a Partner program (MPI Global Partnership in Livestock Emissions Research contract 15810) and involves the direct screening of rumen methanogens in pure culture. This approach will be used extensively in financial year F15 within the PGgRc-NZAGRC programme. There are two main advantages of directly screening methanogens and these are that all essential enzymes of the target methanogen are screened simultaneously (circa 400-500 genes) and that any inhibitor that is found must inhibit a 'druggable' target enzyme. However, the target enzyme or cellular target might not be able to be identified, potentially limiting the ability to use an enzyme structure to guide the further derivatisation of the initial hit compound. For all three of these approaches, any promising compounds that are

identified are ultimately tested in rumen fluid-based *in vitro* assays prior to animal trials. The targeted and phenotypic screening approaches are complementary to each other. Both approaches can facilitate discovery of novel inhibitors independently of each other.

In the last year, good progress has been made in several milestones relating to the programme (animal trial testing, 5.4.1; rumen *in vitro* assay testing, 5.4.2; enzyme preparation and assay development, 5.4.3; enzyme structure development, 5.4.4; *in silico* screening, 5.4.5; and enzyme assay screening, 5.4.6). For example, four different enzymes have had their assays optimised and been screened against a large compound library and a number of hits identified. Furthermore, four enzyme structures/models have been determined and been used to screen over 400,000 compounds *in silico* and nearly one hundred potential hits ordered for testing. A 60 mL rumen *in vitro* assay system has been used to screen >100 compounds derived from either enzymes or pure culture screenings and several of these have been found to cause inhibition. One of these is scheduled for testing *in vivo* animal trials in the first quarter (FY15). Confirmation of various results from enzyme assays, *in silico*-based screenings and pure culture screenings are ongoing and a number of useful inhibitors are expected based on our previous hit success rate.

Key achievements for 2013/14:

- Enzymes have been produced and purified, and assays developed and optimised to enable screening of large compound libraries (Milestones 5.4.3 and 5.4.6).
- Over 100 compounds have been tested in rumen fluid-based assays and several of these have caused inhibition of methane (Milestone 5.4.2). One of these, which shows a good dose-response curve (methane inhibition versus concentration), is scheduled for an animal trial in August (Milestone 5.4.1), and experiments are progressing with several others.
- A number of new enzyme crystals have been produced and will be analysed for diffraction at the Australian Synchrotron in the second quarter of next year (5.4.4). Four new enzyme structures were determined (5.4.4).
- Over 400,000 compounds have been screened in silico and >300 compounds ordered for testing (5.4.5).
- Four manuscripts have been prepared and submitted to the ROI system. One has been submitted to the Proceedings of the National Academy of Sciences.

5.5 - Microbial genomics to underpin methane mitigation

Jointly supported programme

Objective Leader – Drs Eric Altermann and Sinead Leahy (AgResearch)

A comprehensive set of reference genomes is essential to understanding the role of specific groups of rumen methanogens to methane emissions from ruminants and to underpin current and future vaccine and inhibitor development pipelines. Closed genomes are important in this regard to ensure the entire gene complements of methanogens are included in the comparative gene analyses and to enable genome-wide functional studies via protein identification and RNA sequencing-based technologies.

In two years we will have reference genomes for each of the main groups of hydrogenotrophic (*Methanobrevibacter ruminantium* strains M1 & YLM1, *Methanobrevibacter gottschalkii* strains SM9 & D5, *Methanobrevibacter wollini* strain AbM4 and *Methanobacterium* sp. strain BRM9) and methylotrophic rumen methanogens (*Methanosarcina* sp. strain CM1, *Methanosphaera* sp. strain ISO3-F5, and *Methanoplasmatales* spp. strains ISO4-H5, ISO4-G1 & ISO4-G11). Comparative genome analysis will be used to inform target selection in the established vaccine and chemogenomic pipelines and to confirm the presence and variability of the target genes in the methanogen population. Additionally, the information will be used to underpin future mitigation approaches, in particular, the interpretation of metagenomic datasets from high and low methane emitting sheep. The genomes of the three strains which constitute the Rumen Cluster C-like methanogens will be a particular focus as their metabolism and physiology is not well understood, and they are not yet well targeted by either the vaccine or inhibitor pipelines. The current PhD thesis programme on the ISO4-H5 genome to be completed in the next two years will improve knowledge in this area.

To maximize the value of the genome sequences and to improve the international reputation of the PGGRC and NZAGRC methane mitigation programmes, we plan to publish the genomes in peer reviewed journals after IP assessment and where appropriate, patent protection. As part of this objective, Mr Yang Li is completing his NZAGRC PhD thesis programme.

5.5 – Progress in 2013/14

To examine the genetic diversity of selected targets in the vaccine programme and to obtain the required variations in epitopes for effective methanogen inhibition, the genome sequences of rumen methanogens must first be elucidated. This research is essential to allow effective development of inhibitors and vaccines with broad efficacy that will work on farm. The genomes of the following rumen methanogens have been completed this year – *Methanobacterium formicicum* BRM9, *Methanosarcina barkeri* CM1, Rumen Cluster C sp. ISO4-H5, Rumen Cluster C sp. ISO4-G1 and *Methanobrevibacter* sp. D5. Manuscripts detailing the genome sequences of BRM9 and CM1 have been prepared for the journal Standards in Genomics Sciences. BRM9 represents the first report of a genome sequence for a *Methanobacterium formicicum* strain of rumen origin. The genome sequence indicates that this species of rumen methanogen will be amenable to inhibition by the small-molecule inhibitor and vaccine-based technologies being developed for the other genera of methanogens found in the rumen. The genome sequence of CM1 has been found to be similar to that of the freshwater sediment isolate *M. barkeri* Fusaro, but markedly different from the dominant rumen methanogens. CM1 has a much larger genome than previously seen for rumen methanogens and has revealed further insight into the metabolic versatility of rumen methanogens. Although CM1-like methanogens are thought to constitute a small proportion of the methanogen diversity in the rumen, that contribution may change if the more dominant methanogens become displaced by methane mitigation technologies. The genomic differences highlighted from the CM1 genome stress the need to include information from all rumen methanogens in the design of mitigation approaches. Rumen methanogens represented by organisms such as Rumen Cluster C sp. ISO4-H5 and Rumen Cluster C sp. ISO4-G1 make up a significant component of the NZ

ruminant microbiome under certain dietary feeding schemes. However, until now very little has been known about their genetic makeup. The completed genome sequences have for the first time revealed the biochemical pathways by which these methanogens produce methane. This process is different to what had been known previously for rumen methanogens. This type of information is essential if the RCC group of methanogens are to be targeted by current methane mitigation strategies.

To identify and prioritise the best genetic targets for anti-methanogen vaccines, it is necessary to investigate how similar the selected proteins, and their respective epitopes, are across a range of known rumen methanogens. Targets that are highly conserved and show only minimal sequence variability are more likely to be active against a broad range of different methanogens than targets that are very unique to only a few select strains. Similarly, selected targets should be specific against methanogens only, and not affect other members of the rumen microbial community, to avoid negative impacts on rumen function. To provide such critical information, microbial genomics has developed three major software tools: a microbial annotation suite that provides detailed information on gene functionality (this information also informs the chemogenomics programme), a comparative genomics suite that highlights and investigates the differences and similarities between (groups of) genomes, and an integrative analysis tool that analyses the frequency, distribution, and quality of selected targets in methanogens (archaea) and bacteria. Recently, these tools were supplemented with metagenomic and metatranscriptomic databases built from the JGI high-low methane emitting rams datasets. To date, 325 archaeal genomes and plasmids have been incorporated into the comparative genomics database. Based on these data, a subset of rumen methanogens was selected centred on *Methanobrevibacter* sp. D5, a particularly abundant methanogen in NZ ruminants. Comparative genomics identified a suite of 41 possible candidate proteins and a correlation with the metatranscriptomic databases led to eight prioritised targets, five of which feature large extracellular domains. Existing vaccine targets such as GT2 and SecE were subjected to a detailed *in silico* analysis. For GT2, two novel highly conserved protein sequence motifs were discovered that are predicted to be prevalent in most rumen methanogens and are likely to be located on the cell surface. These motifs are currently being investigated in the vaccine programme. Unlike GT2, SecE was found to harbour a much smaller extracellular domain which exhibits a significant degree of sequence variation. In addition, metatranscriptomic analyses may indicate that SecE is present on the cell surface under more select environmental conditions.

To accelerate animal breeding for reduced host methane emissions, the ram rumen microbiome deep sequence metagenomic and metatranscriptomic datasets mentioned above, were compared with CH_4/Kg DMI-linked SNPs from the 10 high or low methane-emitting rams. From the metagenome sequences, 14 microbial genes, with functions mainly related to microbial signal transduction and sugar metabolism, formed two distinct clusters; Cluster 1 was correlated strongly with 43 SNPs, and Cluster 2 with 45 SNPs. The metatranscriptomic data identified 20 transcripts mainly involved in carbohydrate transport/metabolism and protein secretion that associated with 31 SNPs, many of these SNPs being the same as those associated with the Cluster 2 genes. One particularly strong association was found between a rumen microbial mycobactin peptide synthetase gene and a host SNP linked with guanylate cyclase. Guanylate cyclase sits in the host membrane and is stimulated by the guanylin family of bioactive peptides, and some bacterial enterotoxins, to produce cyclic GMP, a second messenger that activates intracellular protein kinases. This in turn induces Cl^- and HCO_3^- secretion into the intestinal lumen, and serves to regulate intestinal fluid and electrolyte secretion. The mycobactin peptide synthetase was strongly correlated in both the metagenomic and metatranscriptomic datasets, opening the possibility that this may be a signalling mechanism between the rumen microbiome and the host influencing secretions into the rumen.

To identify potential rumen microbial proxies for CH_4 emissions in the host, the metagenomic and metatranscriptomic datasets were analysed via sparse Partial Least Squares regression. This analysis selected 371 gene and 266 transcript variables which gave adjusted R^2 of 0.9569 and 0.9734, respectively, with CH_4/Kg DMI. These strong correlations suggest that the abundance of these particular rumen microbial genes and transcripts may be used to predict CH_4/Kg DMI.

Key achievements for 2013/14:

- Identification and prioritisation of 41 new vaccine targets for *Mbb*. sp. D5, 8 of them being prioritised.
- Discovery of two highly conserved motifs in the existing vaccine GT2 extracellular domain target
- Kelly WJ, Leahy SC, Li D, Perry R, Lambie SC, Attwood GT, Altermann E (2014) The complete genome sequence of the rumen methanogen *Methanobacterium formicicum* BRM9. Standards in Genomic Sciences (manuscript currently under journal review).
- Lambie SC, Kelly WJ, Leahy SC, Li D, Reilly K, McAllister TA, Valle ER, Attwood GT, Altermann E (2014) The complete genome sequence of the rumen methanogen *Methanosarcina barkeri* CM1. (manuscript submitted to ROI system, awaiting approval)
- Creation and maintenance of a comprehensive methanogen/archaeal comparative genomics database and creation of metagenomic and metatranscriptomic databases based on the JGI datasets

5.6 - Microbiology to underpin methane mitigation

PGgRc sole funder

Objective Leader – Drs Sandra Kittelmann & Peter Janssen (AgResearch)

The microbiology objective analyses microbial community structure in rumen samples from animal trials to evaluate the effects of methane mitigation strategies on rumen microbial communities.

Ruminant methane is derived largely from the action of the rumen microbial community. Low methane emitting animals have different microbial community structures from high emitting animals, whether through natural variation or feeding different diets. Understanding the differences in microbial community structure within and between mitigations points to common or different mechanisms that drive the differences in methane. In addition, partial effects on the microbial community, in the absence of methane emissions reduction, will inform vaccine and inhibitor objectives of near successes that deserve to be refined further.

To enable testing on larger numbers of trials envisaged in the overall programme, we will also start using higher-throughput technologies (sampling methods, sequencing methods) to collect these data in a timely, cost-effective and non-invasive way. The outcomes of animal trial analyses will be made available to other scientists within the PGgRc-NZAGRC programme. The outcomes of methodology development will be available to the PGgRc-NZAGRC directly.

5.6 – Progress in 2013/14

The Microbiology objective aims at understanding the microbial processes occurring in the rumen during the application of different methane mitigation strategies, thereby guiding the four different research streams within the program. The analysis of rumen samples stemming from various animal trials conducted by the different research streams is accompanied by the constant development of sequencing and analysis pipelines to achieve the highest possible resolution of the community structure in a cost-effective way. We are currently working towards shifting to Illumina sequencing technology and are testing alternative sampling methods. Furthermore, a new database for rumen and intestinal methanogens has been developed which allows high-resolution BLAST-based community structure analysis of pyrosequencing data sets (Seedorf *et al.,* 2014, PeerJ, accepted for publication). Using these latest tools, rumen samples from animal trials undertaken from the different research streams have been analysed on a molecular basis to understand the microbial processes occurring in the rumens of animals that exhibited (Animal Selection, Low GHG Feeds) or were treated for lower methane yields (Vaccination). The results are reported in the context of each respective Research Aim:

Research Aim 1: Sheep feeding on a diet consisting of lucerne pellets showed significant natural differences in methane yields. Analysis of the microbial community in animals with naturally high and naturally low methane emissions revealed the existence of two different low methane bacterial community types and one high methane bacterial community type. Each of the two low methane community types was characterised by a higher relative abundance of certain diagnostic species (*Quinella* spp., or *Sharpea*, *Kandleria* and *Fibrobacter* spp.). Members of these bacterial genera have been shown to (or are postulated to) produce less hydrogen than those species characteristic for samples from animals with high methane yields (such as members of the families Ruminococcaceae, Catabacteriaceae, Lachnospiraceae and the orders Clostridiales and Bacteroidales). It is likely that the two different low methane bacterial community types result from different animal-related mechanisms (genotypes) and they may have different production characteristics. Correlation of microbial community types with genotypes of the animals will allow insights into whether the two low methane bacterial community types have similar or differing production characteristics.

Research Aim 2: Compared with ryegrass, methane emissions from lambs fed forage rape were 20-30% lower, and this difference persisted for at least 3 months. Ruminal microbial communities in forage rape-fed lambs were different from those in ryegrass-fed lambs, with greater proportions of potentially propionate-forming bacteria, and were consistent with less hydrogen and hence less methane being produced during fermentation. Molar proportions of ruminal acetate were lower and those of propionate were higher in forage rape-fed lambs, in agreement with the microbial community structure. Forage rape contained more readily fermentable carbohydrates and less structural carbohydrates than ryegrass, which might result in a fermentation profile towards less acetate and hydrogen and more propionate. The ruminal pH was lower in forage rape-fed lambs, which may inhibit methanogenic activity, shifting the rumen fermentation to more propionate and less hydrogen and methane. The significance of these two mechanisms remains to be investigated.

Research Aim 3: We report the first indication of an impact of candidate vaccine antigens in combination with different adjuvants on the microbial community in the rumens of treated animals. These results suggest that the vaccine formulations were effective against the specific microorganisms they targeted. Future trials have been designed to evaluate the optimal type and route of vaccination against the dominant methanogens in New Zealand ruminants. Once these parameters are established, a large scale animal trial including respiration chamber methane measurements will be conducted.

Key achievements for 2013/14:

- The low methane trait in sheep is linked to two different bacterial community types that produce less hydrogen than those prominent in high-methane emitting animals (Kittelmann *et al*., 2014, PLOS ONE, accepted for publication).
- Rape has been identified as a low GHG feed, favouring the growth of microorganisms that produce less hydrogen than those prominent on ryegrass (Sun *et al*., PLOS ONE, under revision).
- The archaeal taxonomy has been revised to allow for high-resolution community structure analysis of data stemming from next-generation sequencing technologies (Seedorf *et al*., 2014, PeerJ, accepted for publication).
- The dominant methanogens in New Zealand ruminants have been identified (Seedorf *et al*., submitted to the ROI system), and information has been made available to other work streams within the PGgRc-NZAGRC methane program.
- We have obtained evidence for a measurable impact of prototype vaccines on the archaeal communities in the sheep rumen.

5.7 - Understanding the low methane rumen

Jointly supported programme

Objective Leader – Drs Mike Tavendale and Gemma Henderson (AgResearch)

Methane mitigation technologies must be part of profitable farming systems. Their viability will depend on carbon costs, implementation and material costs, and production impacts. In theory, the reduction of methane emissions per unit of feed intake could result in an increase in production through the capture of metabolisable energy that was previously lost as methane formed from hydrogen produced in the fermentation. This could be through increased production of propionate or other reduced fermentation products or an increase in homoacetogenesis to produce acetate from hydrogen and carbon dioxide. Increased production would improve the uptake and economics of mitigation technologies. In addition, users of technologies will want to be informed of production effects before they use them.

This objective will first predict the impacts of methane inhibition using model inhibitors. As soon as viable vaccines (Objective 5.3) or inhibitors (Objective 5.4) are developed, it will switch to using those to measure production effects.

Work in this objective forms part of a PhD programme being conducted by Ms Preeti Raju.

5.7 – Progress in 2013/14

A body of research has been undertaken to address the question: what happens in the animal's rumen when methane is no longer formed? Specifically does the hydrogen, no longer forming methane, become incorporated into microbial products that may be of benefit to animal production, and or does the accumulating hydrogen influence the fermentation of feed components such as carbohydrates?

The short-term microbiological impact of methane inhibition and effects on hydrogen production, utilisation *in vivo*, and identification of microorganisms involved in alternative hydrogen utilisation pathways was undertaken with a five day trial with sheep receiving methanogen inhibitors. The effect of the inhibitors on alternative hydrogen utilising pathways, homoacetogenesis, was assessed by incorporation of labelled carbon dioxide into labelled acetate and shifts in the bacterial and archaeal communities by microbial gene sequencing and enumeration. Results from these studies confirm methanogen inhibition increases ruminal homoacetogenesis and propionate producing microbes become more abundant.

To evaluate long term the effects of inhibiting ruminant methanogenesis an assessment of the suitability of acetylene for reducing methanogenesis long term was undertaken by daily administering acetylene for 21 days to sheep. Methanogenesis returned towards the end of the trial confirming acetylene's unsuitability for use in long-term studies.

An assessment of the rumen metabolite response towards methanogen inhibition was undertaken with NMR spectroscopy in collaboration with Massey University. Analysis of rumen samples via NMR analyses confirmed the major rumen metabolites acetate, propionate and butyrate remained the major metabolites for rumen contents collected from methanogen inhibited animals, only their molar ratios changed. The analysis has given insight to the presence of methanogenic substrates other than dietary carbohydrate derived hydrogen and has indicated the occurrence of biochemical processes, which may explain where some of the hydrogen is disappearing to other than methane production.

Techniques to study the question on whether the accumulation of ruminal hydrogen in response to methanogen inhibition influences ruminal fibre fermentation are currently being developed for use in a continuous flow rumen simulation system. Completion of the modifications required to the

electronic control system for the continuous flow system have been undertaken and tested enabling the fibre studies to proceed.

Key achievements for 2013/14:

- Results from short-term methanogen inhibition trial compiled and reported indicating increased homoacetogenesis and shift to propionate occurs in response in methane inhibition
- Utility of acetylene for long term inhibition of methanogenesis explored and results demonstrated it's not a viable tool
- Data from studies of acetylene inhibition of methanogenesis have been compiled as manuscripts.
- Rumen metabolites successful characterised by rumen fluid NMR analysis and response towards methane inhibition evaluated.
- Modifications to continuous flow fermenters successfully undertaken ensuring studies of ruminal hydrogen on ruminal fibre fermentation can commence.

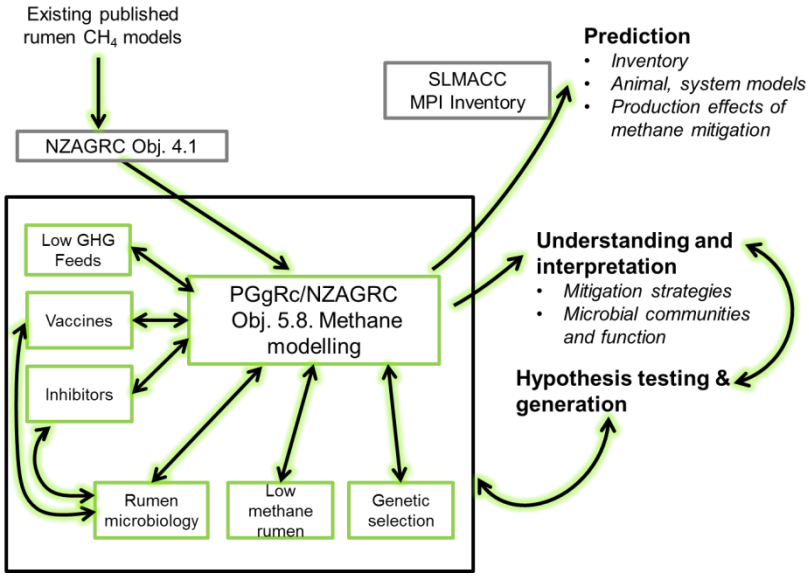
5.8 - Modelling rumen methane production

Objective Leader – Dr David Pacheco (AgResearch)

The development and evaluation of methane mitigation strategies requires a mechanistic understanding of the processes influencing methane formation in the rumen. Work in this objective seeks to improve our ability to predict responses in methane formation from NZ ruminants.

The outcomes from this project will be used by scientists in the NZAGRC/PGgRc programme as a tool to develop hypotheses regarding methanogens growth and activity in the rumen in response to current and future intervention such as feeding practices, inhibitors and vaccines. In the medium term, a predictive model of methanogenesis will be available for the wider scientific community for incorporation into whole animal models, which in turn will be able to generate knowledge on methane production and animal productivity. Ultimately, such models will be used to improve inventories and tools for monitoring the effects of mitigation options on farm practices.

The methane modelling objective will serve as an integration point for knowledge related to the development of methane mitigation strategies. This objective builds on previous work conducted as part of the "Integrated Systems" programme of the NZAGRC (Objective 4.1). The relationships between this objective and the rest of the NZ programme on methane accounting and mitigation are presented in the diagram below. The integrative role of the methane modelling project will be formalised in the form of six-monthly meetings with other objective leaders within the programme, to discuss advances in the modelling capabilities, with the purpose of defining simulations to validate the model with empirical findings. Also, as the model progresses, it is expected that simulations will the conducted to test likely outcomes of empirical research, leading to improved design and power of experiments.



5.8 – Progress in 2013/14

Empirical and mechanistic models of rumen and animal performance can be used to predict the effect of diet on ruminant methane emissions. It is generally recognised that mechanistic models of rumen and animal metabolism have greater accuracy than simpler empirical models and are better equipped to predict the responses to dietary methane mitigation practices. However, there are limitations that need to be addressed before these models can achieve their full potential in research on methane mitigation options in grazing systems. The first one is that existing models have focussed on simulating dairy cattle diets, and need to be re-deployed and validated for other ruminant species. Second, the predictive ability of these models can be improved, not only in terms of methane emission predictions but also in terms of predicting production of intermediate

metabolites, such as VFA and microbial protein, which is an important consideration for prediction of productive performance of the ruminant.

Sheep rumen model. Given the relevance of the sheep industry in New Zealand, we investigated the feasibility of applying the relevant equations in the publicly available version of Molly (Molly95) to predict rumen emissions from sheep. We investigated the ability of a modified version of Molly95 to quantify methane emissions from sheep. The overall objective was to modify the rumen sub-model to interpret existing data and to integrate concepts of underlying function that play a role in determining rumen function and methane emissions. Molly95 does not model the rumen hydrogen pool and implicitly assumes it is constant. However, rumen hydrogen concentration is affected by feed digestibility, passage rate and time after feeding. Additionally, the hydrogen concentrations are important as they provide the basis for thermodynamic control of methane production. Thus we introduced a rumen hydrogen pool as a dynamic variable, along with the re-parameterisation and scaling required for simulation of sheep metabolism. This process was tested against experimental data that included fresh temperate forages offered at a wide range of sheep feeding levels (Hammond et al., 2014: Anim Feed Sci Tech 193: 32-43). We have now an implementation of a mechanistic, mathematical model of the rumen of a sheep, which has an improved predictive ability over the re-scaled version of Molly95. Modelling an explicit hydrogen pool provides the basis for implementation of the model of methanogen-hydrogen interactions developed by Mr James Wang, a PhD candidate working in this programme.

Previous versions of the Molly dairy cow model, including the DairyNZ Whole Farm Model, predicted methane production with acceptable accuracy (as reported by Gregorini et al 2013: J Dairy Sci 96: 5046-5052). However, a shortcoming of the model was that methane yield did not respond to changes in level of intake. Experimental work with cattle and sheep has provided evidence that as feed intake increases, the amount of methane produced per unit of intake decreases. The proposed mechanism behind this relationship is an accelerated passage of feed through the rumen, which the user-defined passage rate previously implemented in Molly did not simulate. The development and implementation of a passage sub-model in Molly has addressed this shortcoming. Further, this new sub-model will allow simulation of the rumen by-pass of soluble dry matter and small particles in fresh forage fed ruminants. The role of this by-pass process as an important contributor to the variation in methane emissions observed from fresh forage diets has been identified as part of objective 5.2 and now can be explored through simulations. Finally, the new development includes a mechanistic representation of ingestion and mastication, which will enhance the application of Molly to explore opportunities for methane mitigation through changes in grazing management.

Key achievements for 2013/14:

- We have improved the predictions of methane from the Molly dairy cow model by developing a submodel of particle and outflow rate from the rumen.
- We have scaled the original Molly95 model to represent the rumen of sheep. This model will be useful to support the analysis and interpretation of animal experiments that use sheep as an animal model.
- The improvement made to the sheep model (an explicit pool of rumen hydrogen), allows implementation of a model of methanogen growth. This leads to an improved predicted tool to simulate methane mitigation strategies.

2.1 – Manipulating N inputs

Objective Leader – Dr Cecile de Klein (AgResearch)

This objective investigates ways to manipulate the N inputs into the pastoral system though plant breeding/selection and plant management options. The plant breeding/selection component focuses on exploring the hypothesis that novel pasture grasses can produce more DM per unit N supplied than existing grass species to produce high yielding plants with low N content. This research combines molecular biology, plant physiology and ecology to alter the fundamental biology of forage plants' strategies for utilising N that has already been captured by the plants and convert it into DM.

High yielding plants with low N content will not only reduce the total N input to the system, but also alter the N concentration of animal urine deposited to pasture. Over 80% of the national N_2O emissions are derived from animal urine and the effect of N concentration on N_2O emissions can have a major impact on total emissions. This objective will establish the relationship between N concentration in the urine and N_2O emissions, to fully assess the effectiveness of this mitigation option (and other options that will impact on the N concentration of animal urine) on reducing the agricultural N_2O emissions.

The plant management component of this objective focuses on understanding and manipulating N_2O emitted by leaves. Previous work has shown that plants can emit N_2O in three ways: 1. N_2O produced by soil micro-organisms is transported to the atmosphere through the plant. 2. N_2O is produced by microorganisms (ammonia oxidizing bacteria (AOB)) on plant leaves 3. N_2O is produced by the plant during photo-assimilation of nitrogen. The work in this objective will quantify the contributions from each of these pathways and screen plants for variation in their potential to emit N_2O.

Key research questions:

1. What are key gene targets that regulate plant growth?
2. Can exogenous growth stimulants be developed that promote plant growth under N limitation?
3. What is the relationship between urine N concentration and the N_2O emission factor?
4. What is the relative contribution of plant canopy N_2O emissions to total pasture emissions?
5. What is the relative contribution of the three different pathways to N_2O emissions from plant canopy?

Significant new knowledge:

- Putative key molecular factors limiting plant growth under N limitation;
- The potential for developing novel pasture species that can produce more DM per unit of N supplied;
- Assessment of the impact of developing these species on N intake, milk yield, N excretion rates and N_2O emissions;
- Assessment of the effectiveness of N_2O mitigation strategies that alter the urine N concentration and N excretion rates;
- Assessment of the relative importance of N_2O emissions through plant canopy in relation to total pasture N_2O emissions;

2.1 – Progress in 2013/14

We have shown that forage plants are not at all times growing to the limit of resource, e.g. nitrogen (N), supply but are limited by evolved mechanisms that commonly restrict their growth rate. These are manipulated by the plant to exact an evolved 'strategy': to boost growth temporarily following defoliation, or during spring to stimulate growth during flowering. This has substantially valuable implications and prospects for mitigation of not only nitrous oxide emission, but for significantly improving multiple aspects of the efficiency of use of N. Put simply, our work redefines the long held understanding of the response of grass yield to fertiliser N inputs (the 'nitrogen response curve') that has been the basis for over a century of fertiliser N recommendations and 'nutrient balance' N input advice. Our work reveals there is significant scope to increase grass growth without the need for substantial increases in N input offering far greater N use efficiency through the plant, animal and soil. Alternatively, and an even greater prospect, is that the same current level of production could be achieved with a reduced input of fertiliser N.

Evidence supporting this paradigm shift is published in a definitive paper from our work in this Objective (see Parsons et al, 2014 Grass and Forage Science) along with practical implications (see Ball et al., 2013 NZGA). To demonstrate that plant growth is not limited at all times by N availability, but by internal 'signalling' and an evolved 'strategy' within these forage grasses, we simply added an external agent that contains no N, and demonstrated that growth was stimulated both at low mineral N supply, and substantially more so at high mineral N. This challenges conventional explanations for what is causing growth at low N, and what is causing growth to appear saturated at high N supply. Adding this external agent demonstrates that a new line can be drawn above the century old line on a yield vs N graph, hence the same level of plant growth could be achieved at a lower N input rate.

It is irrelevant in many ways that the agent we used is a known and commercially available growth stimulant, gibberellic acid (GA). We are not suggesting that the use of exogenous sprays of GA alone is the ultimate mitigation method, simply that we have demonstrated that there is real scope to alter the way plants respond to N. Technically, GA is not a growth stimulant, but an antagonist of a growth repressor (DELLA). Hence growth is stimulated by 'negating a negative' effect. To advance toward mitigation strategies we need, instead of sprays, to seek plants with a genetic disposition for having a weaker natural growth restriction. To that end, we identified variants of the LpDELLA gene in the parents of a mapping population. Those variants are inherited and segregate in the progeny in an expected Mendelian fashion and there is an indication of higher growth amongst plants carrying two versions of the LpDELLA variant compared with those with only one indicating an opportunity for selection.

Our literature review revealed that there is gradually growing recognition, globally, of the mechanism for growth control across multiple plant species, including cereals. But this is the first evidence for function in perennial grasses, and our team has even identified putative genes controlling N use response. What we pursued, strongly in our project, then, is the genetic diversity in the fundamental mechanism by which growth responds to N supply.

The prospects from this work, for global food production, let alone associated with reduced environmental impact, from all N release pathways, are substantial.

Key achievements for 2013/14:

- We confirmed that the growth rate of forage plants is not at all times growing to the limit of resource supply and revealed that this can be manipulated externally
- We identified a set of genes related to GA biosynthesis and degradation that play a critical role in regulating grass regrowth and carbohydrate metabolism and unravelled the molecular mechanisms limiting plant growth under N limitation.
- We identified DELLA as a candidate gene underlying a ryegrass growth quantitative trait locus in a mapping population, supporting the development of molecular tools for breeding higher yielding ryegrass cultivars that require less N.

2.2 – Manipulating nitrification processes

Objective Leader – Prof Hong Di (Lincoln University)

Comprehensive research undertaken in NZ has already demonstrated that nitrification inhibitors (NI) can provide an effective method for reducing N_2O emissions from urine patches deposited on pasture. Research funded by NZAGRC will address the challenge of optimising inhibitor use so that their effectiveness and longevity is increased.

Microbes are the engines driving nitrification in the soil. The performance of NI is affected by soil and environmental conditions. A sound understanding of the quantitative relationships between microbial, soil and environmental factors and processes is critical to improving the performance of nitrification inhibitors. Microbial communities responsible for nitrification in the soil will be studied using molecular biology techniques. Effects of key soil and environmental conditions on the performance of NI will be quantified.

Key questions that this research will address are:

1. What is the relationship between the main soil, microbial and environmental factors and processes, and the effectiveness of nitrification inhibitors in reducing nitrous oxide emissions and increasing pasture yield?
2. How can the use or the formulation of NIs be optimised to ensure increased effectiveness and longevity in the soil?

The research will generate significant new knowledge and understanding of:

- The microbial populations and processes responsible for nitrification and their relationships with nitrous oxide production;
- The effectiveness of NIs in inhibiting microbial populations and affecting processes that contribute to nitrous oxide emissions and N supply;
- Relationships between soil and environmental conditions that affect the efficiency of NI;
- New generation inhibitors and recommendations for their optimal use.

2.2 - Progress in 2013/14

Over the past year, all the milestones of this objective have been successfully achieved.

An incubation experiment on the effect of soil moisture and nitrification inhibitor use on N_2O emissions has been successfully completed. Results show a major effect on N_2O emissions by soil moisture content, with emissions at 130% field capacity being 45 times higher than at 100% field capacity, and 400 times higher than at 60% field capacity. N_2O emissions were related to ammonia oxidising bacteria (AOB) *amoA* gene copy numbers but not to ammonia oxidizing archaea (AOA). DCD reduced N_2O emissions by 44-65%. These results show great potential for the use of DCD to reduce N_2O emissions in wet soils such as those under winter forage grazing. The results also identify AOB as the prime target for inhibition in high-N loading urine patch soils and this is important in the development/evaluation of new nitrification inhibitors.

Field and laboratory experiments have been conducted in collaboration with MBIE-funded research, and results showed that seven years of DCD use did not significantly affect soil microbial communities and key enzyme activities, demonstrating the specificity of DCD for nitrification inhibition while not affecting other non-target soil microbial activities or processes.

Experiments have been carried out, in collaboration with MBIE-funded programme, to determine the effect of a new nitrification inhibitor dimethylpyrozole phosphate (DMPP) and DCD solution on AOB and AOA population growth and on the rate of NH_4^+ oxidation. The results showed that the growth of AOB, as measured by the functional *amoA* gene copy numbers, was effectively inhibited by both DMPP and DCD. There was no significant difference in the NH_4^+ oxidation rates between

the DMPP and DCD treatments. These results indicate that the new nitrification inhibitor DMPP has the potential to inhibit nitrifiers, reduce nitrification rates and mitigate nitrous oxide emissions.

Field trials have been conducted in collaboration with MBIE-funded programme to determine the relative effectiveness of the new inhibitor DMPP versus DCD in reducing N_2O emissions in grazed pasture soils. Results showed that N_2O emissions from dairy cow urine patches were reduced by 62-66% by the application of DMPP and DCD. There was no significant difference between the liquid DMPP and DCD solution in terms of their effectiveness in reducing N_2O emissions. These results suggest that there is no apparent advantage of DMPP over DCD for use in grazed pasture soils.

Results have been published in international peer-reviewed papers (Guo *et al*., 2013; Di *et al*., 2014), presented at the World Congress of Soil Science in Korea, and at the N workshop in Lisbon. Results have also been communicated to national and international visiting scientists and policy makers (MPI), visiting VIPs, dairy and fertiliser industry personnel and farmers, and have been made available to modellers to help optimise NI use.

Key achievements for 2013/14:

- Successfully achieved all the milestones as specified in the contract on schedule and to a high standard.
- This research has significantly improved our knowledge and understanding of soil moisture effect on N_2O emissions and ammonia oxidiser growth, and the results showed great potential for the use of DCD to reduce N_2O emissions in wet soils such as those under winter forage grazing. The results have also identified AOB as the prime target for inhibition in high-N loading urine patch soils and this is important in the development/testing of new nitrification inhibitors.
- This research has significantly improved our knowledge and understanding of the effectiveness of the new nitrification inhibitor DMPP compared with DCD in reducing N_2O emissions, and the results showed that the two NIs were equally effective in reducing N_2O emissions in grazed pasture soils and there was no apparent advantage of DMPP over DCD for use in grazed pastures to reduce N_2O.
- This research has significantly improved our knowledge on the long-term viability of DCD use, and results showed no adverse effect on other microbial communities or enzyme activities after seven years of DCD use, thus demonstrating the longer-term viability of the NI technology.
- Research findings have been successfully published in international peer-reviewed papers (Guo *et al*., 2013; Di *et al*., 2014), presented at international conferences, such as the World Congress of Soil Science in Korea, and N workshop in Lisbon, communicated to our research team at the annual NZAGRC workshop, and presented to end-users, including VIPs, visiting scientists, policy makers (MPI), dairy and fertiliser industry personnel, and farmers.

COMPLETED

2.3 – Manipulating denitrification processes

Objective Leader – Dr Surinder Saggar (Landcare Research)

Landcare Research
Manaaki Whenua

Denitrification is the primary process of N_2O production in New Zealand pasture soils. However, we lack a comprehensive, quantitative understanding of denitrification rates and controlling factors across agrosystems. Denitrification is a facultative anaerobic microbial process producing nitric oxide, nitrous oxide and N_2 from nitrate and nitrite. Abiotic denitrification can occur under some conditions. Understanding those mechanisms (microorganisms; biotic processes and mineral oxide; abiotic processes) and soil & environmental factors that have the potential to reduce the production of N_2O during denitrification is vital to the development of new and effective N_2O mitigation technologies. This objective will test and improve the latest microbiological and tracer techniques to identify pathways to reducing N_2O production during denitrification and develop mitigation technologies that reduce N_2O emissions by lowering N_2O/N_2 ratio during denitrification, including in areas where denitrification is maximised to reduce nitrate leaching losses (e.g. riparian buffer zones). This research objective will contribute to the NZAGRC's objectives of developing novel and effective N_2O mitigation technologies and provide national policy (MAF, MfE) and regional (Regional Councils) land management agencies, and the dairy industry with the ability to determine N_2O mitigation potential from soil denitrification. This will assist end-users with negotiating nitrous oxide emission reductions targets to protect existing and develop new trade initiatives for New Zealand.

Key questions:

1. What is the relationship between soil, microbial and environmental parameters and processes and N_2O/N_2 ratio of denitrification?
2. What is the effectiveness of soil amendments for reducing nitrous oxide production during denitrification?
3. What are the optimum soil and environmental conditions required for maximum nitrous oxide mitigation using the most effective soil amendment?

Significant new knowledge:

The research will generate significant new knowledge and understanding of:

- The soil microbial populations and processes responsible for denitrification, and their relationships with nitrous oxide production;
- The pathways and microbial communities to reducing nitrous oxide production during denitrification by lowering N_2O/N_2 ratio;
- Relationships between soil and environmental conditions that affect the efficiency of soil amendments.

2.3 - Progress in 2013/14

With the NZAGRC decision to put the denitrification research on hold, significant time and efforts were involved to revise the research in this Objective to achieve a successful outcome for the NZAGRC investment in the denitrification by using Landcare Research newly developed automated N_2O/N_2 analysis technique and linking of this work with GPLER funded Denitrification project with the Project Manager and Principal Investigator Sergio Morales (University of Otago); by determining the efficacy of Cu, lime and soluble carbon in modifying denitrifier communities. Sixty soil samples representing 10 dairy pasture soils were analysed for extractable Cu to select soils for Cu manipulations. However, Cu manipulations were not supported because of the non-selective nature of Cu in affecting the soil microbial communities. Finally in March 2014, it was agreed that a new Objective 6.2 with additional funding for 2014-15 will wrap up the previous NZAGRC Objective 2.3 and mitigation option of liming for modifying denitrifier community structure, accelerating complete denitrification and mitigating N_2O emissions from urine will be evaluated.

This report summarises the results of research completed under the Objective 2.3

- Application of cattle urine with and without DCD to three soils of low, medium and high denitrification enzyme activities (DEA) raised soil pH and increased ammonium-N, nitrate-N, soluble carbon and microbial biomass carbon (MBC). This resulted in higher *nos*Z gene copy numbers compared to control samples indicating higher total denitrification. The emissions of N_2O and N_2 from urine application were higher in soils with higher DEA than the soil with least DEA. Our results show that cattle urine application induced changes in denitrification in soils and the addition of DCD with cattle urine did not influence overall denitrifier community structure or abundance. The magnitude of increase in denitrification during incubation and its reduction by DCD application were variable among the three soil types and reflected the differences in their inherent soil properties such as DEA, MBC, Olsen P, total carbon and total nitrogen, denitrifier community structure and their abundance.
- We also assessed the impact of DCD application on microbial communities using gas chromatography, soil chemical analyses, high throughput 16S amplicon sequencing, terminal restriction fragment length polymorphism (T-RFLP) and quantitative PCR (qPCR) on three denitrifier functional genes (*nir*S, *nir*K and *nos*Z). Our results support the current observations that DCD has only a small and transient impact on microbial communities with impacts being comparable to those seen from urine deposition by ruminants.
- We determined the changes in three denitrifier functional genes (*nir*S, *nir*K and *nos*Z), denitrification rate and changes in the production of N_2O and N_2O+N_2 with changes in soil water content (SWC) in 5 soils contrasting in DEA. Our results show that DR increases with increase in SWC but the increase in N_2O and N_2 emissions with increase in SWC varied among the soils. The DR and N_2 production at higher SWC better related to the DEA of the soils, the number of denitrifier T-RFs (representing denitrifier gene richness) and denitrifier gene abundance. These results suggest that the denitrifier functional genes abundance under saturated soil conditions reflects better the ability of the soil to enhance the reduction of N_2O to N_2 than using field moist soils.

Key achievements for 2013/14:

- Using gas chromatography, soil chemical analyses, high throughput 16S amplicon sequencing, terminal restriction fragment length polymorphism (T-RFLP) and quantitative PCR (qPCR) on three denitrifier functional genes (nirS, nirK and nosZ) we observed that both soil type and latitude were powerful regulators of both emissions and the final N gas being emitted (N_2 vs N_2O)
- The results of changes in SWC show that the denitrifier functional genes abundance under saturated soil conditions reflects better the ability of the soil to enhance the reduction of N_2O to N_2 than using field moist soils.
- DCD has only a small and transient impact on microbial communities with impacts being comparable to those seen from urine deposition by ruminants.
- Participation in the "Enhanced N_2O Workshop Invermay 19-21 November" organised to develop the research programme for the GPLER Denitrification project and to discuss the possibilities to achieve a successful outcome for the NZAGRC investment in the denitrification area by linking with GPLER Denitrification project.
- Sergio E. Morales, Neha Jha and Surinder Saggar (2014) [Abstract] Impact of urine and DCD application on microbial communities in dairy-grazed pasture soils In 15th International Symposium on Microbial Ecology, 24-29 August 2014, Seoul, South Korea

COMPLETED

6.2 – Denitrification processes

Objective Leader – Dr Surinder Saggar (Landcare Research)

Denitrification is the primary process of N_2O production in New Zealand pasture soils. However, we lack a comprehensive, quantitative understanding of denitrification rates and controlling factors across agrosystems. Denitrification is a facultative anaerobic microbial process producing nitric oxide, nitrous oxide and N_2 from nitrate and nitrite. Abiotic denitrification can occur under some conditions. Understanding those mechanisms (microorganisms; biotic processes and mineral oxide; abiotic processes) and soil & environmental factors that have the potential to reduce the production of N_2O during denitrification is vital to the development of new and effective N_2O mitigation technologies.

This objective will wrap up the previous NZAGRC Objective 2.3 which focussed on testing and improving the latest microbiological techniques to identify pathways to reducing N_2O production during denitrification and develop mitigation technologies that reduce N_2O emissions by lowering N_2O/N_2 ratio during denitrification, including in areas where denitrification is maximised to reduce nitrate leaching losses (e.g. riparian buffer zones). Mitigation option of liming for modifying denitrifier community structure, accelerating complete denitrification and mitigating N_2O emissions from urine is evaluated.

6.2 - Progress in 2013/14

Summarised under Objective 2.3 (above).

6.1 – Plant Effects on N_2O Emissions

Objective Leader – Dr Saman Bowatte (AgResearch)

It is well established that nitrification rates in soil are strongly influenced by the presence of plants and can differ markedly depending on the plant species. Plants can influence nitrification in soils by a variety of mechanisms: (a) they may secrete inhibitory compounds known as biological nitrification inhibitors (BNI compounds) that directly influence nitrifying organisms, (b) they may compete strongly for nitrogen and thus reduce the substrate for soil nitrifiers, and (c) they may alter the identity of the microbial community and/or microbial activity by altering the soil environment e.g. soil pH and moisture content.

Our previous work has found differences in nitrification between species, between cultivars and between endophyte-grass combinations. This programme will test whether differences apparent in these initial experiments are evident in a field situation, and if so, whether the effect is quantitatively important and whether there are trade-offs (e.g. in forage production) that might reduce the desirability of a low emitting species as a mitigation option. Our broad screening approach will also complement industry testing of alternative pasture species by providing valuable information on the most suitable material for testing in grazing trials.

6.1 – Progress in 2013/14

The main focus of the project is to measure plant effects on N_2O emissions in the field, to provide the evidence to determine whether observed differences in nitrification among soils of different plant species/cultivars are, evident in N_2O emissions in the field under urine application and of sufficient magnitude to warrant further investigation and investment.

To select the plant material to test in the field, we conducted a screening experiment using an extensive range of species and cultivars with agronomic value. Seeds of 103 species and cultivars of grassland plants were sown into pots (200 ml volume) containing soil from the paddock identified for the field experiment. The plants were maintained in a ventilated glasshouse. After 4 weeks of growth we measured potential nitrification of rhizosphere soil as a surrogate for N_2O emission. From the results of this screening we identified following grassland species and cultivars that give a wide range in nitrification potential (Table 1).

The field experimental plots have been established in a paddock close to the AgResearch campus buildings at Grasslands, Palmerston North. The plots area has been marked out, sprayed with herbicide and cultivated. The seeds were sown in mid-April. The experiment will involve 18 plant species/cultivars, 2 rates of cow urine with 4 replicates. Included in the plants will be a bare soil control and a standard pasture mix which will act as a further control or benchmark. The plots will be weeded, fertilised and watered as required to ensure good growth and that the plots remain as monocultures. In the first spring we will apply urine and measure N_2O on all species. Subsequent experiments will look in more detail at species that emerge as 'interesting' from this initial dataset.

Table 1: Grassland species and cultivars to test N_2O emissions in the field

Functional group	Species	Cultivar	Endophyte
Grass	L.perenne	Rely	AR37
Grass	L.perenne	Aber Magic HSG	AR1
Grass	L.perenne	Rohan	LE
Grass	L.perenne	Halo	AR37
Grass	L.multiflorum	Grasslands Moata	
Grass	Westerwolds	Grasslands Tama	
Grass	D.glomerata	Greenly	
Grass	A.capillaris	BR 1940	
Grass	B.willdenowii	Grasslands Matua	
Grass	B.stamineus	Grasslands Gala	

Grass	H.lanatus	Forester	
Legume	T.repens	Weka	
Legume	T.repens	Grasslands Bounty	
Legume	Sulla	Accession AL 4992	
Legume	M.sativa	Grasslands Kaituna	
Forage	Forage rape	Titan	
Forage	Chicory	Choice	
Forage	Plantain	Tonic	

Key achievements for 2013/14:

- Demonstrated the existence of high variation in soil nitrification potentials among grassland species with high agronomic value
- Field trial has been established to test observed differences in nitrification among soils of different plant species/cultivars are evident in N_2O emissions in the field under urine application

3.3 - Process-based modelling of drivers of soil carbon change

Objective Leader – Prof Tony Parsons (Massey University)

Our capacity to manipulate soil C (stocks and sequestration rates) depends on how well we understand the fundamental drivers of C supply, transformations and stability, in the whole of the plant, animal, and soil continuum, and so can evaluate the scope and credibility of manipulating these. The extremely long time frame for measurable changes in soil C stocks, its spatial and temporal variability, and the greater difficulty therefore in measuring changes in the rate of sequestration, means that detailed process-based dynamic models are an inescapable tool for generating insights into the drivers of soil C change. Such models are also essential to foresee what impacts strategies for changing soil C would have on emissions of methane, nitrous oxide, and on agricultural productivity.

Many grassland ecosystem models have been produced, but these differ significantly in suitability for this task. Some detailed 'soils' models lack a dynamic or responsive plant component. Others lack dynamic treatments of the grazing animals' role in C and N cycling. Some whole 'systems' models lack an explicit soil biologically active biomass. Models can differ considerably in the way different 'pools' /forms of organic matter are represented and in how these interact.

We will make progress by revisiting the scope of the few major soil C (but ecosystem wide) models, e.g. Century, (CenW), RothC, Hurley Pasture Model, and adding to a selected model (maybe a different model for different purposes) several essential new components. Of particular interest is to reconsider the enzymatic stoichiometry of different forms of soil micro-organism, notably the requirement for excess C (relative to N) in heterotrophs, compared to the reverse, a substantial requirement for N more than C in chem-autotrophs (typically nitrifiers) obtaining energy not from oxidative respiration (of C), but from their nitrogen transformations of ammonium. The modelling will progress in concert with development of molecular methods for assaying the balance of critical functions in soil, being investigated in FRST SRU C10X0903. Other examples of new components to be added are: changes in plant traits with likely impacts on the 'microbial loop' (+ve feedbacks to plants from soil micro-organisms) and e.g. Priming, and Progressive Nutrient Limitation (-ve feedbacks that may limit C sequestration). Our models include the role of animals in uncoupling, and of legumes in re-coupling, the C and N cycles (Soussana 2008; Schwinning and Parsons 1996). We will be using the original sources of insights in these areas, which are being relied on heavily in other nations (as in Soussanna 2008), to guide 'rules of thumb' for IPCC-type national C commitments.

3.3 – Progress in 2013/2014

Work in Objective 3.3 over 2013-14 involved parallel work with two highly regarded process-based models of pasture production and associated carbon and nitrogen fluxes. The models are the Hurley Pasture Model (HPM), originally developed by John Thornley and refined over the years with Tony Parsons; and the Carbon, Energy, Nutrient and Water (CenW) model developed by Miko Kirschbaum as a modification of the CENTURY model. Both models incorporate a range of physical, biochemical, plant and soil physiological principles and animal grazing and ensure the conservation of mass of the different chemical elements involved in plant growth, animal grazing and cycling of carbon, water and nutrients through plants, animals and the soil. The use of **two** models, in parallel, has reflected the significant requests by the Steering Groups for two contrasting work programmes.

1. Using the HPM model (by A.J. Parsons): This year saw the release in print (Parsons et al., 2013; Science of the Total Environment) of our analysis of the impacts of multiple components of the intensification of pastoral agriculture (as seen in NZ over the last decade) on the numerous

fluxes of C and N; and the resulting consequences in terms of C (and N) sequestration (*cf states*). The analyses explored all combinations of managements (stock numbers; nutrient inputs and animal sector (dry vs dairy), and highlighted the major policy concerns for difficulties in combining three national (and global) goals: increased food supply/production; reduced environmental impact (C and N emissions/losses) and not least, for concerns over global atmospheric GHG content, for C sequestration. The analyses highlighted how the major changes in C sequestration were inextricably coupled to changes in nitrogen use efficiency, and how major advances must depend on altering the fundamental biology of this, in plants, soils and animals. We offered, from global review as well as our own research programmes (see nitrous oxide theme, with Rasmussen), examples with mitigation potential for progress in this area. We referred to a publication (Parsons et al, 2012: Past lessons and future prospects for plant breeding...; Grass and Forage Science) in which we had laid out essential research needs, and new approaches. During 2013/14 we pursued experimental studies of the impact of contrasting novel plant traits (endophyte, legume, high sugar, cultivar/species; and N use) on the metagenomic profile (quantitatively, what organisms – bacteria and notably fungi – are present) in a initially identical soil. This works borrows heavily from commitments in our other non-NZAGRC research programmes, where we have begun advanced analyses of the impacts of altered soil communities on the amounts and nature of soil organic matter (hence C and N) and the role in this of plant trait induced changes in the level of expression of key soil functional genes. To enable this, a substantial collaboration was re-established with Rothamsted, BBSRC, UK (vice Dr Jenni Dungait), and Massey University (vice Parsons) deployed two staff ..one now based in the UK, at their NWFP research centre. Directly within the NZAGRC context, Parsons has pursued, using the HPM, the issued raised in STOTEN (above) of how changes in management give rise to very long term transients in flows, emissions and sequestration (eg losses) of C and N, of the order of 10 to 30 years, during which rates of emission could be up to double what they would ultimately become, and how this would call for caution over the suitability and generality of emissions factors or conclusions about the impacts of land use change (such as those estimated eg by Overseer) on the ultimate sustainable outcome for food production and the environment. This is notably so for 'slow-responding' components such as 'pools' of sequestered C and N, but likewise true even for faster responding fluxes, notably C and N leaching. This work inevitably extends outside and beyond the current contract. Progress on finer, but fundamental components of this 'big picture' were published, eg on the sharing/allocation of resources between plant shoots, roots and mycorrhizae (Thornley and Parsons, J. Theor. Biol). Work in all these areas is terminated by lack of on-going funding, June 2014.

2. Using the CenW model (by Miko Kirschbaum): The work with CenW focused on a detailed comparison between model runs and site observations with eddy covariance (EC) data at a Waikato experimental farm. Analysing grazing systems with EC measurements poses significant challenges as the respiration from grazing animals can result in large short-term CO_2 fluxes. As paddocks are grazed only periodically, eddy covariance observations derive from a mosaic of paddocks with very different exchange rates. This violates one of the key assumptions underlying the use of EC data, and various approaches were trialled to overcome this key methodological challenge. The work ultimately developed a novel approach whereby gas exchange from 26 paddocks around the EC tower was modelled individually based on the detailed grazing history of each paddock. These simulations were then coupled to a footprint analysis which estimates the source are of the gas exchange flux observed at the tower to estimate the net fluxes at the EC tower that provided the appropriate comparison against actual EC observations.

Overall, it was possible to obtain good agreement between modelled fluxes and measurements, especially for daily evapotranspiration rates and gross primary production. The work confirmed that with the use of the novel modelling approach, CenW simulations could adequately model carbon and water exchange in grazed pastures. This work has made it possible to use short-term EC measurements to gain insights into the effect of management changes on changes in productivity and carbon storage. These insights can then be used through the model for longer-term scenario analysis of the effect of unavoidable climatic changes and different management regimes on long-term carbon storage.

This work is being refined still further through the work of Nicolas Puche as part of his Ph.D. work. Nicolas is modifying the CenW model from a daily to a half-hourly time step to allow even better comparison between modelled data and observations, and to check whether the current approach of generating daily fluxes from actual half-hourly observations might have introduced any biases into the derived numbers.

Key achievements for 2013/14

- Journal article submitted. Kirschbaum, MUF, Rutledge, S, Kuijper, IA, Mudge, PL., Puche, N, Wall, A, Schipper, LA, Campbell, DI. Modelling carbon and water exchange of a grazed pasture in New Zealand constrained by eddy covariance measurements. *Science of the Total Environment* (submitted).
- Nicolas Puche successfully completed the first year of his Ph.D., including a successful confirmation hearing and report, and he presented a public seminar about his work: "Modelling the effect of different management regimes on carbon gains and losses from pasture systems in New Zealand", Landcare Research, Palmerston North, 17 June 2014.
- Conference presentation: Kirschbaum, M, Rutledge, S, Kuijper, I, Mudge, P, Puche, N, Wall, A, Schipper, L, Campbell, D. Modelling carbon and water exchange of a grazed pasture in New Zealand constrained by eddy-flux data. Ozflux General Meeting, Cairns, Australia, 8-11 July 2013.
- Journal paper released in print: Parsons, A.J., Thornley, J.H.M., Newton, P.C.D., Rasmussen, S. & Rowarth, J.S. (2013) Soil carbon dynamics: the effects of nitrogen input, intake demand and off-take by animals. Special Issue 'Soil as a Source and Sink for Greenhouse Gases'. *Science of the Total Environment* 465, 205-215.
- Thornley, J.H.M. & Parsons, A.J. (2014) Allocation of new growth between shoot, root and mycorrhiza in relation to carbon, nitrogen and phosphate supply: teleonomy with maximum growth rate. *Journal of Theoretical Biology*, 342, 1-14.

3.4 - Manipulation of carbon inputs, incorporation and retention to protect and enhance soil carbon

Objective Leader – Dr David Whitehead (Landcare Research)

Landcare Research
Manaaki Whenua

Soil carbon storage on land managed by pastoral farmers accounts for 85% of the national carbon storage for all land uses (to a depth of 0.3 m) in New Zealand, so small changes in carbon storage are important for the national inventory. Carbon storage is already high in many soils, so research to develop farm management practices that protect and retain existing soil carbon, possible leading to increases in carbon storage, is a priority for the pastoral industry. Most research so far has concentrated on methodologies to quantify the amounts of carbon stored in soils with much less emphasis on ways to manipulate the rates of carbon input, incorporation and retention in soils and, crucially, how any changes in storage can be verified.

The aim of this objective is to use measurements and models to quantify changes in soil carbon storage following experimental manipulation that could be applied as farm management practices. The research will move beyond quantification of the amounts of carbon in soils (this work is funded by other agencies outside the NZAGRC) to understanding the processes driving soil carbon storage in relation to farm management with a focus on three areas identified as high priority:

- Comparison of farm practices at adjacent grazed sites (*manipulating carbon input*). The experimental variable (eg. addition of nitrogen fertiliser, selection of pasture species) and the sites will be selected following a full review of previous work and discussions with end users
- Effects of the presence or absence of invertebrate earthworms on the amount and distribution of carbon in the soil profile (*manipulating carbon incorporation*)
- Addition of biochar to grassland soils (*manipulating carbon retention*).

We will use a range of approaches and work at a range of scales from mesocosms, field plots and paddocks, employing a range of appropriate methodologies. We will make long-term, continuous measurements of carbon exchange at ecosystem scales at adjacent sites with contrasting management practices using micrometeorological instrumentation and supplementary field measurements. From estimates of seasonal changes in the components of carbon balance, we will calculate changes in soil carbon storage and reveal the processes regulating rates of storage. Our initial work with earthworms will be done using mesocosms in controlled conditions where the earthworms are introduced and this approach will later be extended to three field sites with contrasting soil types. We will use proof of concept lysimeters experiments to test the effects of and biochar addition and rooting depth using different pasture species on biochar stabilisation, then extend this work to field trials with different summer forage crops. We will also develop reflectance spectroscopy techniques to analyse for root density and black carbon concentrations in soils enriched with biochar.

Data from these three approaches will be interpreted in relation to environmental and experimental variables and used with models to inform end users of forecasted changes in soil carbon with manipulation practices.

3.4 – Progress in 2013/2014

We have continued measurements in the laboratory and at field sites and combined these with the use of models to determine the effects of weather and management variables on changes in soil carbon stocks. Our three areas of focus have been:

- Increasing carbon inputs to soil by replacing conventional ryegrass/clover grassland with mixed swards
- Improving the rate of incorporation of carbon from dung into the soil profile by introducing earthworms
- Determining the impacts of biochar additions on total and ‘native’ soil carbon and the stability of soil carbon fractions.

Manipulating carbon inputs using mixed-swards with deeper rooting plants
We continued measurements of the exchange of carbon dioxide for a grazed grassland at a dairy farm in the Waikato and combined these with data on carbon imports from additional feed and exports from meat, milk and methane emissions and timings of relevant farm management events. Averaged over 4 years, the site was a sink for both carbon dioxide exchange (1.65 t C ha^{-1} y^{-1}), and total carbon including imported and exported carbon (0.60 t C ha^{-1} y^{-1}). We created a global compilation of 54 site years of carbon balances made over grazed pastures. This compilation suggested that many of these sites were net carbon sinks when accounting for all imports and exports.

We started continuous measurements of carbon dioxide exchange at three sites at a dairy farm in the Waikato with the three treatments: undisturbed ryegrass/clover, ryegrass replaced with ryegrass/clover and ryegrass replaced with a mixture of species to give a diverse sward incorporating plants with deeper root systems. We showed that biomass production for the first year for the re-grassed sites was about 17.2 t DM for the ryegrass/clover and 16.9 t DM for the diverse sward. Both were greater than the old ryegrass/clover mix which produced 15.3 t DM. This suggests that conversion from ryegrass/clover to mixed swards will increase the herbage yield and increase the amount of carbon entering the soil profile. However, continued measurements of biomass and the components of carbon exchange are needed to quantify the changes and attribute them to treatment effects in relation to changes in weather and management variables.

Introduction of earthworms to increase the incorporation of carbon from dung into soils
We introduced the anecic earthworm *Aporrectodea longa* into three different soils at field sites and showed that they had established successfully and reached abundances of 50 individuals/m^2 at all sites despite differences in soil type and climate. There was little evidence that *A. longa* increased the rate of dung incorporation into the soil, suggesting that earthworm abundances had not yet reached a critical mass, with burrows being too far dispersed to influence bulk soil carbon. At a different site where *A. longa* had been introduced more than 20 years previously, and had reached abundances of 200 individuals/m^2, more dung was detected in the soil profile compared with a soil containing no anecic earthworms. The implications of this work are that introducing earthworms is likely to increase the rate of incorporation of carbon from dung into the soil but the build-up of soil carbon is slow and unlikely able to be detected in periods less than a decade.

Effects of the addition of biochar on soil carbon and its stability
We used a model to estimate the stability of biochar in soils with added plant residues and effect of this amendment on carbon stocks. The carbon lost from both biochar production and decomposition of plant material 'broke even' with that lost from fresh plant residue decomposition by 35 weeks but this was dependent on soil type. These results provide experimental evidence for the potential of biochar to sequester carbon and avoid carbon losses from respiration of plant material while protecting the native soil carbon. However, long-term field studies along with system analysis are needed, prior to decide whether biochar technology is the best option for a particular situation.

Key achievements for 2013/14

- Rutledge S, Mudge PL, Campbell DI, Woodward SL, Goodrich JP, Wall AM, Kirschbaum MUF, Schipper LA. Carbon balance of an intensively grazed temperate dairy pasture over four years. Agriculture Ecosystems and Environment (submitted).
- Schon NL, Mackay AD, Gray RA, Dodd MB, van Koten C. Quantifying dung carbon incorporation by earthworms in three soils under permanent pasture. Agriculture, Ecosystems and Environment (submitted).
- Herath HMSK, Camps-Arbestain M, Hedley M, van Hale R, Kaal J 2014a. Fate of biochar in chemically- and physically-defined soil organic carbon pools. Organic Geochemistry 73:35-46.

- Herath HMSK, Camps-Arbestain M, Hedley M, Kirschbaum MUF, van Hale R, Wang T. 2014b. Experimental evidence for sequestering C with biochar by avoidance of CO_2 emissions from original feedstock and protection of native soil organic matter. Global Change Biology doi: 10.1111/gcbb.12183.

3.5 - Improved soil carbon measurements

Objective Leader – Prof Frank Kelliher (AgResearch)

This objective's goal is improved methods to verify temporal changes in soil carbon (C) storage and accounting rules suitable for a national inventory of agricultural soils.

Soils data in New Zealand are fragmented, geographic coverage limited and most samples have come from a depth < 0.1 m. It will be difficult to verify slow, relatively small and variable changes of C storage rate in pastoral agricultural soils. Improved methods will include the development of data analyses. This objective will begin with two foci.

Firstly, many soil properties change with depth and such relations are called vertical distributions. Analysis proceeds with a (continuous) function, optimised to fit integrated measurements made on samples excavated at discrete depth intervals. For soil C, research questions include:

1. Can C storage in soils be extrapolated from one depth interval to another (e.g., from a shallow depth interval to a deeper one) with sufficient accuracy to detect statistically significant temporal changes?
2. Is the C storage rate in soils related to the vertical distribution of C storage?
3. Do climate change and/or management (e.g., irrigation, deeper-rooted plants) sustainably affect the vertical distribution of C stored in soils?

The second foci will be analysing measurements that partition total organic C into functional fractions (distinguished by different decomposition rates) for a soil carbon cycling model. A system of automated, proxy measurements has been developed by CSIRO. As a case study, proxy measurements were recently done by CSIRO on a set of New Zealand agricultural soil samples collected by Plant and Food Research. Analysing the measurements will quantify parameters in a model to estimate C storage rate over (many) decades and sensitivity of the rate to the measurements (different fractions). Research questions include:

1. Can the partitioning of total organic C into functional fractions improve the estimation of C storage rate in soils?
2. Does the partitioning of total organic C into functional fractions reveal differences between soils that can be used to improve sustainable management of the C storage rate?
3. Can a functional fraction measurement/model system help to develop accounting rules suitable for a national inventory of agricultural soils?

The development of data analyses can improve methods to verify temporal changes in soil C storage. This can contribute to the development of soil C storage rate accounting rules for agricultural land managers. This can connect them to the national inventory of agricultural soils and improve its performance, the estimation of C storage rate in soils.

3.5 - Progress in 2013/2014

The research led to the completion of two manuscripts which were submitted for publication.

Kelliher, Curtin and Condron (2014. Soil carbon stocks in particle-size fractions under seasonally irrigated, grazed pasture. New Zealand Journal of Agricultural Research 56: 239-244) showed that carbon (C) in the clay-size (< 5 μm) fraction, commonly regarded as the most stable, was as responsive to irrigation at Winchmore as C in the in the sand-size (> 53 μm) fraction. This observation did not support our hypothesis. While the results of measuring soil C in clay-, silt- (5 - 53 μm) and sand-size fractions suggested such partitioning had not been a driver of responsiveness to irrigation in this study, further research is warranted about irrigation effects on C stocks in grassland soils and the processes determining C stability under field conditions.

Curtin, Beare and Qiu (2014. Texture effects on carbon stabilisation and storage in New Zealand soils containing predominantly 2:1 clays. Soil Research. Submitted for publication) examined the role of the fine mineral fraction in carbon (C) storage in sedimentary soils. While there was no correlation between the C concentration in whole soil and the clay content, the majority of the soil C was stored in the clay fraction. The C concentration in the clay and fine silt fractions decreased as the mass proportion of these fractions increased. Thus, there was little change in the amount of stable C as the mass proportion of fine particles increased. It was concluded such soils may be under-saturated and there may be the potential to store additional stable C in them.

Key achievements for 2013/14:

- Kelliher et al. (2014) showed that while the partitioning of soil carbon into clay-, silt- and sand-size fractions had not been a driver of responsiveness to irrigation at Winchmore, further research is warranted on the processes determining carbon stability in soils
- Curtin et al. showed sedimentary soils containing predominantly 2:1 clays may be under-saturated and there may be potential to store additional stable carbon in them.

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APPENDIX 3 – NZAGRC INTERACTIONS AND OUTPUTS

Meetings and Presentations (New Zealand)

- Meeting with Nathan Guy, Minister for Primary Industries: 24 July, 2013
- NZAGRC Science Leadership Team Meeting: 02 August, 2013
- NZAGRC Science Programme: Integrated Systems Workshop: 07 August, 2013
- NZAGRC Science Programme: Nitrous Oxide Workshop: 12 August, 2013
- NZAGRC Science Programme: Soil Carbon Workshop: 16 August, 2013
- NZAGRC Steering Group Meeting: 21 August, 2013
- Meeting with William Rolleston, Federated Farmers: 29 August, 2013 - Wellington
- MPI Policy Team: Visit to Palmerston North: 4 September, 2013 - Palmerston North
- PGgRc-NZAGRC science programme planning: methane : 4 September, 2013 - Palmerston North
- NZCCC Executive Meeting: 9 September, 2013 - Wellington
- Appointment in Auckland with Interbrand-PGgRc and NZAGRC: 9 October, 2013 - Auckland
- Massey Agricultural End of Year Dinner : 11 October, 2013 - Palmerston North
- Parliamentary briefing on IPCC AR5 WGI: 16 October, 2013 - Wellington
- Follow up meeting with William Rolleston, Federated Farmers: 17 October, 2013 - Palmerston North
- PGgRc Board Strategic Day: 11 November, 2013 - Palmerston North
- Agricultural Inventory Meeting: 12 November, 2013 - Wellington
- NZAGRC Science Leadership Team Meeting: 13 November, 2013 - Palmerston North
- Meeting with Simon Wear, MPI re Dairy Effluent with Massey University: 16 November, 2013
- IGPS climate change roundtable: 5 December, 2013 - Wellington
- Climate Change Impacts and Implications Local Government workshop: 11 December, 2013 - Wellington
- NZAGRC SLT Meeting: 11 December, 2013 - Palmerston North
- PAC chamber and SF6 measurements on methane selection - Review: 12 December, 2013 - Palmerston North
- NZoNet and Methanet meeting: 13 December, 2013 - Wellington
- NZAGRC SLT Meeting: 10 February, 2014 - Palmerston North
- Meeting with AgResearch Portfolio Leader-Maori Agribusiness: 14 February, 2014 - Palmerston North
- Meeting with Cecile de Klein and Liz Wedderburn: 18 February, 2014 - Palmerston North
- Climate Science-Policy Discussions: IPCC WGII & WGIII: 3 May, 2014 - Wellington
- Intellectual Property legal opinion: Greenfeed: 3 May, 2014 - Wellington
- Workshop: Mitigating methane emissions from dairy effluent ponds with floating biofilters: 5 March, 2014 - Palmerston North
- Discussion with MPI Policy re contracting new policy work: 7 March, 2014 - Wellington
- Visit from Green Party MPs: 10 March, 2014 - Palmerston North
- IPCC AR5 Working Group II and III Journalist Briefing with Science Media Centre: 12 March, 2014 - Wellington
- HSG/Methane Steering Group Meeting: 12 March, 2014 - Palmerston North
- Presentation to AgResearch Board: 13 March, 2014 - Dunedin
- NZAGRC-PGgRc Science Planning Workshops: 27 March, 2014 - Palmerston North
- Workshop on DCD research: 9 April, 2014 - Wellington
- NZAGRC-PGgRc Methane Programme Review: 5 May, 2014 - Palmerston North
- Visit by MPI Team (Biosecurity, Food and Animal Welfare Directorate): 8 May, 2014 - Palmerston North
- NZAGRC Contract Review update: 9 May, 2014 - Wellington
- NZAGRC/PGGRC meeting day: 13 May, 2014 - Wellington
- Presentation: Wellington City Council, Greater Wellington Regional Council on climate change: 8 May, 2014 - Wellington
- Presentation: Auckland City Council on climate change: 28 May, 2014 - Auckland
- Presentation: Dunedin City Council, Otago Regional Council on climate change: 16 June, 2014 - Dunedin
- Workshop: Managing GHG emissions (DairyNZ PGP project): 6 August, 2014 - Hamilton
- Workshop: Ag Emissions White Board session (DairyNZ): 11 August, 2014 - Wellington

Meetings and Presentations (International)

- IPCC Lead Author Meeting: 1 July, 2013 - 5 July, 2014
- IPCC Lead Author Meeting: 15 July, 2013 - 19 July, 2013
- Filling the Research Gap - Feedback Meeting: 22 July, 2013
- FACCE-JPI Science Advisory Board: 10 September, 2013 - London
- FACCE-JPI SAB Meeting: 11 September, 2012 - 12 September, 2012
- Presentation to Sustainable Intensification Conference, Edinburgh: 24 September, 2013 - Edinburgh
- World Agricultural Forum, Hyderabad: 4 November, 2013 - Hyderabad, India
- FACCE JPI CSC meeting: 5 November, 2013 - Berlin, Germany
- Science and Technical meeting for the Ruminant Pangenome Project: 25 November, 2013 - Perth
- IPCC SYR meeting: 6 January, 2014 - The Netherlands
- FACCE-JPI Science Advisory Board: 10 January, 2014 - London
- FullCAM and Agriculture Inventory Meeting: 6 February, 2014 - Palmerston North
- Climate Smart Agriculture FACCE-ERANET+ Meeting: 1 April, 2014 - Paris
- CCAFS/GRA/IPCC Outreach event: 16 April, 2014 - Washington
- Global Animal Nutrition Conference: 21 April, 2014 - Bangalore
- Conference: Livestock, Climate Change and Food Security: 19 May, 2014 - Madrid, Spain
- Meeting: AGM of Animal Change project participants: 22 May, 2014 - Madrid, Spain
- Meeting: Scientific Evaluation Committee for FACCE-ERANET+: 23 June, 2014 - Paris
- Meeting: IPCC AR5 Synthesis Report: 29 June, 2014 - Kuala Lumpur

International Visitors and Groups

- Visit from CIAT, BecA & Kenya Agricultural Research Institute, Kenya to discuss collaborative research between NZ & Kenya: 23 September, 2013 - Palmerston North
- Meeting with Sjoerd Croque, Vice Chair of the Global Research Alliance (The Netherlands): 16 December, 2013 - Wellington
- Meeting with Bruce Campbell, CGIAR/CCAFS: 17 March, 2014 - Wellington / Palmerston North
- Visit by Irish Minister of Agriculture, Food and Marine: 19 March, 2014 - Wellington
- Martin Scholten, Wageningen UR: 28 April, 2014 - Palmerston North
- Visit: Ecuadorian Minister of Agriculture: 10 June, 2014 - Palmerston North

Global Research Alliance related interactions

- MPI/NZAGRC Quarterly Meeting: 22 August, 2013 - Palmerston North
- FACCE-JPI International Evaluation Committee: 24 October, 2013 - Frankfurt, Germany
- Animal Change Training Module Meeting: 29 October, 2013 - Brussels
- MPI/NZAGRC Quarterly Meeting: 28 November, 2013 - Wellington
- CCAC Agriculture Initiative's new Livestock and Manure Management work stream meeting: 22 January, 2014 - Rome, Italy
- Grassland Research Network teleconference: 24 January, 2014 - Wellington
- FACCE-JPI Workshop on animal health and GHG emissions: 31 January, 2014 - Palmerston North
- LRG Co-Chairs Teleconference: 10 February, 2014 - Palmerston North
- LRG Research Network Coordinators Teleconference: 13 February, 2014 - Palmerston North
- Meeting with Matt Hooper, MPI Rome: 25 February, 2014 - Palmerston North
- MPI/NZAGRC Quarterly Meeting: 25 February, 2014 - Palmerston North
- GPLER Technical Assessment Panel Meeting: 6 March, 2014 - Wellington
- Workshop: Stakeholders Adoption and Change Monitoring: 13 May, 2014 - Hamilton
- Workshop: Animal Health and Disease and GHG Mitigation: 21 May, 2014 - Madrid, Spain
- Meeting: GRA Quarterly Report: 3 June, 2014 - Wellington

- Meeting: UNFCCC SBSTA (attendance on behalf of MPI): 4 June, 2014 - Bonn, Germany
- Meetings: Global Research Alliance (Council, Co-Chairs) : 13 June, 2014 - Netherlands
- Conference: RUFORUM (on behalf of MPI): 20 July, 2014 - Mozambique

Media Interactions

A number of NZAGRC's interactions with media this year have been in response to the release of the IPCC AR5 WGII & WGIII reports in 2014, in which the Director and Deputy Director (International) had authorship, and coordination and authorship roles, respectively. Interactions were in a variety of media including television, radio, newspaper, media opinion pieces and presentation format.

Dr Harry Clark was also inducted as a member of the New Zealand Order of Merit in New Years' Day 2014 honours list, which initiated some media interaction.

- Television: Keeping it Pure - Harry Clark and Peter Janssen: 26 January, 2014 - Palmerston North
- HSG/Methane Steering Group Meeting: 12 March, 2014 - Radio New Zealand
- "Scientists fear 4degC rise possible" -- Andy Reisinger: 12 March, 2014 - Radio New Zealand
- "Climate Change Impacts - Our Changing World" -- Andy Reisinger: 27 March, 2014 - Radio New Zealand
- "NZ to expect more floods and rising seas" -- Andy Reisinger: 31 March, 2014 - Radio New Zealand
- "NZ facing greater weather extremes: international report" -- Andy Reisinger: 31 March, 2014 - Press Release from NZCCC
- IPCC WGII & WGIII media statements/interactions: 1 April, 2014 - Various
- "Opinion: NZ must face climate change flexibly" -- Harry Clark, Andy Reisinger: 1 April, 2014 - The Dominion Post
- "Farming upside to climate change" -- Andy Reisinger: 7 April, 2014 - Farmers Weekly
- Interview: Andy Reisinger, NZAGRC discusses latest IPCC report: 10 April, 2014 - 95bFM
- "Opinion: Financial imperatives in reducing emissions" -- Andy Reisinger: 14 April, 2014 - The Dominion Post
- "Report shows need for climate agreement, says Groser" -- Harry Clark: 14 April, 2014 - Newstalk ZB
- Interview: Andy Reisinger, NZAGRC discusses latest IPCC report: 22 April, 2014 - Radio Live Sport
- Meeting Science Media Centre: 7 May, 2014 - Palmerston North
- Media: City 'not exempt' from climate change: 17 June, 2014 - Otago Daily Times

Conference Presentations

- Iris Vogeler, Rogerio Cichota, Donna Giltrap, 'Nitrogen cycling under urine patches: Model comparison and sensitivity analysis' - Modsim - 08/07/13
- Neil Wedlock, Dairu Shu, Supatsak Subharat, Peter Janssen, Bryce Buddle, 'Development of vaccines against rumen methanogens' - 10th International Veterinary Immunology Symposium, Milan 2013 - 22/08/13
- Susanne Rasmussen, 'Is perennial crop growth resource or strategy limited? Insights from grass responses to gibberellin and nitrogen' - 7th EPSO conference - 02/09/13
- Neha Jha, Surinder Saggar, Julie Deslippe, Russ Tillman, Donna Giltrap, Saman Bowattte, 'Changes in denitrification rate, bacterial denitrifier community structure and abundance in dairy grazed pasture soils treated with cattle urine and DCD' - FLRC Workshop 2013 - 10/09/13
- David Pacheco, Garry Waghorn, Peter Janssen, 'Lowering methane from grazing ruminants: a fit with productive and financial realities?' - 2014 ISNH/ISRP Conference - 13/09/13
- Ron Ronimus, LR Schofield, V Carbone , Y Zhang, C Sang, GM Cook, AJ Sutherland-Smith, 'Using methanogen enzyme screening assays and enzyme structures to discover novel inhibitory compounds for controlling ruminant methane emissions' - ComBio 2013, Perth - 25/09/13
- Wendy Bain, Louret Bezuidenhout, Neville Jopson, Cesar Pinares-Patino, John McEwan, 'Rumen Differences between Sheep Identified as being Low or High Emitters of Greenhouse Gas' - AAABG - 16/10/13
- Hong Di, Keith Cameron, Andriy Podolyan, Bruce Ball, Jizheng He, 'Efficacy of a nitrification inhibitor mitigation technology for nitrate leaching and nitrous oxide emissions in winter forage grazing systems' - 20th World Congress of Soil Science - 31/10/13
- John McEwan, 'Future Challenges: Ruminant Genomics' - AAABG - 08/11/13
- Neil Wedlock, 'Evaluation of salivary antibody and potential adjuvants for anti-methanogen vaccination in New Zealand sheep' - Australasian Society for Immunology annual conference 2013 - 28/11/13
- Neil Wedlock, 'Development of a sub-unit vaccine to reduce methane emissions in ruminants' - Australasian Society for Immunology - 28/11/13
- Nicole Schon, Alec Mackay, Ross Gray, 'Earthworms in hill-country pastures' - FLRC 2014 conference - 24/01/14
- Nicole Schon, Alec Mackay, Ross Gray, 'Earthworms in sheep-grazed pastures' - FLRC 2014 conference - 14/02/14
- Sam McNally, Louis Schipper, Daniel Laughlin, Susanna Rutledge, Johan Six, Mike Dodd, 'Comparative root C inputs under a mixed sward and conventional ryegrass-clover pasture' - Fertiliser and Lime Research Conference - 18/02/14
- Iris Vogeler, Tony van der Weerden, Rogerio Cichota, 'A boundary line approach for estimating the risk of N2O emissions from soil properties ' - FLRC conference - 18/02/14
- Arjan Jonker, Katherine Lowe, Stewart Ledgard, David Pacheco, 'Substitution of Lucerne Silage by Increasing Levels of Maize Silage or Maize Grain Results in a Quadratic Response in Methane Emissions from Sheep' - ISNH-ISRP joint meeting - 25/04/14
- Sara Elmes, Wendy Bain, Gordon Greer, Sharon Hickey, Emily Young, Natalie Pickering, Suzanne Rowe, Kevin Knowler, Cesar Pinares-Patino, John McEwan, 'Brief Communication: An exploratory investigation of the effects of selection for altered methane emissions on rumen physiology and carcass traits in sheep' - New Zealand Society of Animal Production (NZSAP) - 30/06/14
- Peter Janssen, 'Rumen microbial community profiling as a tool to study ruminant production' - New Zealand Society for Animal Production Conference - 30/06/14

Journal Articles

Submitted

- Natalie Pickering, Sharon Hickey, Cesar Pinares-Patino, Suzanne Rowe, Ken Dodds, John McEwan, 'Genome wide association study for methane emissions in New Zealand dual purpose sheep, utilising the HD chip' - PLoS ONE - 30/06/14
- Natalie Pickering, Eileen Wall, Georgios Banos, Mizeck Chagunda, Raphael Mrode, John McEwan, 'Genetic parameters for predicted methane production and laser methane detector measurements' - Journal of Animal Science - 30/06/14
- Cesar Pinares, John Mcewan, Natalie Pickering, German Molano, Sarah MacLean, Sharon Hickey, Ken Dodds, Kevin Knowler, Holly Kjestrup, Edgar Sandoval, Emily Young, 'Associations of blood plasma and ruminal volatile fatty acids with methane emission in sheep fed on lucerne pellets' - Journal of Animal Science - 31/03/14
- Xuezhao Sun, Sarah McLean, Dongwen Luo, David Pacheco, 'Sheep Fed Five Different Summer Forage Brassicas Emitted less Methane than Sheep Fed Perennial Ryegrass/White Clover Pasture ' - Animal Production In Australia - 25/04/14
- David Pacheco, Garry Waghorn, Peter Janssen, 'Decreasing methane emissions by feeding grazing ruminants: a fit with productive and financial realities?' - Animal Production Science (CSIRO Publishing) - 21/03/14
- Xuezhao Sun, Linda Krijgsman, Holly Kjestrup, John Koolaard, David Pacheco, 'A note on digestibility trial with sheep fed fresh forage' - Animal Production Science - 13/03/14
- Sinead Leahy, William Kelly, Suzanne Lambie, Dong Li, Kerri Reilly, Graeme Attwood, Eric Altermann, 'The complete genome sequence of the rumen methanogen Methanosarcina barkeri CM1.' - Standards in Genomic Sciences (SIGS) journal - 30/06/14
- Sandra Kittelmann, Peter H. Janssen, John C. McEwan, Michelle R. Kirk, Henning Seedorf, Cesar S. Pinares-Patino, 'Natural variation in methane emission of sheep fed on a lucerne pellet diet is unrelated to rumen ciliate community type' - Environmental Microbiology Reports - 16/05/14
- Saman Bowatte, Paul C D Newton, Shona Brock, Phil Theobald, Dongweng Luo, 'Bacteria on leaves: a previously unrecognised source of N2O in grazed pastures' - ISME Journal - 11/02/14
- Hong Di, Andriy Podolyan, Keith Cameron, Cecile de Klein, Surinderr Saggar, 'Abundance, dynamics, transcriptional activity and spatial distribution of ammonia oxidising populations in three NZ soils, and relationships with N2O emissions and DCD effect: the field-plot experiments' - New Zealand Journal of Agricultural Research - 29/01/14
- Hong Di, Keith Cameron, Andriy Podolyan, Aimee Robinson, 'Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil ' - Soil Biology and Biochemistry - 19/12/13
- Sergio Morales, Neha Jha, Surinder Saggar, 'Biogeography and biophysicochemical traits link N2O emissions and microbial communities across New Zealand pasture soils' - ISME (International journal in Microbial Ecology) - 01/07/14
- Susanna Rutledge, Paul Mudge, Dave Campbell, Sharon Woodward, Jordon Goodrich, Aaron Wall, Miko Kirschbaum, Louis Schipper, 'Carbon balance of an intensively grazed temperate dairy pasture over four years' - Agriculture Ecosystems and Environment - 12/05/14
- Louis Schipper, Sam McNally, Daniel Laughlin, Susanna Rutledge, Mike Dodd, Johan Six, 'Comparative root C inputs under a mixed sward and conventional ryegrass/clover pasture' - Proceedings Fertiliser and Lime Research Workshop - 27/03/14
- Nicole Schon, Alec Mackay, Ross Gray, Mike Dodd, C van Koten, 'Quantifying dung carbon incorporation by earthworms ' - Biology and Biochemistry - 13/12/13
- Roberto Calvelo Pereira, Erwin Wisnubroto, Mike Hedley, Marta Camps Arbestain, Steve Green, Surinder Saggar, 'Influence of biochar amendment on greenhouse gas emissions immediately following ammonium sulphate application to different pasture species grown on two contrasting New Zealand soils ' - Agriculture, Ecosystems & Environment - 23/08/13
- Denis Curtin, Mike Beare, Weiwen Qiu, 'Texture effects on carbon stabilisation and storage in New Zealand soils containing predominantly 2:1 clays' - Soil Research - 11/07/14
- Francis M. Kelliher, Peter J.S. West, James L. Moir, 'Soil carbon stock beneath an established irrigated pasture grazed by dairy cattle' - New Zealand Journal of Agricultural Research - 31/03/14

Published

- Weibing Shi, Christina Moon, Sinead Leahy, Sandra Kittelmann, Jeff Froula, Dongwan Kang, Christina Fan, Samuel Deutsch, Dragana Gagic, Henning Seedorf, William Kelly, Renee Atua, Carrie Sang, Priya Soni, Dong Li, Cesar Pinares-Patino, Feng Chen, Axel Visel, John McEwan, Peter Janssen, Zhong Wang, Graeme Attwood, Edward Rubin, 'Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome' - Genome Research - http://dx.doi.org/10.1101/gr.168245.113
- Henning Seedorf, Sandra Kittelmann, Gemma Henderson, Peter Janssen, 'RIM-DB: A Taxonomic Framework for Community Structure Analysis of Methanogenic Archaea from the Rumen and other Intestinal Environments' - PeerJ - http://dx.doi.org/10.7717/peerj.494
- Sandra Kittelmann, Cesar S. Pinares-Patino, Henning Seedorf, Michelle R. Kirk, Siva Ganesh, Jeffrey I. Gordon, John C. McEwan, Peter H. Janssen, 'Two Different Bacterial Community Types Are Linked with the Low-Methane Emission Trait in Sheep' - Plos One - http://dx.doi.org/10.1371/journal.pone.0103171
- Mike Beare, Stephen McNeill, Denis Curtin, Roger Parfitt, Haydon Jones, Joanna Sharp, 'Estimating the organic carbon stabilisation capacity and saturation deficit of soils: A New Zealand case study' - Biogeochemistry - http://dx.doi.org/10.1007/s10533-014-9982-1
- J.H.M. Thornley, A.J. Parsons, 'Allocation of new growth between shoot, root and mycorrhiza in relation to carbon, nitrogen and phosphate supply: teleonomy with maximum growth rate.' - Journal of Theoretical Biology - http://dx.doi.org/10.1016/j.jtbi.2013.10.003
- Roberto Calvelo Pereira, Marta Camps Arbestain, Joeri Kaal, Marcos Vazquez Sueiro, Marta Sevilla, Jason Hindmarsh, 'Detailed carbon chemistry in charcoals from pre-European Maori gardens of New Zealand as a tool for understanding biochar stability in soils' - European Journal of Soil Science - http://dx.doi.org/10.1111/ejss.12096
- Nicole Schon, Alec Mackay, Ross Gray, Mike Dodd, 'The action of an anecic earthworm (Aporrectodea longa) on vertical soil carbon distribution in New Zealand pastures several decades after their introduction' - European Journal of Soil Biology - http://dx.doi.org/10.1016/j.ejsobi.2014.03.002
- Saman Herath, Marta Camps Arbestain, Mike Hedley, 'Fate of biochar in chemically- and physically-defined soil organic carbon pools' - Organic Geochemistry - http://dx.doi.org/10.1016/j.orggeochem.2014.05.001
- Saman Herath, Marta Camps Arbestain, Mike Hedley, Miko Kirschbaum, Robert van Hale, 'Experimental evidence for sequestering C by avoidance of CO2 emission from feedstock and enhanced protection of native soil organic matter with the use of biochar' - Global Change Biology - http://dx.doi.org/10.1111/gcbb.12183
- Tao Wang, Marta Camps-Arbestain, Mike Hedley, 'Predicting C aromaticity of biochars based on their elemental composition' - Organic Geochemistry - http://dx.doi.org/10.1071/SR13177
- Tao Wang, Marta Camps-Arbestain, Mike Hedley, Bhupinder Pal Singh,, Roberto Calvelo-Pereira, Congying Wang, 'Determination of carbonate-C in biochars' - Soil Research - http://dx.doi.org/10.1071/SR13177

Other interactions/publications

- John McEwan, Cesar Pinares, Sharon Hickey, Emily Young, Ken Dodds, Sarah MacLean, G Molano, E Sandoval, H Kjestrup, R Harland, Suzanne Rowe, Natalie Pickering, 'Genomic selection as a tool to decrease greenhouse gas emission from domestic ruminants (Abstract submission)' - Genetic Resources for Food and Agriculture in a Changing Climate - 17/01/14
- Arjan Jonker, Katherine Lowe, Stewart Ledgard, David Pacheco, 'Graded substitution of lucerne silage by maize silage or maize grain results in a quadratic response in methane emissions from sheep (Abstract submission)' - ISNH-ISRP Joint meeting - 13/12/13
- Neil Wedlock, 'Evaluation of salivary antibody and potential adjuvants for anti-methanogen vaccination in new zealand sheep (Abstract submission)' - Australasian Society for Immunology conference 2013 - 27/08/13
- Neil Wedlock, Subatsak Subharat, Sandra Kittlemann, Dairu Shu, Debjit Dey, Bryce Buddle, Peter Janssen, 'Development of a sub-unit vaccine to reduce methane emissions in ruminants (Abstract submission)' - Australasian Society for Immunololgy (ASI) conference 2013 - 27/08/13
- Vince Carbone, 'Fast start Marsden proposal (Abstract submission)' - Marsden fund council by the Royal Society of New Zealand - 24/06/14
- Vince Carbone, 'Australian Synchrotron Beamline Proposal: Structural characterisation of archaeal methanogen enzymes. (External Report)' - Australian synchrotron - 05/08/13
- Ron Ronimus, Linley Schofield , Vince Carbone, Greg Cook, Yanli Zhang, Carrie Sang, Debjit Dey, Andrew Sutherland-Smith,

'Using methanogen enzyme screening assays and enzyme structures to discover novel inhibitory compounds for controlling ruminant methane emissions (Abstract submission)' - ComBio 2013 - 29/09/13

- Yang Li, 'The genome sequence of the rumen methanogen Rumen Cluster C sp. ISO4-H5 (copy) (Abstract submission)' - Massey University - 13/06/14
- Henning Seedorf, Sandra Kittelmann, Gemma Henderson, Peter Janssen, 'Survey of the New Zealand rumen methanogen community by using the novel rumen and intestinal methanogen database RIM-DB (Abstract submission)' - International Symposium on Microbial Ecology (ISME15) - 22/03/14
- Wendy Bain, Louret Bezuidenhout, Neville Jopson, Cesar Pinares-Patino, John McEwan, 'Rumen Differences between Sheep Identified as being Low or High Methane Emitters (Abstract submission)' - World Congress of Genetics and Livestock Production - 10/03/14
- Graeme Attwood, 'Release of Methanobrevibacter AbM4 paraformaldehyde-fixed cells and genomic DNA (Material Transfer)' - Zhipeng Li, Chinese Academy of Agricultural Sciences, China, Marissa Hunt, University of California - 24/07/13
- Bo Lin, 'Characterization of rumen microbial community composition of buffalo feeding on diets varying in forage:concentrate ratio (copy) (Abstract submission)' - The 2014 Joint Annual Meeting (JAM) of the American Dairy Science Association (ADSA), the American S - 21/02/14
- Savannah R. Devente, 'Verification of 18S rRNA gene sequence types obtained from single cells of the rumen ciliate Charonina ventriculi by means of fluorescence in situ hybridization (UG Thesis)' - Bachelor Thesis to obtain degree from University of Applied Sciences Leiden, The Netherlands - 30/05/14
- Sandra Kittelmann, Cesar S. Pinares-Patino, Wendy E. Bain, Henning Seedorf, Michelle R. Kirk, Siva Ganesh, John C. McEwan, Peter H. Janssen, 'Bacterial community types linked with low- and high-methane emissions from sheep (Abstract submission)' - International Symposium on Microbial Ecology - 12/03/14
- Tim McAllister, Leluo Guan, Gemma Henderson, Graeme Attwood, Peter Janssen, 'Use of genomics and transcriptomics to identify strategies to lower ruminal methanogenesis (Abstract submission)' - American Society of Animal Science (ASAS) and the Canadian Society of Animal Science (CSAS) Joint An - 04/03/14
- Pierre Noziere, Emilie Ollion, David Pacheco, Daniel Sauvant, 'Evaluation of a bovine VFA prediction model using data on sheep (Abstract submission)' - ModNut 2014: 8th International Workshop Modelling Nutrient Digestion and Utilization in Farm Animals - 16/05/14
- Sergio Morales, Neha Jha, Surinder Saggar, 'Impact of urine and DCD application on microbial communities in dairy-grazed pasture soils (Abstract submission)' - 15th International symposium on Microbial Ecology
- Sergio Morales, Neha Jha, Surinder Saggar, 'Biogeography and biophysicochemical traits link N2O emissions and microbial communities across New Zealand pasture soils (Abstract submission)' - 15th International symposium on Microbial Ecology