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"Harnessing soil biology to improve the productivity of the new sugarcane farming system"

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Contents

1. Executive summary

This project addresses the knowledge gap of how management affects soil biological processes. This is important because management has to maximise soil health and nutrient relations. Knowledge of soil biology in context of management strategies will allow optimising economic and environmental outcomes for the sugar industry.

The project assessed how management options of the 'new sugarcane farming system' (reduced tillage, legume break crop, trash blanketing, and reduced nitrogen (N) fertiliser application), impact soil biology. We examined the functional groups and activity of soil microbes in context of soil N availability and gaseous emissions. Sugarcane soils in North and Southern Queensland, including the Yield Decline Joint Venture site in Ingham and two commercial farms with contrasting management practices in Bundaberg, were used for this research. A suite of well established and new methods were applied to analyse soil biological processes.

A focus on soil microbiological processes is justified because microbes are the main drivers of N turnover in soil. Microbes supply N to crops by breaking down complex organic matter and soil-bound N, but also compete with plants for more easily-accessible N. Microbes convert N into easily leachable nitrate and gaseous N forms and are drivers of carbon (C) turnover in the soil. These microbial processes have not been comprehensively studied in sugarcane soils with different management. A special focus of this study was the development and application of novel molecular techniques to monitor soil microbial gene expression. This approach allows microbial functional analysis by treating soil as a "super organism" rather than deducing function from the presence of particular microbial taxa which is biased towards known microbial taxa.

A microarray containing >10,000 putative genes (known and unknown) was constructed from sugarcane soils. Considerable technical difficulties were encountered with microarray analysis. Hence, efforts were directed to

advanced rapid sequencing technologies which have recently become available to overcome the limitations of microarrays.

The molecular methods developed in the project allow isolating soil microbial RNA for gene expression and other genomic analysis. Outcomes of this study will allow a comprehensive analysis of all expressed genes, and hence unprecedented insight into the functional aspects of soil microbes.

In brief, the results show that sugarcane management affects soil microorganisms with respect to their biomass, functional community composition and gene expression. These changes were mostly short-lived and had little or no effect on the overall availability of N, N cycling, N losses and sugarcane yield. This response of the studied sugarcane production systems supports the notion that less input, as implied by the new sugarcane farming system, is not detrimental to sugarcane yield and reduces environmental impacts. Additionally, this project has opened new fields in soil ecological research in sugarcane agro-ecosystems. The development of novel approaches for monitoring soil microbial gene expression, especially 454-sequencing, will provide insights into the complexity of microbial responses to management options and environmental conditions and facilitate more detailed understanding of microbe-nutrient relations and other soil biological interactions in the future.

The outcomes of this project support the application of the ‘new farming system’ in Australian sugarcane production, especially opportunities for further reduction of N fertiliser rates. Recommendations for further improvement of the new farming systems have been identified and listed in this report (see chapter 9).

2. Background

Over the last 25 years, the sugarcane production underwent significant changes in Australia. The traditional way of land management comprising long-term monoculture of sugarcane with conventional tillage, burning the crop prior to harvest and the application of high rates of N fertiliser has changed to new farming system to maintain yields, adapt to economic pressure and technical developments and to address environmental concerns. Triggered by the occurrence of yield decline, i.e. the loss of productive capacity under long-term sugarcane monoculture, intensive research activities in Australia over the past decades have yielded strong support for the benefits of practices including green cane harvesting, break cropping, minimum tillage and controlled traffic (Sugar Yield Decline Joint Venture; Garside *et al.*, 2005), and paved the way for their introduction across the industry. Application of these practices is not only beneficial in economic terms due to substantial labour and cost savings, but they also significantly improve a multiplicity of soil physical, chemical and biological properties, which were implicated in soil degradation due to long-term sugarcane monoculture (Wood, 1985; Garside *et al.*, 2005).

In recent years, the application of synthetic N fertiliser in sugarcane production systems has been under scrutiny, due to the rising costs of fertiliser and environmental concerns. Fertiliser recovery by the sugarcane crop is estimated to be only 6 to 45% (Kingston *et al.*, 2008). A significant part of the applied fertiliser in sugarcane and other crop systems is lost into the environment and N pollution is currently viewed as one of the major global threats to ecosystem health and biogeochemical cycles (Gruber & Galloway, 2008; Rockström et al., 2009). Most concerning is N pollution via nitrate leaching (Southwick *et al.*, 1995; Thorburn *et al.*, 2003; Ghiberto *et al.*, 2009) and emission of N gases, especially the potent greenhouse gas nitrous oxide which has >300-times the global warming potential of CO_2. Nitrate is either derived from nitrate-containing synthetic N fertiliser or from ammonia- or urea-based fertilisers, which are microbially converted to nitrate in the soil. Nitrate is also the precursor for the denitrification pathway, a chain of microbial

processes that produce various N gases, which is the major source for nitrous oxide from agricultural soils. A considerable proportion of fertiliser-N is converted into nitrous oxide in sugarcane systems (Weier, 1998; MacDonald *et al.,* 2009; Allen *et al.*, 2010).

Since microbial processes are the major reason for N fertiliser losses from sugarcane farming systems, more knowledge about soil biology and soil N cycling as affected by sugarcane management is required to identify management options that alleviate the environmental footprint of sugarcane production, reduce economic losses and increase sustainability of the Australian sugar industry. Management options including trash blanketing and legume break cropping reduce the amounts synthetic N fertilisers needed by providing organic N sources to the crop (Meier *et al.*, 2006; Robertson & Thorburn, 2007; Dourado-Neto *et al.*, 2010) and N losses from soil through wind and soil erosion (McLaughlin, 2006). Organic N forms also increase N retention in the soil via microbial immobilisation, since they are more attractive for microbial communities and subsequent immobilisation as microbial biomass than inorganic N compounds generated from synthetic N fertilisers (Jones *et al.*, 2005).

Past research has investigated some effects of modern management on soil biology in sugarcane systems, but these investigations either focussed on the general presence of microbes (total microbial biomass) or on microbial pathogens (Wood, 1991; Garside *et al.*, 1997; Magarey *et al.*, 1997; Holt & Mayer, 1998; Pankhurst *et al.*, 2003, 2005a,b,c; Stirling *et al.*, 2003; Stirling, 2008). No detailed information is available about the soil microorganisms involved in N cycling in sugarcane soils and responses of microbial communities to management practices and was addressed in this project.

3. Objectives:

This project focuses on soil biology in relation to N cycling in the new farming system which aims to improve yield and sustainability of sugarcane production with rotation breaks, legume break crops, trash blanketing, and reduced tillage. The project's objectives are to improve knowledge of soil biology and nutrient status. Research for this project was conducted at two experimental sites.

1 & 2) Advance knowledge of soil biology using powerful molecular techniques and develop quantitative tools to monitor the functional diversity of soil organisms

We advanced techniques and built capacity to monitor functional diversity of soil organisms using well-established and novel techniques. Molecular techniques to investigate the community composition of selected N-related functional groups of soil microorganisms were applied at the Yield Decline Joint Venture (YDJV) site in Ingham and at three sites at Bundaberg.

The functional characteristics of the soil microbial community was monitored via a custom-made microarray in differently managed plots in Ingham, similar to microarray analysis used in modern medicine. Specific genes and functional groups of genes expressed in soils were analysed. A publication on this topic is being published in an international scientific journal (McGrath *et al.*, 2010; manuscript accepted for publication) and demonstrates that microbial gene expression differs between cane-soy and cane-monoculture sites, although the function of most genes remains unknown due to the laborious cloning required for each identified gene which was carried out for ≈800 genes.

To advance knowledge of microbial function, we took advantage of the rapid advances in molecular techniques, and planned to analyse gene expression of soil microbes using so-called '454-sequencing' approach to compare soil microbial function at the Bundaberg sites. This latest break-through

technology has never been performed on soil microbial genes prior to our project, and represents the most powerful approach to analyse microbial function in soil. These techniques are clearly the future for comprehensive analysis of soil microbial function, and our project contributes valuable knowledge to this rapidly expanding field.

Considerable effort was therefore invested in establishing extraction and purification procedures to ensure quantity and quality of the samples. Sample sequencing will take place in August 2010 and bioinformatics analysis will ensue as part of Richard Brackin's PhD, continuing the project.

In addition, other techniques, including ^{15}N pool dilution, which do not rely on microbial DNA or RNA analysis, but which are nevertheless informative, have been assessed for their use for soil biology research. Several techniques are now established in our laboratory and will be used in Richard's PhD research.

3) Gain detailed information about soil nitrogen and carbon dynamics in relation to crop development

The project produced knowledge on soil N and C dynamics in context of crop management, crop development and in comparison to a natural reference site, a nearby forest ecosystem on the same soil type.

Analyses at both sites, Ingham and Bundaberg, were performed a high-interval sampling early in the growing season during the times of high turnover of N and C. Plant-available N forms, including ammonium, nitrate and amino acids, were intensively monitored in the upper soil over the entire crop cycle of plant crop for eight management types at the YDJV site Ingham. For selected plots, microbial biomass was also determined. Crop development and N acquisition of the crop was monitored by collaborators (Bell *et al.*, 2010) as part of another project and linked to the findings in this project.

At Bundaberg, comprehensive soil analysis was complemented with analysis of a sugarcane plant crop at adjacent farms which are managed

conventionally and with the ‘new farming system’. In addition, a natural reference site consisting of a nearby forest remnant was included in our investigations. Both sugarcane sites had been managed for >10 years with current management allowing discerning long-term trends. In addition to plant-available N forms, the availability of soil protein was investigated, the precursor-N for amino acids and inorganic N forms. Microbial biomass, microbial gross N turnover rate, nitrous oxide emission and crop N acquisition were monitored throughout the crop cycle, and N turnover processes were compared to the forest site. Since high inorganic N concentrations can reduce the uptake of greenhouse gas methane by soil microbes, we investigated methane fluxes and the community composition of methanotrophic (methane-consuming) bacteria. The depth distribution of light fraction organic matter was analysed to compare the effects of conventional sugarcane management and the new farming system on soil organic carbon levels.

4) Develop framework for effective management of soil biology and nutrient relations

New insights into soil N turnover processes were gained by combining analysis of fast-turnover soluble and gaseous N and C as well as longer term N and C pools. Analysis of microbial N turnover has provided insight into the conversion of N forms in soils. Microbial gene analysis has mostly complemented other analyses performed in the project and more detailed gene expression analyses are ongoing. The combination of techniques used here has generated knowledge for discerning processes in current sugarcane systems. An initial framework on soil biology and N and C processes has been developed with recommendations for improving nutrient relations. A key finding of this project is that soil biology and related processes in the studied sugarcane systems differ surprisingly little, and it is discussed in detail below.

5) Provide recommendations for crop management

The outcomes of this project support the application of the new farming system as a step towards improved crop management. Break crop, trash

blanketing, reduced tillage and lower N fertiliser applications deliver higher or similar sugarcane yields and are thus advantageous in economic and environmental terms. However, further improvements to management are required to reduce the generation of ammonium and nitrate as these N forms undergo rapid conversion and are subject to leaching and gaseous loss in conventional and new farming systems. Similarly, we did not find evidence that the new farming system enhances carbon sequestration despite low tillage and trash blanketing, which are considered advantageous for carbon storage. Clearly, further improvements to crop system management are needed to enhance N retention and C storage in sugarcane crop systems.

4. Methodology:

The project consisted of both field and laboratory-based activities. The project aimed to complement and enhance existing research activities which focus on management in context of sugarcane yield or pathogen levels. Detailed investigations of management effects on the abundance, diversity and activity of soil microorganisms, and the associated availability and cycling of nitrogen and carbon in sugarcane soils have not been conducted previously and were the focus of this project (**Fig. 1**).

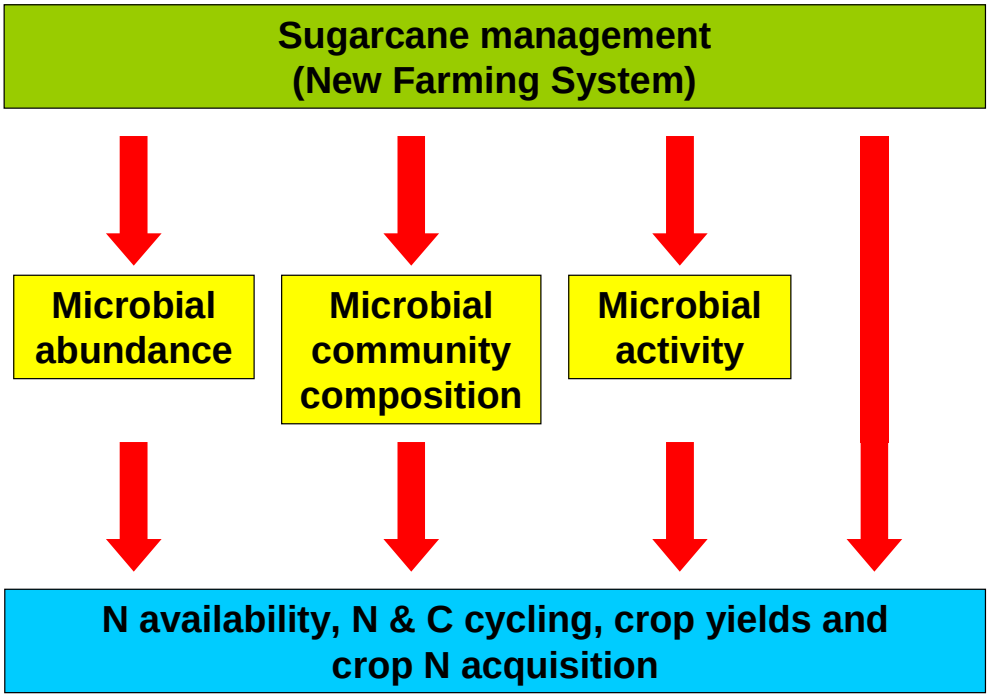


Fig. 1: Principal areas of investigations in the project. Soil biological investigations focussed on microorganisms involved in N and C cycling.

Intensive sampling was conducted at the YDJV site at Ingham in North Queensland (9 field campaigns between May 2007 and October 2008) and three sites near Bundaberg in Southern Queensland (9 field campaigns between November 2008 and September 2009). All investigations were conducted in close cooperation with sugarcane growers (M. Raiteri, Ingham; J. & P. Bonaventura, T. Chapman, Bundaberg) and project partners (G. Park, BSES Ingham; A. Garside, BSES Townsville, M. Bell, DEEDI Kingaroy). The

YDJV site in Ingham was chosen because of the diverse management implemented and complementary research activities, which were conducted simultaneously by partners, including yield measurements. The experimental sites near Bundaberg were chosen because of comparable soil type and long-term application of different management practices (>10 years) under commercial conditions. Such long term studies have been rare and our rationale was that stronger differences in soil processes could be expected, which were additionally compared to a natural reference site.

Soil samples were processed in our laboratory and a short description of applied methods is provided in chapter 5. Briefly, soil microbiological methods included the determination of soil microbial biomass (fumigation-extraction), microbial community composition (DGGE, Denaturing Gradient Gel Electrophoresis, cloning, sequencing), analysis of the expression of functional genes (microarray, 454-sequencing) and microbial N turnover (^{15}N-pool dilution technique). Soil investigations included the measurements of available soil N forms (ammonium, nitrate, the 20 amino acids contained in proteins using Ultra Pressure Liquid Chromatography, nitrate and protein assays) throughout a sugarcane plant crop, trace gas exchange (nitrous oxide, methane, carbon dioxide using gas chromatography) and crop N and carbon acquisition (sequential harvest and element analysis). Soil properties were determined, including soil texture, ion exchange capacities, pH, electrical conductivities, element contents, nutrient analyses and the investigation of aluminium and manganese levels. Soil organic matter analyses were conducted using density fractionation.

Capacity building during this project included the training of undergraduate students and scientific staff, who have developed skills for microbiological research in sugarcane systems. Four undergraduate students participated in field campaigns, three research assistants (2 RA part-time, 1 RA 2 years full time), one Master student and one Honours student (Rhiannon Mondav, 2009) were involved in the analysis of nutrients, soil microbial community composition and soil microbial gene expression, using a variety of molecular approaches. New procedures for the extraction and purification of genetic

material and preparation for 454-sequencing were developed by Honours student Rhiannon Mondav who is now working in the project as an RA (Mondav, 2009-2010). The post-doctoral fellow (Jirko Holst) led the investigations of soil N cycling, analysis of microbial biomass, community composition and gene expression, soil organic matter, greenhouse gas exchange, the determination of soil properties and soil nutrient analyses. The former RA Richard Brackin is continuing the project as an SRDC-funded PhD student (since January 2010) and current RA Rhiannon Mondav is considering PhD studies as a next career step. The methods developed in this project will form the foundations for Richard's PhD research. To further enhance capacity building, an additional PhD student and Honours students, will be sought.

Outcomes of this project were communicated to the sugar industry through ASSCT meetings (McGrath *et al.*, 2008b; Holst *et al.*, 2009, 2010) and international scientific journals (McGrath *et al.*, 2008, 2010). The remaining results are in the process of publishing in international scientific journals in the immediate future. Project developments were discussed with research and industry partners within annual project reviews (A. Sudgen, R. Troedson, SRDC; R. Dalal, DERM Queensland; B. Granshaw, R. Quirk, growers). The final project meeting (13 August 2010) will outline the communication strategies and the next steps required.

Strategic linkages within Australia and overseas have been established during the project, including Dr Pauline Mele (DPI Victoria) who leads a soil biology initiative in Australia and Prof Ingrid Koegel-Knabner (Technical University Munich-Weihenstephan), an expert on soil carbon processes. Since 2009, we worked in close association with Dr Gene Tyson (UQ) and Dr Frank Stewart (Massachusetts Institute of Technology, Boston, USA) on the microbial mRNA extraction and purification methods. A satellite conference on soil biology (Soil Science Congress, Brisbane 2 August 2010) will be attended by R. Brackin, R. Mondav and S. Schmidt (9-11 August 2010, Sunshine coast). Further collaborations with soil biologists are planned once the current 3 year project is completed and publications are submitted to international scientific journals.

5. Output:

5.1 Layout, management and general soil properties of the studied experimental sites

The YDJV site near Ingham was set up as a split plot design and consisted of four replicate blocks (Bell *et al.*, 2010), of which one block (No. 1) was excluded from our investigations due to substantial and persistent soil wetness. From the remaining blocks, only plots with sugarcane monoculture and legume break cropping were part of our investigations (**Fig. 2**). Thus, for the purpose of this study each replicate block was divided into two plots, on which sugarcane was either grown as monoculture (Cane) or in rotation with a soybean break crop (Soy). These crops were cut down in May 2007 and the whole soybean biomass (incl. seeds) or the sugarcane trash remained at the site for decomposition (trash blanketing). The plots were then split and either conventionally tilled (+Till) or zero-tilled (-Till) in July 2007. In August 2007, the new sugarcane plant crop was planted on all plots with a row spacing of 1.8 m. During planting, the sugarcane received a starter fertilisation of 20 kg N ha^{-1} (DAP). The subplots were again divided and received an additional urea fertilisation of 129 kg N ha^{-1} (+N) or not (-N). Thus, in total eight different managements (Cane+Till+N, Cane+Till-N, Cane-Till+N, Cane-Till-N, Soy+Till+N, Soy+Till-N, Soy-Till+N, Soy-Till-N) were investigated, each three times replicated. Research was conducted between May 2007 to September 2008, with in total seven field campaigns for soil sampling during the sugarcane plant crop (May, Nov, Dec 2007, Feb, Apr, May, Jul 2008) and two after its harvest (Sep, Oct 2008).

The second experimental area was situated south of Bundaberg. It consisted of two commercial sugarcane and one forest site. The first site is conventionally managed for more than 20 years (Bonaventura farm). Sugarcane is grown as monoculture, with a high N fertiliser application rate and burning of trash after the last ratoon. Prior to planting the next sugarcane

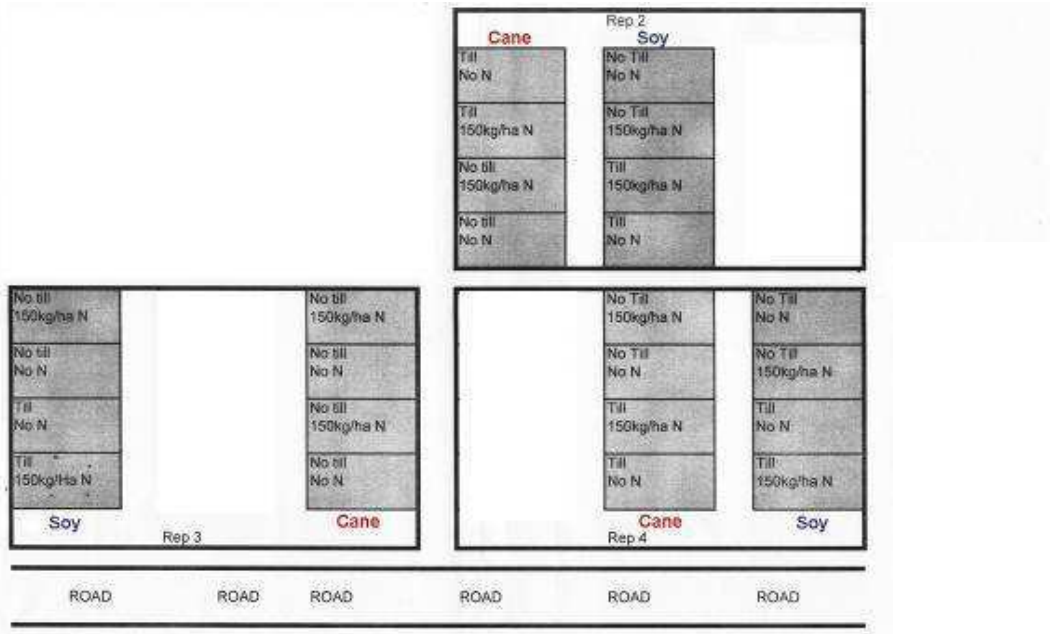


Fig. 2: Investigated plots and experimental layout of the YDJV site near Ingham for the purpose of this study.

crop, the soil is conventionally tilled and fallowed, and water application occurs as intermittent flood irrigation. The distance between sugarcane rows is 1.54 m. The second sugarcane site was approximately 700 m from the conventional site and belongs to another grower (Chapman farm). It is managed according to the new farming system, including legume break cropping (peanuts), consistent trash blanketing (no burning), lower N fertiliser application rates, overhead irrigation and controlled traffic (row distance: 1.8 m). Prior to planting the new sugarcane crop, the site is subjected to zonal tillage (only sugarcane beds are tilled). A third site, a forest remnant was included into the investigations to compare agricultural sites with a natural reference site. The forest site provides a base-line for soil biological, chemical and physical parameters under near natural conditions. Such knowledge is useful especially when considering microbial process, nutrient cycling and carbon sequestration because currently there is scant information on how natural and matching sugarcane soils differ in these traits. The forest is situated on crown land approximately 3 km away from the two sugarcane sites. There is no apparent anthropogenic use, however the site cannot be considered as fully undisturbed. Burnt tree trunks indicated previous fires in the studied forest.

Since both sugarcane sites belonged to different growers, the management at both sites could not completely harmonised (e.g. irrigation, sugarcane varieties). However, sugarcane was planted at both sites in the third week of August 2008. At the conventionally managed site, sugarcane (Q117) was fertilised with ammonium-urea (TB51/51, Hi-Fert Pty. Ltd.; 107.4 kg N ha^{-1}) on 12 December 2008 and several times furrow irrigated (2x in Dec 08, 1x in Jan, 2x Feb, 1x May 09). The fertiliser also contained potassium and sulphur. Sugarcane harvest was on 4 September 2009. At the new farming site, sugarcane (Q208) followed a peanut break crop (harvest in May 2008, re-growth sprayed) and was fertilised with ammonium-nitrate (CAL-AM, Incitec Pivot; 66 kg N ha^{-1}) on 26 November 2008, just after the first soil sampling. It received several overhead irrigations during the growing season. The fertiliser additionally contained potassium and calcium. The sugarcane was harvested on 27 August 2009. Soil investigations were conducted in total eight times (Nov, Dec 2008, early and late Jan, Feb, Mar, May, Jul, Sep 2009) during the growth of the sugarcane plant crop.

Soil investigations

Most microorganisms, including bacteria, are <1 µm and operate ecologically on a tiny scale when compared to the whole field. Special care is therefore needed since relative minor changes in soil properties maybe of greater importance for the microbial presence, community composition and activity than the applied sugarcane management. Ion exchange capacities also affect the retention of nutrients like ammonium and nitrate in the soil. Therefore, detailed soil physical/chemical investigations were conducted at all sites to complement soil biology research.

The soil at the YDJV site in Ingham was identified as an oxyaquic Hydrosol (Australian Soil Classification; Isbell, 2002), consisting of a topsoil (0-25 cm depth, field texture: silty loam, Munsell colour, wet: 2.5Y 3/2) and subsoil layer (field texture: sandy clay loam, Munsell colour, wet: 2.5Y 4/3; **Fig. 3**). The subsoil contained small amounts of charcoal. The soil is acidic (pH_{CaCl2} ~5) and has low carbon (0.2-1.0%) and nitrogen contents (0.02-0.08%) (**Fig. 3**).

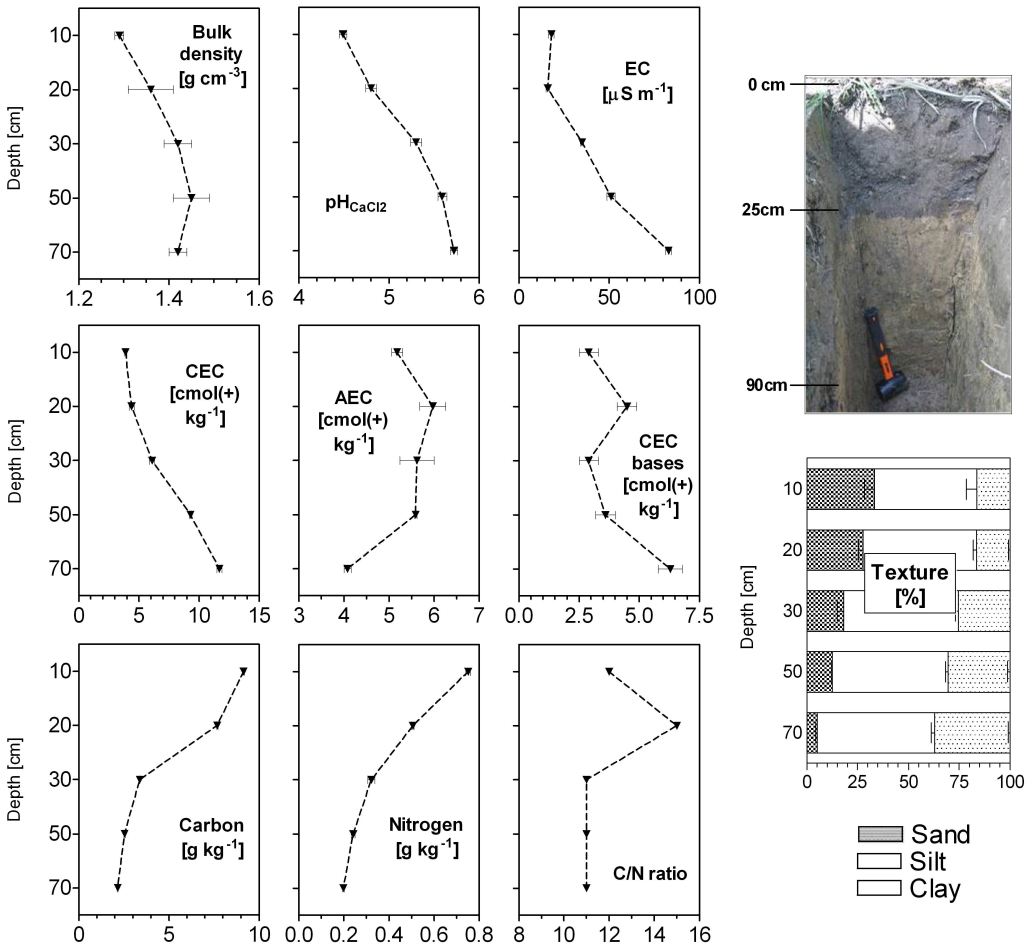


Fig. 3: Soil properties (±s.e.) at the Yield Decline Joint Venture site Ingham. Soil pit was opened at a zero-tilled sugarcane monoculture plot with reduced fertiliser application on 1 May 2008.

The three Bundaberg sites had the same soil type, a Yellow to Brown Dermosol (Australian Soil Classification; corresponds to Kepnock soil in the BSES soil classification; Schroeder *et al.*, 2007) with topsoil (0-25 or 0-20 cm; field texture: sandy loam, Munsell colour, wet: 2.5Y 2.5/1 to 3/1) and subsoil layers (field texture: clay loam to sandy loam, Munsell colour, wet: 2.5Y 5/4 to 10YR 5/6; **Fig. 4**). Carbon and nitrogen contents varied between 0.2-2.4% and 0.01-0.1%, respectively (**Fig. 5**).

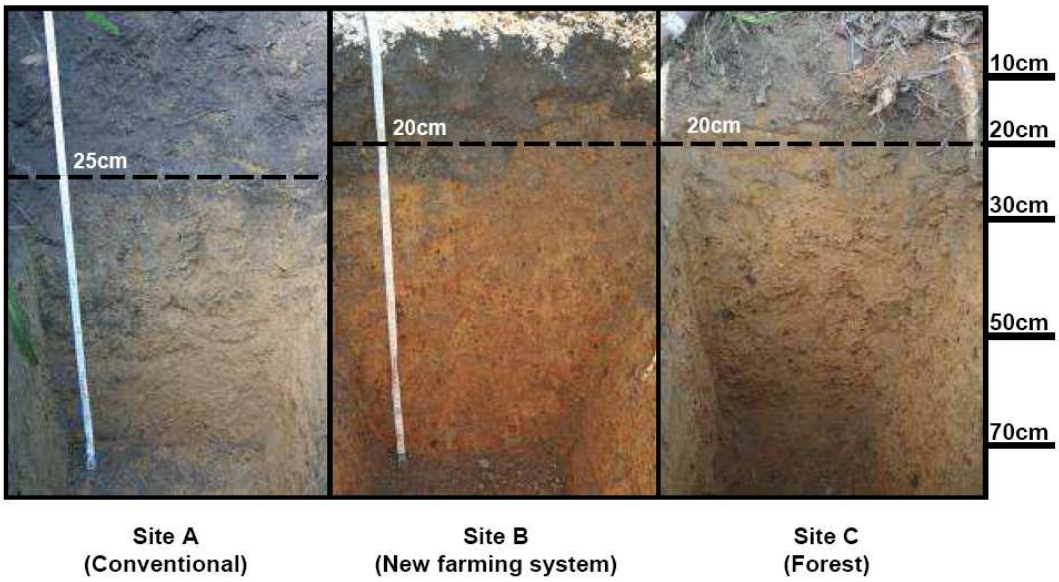


Fig. 4: Soil at the experimental sites near Bundaberg.

Soil texture differed between the Bundaberg sites, with the forest site having significantly higher sand contents than both agricultural sites (**Fig. 5**). As a result of fertiliser additions, both agricultural soils had higher electrical conductivities (EC) and phosphorous concentrations compared with forest soil (see below). A number of parameters reflected the effects of liming at the new farming systems site, e.g. the high pH (>6), high ion exchange capacities and concentrations of calcium. In contrast, the soil at the conventional sugarcane site had the lowest pH (pH_{CaCl2}<5) due to high N fertiliser additions and no liming. The acidic soil reaction may also increase the likelihood of aluminium and manganese toxicity, indicated by increased extractable aluminium and manganese concentrations at the conventional site (**Fig. 6**).

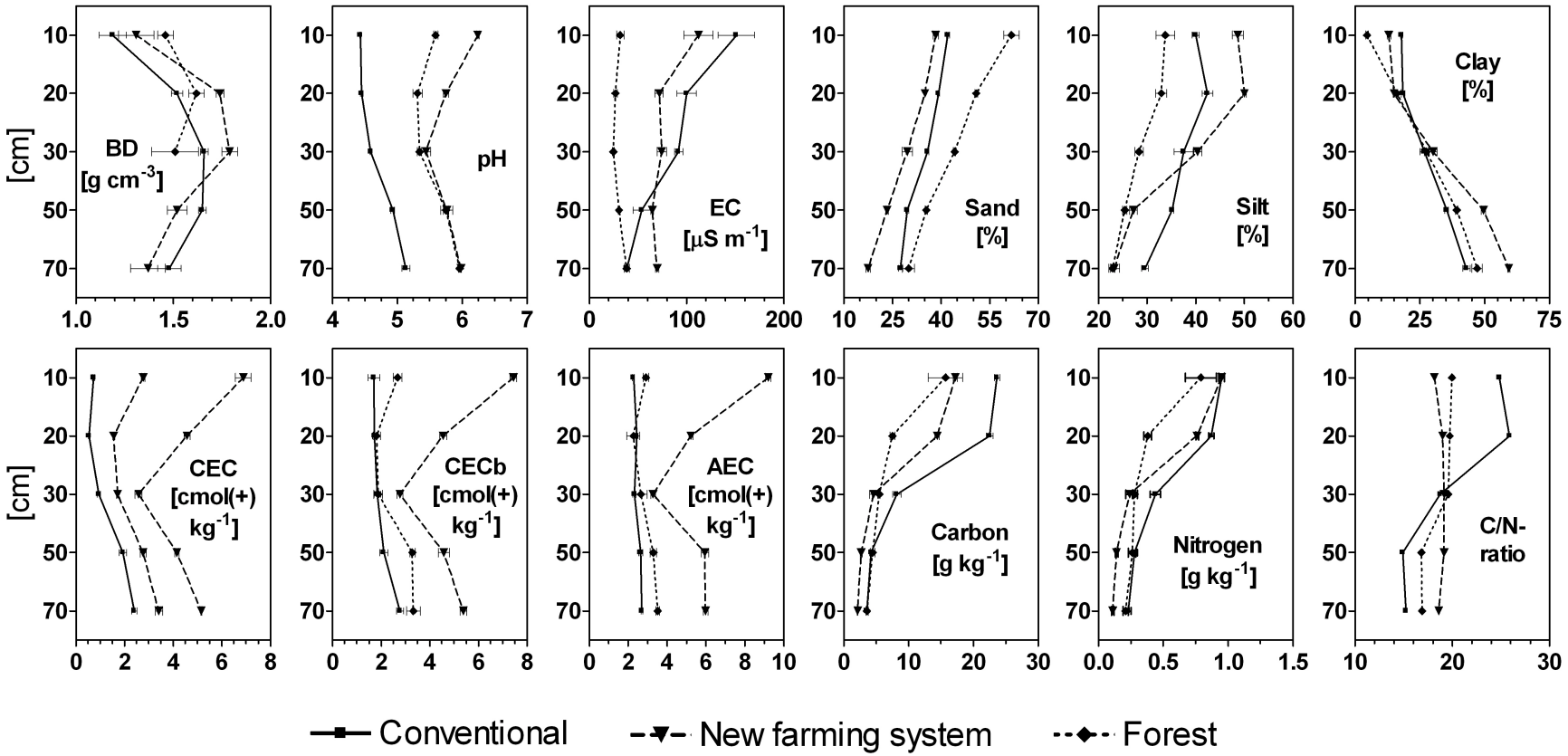


Fig. 5: Soil properties (±s.e.) over depth at the three Bundaberg sites. Soil pits were opened in late January 2008.

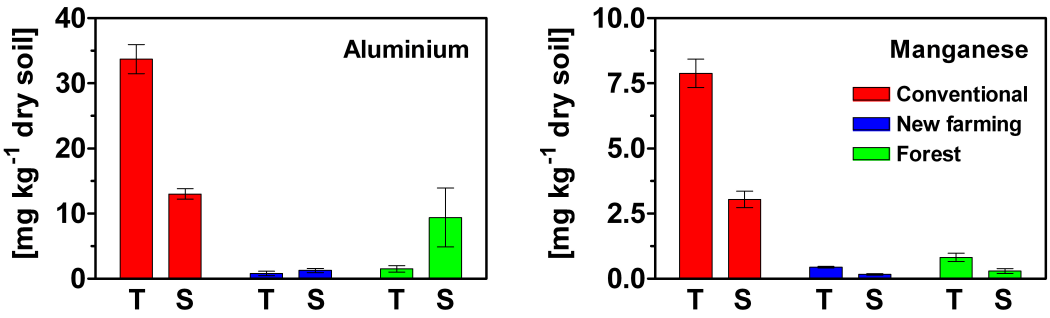


Fig. 6: Readily available aluminium and manganese (±s.e.) in topsoil (T) and subsoil (S) of the Bundaberg sites. Soil samples collected in November 2008 were extracted with 0.02 M $CaCl_2$ (Soon *et al.*, 2008).

Although sugarcane tolerates high levels of extractable aluminium (20-53 mg kg^{-1} soil; Hetherington, 1986; Slattery *et al.*, 1999) and manganese, microorganisms may be subjected to toxicity (Piña & Cervantes, 1996).

Assessment of the general nutrient status of the three soils, measuring extractable concentrations of plant nutrients in November 2008, showed some differences between sites (**Fig. 7**). Comparing the measured values with the guidelines of the Australian sugarcane nutrition manual (Calcino, 1995), the conventional site was deficient in calcium, magnesium and zinc. The new farming site was deficient in potassium, which was however supplied during the N fertilisation. Iron was in the typical range of Australian sugarcane soils. Phosphorous levels were sufficient at both sugarcane sites. Although sodium availability was higher at the new farming site compared with the conventional site, the percentage of cation exchange capacity occupied by exchangeable sodium (exchangeable sodium percentage, ESP) was greater in topsoil of the latter site (conventional: 6.6; new farming: 2.4). An ESP of 5-15 indicates some breakdown of soil structure with negative effects on aeration and water penetration (Calcino, 1995), thus the soil at the conventional site may be subject to degradation.

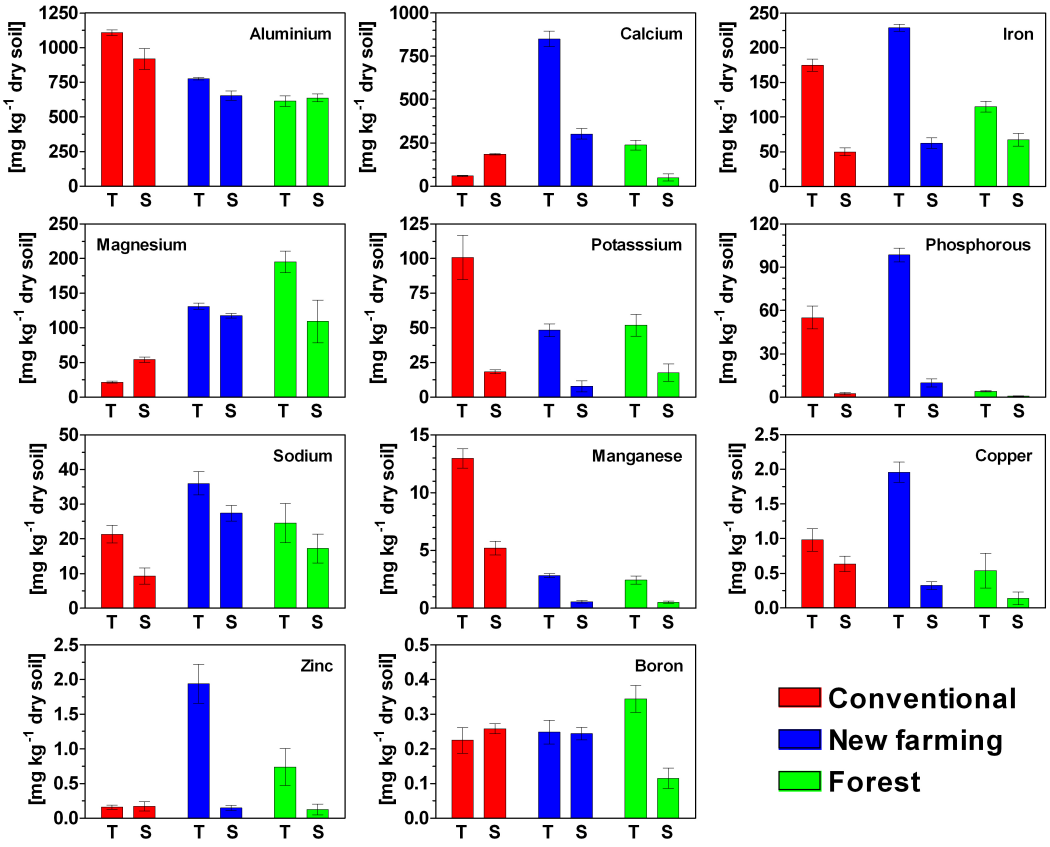


Fig. 7: Soil nutrient status (±s.e.) in topsoil (T) and subsoil (S) of the Bundaberg sites. Nutrients were extracted with Mehlich-3 solution from samples collected in November 2008 (Ziadi & Tren, 2008).

5.2 Soil microbial biomass

Microbial biomass was quantified using the fumigation-extraction method (Joergensen & Brookes, 2005; Voroney *et al.*, 2008).

At the YDJV site in Ingham, soil microbial biomass was investigated at selected plots with reduced N fertiliser application. It was in a range of 1.9-21.1 mg N kg^{-1} soil, which is in agreement with previous investigations of sugarcane soils in Queensland (Holt & Mayer, 1999; Wood, 1991) and our investigations at the Bundaberg sites (see below). Microbial biomass was significantly higher in topsoil than subsoil in all treatments (**Fig. 8**). In April 2008, topsoil microbial biomass did not differ between Monoculture (Cane) and Break crop plots (Soy), but was significantly lower in soil of tilled versus zero-tillage break crop plots. In October 2008, microbial biomass was similar in tilled and zero-tilled break crop plots indicating a recovery of microbial biomass in tilled plots. Microbial biomass in subsoil was similar between managements on both sampling occasions.

These results suggest that
(a) tillage reduces microbial biomass in the biologically important topsoil layer for a period after which microbial biomass recovers, and
(b) none of the investigated managements affects microbial biomass in the subsoil.

At the Bundaberg sites, microbial biomass was in a range of 0.5-44.8 mg N kg^{-1} dry soil. In topsoil, microbial biomass was similar in the new farming system and the forest site (**Fig. 9**). At both sites, it was significantly higher as compared to the conventional sugarcane site. Subsoil microbial biomass was similar between both sugarcane sites. The observation of higher microbial biomass under trash blanketing is in line with previous investigations (Wood *et al.*, 1991). Our findings indicate that the new farming system obtains the microbial biomass at natural levels and that the microbial biomass in the subsoil is largely unaffected by both agricultural managements.

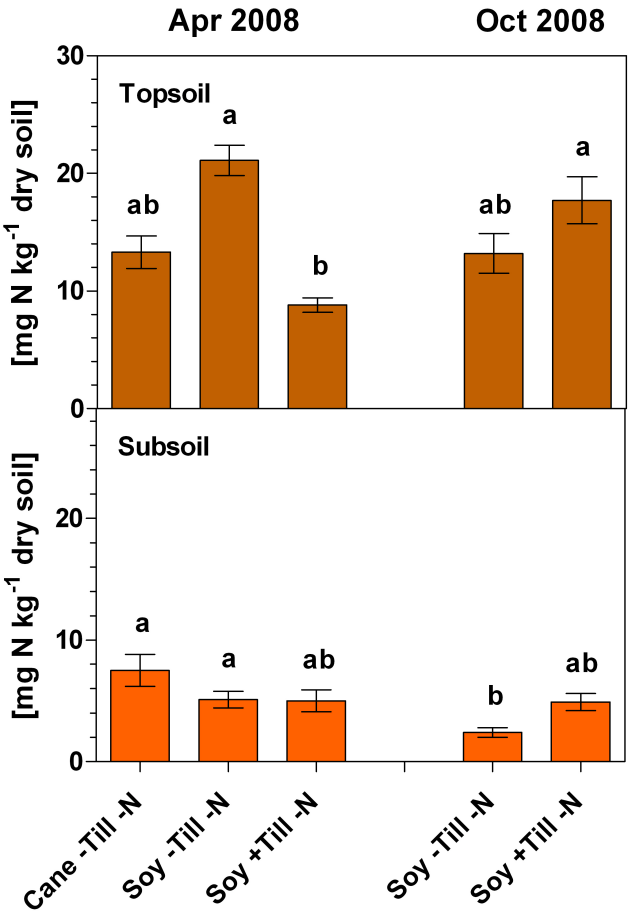


Fig. 8: Microbial biomass (±s.e.) at the YDJV site, Ingham. Different letters indicate significant differences between managements ($p<0.05$).

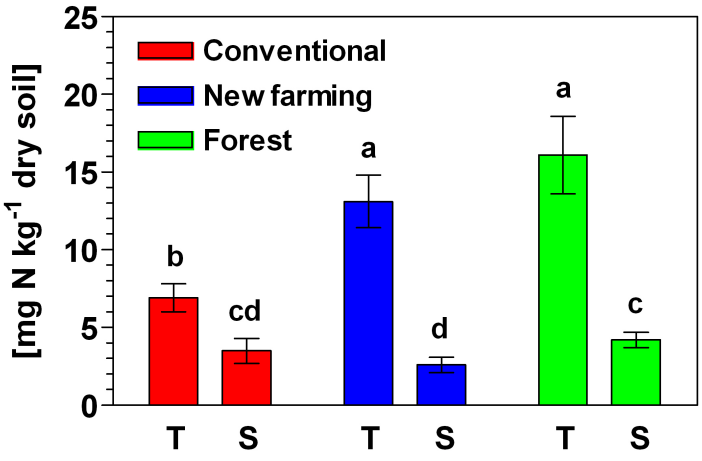


Fig. 9: Soil microbial biomass (±s.e.) in topsoil (T) and subsoil (S) at the Bundaberg sites, determined between November 2008 and September 2009 (9 sampling times). Different letters indicate significant differences between means ($p<0.05$).

All soil samples analysed for microbial biomass were also analysed for a range of soil parameters, including pH, EC, texture, soil C and N contents, moisture, soluble ammonium, nitrate and amino acid concentrations. There were no significant correlations between these environmental parameters and microbial biomass in topsoil and subsoil. No seasonal trend in soil microbial biomass was observed (**Fig. 10**).

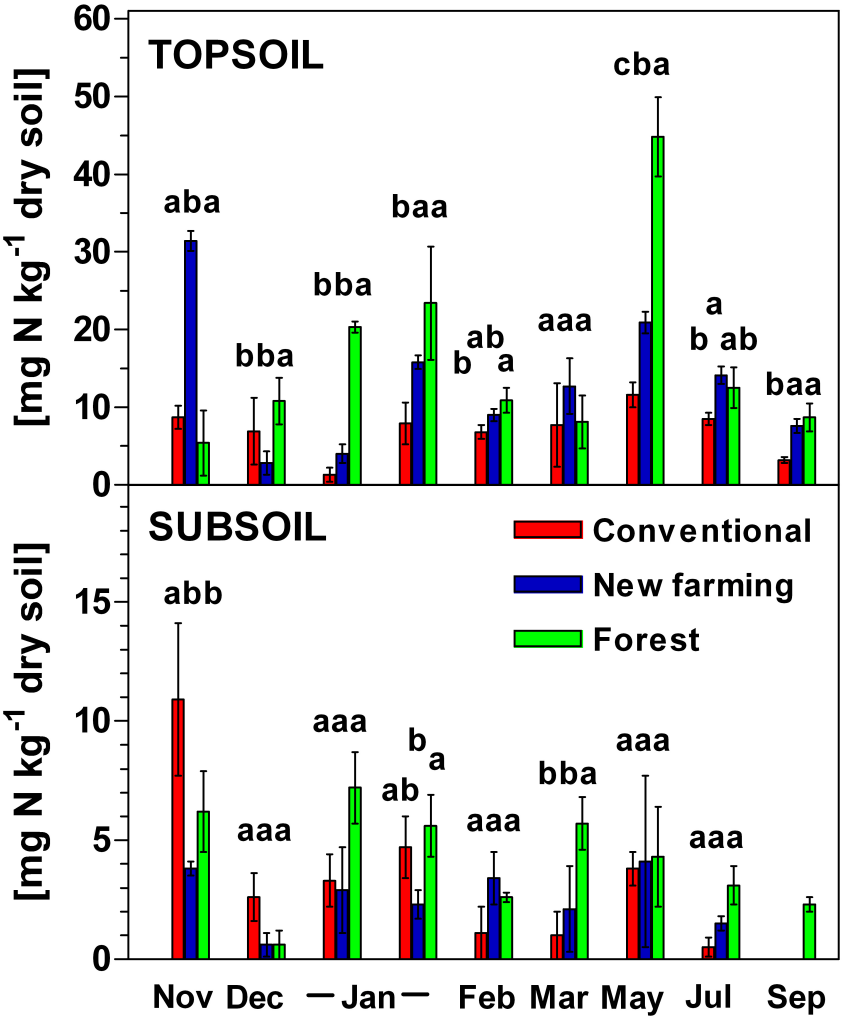


Fig. 10: Soil microbial biomass (±s.e.) at the Bundaberg sites. Different letters indicate significant differences between sites at the same sampling date ($p<0.05$). No subsoil data available for sugarcane sites in September 2009.

5.3 Soil microbial community composition

The functional community composition of N-converting microorganisms (**Fig. 11**) was assessed through amplification of a specific target gene using polymerase chain reaction (PCR) and the subsequent separation of the amplicons from different species with denaturing gradient gel electrophoresis (DGGE; Nakatsu, 2007). Amplicons were cloned and sequenced and sequences were used to identify microbial species.

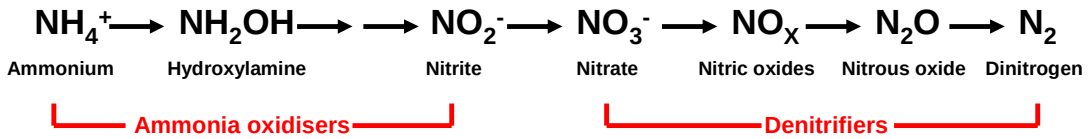


Fig. 11: Overview about the involvement of different functional groups of microorganisms in soil N cycling.

At the YDJV site in Ingham two key functional groups of microorganisms in the N cycle were investigated: ammonia-oxidising and denitrifying bacteria. Ammonia-oxidising bacteria mediate the first step of the nitrification (conversion of ammonia to nitrate) and denitrifying bacteria are responsible for the conversion of nitrate into several gaseous N compounds during the denitrification including the greenhouse gas nitrous oxide (**Fig. 11**). Ammonia oxidising bacteria were detected using 16S rDNA-gene (Kowalchuk et al., 1997) and denitrifying bacteria using nirS-gene target sequences (Oros-Sichler et al., 2007) from samples taken from sugarcane monoculture (Cane) and soybean break crop plots (Soy) in May 2007.

The analysis showed for both functional groups that some microbial species were common to all samples of both managements, whereas few species were characteristic to one or the other management (**Fig. 12**). This indicates that the introduction of legume break crops into the sugarcane cycles changed the community composition of these N-related microbial groups.

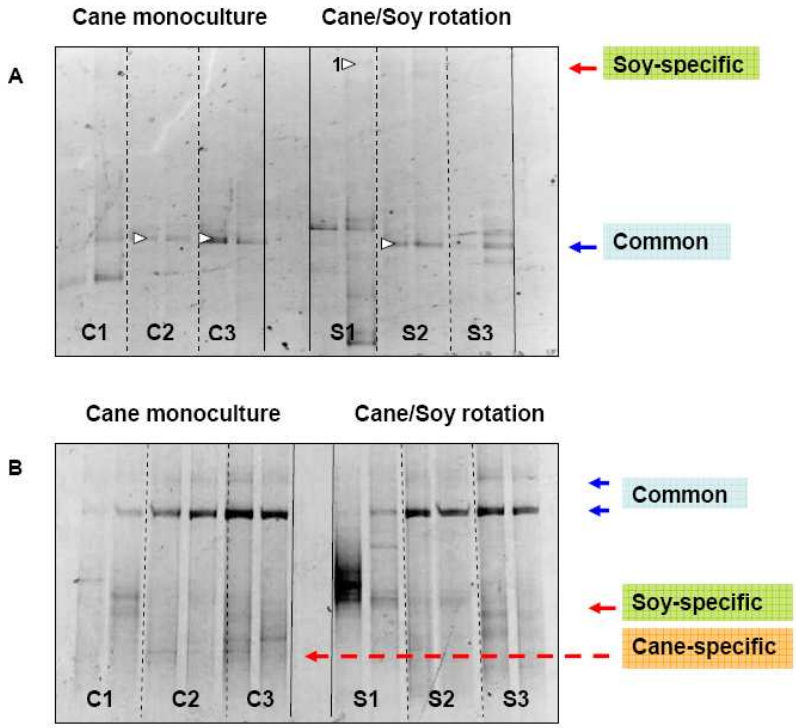


Fig. 12: Results of the DGGE microbial community structure analysis for a) ammonia oxidising bacteria and b) denitrifying bacteria. Two samples per replicate block (C1-C3; S1-S3) were analysed. Arrows show species which are present either in all samples ("Common") or specific to break crop ('Soy-specific") or monoculture plots ("Cane-specific").

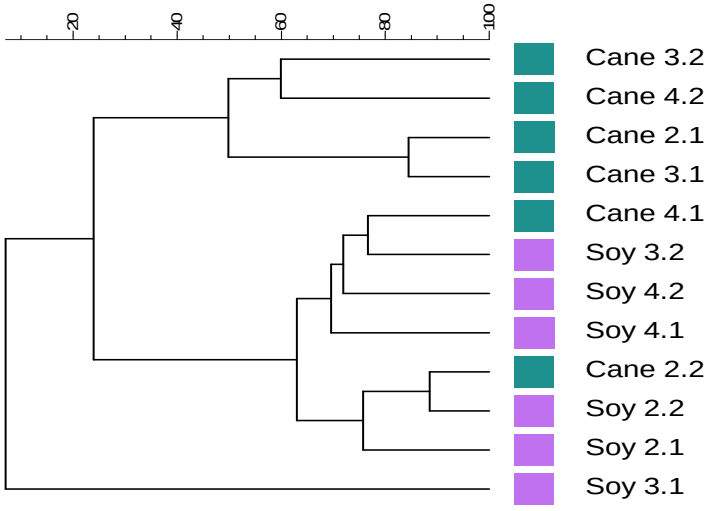


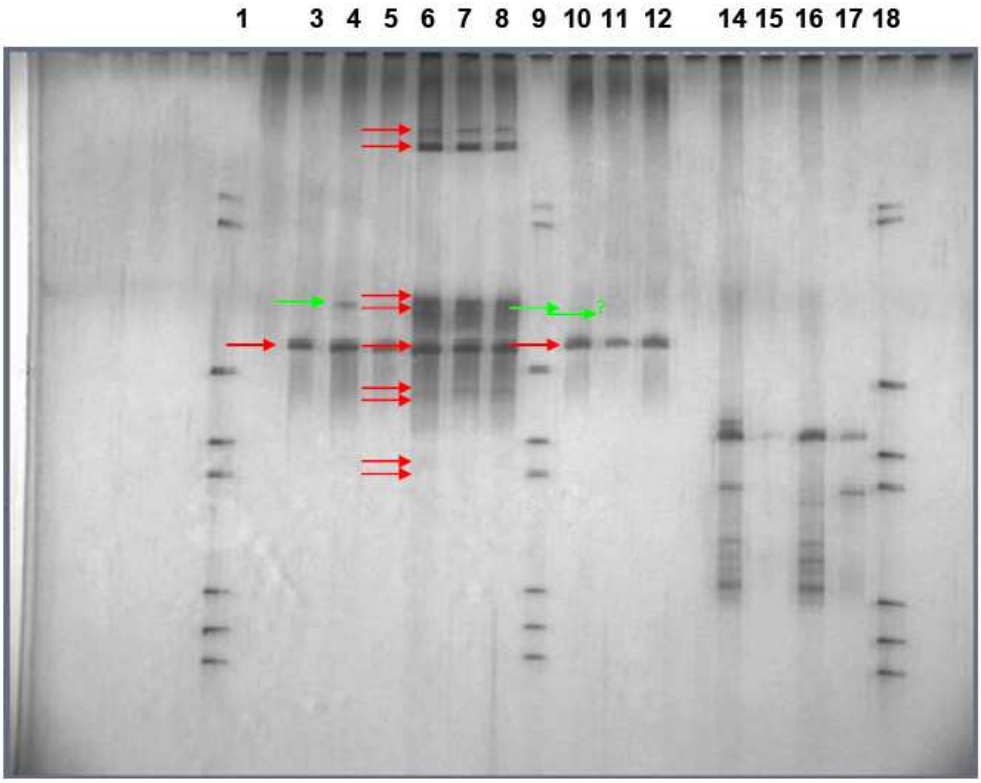
Fig. 13: Similarity analysis of different samples based on the community composition of ammonia-oxidising bacteria (16S rDNA). Labelling: management, replicate block, sample number (e.g. Soy 4.1). From Mondav (2009).

Effect of management on the composition of the microbial community is also supported by a similarity analysis using the obtained 16S rDNA-data, which separated most individual soil samples into two major groups, one corresponding to monoculture, the other to the break crop management (**Fig. 13**; Mondav, 2009).

The PCR amplicons of the different microbial species were additionally purified from the DGGE gel and sequenced. Databank search verified that the identified ammonia-oxidising bacteria belong to the genus *Nitrosospira*, whereas the taxonomy of the denitrifying bacteria is unknown.

At the Bundaberg sites, ammonia-oxidising bacteria and ammonia-oxidising archaea were investigated. Archaea, a distinct group of microorganisms that differ from bacteria, are considered to be the most abundant ammonia-oxidising prokayotes in soil (Leininger *et al.*, 2006). Target genes were the archaeal and bacterial ammonia monooxygenase genes (Rotthauwe *et al.*, 1997; Schauss *et al.*, 2008).

DGGE analysis showed the greatest diversity of archaea in soil of the new farming system (**Fig. 14**). The data indicate that bacteria were absent from the conventional sugarcane and the forest sites, since amplicons were only produced from soils of the new farming system. Subsequent cloning and sequencing of amplicons worked well for archaea, but was not successful for the bacterial amplicons. Several attempts to repeat PCR using bacterial primers did not yield any amplicons, and we conclude that the previously produced ‘bacterial’ amplicons are possible artefacts. Thus, we conclude that archaea are the likely ammonia-oxidisers in the studied soils.



Lanes 1, 9, 18	Standard
Archaeal amoA:	
Lanes 3-5	Conventional sugarcane management
Lanes 6-8	Sugarcane new farming system
Lanes 10-12	Forest
"Bacterial" amoB:	
Lanes 14-17	Sugarcane new farming system

Fig. 14: DGGE analysis of the community composition of ammonia-oxidising archaea and bacteria at the Bundaberg sites.

5.4 Soil microbial gene expression

Different agricultural management not only impacts on the species composition of microbes, but also on the activity of soil microorganisms which is detected by analysis of microbial mRNA in the soil ("microbial gene expression'). mRNA is the transcribed copy of a DNA-encoded gene that leads to the making of the corresponding proteins to carry out physiological functions. Thus, higher amounts of particular mRNA-transcript encoding a protein are indicative that this particular process is occurring. Analysis of the mRNA transcripts of whole microbial communities (metatranscriptomics) is considered the most advanced technique in microbial studies because it provides a snapshot of the active processes occurring irrespective of microbial species present.

To measure soil microbial gene expression at the YDJV site, RNA was isolated from soil, reversely transcribed and hybridised on a custom-made microarray. The microarray was developed as part of this research project and contained 13,056 unknown functional sequences (probes) from diverse soil and other relevant microbial communities. The probes were derived from soil mRNA, which was isolated using a novel method (McGrath *et al.*, 2008a). For analysis of overall soil microbial gene expression, samples from monoculture (Cane) and soybean break crop (Soy) plots (both zero-tilled and with reduced N fertiliser application) were collected in May and December 2007 and in April 2008. In May 2007, we found distinct differences in the gene expression between Cane and Soy treatments, described as a reduction in correlation (r^2 value) between expression levels of all expressed genes from Cane and Soy plots as compared to correlations between samples from either Cane or Soy plots (**Fig. 15**). This suggested that there was a distinct and large-scale difference in soil microbial gene expression between soils under sugarcane monoculture or soybean break cropping. In December 2007 and April 2008, we observed a further reduction in the correlation between Cane and Soy samples as compared with May 2007, indicating that the gene expression levels were even more different than in May 2007 (**Fig. 16**).

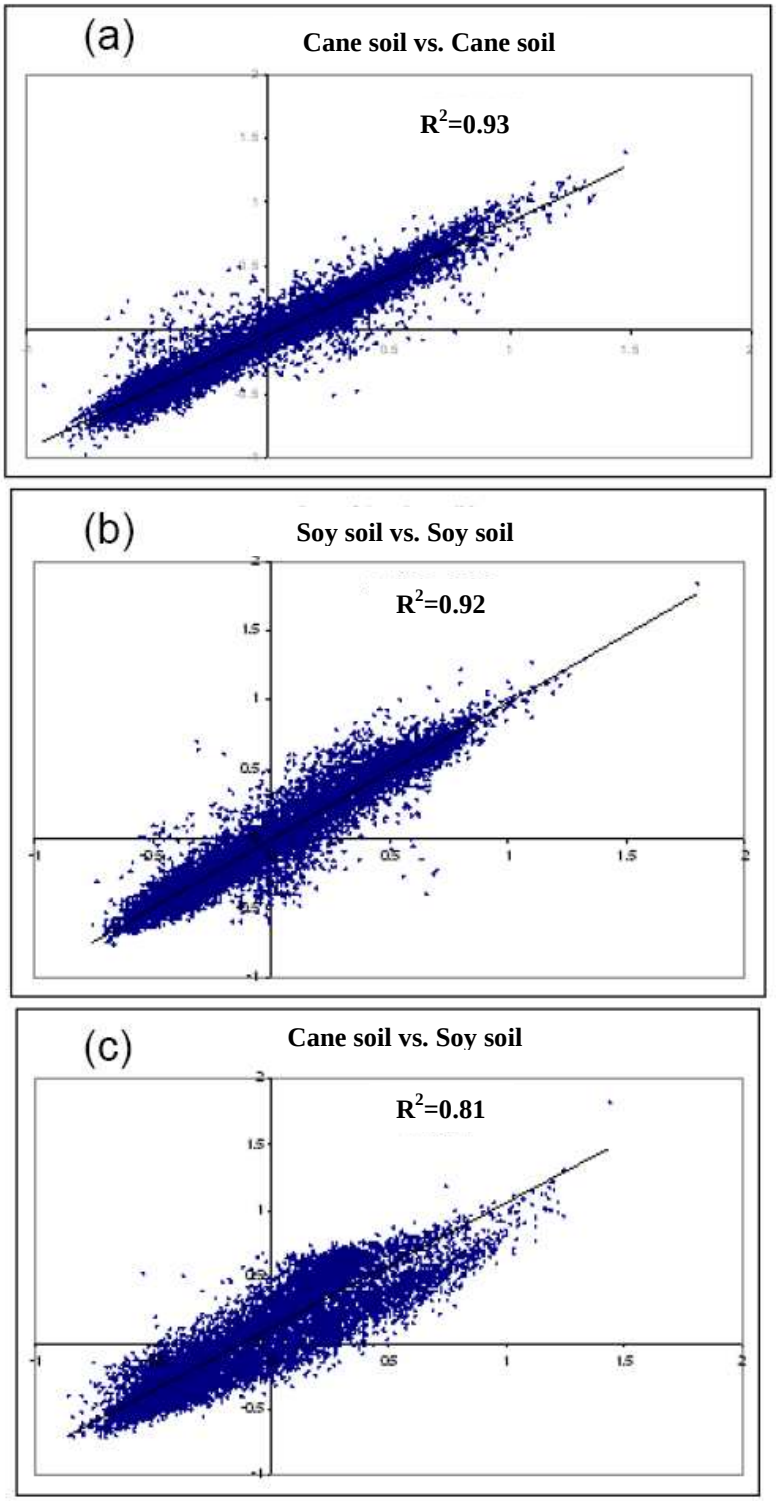


Fig. 15: Scatterplots of average gene expression levels compared between different soil microbial communities. Relative ratios of gene expression are given (both x and y axis). For both graphs, n=13,056 data points, which represent the activity of anonymous microbial genes across two soil samples. From McGrath *et al.* (2008b).

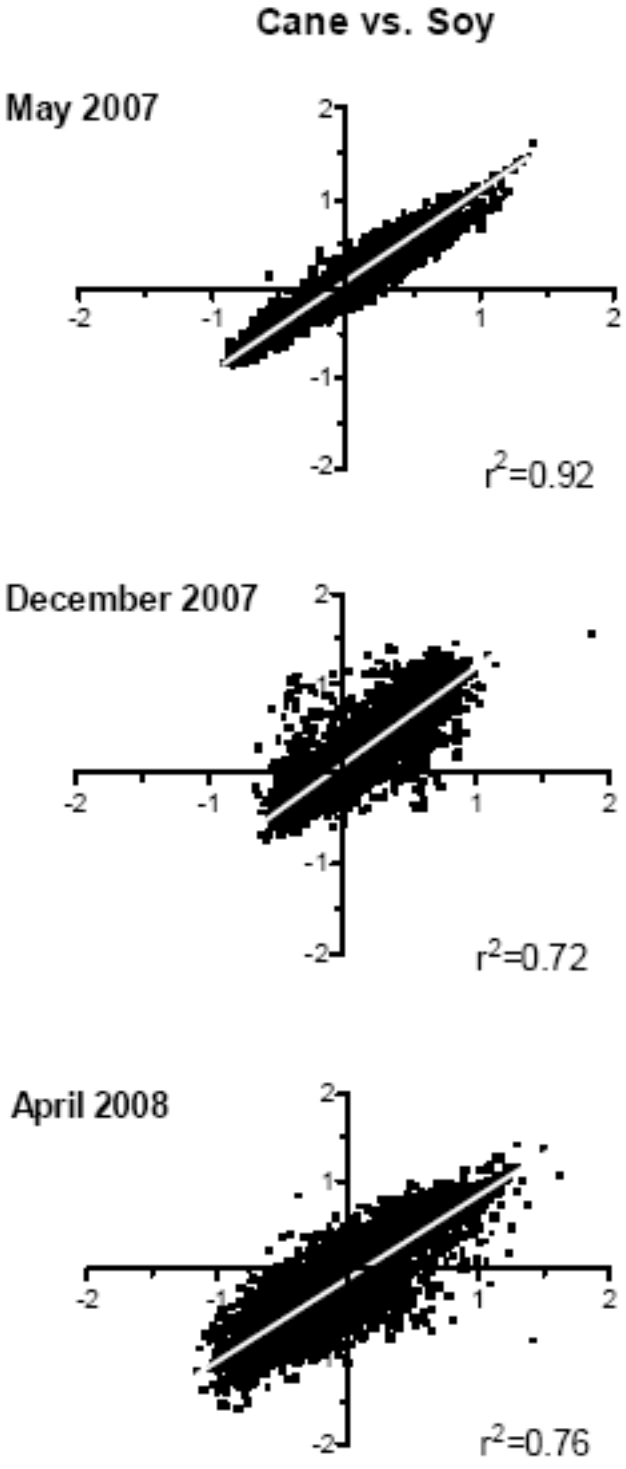


Fig. 16: Scatter-plots of gene expression levels of soil samples from different treatments (Cane vs. Soy) in Ingham. Relative ratios of gene expression are given on both, x- and y-axis. Data represent averages for three or more independent soil extractions and microarray analyses.

However, also samples of the same treatment (Cane vs. Cane or Soy vs. Soy) showed a markedly decreased correlation. This indicated that there was a strong small scale spatial heterogeneity in the soil microbial activity in December 2007 and April 2008 as compared to the fallow period in May 2007. The reason for this overall increase in functional diversity maybe the presence of sugarcane plants on all plots in December 2007 and April 2008. This plausible because microbial activity in terrestrial ecosystems is largely dependent on the exudation of recently produced photosynthates (Högberg & Read, 2006). The presence of sugarcane plants, their physiological activity and the distribution of roots within the soil likely creates a highly heterogenic environment with respect to nutrients, oxygen, water availability etc., affecting microbial activity and thus microbial gene expression. Therefore, we conclude that management effects on the overall soil microbial gene expression were comparatively short-lived as indicated by similar gene expression in December 2007 and April 2008.

However, there could be a management effect on the expression of individual genes, which are involved in N cycling and associated processes like carbon and phosphorous metabolism or energy production. To evaluate this, we analysed the expression of identifiable clones over time. Approximately 900 microarray clones underwent sequencing and around 200 sequences were linked to transcript homologues, thus providing a glimpse into microbial gene expression in soil. Many of the sequenced clones could not be identified due missing functional matches with available genetic databases. This is not surprising, since the anonymous microarray contains many sequences which have not been described prior to our analysis. A selection of identified genes is shown in **Tab. 1**.

The expression of these clones was compared between different managements and time points, using a simplified matrix system. Some data were unavailable because of microarray manufacturing errors, sample loss during extraction of genetic material or were excluded during quality control. Investigated were a) break crop and monoculture plots with reduced N fertiliser application (Soy-N vs. Cane-N), b) monoculture plots with full and

reduced N fertilisation (Cane+N vs. Cane-N) and c) break crop plots with full and reduced N fertilisation (Soy+N vs. Soy-N) for three or two sampling days each (**Tab. 2**).

For example, management comparison A is explained in **Table 2**: when statistical analysis revealed that the expression of a certain clone was higher under the first management (Soy-N) than under the second management (Cane-N), the clone was assigned with “1”, when it was similar under both managements it received a “0” and when lower a “-1”. The algebraic sign of the final sums indicates under which management soil microbial gene expression was higher and the new data matrix was used to test a null hypothesis (using paired t-test), i.e. that the microbial gene expression was similar under both managements (all clones with “0”). Management comparisons B and C were processed similarly.

The analysis showed that the expression of these selected genes was higher under soybean break cropping than under sugarcane monoculture in December 2007, and similar in May 2007 and April 2008 (comparison A; **Tab. 2**). These different gene expression in December 2007 seems not strongly linked to the availability of N, since N availability was similar between managements on all sampling dates, and rather C availability or other factors maybe more important for microbial activity. On zero-tilled sugarcane monoculture plots (comparison B), the microbial gene expression was higher under fully fertilised conditions in December 2007 and similar in April 2008 as compared to reduced N fertiliser application (**Tab. 2**), which coincided with a higher ammonium availability on fully fertilised plots in December 2007. All other N forms were in similar concentrations available. On zero-tilled break crop plots (comparison C), microbial gene expression was lower under full N fertilisation in December 2007, and similar in April 2008 as compared to break crop plots with reduced N fertilisation (**Tab. 2**). For both time points we did not observe significant differences in the availability of N between both managements.

Table 1: Selection of identified microarray clones, involved in nitrogen and carbon metabolism, energy production or phosphorous cycling.

Clone	Gene function
Nitrogen metabolism	
004h12	predicted amino-acid transporter
014h3	probable glycine betaine/L-proline ABC transporter, subst...
015g3	73 bp at 5' side: glutamate/aspartate:proton symporter; 346 bp at 3' side: conserved protein
017e6	putative glutamate permease
018b6	neutral amino-acid efflux system
018c11	186 bp at 5' side: glutamate/aspartate:proton symporter, 233 bp at 3' side: conserved protein
018c6	412 bp at 5' side: glutamate/aspartate:proton symporter, 7 bp at 3'side: conserved protein
019b9	132 bp at 5' side: homoserine/threonine efflux pump, 408 bp at 3' side: conserved hypothetical protein
020a9	dipeptide transporter; periplasmic-binding component of A
024d10	dipeptide transporter or 4-phytase
041e3	transposase (IS4 family), sodium/glutamate symporter
042d5	nitrate reductase, periplasmic, large subunit, assembly protein for periplasmic nitrate reductase
094e11	putative high-affinity branched-chain amino acid transporter
101g5	putative branched-chain amino acid ABC transporter, permease
087g9	periplasmic nitrate reductase subunit NapA apoprotein
121g8	nitrate reductase (narG)
Carbon metabolism	
001b11	maltose transporter subunit; periplasmic-binding component
001d8	pyruvate kinase II
021c6	2-deoxyribose-5-phosphate aldolase
131h3	putative polysaccharide biosynthesis protein (capD-like)
Energy production	
023h12	cytochrome c assembly protein
046c5	cytochrome P450-like protein
Others	
018g2	phosphate uptake regulator, PhoU

Tab. 2: Analysis of temporal expression analysis of identified microarray genes. All investigated managements were zero-tilled, main N fertiliser application was in November 2007. Stars indicate the rejection of the null hypothesis (i.e. no management effect on microbial gene expression; $p<0.05$). Numbers indicate different or similar expression levels between managements (M). M1>M2: 1, M1=M2: 0; M1<M2: -1. For further explanations see text.

Comparison		A		B		C	
Management 1 vs. Management 2		Soy-N vs. Cane-N		Cane+N vs. Cane-N		Soy+N vs. Soy-N	
	May-07	Dec-07	Apr-08	Dec-07	Apr-08	Dec-07	Apr-08
Nitrogen metabolism							
015g3	0	-	0	1	0	-	0
018c11	1	-	0	1	0	-	0
019b9	0	1	-	1	-	-1	1
020a9	1	0	-1	0	1	-1	1
101g5	0	-	0	1	-	-	0
087g9	0	1	0	1	-	0	0
121g8	0	-	-1	0	-1	-	0
Carbon metabolism							
001b11	1	1	0	0	0	-1	0
001d8	1	1	0	0	0	-1	0
021c6	1	-	-1	0	-	-	0
131h3	-1	0	0	0	0	0	0
Energy production							
023h12	0	1	-	1	-	0	-
046c5	0	1	0	0	0	-	0
Phosphor metabolism							
018g2	1	1	0	1	-	-	0
SUM	5	7*	-3	7*	0	-4*	2

Although it remains difficult to evaluate differences in microbial gene expression between managements and their causes due to the small number of genes studied, these examples suggest the observed differences are not strongly linked to the availability of N. This supports that microbial activity is not just the response to one, but to a multiplicity of environmental factors and they underline the validity of the chosen research approach, because microarray analyses allow the simultaneous investigations of thousands of different microbial genes, involved in very different physiological activities. This technique complements other emerging technologies, such as next generation sequencing but offers the advantages (1) that only differentially expressed genes require sequencing, (2) cross-hybridization reveals transcriptional activity of entire gene families (rather than individual genes) and (3) relatively low costs. This metatranscriptomics approach provides a valuable addition to elucidate microbial function in agricultural soils and other environmental matrices. With rapid reduction of costs for microbial sequence analyses and ever increasing microbial sequence databases, it can be anticipated that microbial activity profiles obtained from gene expression signatures as developed in this project will contribute towards a complete modeling of biochemical processes in sugarcane soils.

To investigate the expression of soil microbial genes at the Bundaberg sites, another approach was used because technology had moved on from microarray to independent sequencing of transcripts. The recent developments in sequencing technologies offer new opportunities with respect to sensitivity, number of sequences and sequencing speed (Morozova & Marra, 2008). The 454-sequencing technology relies on real-time ‘pyro-sequencing’ where the extension or removal of a nucleotide in a DNA strand is coupled to a light emitting reaction (‘pyro’) allowing identification of the sequence, base by base. Several attempts have been made in this project to produce material sufficient for a single run on a next-generation sequencing platform (Roche 454 FLX Titanium), again a world first using soil as the matrix. The main problems for this approach were related to the quality and quantity of RNA, and the removal of ribosomal RNA. To overcome these obstacles, we were assisted by leaders in the field, Dr Gene Tyson (UQ) and

Dr Frank Stewart (MIT, Boston) who shared their unpublished protocols and provided advice. Several attempts to extract high-quality mRNA from soil were made over a period of 18 months by optimising previously used sampling protocols and testing various commercially available molecular kits for purification and transcription of mRNA into cDNA. None of these procedures produced sufficient cDNA for sequencing, but we were able to optimise the protocol including omission of the initial freezing step which led to degradation of RNA.

To overcome the consistent problem of low mRNA yields, re-sampling at Bundaberg was conducted in February 2010 and our new methods for sample preparation were applied (**Fig. 17**).

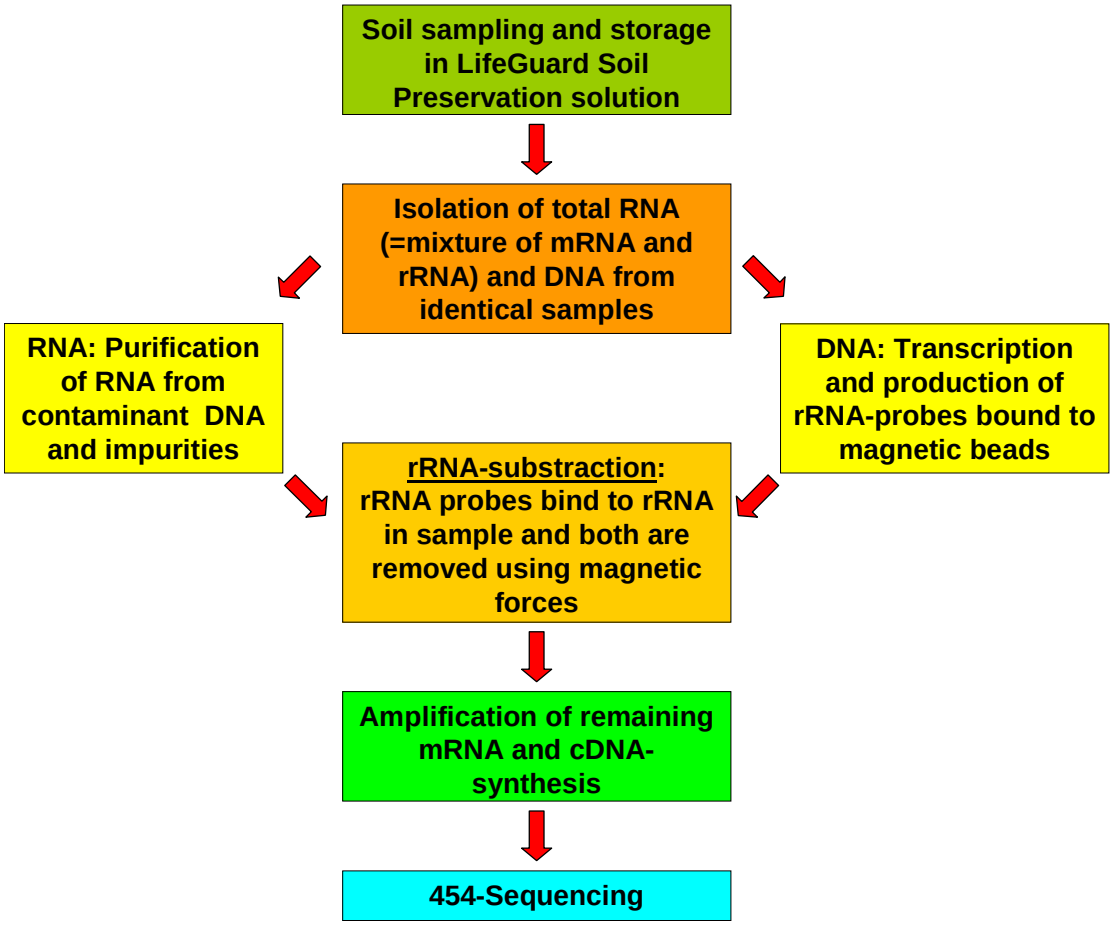


Fig. 17: Flow chart of experimental procedures to prepare soil samples for 454-sequencing (analysis of all microbial mRNA).

The sampled soil was stored in a novel solution (LifeGuard Soil Preservation solution, MoBio), which became available in late 2009 and which prevents RNA degradation, but preserves the transcript profile without the need for freezing. After removal of contaminant DNA and humic substances, the extracted total RNA was in excellent condition (**Fig. 18**). In order to exclude ribosomal (rRNA) from the total RNA, a unpublished rRNA-subtraction protocol was provided by the Massachusetts Institute of Technology, Boston, USA. The protocol uses DNA extracted from the same soil samples to produce rRNA-probes. The probes are attached to magnetic beads and bind to rRNA-molecules in the total RNA samples, which is then removed using magnetic forces. Since rRNA usually contributes >80% to the total RNA, the remaining amounts of mRNA were very low with 0.02 to 0.1 µg per sample and had to be amplified (MessageAmp Bacteria II kit, Ambion) prior cDNA-synthesis. After additional quality checks, samples were submitted to the Australian Genome Research Facility (AGRF) with a sufficient mass of ~1.0 µg cDNA per sample and has been accepted through all quality control stages. Sequencing is expected to be completed early August with data expected to be available in mid September 2010.

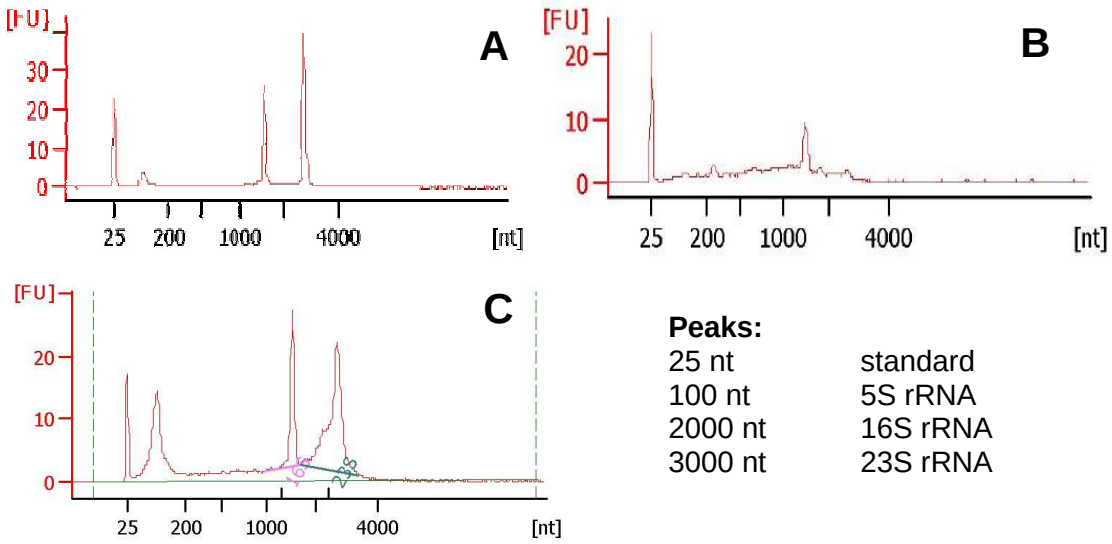


Fig. 18: Analysis of RNA-quality using the BioAnalyzer. A) RNA standard, B) RNA extracted from frozen soil samples and C) from fresh soil. Significant degradation of rRNA molecules has occurred in the stored soil samples, whereas distinct peaks at the lengths expected of rRNA molecules indicate high quality RNA and improved yields from freshly extracted soil samples.

5.5 Management effects on the availability of nitrogen forms in soil and on sugarcane yield

Soluble N compounds were extracted with 1.5 M KCl-solution from fresh soil and filtrates were analysed for ammonium and 20 amino acids with liquid chromatography using an UPLC (Ultra Pressure Liquid Chromatography Unit, Waters, Milford, USA) and for nitrate and proteins using spectrophotometric assays (Miranda *et al.*, 2001). Soluble N compounds of low molecular mass are considered to be easily available to plants (Ros *et al.*, 2009).

At the YDJV site in Ingham, soil was sampled from topsoil and subsoil layers close to the cane rows at eight differently managed plots and analysed for soluble N compounds (ammonium, nitrate, amino acids). Mean concentrations ranged between 0.37-3.61 (amino acids), 0.2-60.5 (ammonium), and 0.1-18.5 mg N kg^{-1} dry soil (nitrate) in topsoil and subsoil over the sampling period. The temporal pattern of these N nutrients is summarised for the conventional management Cane+Till+N (**Fig. 19)**, which is typical for the Ingham area. Amino acids are acquired by sugarcane and used as a N source for growth (Vinall, 2010) and constituted a significant part of the soluble soil N pool (up to 64% of the soluble soil N pool, here defined as the sum of ammonium, nitrate and amino acids). The most abundant soil amino acids were glycine, arginine, serine, alanine, asparagine, glutamine, aspartic acid, glutamic acid and histidine (**Fig. 20**).

All eight investigated managements were compared and no significant differences were found when averaging soil N availability over the sampling period. Differences were however detected for individual sampling dates and are summarised in **Tab. 3**. Applied treatments including break cropping, tillage or N fertilisation affected only the availability of inorganic N compounds (ammonium, nitrate), but not soil amino acid concentrations. Changes in amino acid concentrations in response to management could be disguised by the rapid consumption of amino acids by microbes and plants as well as mineralisation to inorganic N compounds.

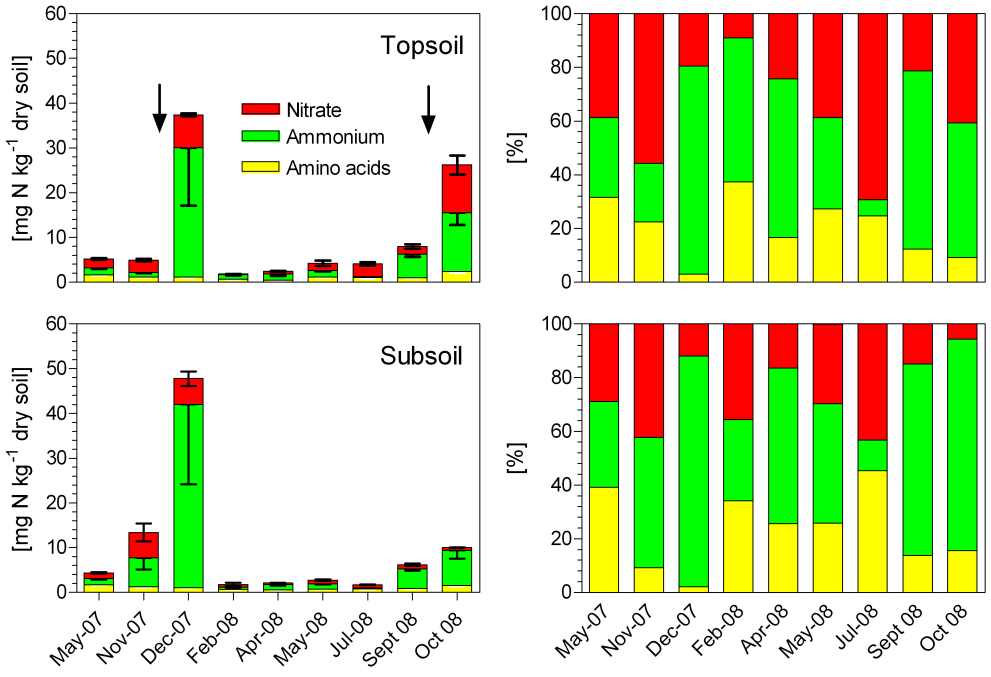


Fig. 19: Average concentrations (±s.e.) of amino acids, ammonium and nitrate and their percentage contribution to the total soluble N pool for the conventional management Cane+Till+N. Arrow indicate main N fertilisation events for the plant crop and the first ratoon.

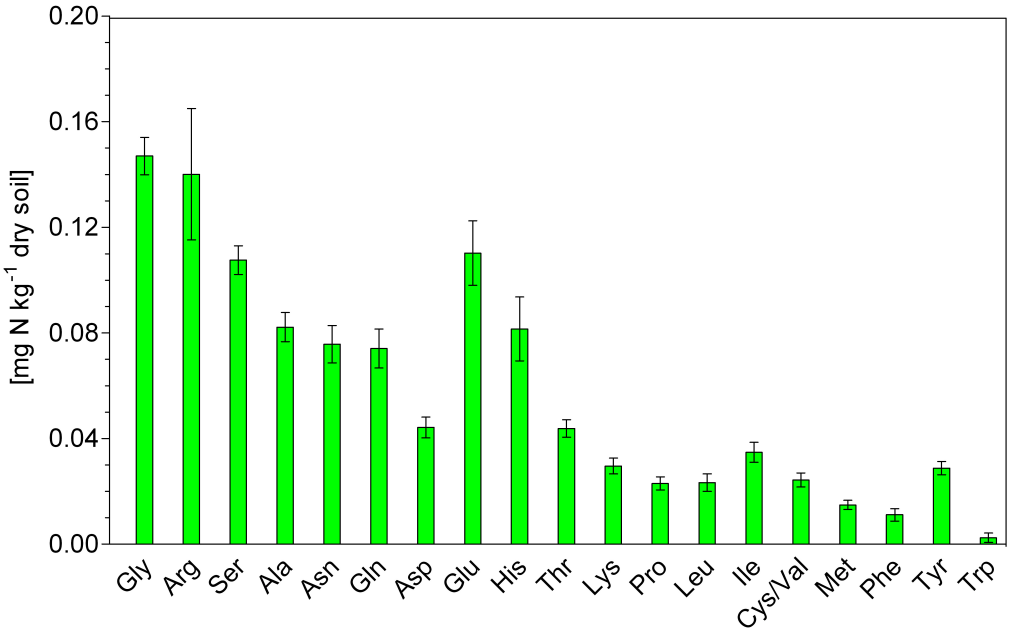


Fig. 20: Average concentrations (± s.e.) of soluble soil amino acids over all sampling dates for the upper 40 cm of the soil profile under Cane+Till+N.

Table 3: Significant effects of management on the availability of ammonium and nitrate during 2007 and 2008. There was no effect of management on amino acid concentrations.

Date	Soil layer/ N compound	Compared treatments/ Concentrations of N compounds [mg N kg-1 dry soil]	
Nov 07	**Topsoil**	**Tillage + Break crop**	**All other***
	Nitrate	8.58±1.28	3.08±0.41
	Subsoil	**Tillage**	**Zero-Tillage**
	Ammonium	8.75±1.85	2.10±0.46
	Nitrate	9.84±2.03	2.06±0.25
Dec 07	**Topsoil**	**Full N**	**Reduced N**
	Ammonium	34.32±8.04	3.51±0.87
	Topsoil	**Tillage + full N**	**Zero-Tillage + full N**
	Ammonium	17.85±7.00	48.05±12.84
	Topsoil	**Tillage**	**Zero-Tillage**
May 08	Nitrate	1.18±0.16	0.79±0.04
Jul 08	Nitrate	2.13±0.16	1.51±0.10

* includes Tillage + Monoculture, Zero-tillage + Break crop, Zero-Tillage + Monoculture

The urea fertilisation caused expectedly a peak in soil ammonium concentrations, which was however short-lived. Tillage increased the availability of N in the subsoil (Nov 07), led in combination with legume break cropping to higher nitrate concentrations in topsoil probably by supporting the decomposition of N-rich soybean biomass (Nov 07) and seemed to accelerate the plant uptake or loss of ammonium after the urea fertilisation as compared to zero-tillage (Dec 07; **Tab. 3**). Nitrate concentrations were higher in tilled soils as compared to zero-tilled plots not only in November 2007, but also in May and July 2008, however the differences are low and no differences were observed during the during six month before (Dec 07 to Apr 08).

Most management actions resulted in responses of the soluble soil N pool but effects ceased before or during the wet season. Although tillage caused the most effects on N availability, it had no effect on sugarcane yields (Bell et al., 2010). The nutrient release from previously inaccessible soil aggregates due to tillage maybe irrelevant for the crop, because the sugarcane was planted only one month after tillage and was likely incapable of taking advantage of these nutrients when their availability was highest.

In contrast, the full N fertilisation and legume break cropping increased yields as compared to reduced N fertilisation and sugarcane monoculture. The urea addition increased yields as compared to plots with reduced N fertilisation, but the size of this yield advantage attests very poor N use efficiency. The applied 129 kg urea-N ha^{-1} yielded only an additional 7-17 tons sugarcane ha^{-1} (Bell *et al.*, 2010). A significant part of the urea application was probably lost during the wet season via N gas production and the sugarcane crop was reported to be N limited even on fully fertilised plots (Bell *et al.*, 2010).

A yield advantage of 11-32 tons ha^{-1} in the plant crop and a higher crop N acquisition of ~5-20 kg N ha^{-1} were observed after legume break cropping (Bell *et al.*, 2010). We did not observe increased N availability in May 2007, but higher nitrate concentrations were measured at conventionally tilled break crop plots in November 2007, thus 6 months after the end of the break crop period. On the same plots, the additional acquisition of N by the sugarcane was highest, however the yield advantage over tilled monoculture was lower (11-13 tons ha^{-1}) than the additional yield produced by zero-tilled break crop management over zero-tilled monoculture (22-32 tons ha^{-1}; Bell *et al.*, 2010). These observations indicate that the yield advantage after legume break cropping is not mainly due to a higher availability of N and other legume break crop effects should be considered more relevant. Legumes may decrease pathogen levels, “fertilise” the soil with hydrogen during biological dinitrogen fixation and have other positive effects on subsequent yields (Dong *et al.*, 2003; Kirkegaard *et al.*, 2008).

It should be highlighted that the significant amounts of N of the mulched soybeans were to a large extent not recovered by the sugarcane plant crop. Bell *et al.* (2010) determined that 210 kg N ha^{-1} was contained in soybean trash at the soil surface, a further 42 kg N ha^{-1} can be estimated to be contained in belowground biomass (~20% of total N; Laberge *et al.*, 2009). Thus, 250 kg N from soybean plus 20 kg N ha^{-1} from the starter-N fertilisation have to be compared with 47-63 kg N ha^{-1} acquired by the subsequent sugarcane crop, and an excess of 220 kg N are therefore unaccounted for. On break crop plots with full N fertiliser application, the imbalance between N supply and cane-N acquisition is even more worrying: ~400 kg soybean-N and urea-N produce 60-85 kg sugarcane biomass-N (Bell *et al.,* 2010). It appears likely that some of the soybean-N will be immobilised by the soil, but numerous reports about elevated nitrous oxide emissions from decomposing soybean mulch (Millar *et al.*, 2004; Gomes *et al.*, 2009; Golding & Dong, 2010) raise concern that a considerable proportion of soybean-N is emitted as nitrous oxide. As for tillage, there was a significant lack of synchrony between N release from the break crop trash and the N demand by the crop. Bell *et al.* (2010) showed for zero-tilled break crop plots that 80% of soybean surface trash-N was already depleted at the time of sugarcane planting, thus the sugarcane crop was likely unable to take advantage of ~170 kg N ha^{-1} released from soybean mulch.

With respect to the supply of N nutrients to the sugarcane crop, the application of the new farming system is advantageous in economic and environmental terms as compared to the conventional management. While tillage increased N availability, this increase had no positive effect on yields. The full N fertiliser application increased yields, however the N use efficiency was extremely poor and a significant reduction of N fertiliser should be considered for environmental and economic reasons. Legume break cropping produced a clear yield advantage in the plant crop, but not primarily caused by increased N availability. However, a better synchrony of N release from legumes and sugarcane N demand by changing harvest and planting dates, dramatic reductions in N application rates, and/ or the replacement of

legumes with non-legumes as break crops may significantly reduce environmental impacts of N losses, while maintaining yield benefits.

At the Bundaberg sites, the availability of soluble N forms (ammonium, nitrate, amino acids) and organic precursor (soil proteins) was investigated over the temporal course of a sugarcane plant crop (**Fig. 21, 22**). Mean concentrations ranged between 0.44-7.03 (amino acids), 0-52.3 (ammonium), 0-42.4 (nitrate) and 0.34-12.5 mg N kg^{-1} dry soil (protein) in topsoil and subsoil over the sampling period. Ammonium and nitrate dominated the soluble soil N pool of the sugarcane sites after fertilisation, whereas the soluble N pool of forest soil consisted mainly of organic N. The inorganic N concentrations reached pre-fertiliser levels at the agricultural sites in February 2008, thus 2-3 months after fertiliser application. Soil amino acid concentrations were lower at the agricultural sites as compared to the forest and similar between both sugarcane systems (**Tab. 4**).

In contrast, the concentrations of ammonium and nitrate were lowest in forest soil compared to sugarcane soils. Soil ammonium concentrations were different between both sugarcane sites due different N fertiliser types and fertiliser application dates. The concentrations of all investigated N compounds were usually lower in subsoil, however, differences in N availability between sites were reflected in both soil layers.

Averaged across all sampling dates, concentrations of soil proteins were significantly lower in the new farming system than under conventional sugarcane management and forest (**Fig. 22, Tab. 4**). This is an interesting finding which warrants further analysis across more soils because it could indicate that the new farming system diminishes the soluble pool of nitrogen which would be available for microbial breakdown to deliver N to the crop. There may be other reasons though. The sensitivity of the used Bradford assay for protein detection is dependent on the protein composition of the investigated samples (Sapan *et al.*, 1999), and different soil protein composition can affect the protein quantification. Differences in soil pH can

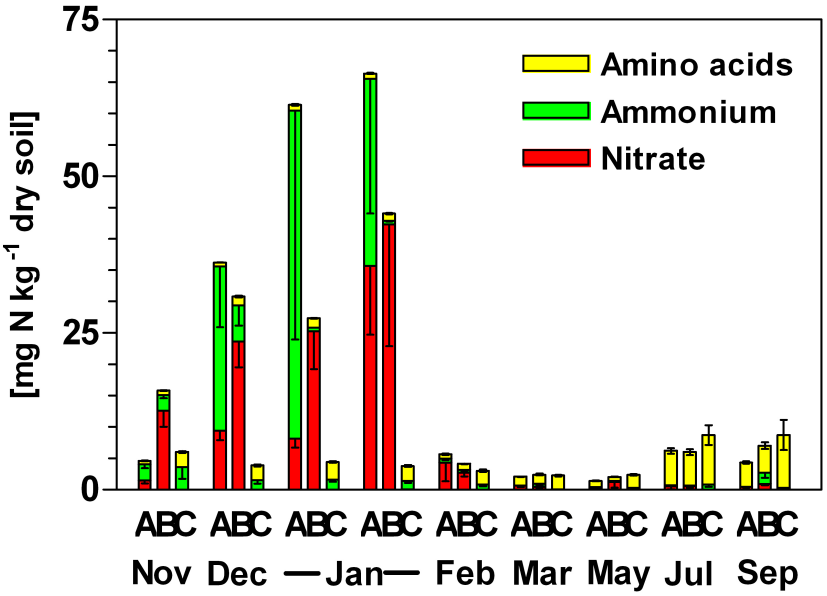


Figure 21: Soluble soil N compounds in the topsoils of the three Bundaberg sites between November 2008 and September 2009. A – Conventional sugarcane; B – Sugarcane new farming system; C – Forest.

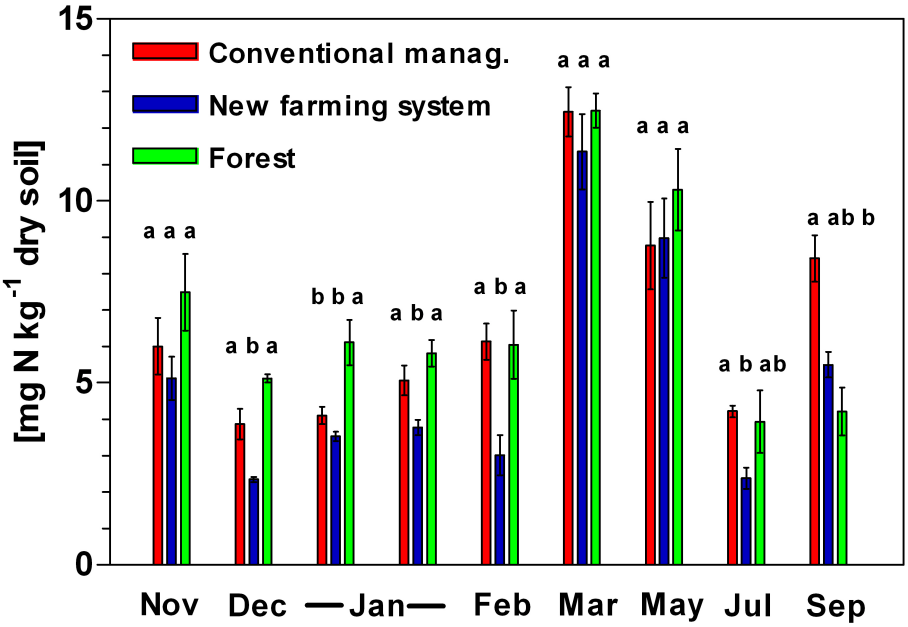


Figure 22: Concentration of soluble soil protein (±s.e.) in topsoils of the Bundaberg sites between November 2008 and September 2009. Different letters indicate significant differences between sites on the same sampling date ($p<0.05$).

change protein concentration at the two sugarcane sites (**Fig. 5**) since organic matter is less soluble under low pH (Kalbitz *et al.*, 2000; Marschner & Kalbitz, 2003) and precipitates with aluminum or iron (Scheel *et al.*, 2007, 2008), which could stabilise organic substances against microbial degradation at the conventional site. Similarly, complexing with tannins may protect proteins from degradation in forest soil (Kraus *et al.*, 2003). In summary, the lower protein concentration in soil of the new farming systems warrants further investigation to determine how soil protein concentrations are linked to plant-available N.

Table 4: Average concentrations (± s.e.) of soluble amino acids and proteins in soil of the Bundaberg sites. Different letters indicate significant differences between sites within each soil depth ($p<0.05$).

	Conventional sugarcane [mg N kg^{-1} dry soil]	**Sugarcane new farming system** [mg N kg^{-1} dry soil]	**Forest** [mg N kg^{-1} dry soil]
Soil proteins			
Topsoil	6.56 ± 0.45 ^{a}	5.11 ± 0.48 ^{b}	6.83 ± 0.46 ^{a}
Subsoil	2.26 ± 0.22 ^{a}	1.42 ± 0.18 ^{b}	3.21 ± 0.35 ^{a}
Soil amino acids			
Topsoil	1.71 ± 0.25 ^{b}	1.95 ± 0.25 ^{b}	3.38 ± 0.34 ^{a}
Subsoil	1.64 ± 0.24 ^{b}	1.44 ± 0.19 ^{b}	2.33 ± 0.32 ^{a}
Soil ammonium			
Topsoil	12.46 ± 5.14 ^{a}	1.38 ± 0.43 ^{b}	1.15 ± 0.26 ^{b}
Subsoil	1.23 ± 0.26 ^{a}	0.83 ± 0.20 ^{ab}	0.41 ± 0.06 ^{b}
Soil nitrate			
Topsoil	6.76 ± 2.00 ^{a}	12.20 ± 3.01 ^{a}	0.02 ± 0.01 ^{b}
Subsoil	3.66 ± 0.53 ^{a}	4.50 ± 0.67 ^{a}	0.02 ± 0.01 ^{b}

In addition to soil investigations, biomass and N acquisition of the sugarcane crop were monitored (**Fig. 23**). We only took small biomass samples, and the data therefore cannot be extrapolated to calculate total crop N accumulation per hectare. However, nearly 50% of total biomass-N was acquired after the end of the wet season (late January), when the high concentrations of fertiliser-derived inorganic N decreased to pre-fertiliser levels (see **Fig. 21**). This finding highlights the contribution of soil-derived N to the crop N demand, either from longer or shorter term N-stores. Organic N is the main soil N reservoir and the likely source of N for the crop once the high concentrations of inorganic N post-fertiliser application have been depleted. Our investigations also show that soil N reserves have to be managed carefully, e.g. via trash retention, since biomass-N of one sugarcane crop corresponds to ~15% of total N in the biologically important topsoil layer (**Fig. 24**). Although sugarcane roots can access deeper soil layers, the topsoil is most important for plant nutrition, because overall N availability declines steeply with depth (**Tab. 5**).

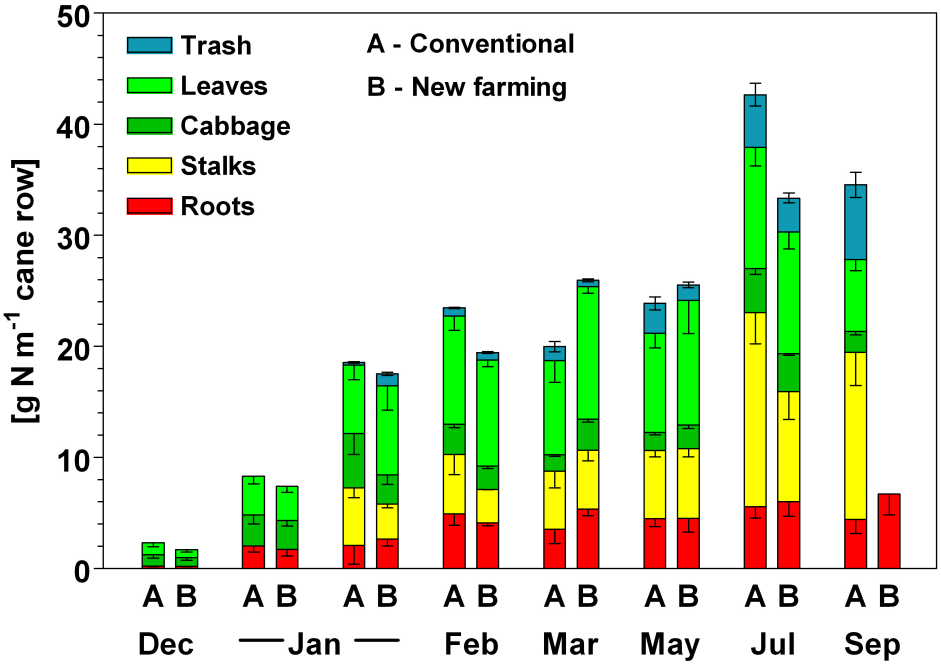


Fig. 23: Crop N accumulation across the growing season at Bundaberg. Shown are averages (with ±s.e.) for three replicates per date and site. Crop N acquisition was similar between both managements on all sampling dates ($p<0.05$). The new farming site was harvested prior the September sampling.

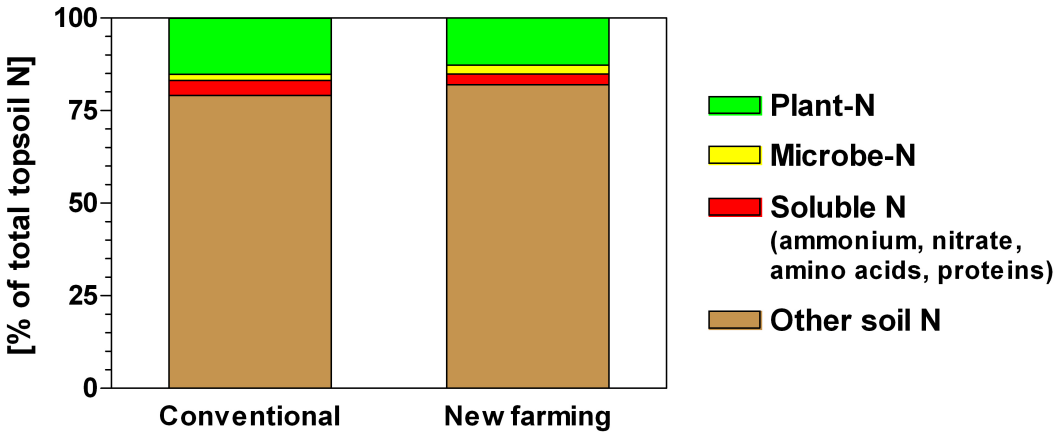


Fig. 24: Proportional amounts of N in different pools compared to total N in topsoil (0-20 cm depth) of both sugarcane sites in Bundaberg. Given are averages over the crop cycle, in case of plant biomass the amount of crop-N at harvest. Estimations based on 2 m x 0.5 m sugarcane row.

Final yields for both sites were 104.8 tons (conventional) and 90 tons fresh weight ha^{-1} (new farming), with a similar CCS of 13.13 and 14.08. Accounting for the different row distances of the two systems, yields were similar per unit cane row (1.6 tons fresh weight per 100 m cane row). This shows that a similar yield was achieved in the new farming system with ~30% less synthetic N fertiliser application.

5.6 Soil microbial nitrogen turnover and nitrous oxide generation

Microbial gross turnover rates of ammonium and nitrate in the soil were determined at the three Bundaberg sites for eight time points (Dec 2008, early and late Jan, Feb, Mar, May, Jul and Sep 2009) to assess how different sugarcane management and land use affects ammonification and nitrification processes in the soil. Both processes are cogs of the soil N cycle and affect the availability of N for plants, but also losses of N from the soil. Nitrogen turnover rates were determined using the ^{15}N-pool dilution technique (Murphy *et al.*, 2003), in which changes in size and isotopic ratio of labelled soil ammonium and nitrate pools determine microbially mediated gross N fluxes.

At the sugarcane sites, gross ammonification and nitrification rates were highest in December 2008 and early January 2009 (**Fig. 25**), coinciding with elevated soil ammonium and nitrate concentrations following N fertilisation. During the remainder of the sampling period, rates of both processes were low and in range of gross ammonification and nitrification rates of the natural forest system. Average gross ammonification rates between December 2008 and September 2009 were 3.4, 10.3 and 2.5 mg N kg^{-1} dry soil day^{-1} at conventional, new farming and forest sites, respectively. Average gross nitrification rates were 6.5 (conventional), 12.6 (new farming) and 1.3 mg N kg^{-1} day^{-1} (forest). Thus, ammonification rates were similar at all sites, while nitrification rates were significantly higher at sugarcane sites (**Tab. 5**) indicative of a higher nitrification potential in agricultural soil. In December, significantly higher gross ammonification rates were observed at the new farming site compared to conventional site, but this is likely due to different timing of irrigation and fertilisation (conventional site: just fertilised and irrigated; new farming site: fertilised one month earlier; dry).

With few exceptions (conventional: Mar 09; new farming: Sep 09), ammonification and nitrification rates were statistically similar at both

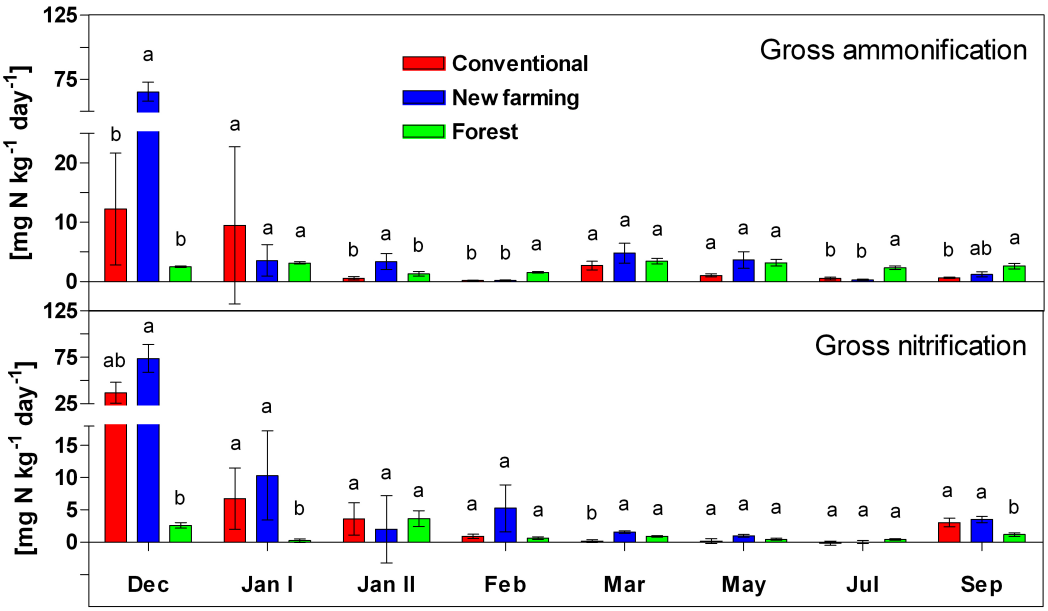


Fig. 25: Average gross ammonification and nitrification rates (±s.e.) in the soil of the three Bundaberg sites over the sugarcane cultivation cycle (December 2008 to September 2009). Letters below bars indicate significant differences between sites separately for each sampling day ($p<0.05$).

Table 5: Average gross ammonification and nitrification rates (±s.e.) at Bundaberg sites Different letters within columns indicate significant differences ($p<0.05$). Data are averages of nine (Dec-Sep) measuring times.

Dec-Sep	Average gross ammonification rates	Average gross nitrification rates
	[mg N kg^{-1} dry soil day^{-1}]	
Conventional system	3.4±2.0 ^{a}	6.5±2.3 ^{a}
New farming systems	10.3±3.2 ^{a}	12.6±4.1 ^{a}
Forest	2.5±0.2 ^{a}	1.3±0.2 ^{b}

sugarcane sites at a given sampling date. In contrast, ammonification rates were significantly higher than nitrification rates at the forest site, with the exception of December 2008 and late January 2009, when the rates of both processes were similar. These results indicate that available ammonium,

delivered from break-down of organic matter or N fertiliser, is rapidly converted to nitrate in both sugarcane soils. In contrast, >50% of available ammonium is immobilised in the forest soil, either through biological (uptake by microbes or plants) or abiotic processes (binding to soil exchange sites) confirming that N turnover in forest soil differs from sugarcane soils. Both sugarcane management systems were characterised by similar N cycles

Ammonification and nitrification rates were positively correlated with soil ammonium and nitrate concentrations and electrical conductivity (indicator for nutrients), but not with other environmental parameters (soil moisture, amino acid concentrations, texture, pH, carbon and N content). There was no correlation with soil microbial biomass (data not shown), which was determined in the same soil samples. Thus, any observed differences in soil microbial biomass at the Bundaberg sugarcane sites are not affecting microbial turnover rates of inorganic N.

During nitrification, N gases including nitric oxides (NO_X) and nitrous oxide (N_2O) can be produced and lost from soils which is of major significance because N is lost and gases of major environmental concern are produced. Nitrate is susceptible to leaching and may lead to aquifer pollution, but is also the precursor for denitrification. Denitrification is a microbially mediated chain of processes that convert nitrate into various N gases (NO_X, N_2O and dinitrogen, N_2; **Fig. 11**) under oxygen limitations. Apart from requiring the presence of nitrate, denitrification requires a high moisture status and availability of carbon, conditions which are provided by strongly fertilised agricultural soils with high organic matter retention including roots and trash and that receive high rainfalls or irrigation. Thus, production of greenhouse gas nitrous oxide is likely to be affected by management supplying different N fertiliser amounts, tillage, irrigation methods, and type and retention of trash. To complement the research on soil biology and management, trace gas measurements were conducted at the Bundaberg sites during each sampling trip (4 measuring positions per site; **Fig. 26**).

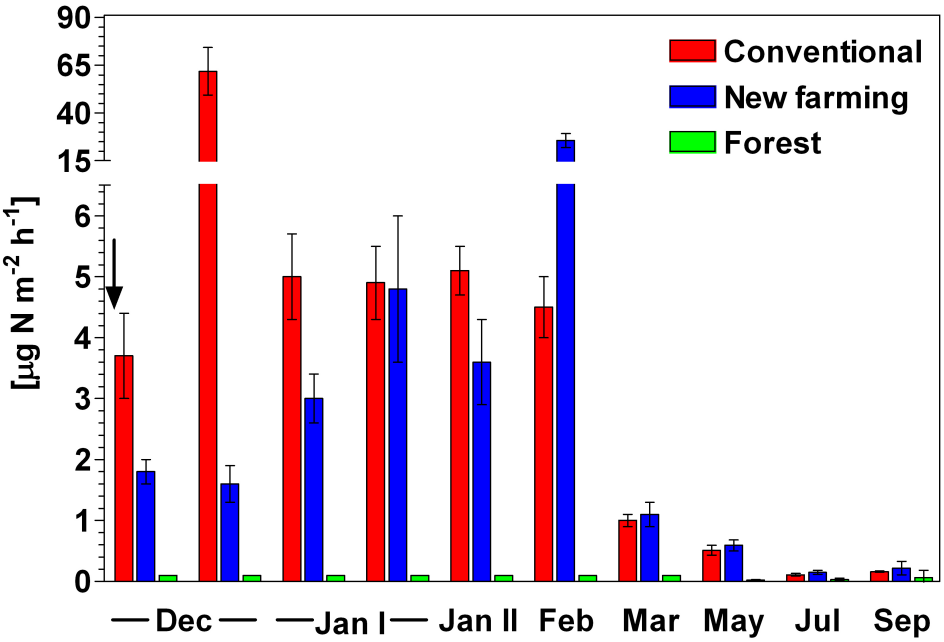


Fig. 28: Average nitrous oxide fluxes (with s.e.) at the Bundaberg sites. In December 2008 and early January 2009, nitrous oxide was measured on two successive days. Arrow indicates furrow irrigation at the conventional site, applied shortly after the N fertilisation.

Averaged over all sampling times, nitrous oxide fluxes (with s.e.) were 8.9±3.1, 4.3±1.2 and 0.1±0.0 µg N m^{-2} h^{-1} at the conventional, new farming and forest sites, respectively. Although all three sites were sources for N_2O, the forest site had significantly ($P<0.05$) lower emissions than both sugarcane systems. Average emissions from both sugarcane systems were similar, despite strong flux differences on some sampling occasions, which were likely to be caused by different timing of management, including fertilisation and irrigation, at the two farming systems. N_2O emissions were correlated with soil moisture and inorganic N (ammonium + nitrate) concentrations (**Fig. 29**).

In summary, no obvious differences in N_2O emission were detected in the two sugarcane soils. Fluxes were in the lower range of previous observations from sugarcane fields (e.g. 3-1100 µg N_2O-N m^{-2} h^{-1}; Allen *et al.*, 2010). More detailed measurement campaigns would be required to calculate annual estimates for the two farming systems including high-frequency sampling

intervals during the 2-3 months post fertilisation when most trace gas emissions occur (Allen *et al.* 2010 and papers cited therein).

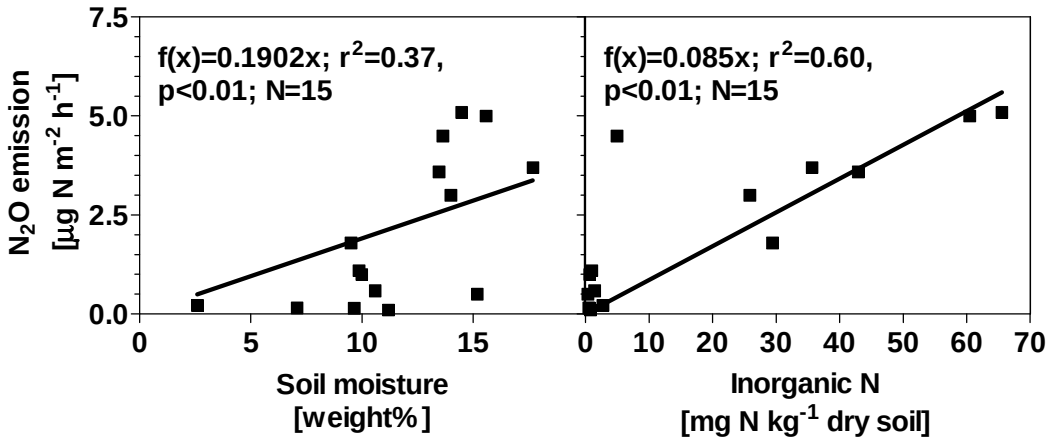


Fig. 29: Linear regression analysis between environmental parameters and N_2O emission for the sugarcane sites in Bundaberg (10 measuring times, 4 replicate measurements per site).

5.7 Link between carbon and nitrogen cycles: trace gas exchange and soil organic matter

Carbon and nitrogen cycles are intricately linked. In soils, organic C compounds provide the energy for microbial activity and their availability thus affects microbial turnover of N or vice versa. Two aspects of carbon-nitrogen interactions were investigated at the Bundaberg sites: the exchange of greenhouse gas methane and the availability of soil organic matter as light fraction carbon.

We chose to study methane because it is the second most potent greenhouse gas after nitrous oxide and can be measured simultaneously with N_2O. Most aerobic soils of natural ecosystems are sinks for methane (Dalal and Allen 2008) as the result of the activity of methanotrophic bacteria, which oxidise methane as a source of energy. In agricultural soils methane consumption is often compromised due to the addition of urea or inorganic N fertilisers (see below). Low methane sink strength was observed in sugarcane soil at the Bundaberg sites (**Fig. 30**).

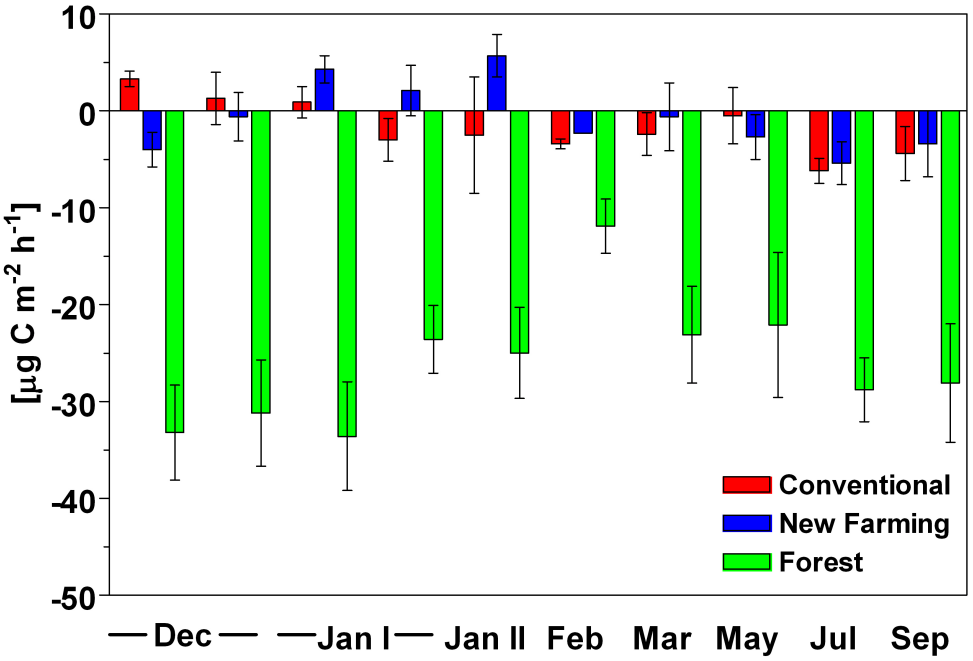


Fig. 30: Average methane fluxes (with s.e.) at the Bundaberg sites.

Mean methane fluxes (with s.e.) were -1.6±0.8, -0.6±0.9 and -25.1±1.9 µg C m^{-2} h^{-1} for conventional, new farming and forest soils, respectively. Overall methane fluxes were similar at the sugarcane soils and a significant methane uptake was observed only into forest soil.

Several reasons have previously been identified which cause the reduction of methane uptake associated with agricultural soils, including (a) ammonium can compete with methane for the microbial enzyme methane monooxygenase, which oxidises ammonium as well as methane; (b) the intermediate compound hydroxylamine (**Fig. 11**) is toxic for methanotrophic bacteria, and (c) fertilisation causes salt stress to which methanotrophic bacteria are particularly sensitive (Bodelier & Laanbroek, 2004). Additionally, it has been proposed that changes in the microbial community composition may be responsible for the reduction of methane uptake, but evidence for this notion is still missing (Bodelier & Laanbroek, 2004). At the sugarcane sites, we observed no significant recovery of soil methane uptake even four months after the soil N pool was depleted of readily-available fertiliser-N, thus other reasons than solely the inhibition, salt or toxicity effects are likely to be involved in the loss of methane uptake. It is conceivable that long-term land-use has caused changes in the community of methanotrophic bacteria.

To address this question, and to explore the diversity of methanotrophic bacteria in sugarcane soils, the community composition of methanotrophic bacteria was investigated, using primers for the 16S rDNA-gene (Chen *et al.*, 2007) and subsequent DGGE analysis. Two types of methanotrophic bacteria (Type I & II) were investigated, which are commonly present in aerobic soils (Hanson & Hanson, 1996). Amplicons were cloned and sequenced. No differences in the community composition of the investigated microbial groups were observed (**Fig. 31**).

The similarity in the composition of the microbial community of methanotrophic bacteria type I and II indicates that these organisms are not responsible for the decreased methane uptake at the two sugarcane sites.

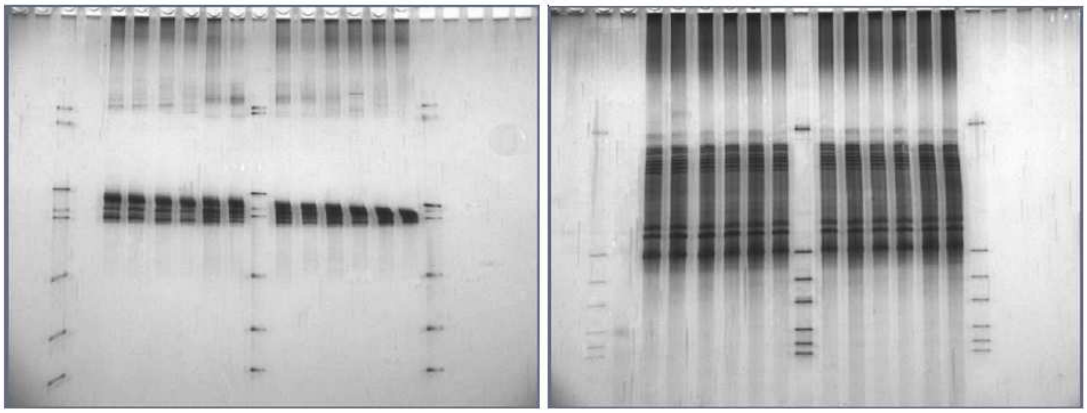


Fig. 31: DGGE analysis of the community composition of methanotrophic bacteria type I (left) and type II (right). On each image, the first four lanes are from the conventionally managed site, followed by two + two lanes from the new farming site (standard in-between) and the last four lanes contain samples from the forest site (from left to right).

The amounts of methanotrophic species may differ between sites, but this could not be assessed further because the 16S rDNA-target gene is a multi-copy gene (several copies per microbe) preventing its use for quantification of methanotrophic microbes. Other studies have suggested that the methane uptake in forest soils may be carried out by unknown microbial species that are only distantly related to the known methanotrophic bacteria (Henckel et al., 2000), and further research has to verify this notion.

Soil organic matter (SOM) is key to soil quality and regulates soil structure, moisture and microbial activity (McLaughlan, 2006). Agricultural management can decrease or increase SOM contents via input or removal of plant material (biomass retention, crop removal) by disturbing the physical structure (tillage) and increasing wind and water erosion. These mechanisms affect the availability of nutrients, their accessibility to microorganisms and plants, microbial turnover and loss from or retention in the agricultural system (McLaughlan, 2006).

For quantification, SOM is separated into mineral-associated SOM and physically uncomplexed SOM (Gregorich & Beare, 2008). Uncomplexed SOM is most sensitive to land management and important for microbial activity and was studied here. A total of 45 samples from soil pits at the three Bundaberg sites were sieved (6 mm) and subjected to a density fractionation using lithium polytungstate (LST; Heavy Liquids) with a density of 1.6 g cm^{-3}. The floating material ("light fraction"; LF) was removed and quantified. The remaining solution underwent ultra-sonication to release organic material occluded in soil aggregates; however this step released extremely fine charcoal from soil aggregates, which could not be recovered from the liquid and measurement of this fraction and the re-use of the heavy liquid (which is very expensive) for further fractionations were impossible.

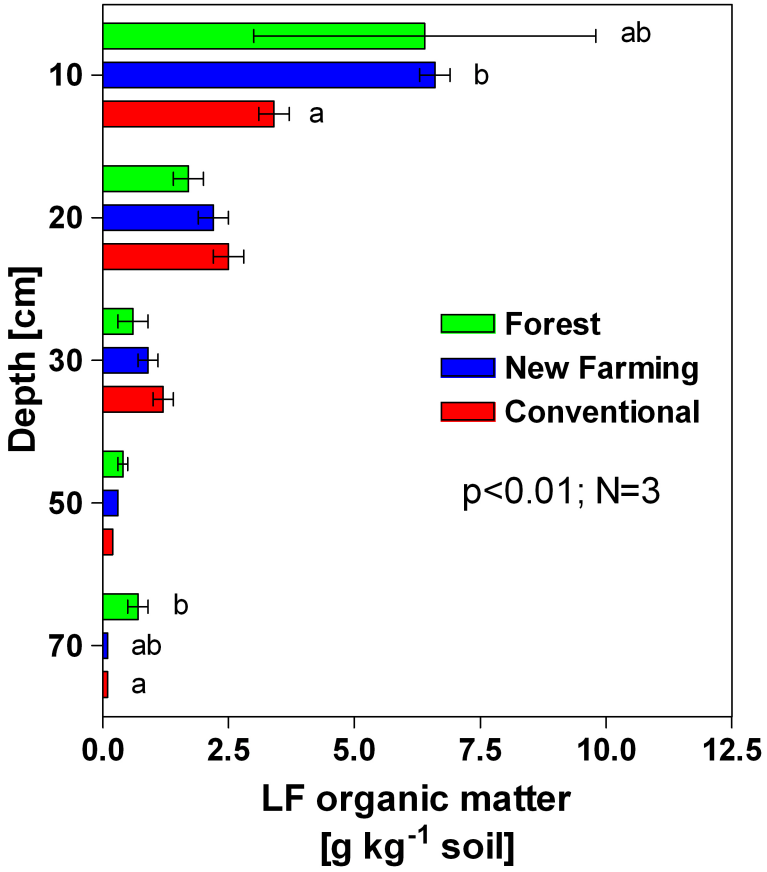


Fig. 32: Quantities of physically uncomplexed soil organic matter (light fraction, with s.e.) from 10 to 70 cm soil depth at Bundaberg sites.

Average amounts of LF matter (±s.e.) were in a range of 0.1 to 6.6 g kg^{-1} dry soil and decreased significantly over depth at all sites (**Fig. 32**). The amounts in the upper 20 cm depth are significantly higher than those in subtropical hoop pine plantations (~0.2 g kg^{-1}; Richards *et al.*, 2007), but lower than in other forest ecosystems in Queensland (14.3-43.4 g kg^{-1}; Golchin *et al.*, 1994). However, amounts of LF matter are in agreement with previous observations from pasture and native grassland soils in Queensland (5.9-8.8 g kg^{-1}; Golchin *et al.*, 1994).

The amount of LF matter between the two sugarcane sites was similar at all depths apart from the top 10 cm of the soil profile where the new farming site had significant higher amounts of LF matter than the conventionally managed site. The new farming site had LF matter contents similar to the forest site in the uppermost soil layer (10 cm). Apart from the 70 cm depth where the forest soil contained significantly higher amounts of LF matter than the conventionally managed site, forest soil and both sugarcane soils had similar LF contents. Although the soil pits are un-replicated due to labour constraints, the observations indicate that (i) the three soils are comparable with respect to LF levels, and (ii) sugarcane management affects only the upper 10 cm of the soil, with the new farming system retaining more LF material than the conventional system. Most of LF material consists of recent plant residues and the new farming system therefore contains more readily decomposable material to support the activity of heterotrophic microorganisms, and supplying or binding nutrients of LF may be important for the storage of carbon in soil (Gregorich *et al.*, 2006). Future research should examine carbon relations, including the fate of LF material and overall carbon fate in Australian sugarcane soils to assess how management, climate and soil type impact soil C relations.

5.8 Synthesis

The investigations of this project have shown that sugarcane management affects soil biology. Microbial biomass, the community composition of N-cycling microorganisms and microbial gene expression were affected by management, but had little or no effect on the availability, cycling, temporal crop acquisition or losses of N from sugarcane soils. When responses to management occurred, e.g. with respect to the availability of inorganic N due to tillage, N fertilisation or soybean break cropping, this had either no effect on sugarcane yields (tillage), the effect was small (N fertilisation), or the effect was primarily not related to N (legume break cropping).

Based on our observations, the new sugarcane farming system is superior to conventional management and practices (tillage, no break crop, full N fertilisation), with better general nutrient status and less soil degradation, more microbial biomass, higher microbial diversity and more light fraction organic matter in topsoil. Yields were similar with a reduced N fertiliser application as compared to high N fertilisation rates. However, both systems were similar when considering the negative effects of low uptake of methane into soil and considerable emissions of N_2O.

The investigations here suggest that, with respect to the supply of N to the crop, currently recommended N fertiliser rates can be further reduced. If tillage and legume break cropping are applied to increase N availability for sugarcane plants, the timing of N supply and plant N demand should be more closely matched (Crews & Peoples, 2005), e.g. through application of tillage and break crop mulching just prior to planting the subsequent sugarcane crop. Legume intercropping rather than break-cropping may also be considered, if possible, to ensure consistent N supply during the early stages of sugarcane development although benefits of N supply and greater water demand have to be considered. Legumes may be shaded out by the mature sugarcane and N will be released from the legume trash.

Environmental concerns about increased nitrous oxide emissions during the decomposition of legume mulch may lead to the use of non-legume break crop if similar yield advantages for subsequent sugarcane crops are the key consideration. Since nitrous oxide emissions from decomposing mulch are strongly related to the residue-N content (Millar *et al.*, 2004), the harvest of the soybean grains can also be considered, because they contain up ~90% of total soybean biomass-N (Toomsan *et al.*, 1995). Use of legumes with less easily degradable plant material could also be considered, such as tannin- and lignin-rich species.

6. Intellectual Property and Confidentiality:

The molecular tools developed for soil microbiological analyses are based on generic gene technology platforms developed for biomedical and biotechnological uses. No protectable intellectual property has been developed this project.

7. Environmental and Social Impacts

The research conducted in this project will have no adverse environmental impact.

8. Expected Outcomes

The main outcomes are:

- Microarray-based analysis demonstrates that microbial gene expression can differ between different sugarcane managements, although insufficient resolution of identified genes prevented comprehensive functional analysis. Greater resolution will be achieved with pyro-sequencing and initial results from this analysis are imminent.

- Techniques for soil biology research have been advanced and will facilitate unprecedented insights into soil biological processes. Further fine-tuning is required to take the new pyro-sequencing approach to application stage, and some of this research is occurring as part of the on-going PhD project.

- Although management affected the presence and community composition of soil microbes, only minor differences were observed in N cycling, N availability and sugarcane yield in the studied soils. Ammonium and nitrate turnover was similar in the new and conventional farming systems, which demonstrates that long-term

differences in management do not fundamentally alter the soils' capacity to cycle N. However, more research is needed to quantify N retention and N losses as result of different management. We conclude that new and conventional management results in similar N and C processes and additional management options should be considered to improve N retention and greenhouse gas relations.

- The new farming system in Bundaberg had more light fraction organic matter in the uppermost soil layer (0-10 cm), which may be of importance for improved carbon sequestration, but could also fuel microbial activity and result in reduced C sequestration into soil. Soils of new and conventionally managed sites in Bundaberg had low uptake capacity for the potent greenhouse gas methane and alternative management approaches should be developed to support a greater methane sequestration.

9. Future Research Needs and Recommendations

The following topics are recommended for further research

- Linking soil biology research with the research needs and recommendations listed below to gain causal understanding of processes in sugarcane soils

- Identification of mechanisms through which legumes improve sugarcane yield, since the positive effect of legume-break crops are not solely related to improved N availability

- Better synchronisation of N availability in soil and plant N demand including release of N from break-crop. Legume intercropping with sugarcane should be considered to avoid rapid N loss due to mismatch of N demand of crop and conversion of trash into N forms vulnerable to loss

- Investigate use of other N forms and soil amendments for their suitability to supply N and other nutrients to the sugarcane crop. Comparison of environmental footprints of alternative N forms compared to currently used fertilisers (urea, inorganic N) should be a priority

- Detailed analysis of the timing, form and quantity of N acquired by sugarcane crop from soil, and assessment of the competition between crop and soil microorganisms for N and other nutrients. Comprehensive analysis of the fate of applied N fertiliser in sugarcane agro-ecosystems

- Identification of best management to minimise nitrous oxide emissions from sugarcane soils and to maximise sequestration of methane into sugarcane soils

- Short- and long-term monitoring of soil carbon stocks under different managements and the interactions with N cycle.

10. List of Publications

McGrath, K.C., Mondav, R., Sintrajaya, R., Slattery, B., Schmidt, S. & P.M. Schenk (2010) Development of an environmental functional gene microarray for soil microbial communities. *Applied and Environmental Microbiology*, accepted for publication.

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12. Appendix

Abbreviations

DNA	Deoxyribonucleic acid
DGGE	Denaturing gradient gel electrophoresis
cDNA	Complementary deoxyribonucleic acid
mRNA	Messenger ribonucleic acid
LF	Light fraction carbon, soil organic matter derived from density fractionation
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid