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Improving management practices of legume crop residues to maximize economic and environmental benefits

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ABSTRACT

Growing a soybean break crop during the fallow period between two cane crop cycles could add 70-280 kg nitrogen/ha into the cropping systems. About 74-88% of the soybean biomass nitrogen derived from biological N_2 fixation and 22-26% from soil nitrogen recovery.

Mineral nitrogen accumulated in bare soil during the fallow period could be lost substantially following high rainfall events. Legume crop rotation provided an effective means to retain the soil mineral nitrogen in plant biomass. Compared to bare fallow, legume cropping significantly increased abundances of total archaeal and bacterial microbes, but decreased abundances of the nitrifying microbes and the *amo*A gene related to nitrification.

Soybean crop residues contained two to three times more nitrogen than peanut crop residues. However, with grain or pod harvest, farming profit was markedly higher for growing peanut than soybean thanks to higher market values of peanut pods.

Rapid release of legume residue nitrogen occurred in the first 2-3 months after harvest or spray out. Nitrogen release was significantly faster from the legume residues incorporated into soil than those retained on the soil surface. If sugarcane is not planted for a prolonged period after the legume cropping, no-till could help slow down nitrogen release thus reduce the risk of nitrogen loss after rainfall. Allowing volunteer soybean to re-grow after grain harvest or growing a nitrogen catch crop significantly decreased mineral nitrate accumulation in soil thus could potentially reduce nitrogen loss. The effects of legume residue management practices on sugar yield and crop nitrogen uptake varied with sites.

EXECUTIVE SUMMARY

Legume crop rotation in sugarcane cropping systems may add extra income from grain harvest. It can also improve soil health, add nitrogen (N) into soil through biological N fixation, and conserve soil mineral N in the biomass to reduce the risk of N loss into the environment during the fallow period. Legume residues generally release N rapidly, especially after tillage. If the available N is not taken up by the subsequent cane crops, it can be lost into the environment. As legume crop rotation is increasingly adopted in the Australian sugar industry, inefficient legume N use becomes a significant issue. This project aimed to improve our understanding of N status and dynamics under different fallow management practices and identify better legume residue management strategies to improve N use efficiency.

Field trials were conducted in the Herbert, Burdekin and Bundaberg regions from 2015 to 2017. Soybean crops were grown during the fallow period and then sprayed out as green manure at the Herbert and Burdekin sites. Soybean and peanut were planted with grain/pods harvested at the Bundaberg site. Following harvest or spray out of the legume crops, different legume residue and N fertiliser management scenarios were trialled in consultation with local growers and extension officers. A field litter bag incubation and a laboratory incubation were also undertaken to investigate soil N transformations in response to different management methods.

Results from the field trials indicated that growing soybean break crops during the fallow period could add 70-280 kg N/ha to the farming systems. The amount of soybean biomass N increased with increasing length of growth. Therefore, as long as site, weather and operations permit, the cropping season should be extended by sowing early and terminating late. About 74-88% of the soybean biomass N derived from biological N_2 fixation and 22-26% from uptake of soil N. The N inputs from soybean residues could save fertiliser cost by approximately $75-215/ha.

There was clear evidence that substantial amounts of mineral N in bare soil were lost following rainfall. Legume crop rotation provided an effective means to retain soil mineral N in plant biomass. In addition, 16S DNA analysis indicated that abundances of soil microbes were significantly higher in soybean or peanut cropping soil than in bare soil at the maximum biomass stage. However, abundances of nitrifying archaea and bacteria and the microbial gene related to nitrification were significantly lower in the legume cropping soil than the bare soil.

With grain or pod harvest, mature soybean crop residues contained 3 times more N (280 kg N/ha) than peanut residues (88 kg N/ha). However, farming profit was markedly higher for growing peanut than soybean thanks to higher market values of peanut pods. If circumstances do not allow the legume crops to grow to maturity, soybean is a better green manure crop because of its greater N benefit.

Rapid decomposition of soybean residues occurred in the first 2-3 months. Therefore, N-efficient management of legume residues is critical during the early months after termination of the legume crops. However, incorporation of legume residues caused 45-65% of the initial isotopically labelled nitrate in soil to be immobilised into non-exchangeable soil N pools after 302 days. Only 23% of the peanut residue N and 27-35% of the soybean residue N were counted in the exchangeable mineral N after 302 days of incubation at 25 ^{o}C and optimal soil moisture. These findings suggested that care should be taken to avoid overestimating N supply from legume residues.

No-till could slow down N mineralisation compared to tillage. If sugarcane is not planted for a prolonged period with possible high rainfall after legume cropping, no-till provides an effective strategy to reduce the risk of N loss. If cane is planted shortly after the legume cropping, tillage can improve N supply to the cane and may be of benefit.

Spraying nitrification inhibitor onto legume residues before tillage slowed down nitrification by at least 10-30 days. However, this did not translate into higher cane or sugar yield and crop N uptake.

Allowing volunteer soybean to re-grow after grain harvest significantly decreased mineral N accumulation in soil profile and thus could potentially reduce N loss. This technique also resulted in an increase in sugar yield by 2 t/ha. If sugarcane cannot be planted for a prolonged period during a high rainfall season, allowing the volunteer legume crop to grow or delaying spray out of the legume crop for green manure can minimise risks of N loss.

This project and the results have been communicated to end users through demonstrations on field days and presentations in the 2018 ASSCT conference. A research paper has been published in Scientific Reports. The research finding and the data sets should be of great value for refinement of the current nutrient management tools and guidelines for the Australian sugar industry.

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1. BACKGROUND

1.1. Benefits of legume crop rotation

Legume crop rotation has been recognised as a key measure for sustainable sugarcane production in Australia (Garside et al. 2005). Growing a grain legume during the fallow period between two cane crop cycles may provide an extra stream of income for cane growers. In addition, legume cropping can reduce the population of sugarcane root pathogens, add extra amounts of nitrogen (N) into soil through biological N fixation, and help conserve soil mineral N in the biomass that can otherwise be lost through leaching, lateral runoff or denitrification (microbial decomposition of nitrate into gases) during the fallow period (Garside et al. 2005). The legume biomass at maturity can contain 50-300 kg N per hectare, with 50-60% of the N coming from symbiotically fixed N and the remaining part from soil N uptake (Garside and Bell 1999). Therefore, following legume cropping during the fallow period, N fertiliser application to the subsequent plant cane can be substantially reduced or even eliminated. For example, the Australian Sugarcane Nutrient Management Guidelines, SIX EASY STEPS (6ES), recommends reducing fertiliser N application rate by 90 kg/ha for plant cane after a 'good' soybean crop with grain (6 t/ha) harvested (Schroeder et al. 2005).

1.2. Challenges with management of legume crop residues

While the 6ES takes into account the potential N contribution from legume residues, it remains difficult to predict the actual amount of legume N available to the cane. Dynamics of the crop residue N release into soil through microbial decomposition (mineralisation) are hard to predict due to variability in decomposability of different plant materials under different soils and weather conditions (Schroeder and Moody 2013). More importantly, substantial amounts of the mineralised legume N can be lost from soil (see below) before being taken up by the following cane crops. As a result, variable N benefits of legume cropping have been reported (Bell and Moody 2014).

With high N contents and low carbon (C) to N ratios (C/N), leguminous crop biomass is more readily mineralisable by microbes to release ammonium-N (NH_4^+-N) into soil than other crop residues (e.g. cane trash), especially after incorporation of the biomass into soil by tillage. Subsequently, the NH_4^+ is transformed into nitrate (NO_3^-), also by soil microorganisms, in a process known as nitrification. Nitrate is the most mobile form of N in soil because it is a negatively charged ion and cannot be adsorbed by negatively charged clay particles and organic matter. If not used by plants, nitrate can be easily washed out of the root zone through leaching or runoff. Nitrate ions are also highly susceptible to denitrification under wet (less aerobic) soil conditions upon heavy rainfall or irrigation (Wang et al. 2012;Weier et al. 1993). Denitrification is a microbial process in which nitrate is decomposed into gases such as nitrous oxide (N_2O) and dinitrogen (N_2). The wet and warm weather conditions of Australian sugarcane production regions combined with green cane trash blanketing (a source of labile carbon) are highly inductive to N losses through denitrification.

As legume crop rotation is increasingly adopted in the Australian sugar industry, inefficient utilisation of legume N becomes a significant issue across almost all sugarcane cropping regions. Weather conditions in the Australian sugarcane cropping regions often do not allow cane planting until several months after the legume crop maturity. During this period, there is essentially no plant uptake of soil mineral N (NH_4^+ and NO_3^-). As a result, the mineral N (particularly nitrate) produced from legume residue mineralisation can be easily lost upon heavy rainfall through leaching, runoff or denitrification (Bell et al. 2006;Garside and Berthelsen 2004). This situation with high risk of N loss due to N supply exceeding crop demand can continue until a few months after cane planting when the crop N uptakes are small. The N losses result in significant economic loss and serious environmental problems including mineral N pollution to water in the Great Barrier Reef (Kroon et al. 2016) and emissions of the potent greenhouse gas nitrous oxide (Wang et al. 2012;Wang et al. 2015).

1.3. Management options, knowledge gaps and project rationale

Improved legume crop residue management practices are required to minimise N losses and thus maximise the economic and environmental benefits of legume rotation in sugarcane cropping systems. Grain (e.g. soybean) harvest, if possible, helps reduce the amount of N retained in the legume residues and provides an extra stream of income for growers. Leaving the mature biomass standing, or slashing and retaining the legume crop residues on the soil surface with minimum or no tillage before cane planting, could slow down decomposition of the crop residues and thus reduce NO_3^- accumulation and potential losses before being taken up by the subsequent sugarcane crops (Bell et al. 2006;Garside & Berthelsen 2004). However, these strategies are not always satisfactory, and more innovative solutions are required because:

a. Grain harvest may not be feasible in some circumstances, for example, due to continued wet weather or low yield;

b. The crop residues remaining after grain harvest can still contain up to 200 kg N per hectare following a 'good' legume crop. Thus, large amounts of mineral N can still be produced from mineralisation of the legume N during the months before peak N demand by the sugarcane crop;

c. Leaving the legume crop residues on the soil surface may not be practicable in some circumstances such as where sugarcane beds have to be re-formed;

d. The surface management of legume crop residues may prompt N loss via ammonia (NH_3) volatilisation; and

e. In a dry year, decomposition of the legume residues laid on the soil surface may be too slow to release adequate amount of N for the subsequent sugarcane crop. A pot trial demonstrated that crop recovery of legume N was significantly lower after surface application compared to incorporation of the legume biomass before cane planting. (Schroeder & Moody 2013).

A range of management strategies have the potential to minimise legume residue N losses, improve the efficiency of N use by the sugarcane crop and thus maintain the sugarcane yield with no or much less need for N fertiliser application. These strategies generally centre around three principles:

a. Manipulating N mineralisation of the legume residues: Leaving the legume residues on the soil surface rather than ploughing in (as mentioned above) is an example of the strategies based on this principle. In addition, different legume species with different degradability (e.g., C/N ratios and lignin contents) also affect the rates of N mineralisation and immobilisation (Crews and Peoples 2005). Interestingly, Subbarao et al. (2007) detected biological nitrification inhibition compounds in the root exudates of peanut. It is worthwhile to investigate and explore the natural nitrification inhibition capacity of this leguminous crop;

b. Holding the mineral N in the relatively stable NH_4^+ form as much as possible (i.e. reducing nitrification). Nitrification inhibitors such as 3, 4-Dimethylpyrazole phosphate (DMPP) have been used with nitrogenous fertilisers to retard the microbial transformation of NH_4^+ into NO_3^- and thus reduce the risk of NO_3^- losses from denitrification and leaching (Chen et al. 2008). However, few studies have examined the effectiveness of nitrification inhibitors on reducing NO_3^- accumulation following retention of legume crop residues; and

c. Capturing the mineral N before it is lost: Growing N catch crops, either legume or non-legume, was found to offer an effective means in areas with a precipitation surplus between

two crops for conserving soil N and later releasing N from decomposition of the catch crop residues to the following main crop (McLenaghen et al. 1996;Vos and van der Putten 2001). Ideally, the N catch crop should be fast-growing with deep roots to maximise capture of the excessive mineral N accumulated in the soil profile. Deep-rooted crops can hold the mineral N in the surface soil into the biomass and capture part of the NO_3^- already moved into deep soil and then transport them to the main rooting zone (usually 0-20 cm) of the subsequent sugarcane crop.

There is a lack of research in the Australian sugarcane industry on the above potentially N-efficient legume management approaches. However, a recent study at Bundaberg (Wang et al. 2015) indicated that leaving soybean residues on the surface by practising no-till reduced annual cumulative N_2O loss by 22% during the sugarcane cropping season compared to practising conventional tillage that incorporated the soybean residues into soil. Furthermore, growing an N catch crop (triticale) under no-till and spraying the legume crop residues with a nitrification inhibitor (DMPP) before tillage reduced N_2O loss by 36% and 44%, respectively, compared to the conventional tillage. As N_2O is mainly produced during the process of denitrification under wet soil conditions which may also lead to NO_3^- leaching, we hypothesise that these techniques should hold great promise to reduce N losses through denitrification and leaching during the period prior to peak N uptake by the sugarcane crop. However, there is a knowledge gap with regards to when, where and how these techniques perform best in the Australian sugar industry.

Before making viable recommendations, research is required to investigate and refine these techniques to improve the synchrony of N supply from soil, legume residues and, if necessary, fertiliser to the N requirements of the following sugarcane crop. This is supported in the recent "Review of Nitrogen Use Efficiency in Sugarcane', in which Bell and Moody (2014) suggested that "there may be opportunities to improve the synchronization of release of N from legume residues by use of nitrification inhibitors applied onto legume residues and further research in this area would be of interest".

In this project, we attempted to identify and develop N-efficient legume residue management practices for different regions with an aim to maintain or improve sugar yield, minimise the environmental impacts, and increase profitability. The results should be of great value for refinement of the current nutrient management tools such as NutriCalc, SafeGauge and SIX EASY STEPS developed for the Australian sugar industry.

2. PROJECT OBJECTIVES

We investigated a suite of novel legume residue and nitrogen management strategies that aimed to minimise the amount of supplementary fertiliser application, reduce legume N losses, enhance the N availability to the subsequent sugarcane crop, maintain cane tonnage and sugar yield, and mitigate the impacts of N losses to the environment such as the Great Barrier Reef. Specific project objectives were to:

- Investigate dynamics of soil mineral N and net N mineralisation of different legume residues, including possible biological nitrification inhibition by legume (e.g., peanut) cropping;
- Quantitatively examine the effects of growing an N catch crop between legume maturity and cane planting on soil mineral N and subsequent N uptake by the following sugarcane;
- Verify the efficacy of spraying a nitrification inhibitor onto the legume residues before incorporation into soil for N conservation;
- Assess usefulness and practicality of pre-planting/fertilisation testing of soil mineral N stock, after a long fallow period, as a tool for determining the optimal fertiliser N application rate to maintain sugar yield; and

- Understand the economic benefits of legume crop rotation and different legume residue management practices.

3. OUTPUTS, OUTCOMES AND IMPLICATIONS

3.1. Outputs

3.1.1. Field demonstrations of the new management strategies to the industry (Year 1&2).

- 15/03/2016: The Bundaberg trial was demonstrated and explained to 60 growers, agronomists and agribusiness people on a Peanut and Soybean Field Day.
- 21/04/2016: The Herbert trial site was demonstrated and introduced to 100 sugarcane industry growers, representatives and stakeholders during the annual Herbert Walk & Talk Day. The group included Senator Matt Canavan (minister for Northern Australia) and his advisors.
- 27/04/2016: The Herbert site was shown to Alvean sugar marketers.
- 05/05/2016: The Herbert trial was demonstrated to about 30 growers and industry stakeholders including staff from other productivity boards.
- 06/2016: There was an on-site meeting with Isis Cane Productivity Services staff and management to talk through trial objectives at the Bundaberg trial site.
- The project concept and results at Burdekin were communicated at the bimonthly BBIFMAC General Meetings & the BCEG (Burdekin Cane Extension Group) meetings. Local extension officers and growers were engaged through recurrent face-to-face discussions and site visits.

3.1.2. Progress and final report to SRA (Year 1,2&3).

- Research project reports for milestone 1&2, 3, 4, 5, 6, and 7, was submitted to SRA and ex-EHP on 01/01/2016, 01/05/2016, 01/07/2016, 01/09/2016, 01/07/2017, and 01/12/2017, respectively.
- The final report was submitted to SRA on 31/05/2016.

3.1.3. Knowledge of the efficacy of legumes species, N catch crops, nitrification inhibitors and tillage management strategies on reducing legume N losses and improving N availability to the sugarcane crop

- Refer to Sections 6 & 7: Results and Conclusions.

3.1.4. A user-friendly 'decision tree' guideline detailing sustainable legume residue management strategies based on soil properties, climate conditions, other management practices, farm machinery availability, market of produce etcetera in the Herbert, Burdekin and Bundaberg regions

- Refer to Section 8. A decision tree for legume crop rotation and residue management

3.1.5. Data and science-based evidence for updating decision support tools such as NutriCalc for determining optimum fertiliser N application rate following a legume fallow based on pre-planting/fertilisation soil mineral N tests (Year 3).

- Refer to Sections 6 & 7 and Appendix 1.

3.1.6. Presentations and papers in workshops/conferences on sustainable management of legume crop residues in the sugar industry such as the ASSCT conferences (Year 3).

- The Bundaberg trial was communicated at the 'Southern Group' meeting organised by SRA in Childers on 23/08/2016. There were approx. 25 people in attendance.

- S. Reeves, W. Wang, M. Heenan, N. Halpin, T. Mcshane, A. Rickert, A. Royle. 2018. Nitrogen mineralisation of legume residues, oral presentation in the Australian Society of Sugar Cane Technologists held at Mackay, 18-20 April, 2018. The paper was published in the conference proceedings V40:219-228
- W. Wang, N. Halpin, S. Reeves, W. Rehbein, M. Heenan. 2018. Legume crop rotation and residue management to improve nitrogen efficiency and sugarcane yield, poster presentation in the Australian Society of Sugar Cane Technologists held at Mackay, 18-20 April, 2018. The poster abstract was published in the conference proceedings V40: 256.

3.1.7. Two to three peer-reviewed papers to scientific journals (Year3).

- C. Paungfoo-Lonhienne, W. Wang, Y. Yeoh and N. Halpin. 2017. Legume crop rotation suppressed nitrifying microbial community in a sugarcane cropping soil. Scientific Reports 7: 16707, DOI:10.1038/s41598-017-17080-z
- C. Paungfoo-Lonhienne, W. Wang, Yun. Yeoh, S. Reeves, N. Halpin, J. Daly. 2018. Soil quality attributes as altered by different management practices of legume crop residues in sugarcane farming. Prepared for Soil Biology and Biochemistry (under revision).
- S. Reeves, W. Wang, M. Heenan, N. Halpin, T. Mcshane, A. Rickert, A. Royle. 2018. Nitrogen mineralisation of different legume crop residues in relation to soil properties, application methods and nitrification inhibitor (under preparation).
- Wang, WJ, Halpin, N, McShane, T., Di Bella, L., Reeves, S., Royle, A., Richert A. 2018. Effects of legume crop rotation and residue management practices on nitrogen sugarcane nitrogen uptake, yield and profitability (to be prepared).

3.1.8. Data for researchers interested in testing and calibrating complex process-based models such as APSIM (Year3).

- Refer to Appendix 1.

3.2. Outcomes and Implications

The project team involved researchers, consultants and farmers from Queensland government departments (DES and DA), BBIFMAC and HCPSL who have good contacts with the sugar community, particularly in the Bundaberg, Burdekin and Herbert regions where the field trials were conducted. Through close collaboration, the team have communicated the tested techniques and knowledge on sustainable management of legume residues to cane growers, consultants, extension officers and researchers through field days and visits, workshops, industry meetings, publication and daily business (refer to Sections 3.1.1, 3.1.6 and 3.1.7). Given the relatively short project life, we will need to continue working closely with organisations such as SRA, Canegrowers and other productivity service agencies to promote adoption of legume crop rotation with improved legume residue techniques in the sugar industry.

As these techniques can significantly reduce N fertiliser input (~70-100 kg N/ha) and increase net farming profit (up to $600-1600/ha), cane growers are encouraged to adopt if conditions are suitable. Assuming the legume rotation and residue management strategies be adopted by half of the cane growers, the potential benefits to the industry would be considerable, although accurate estimation looks difficult at this stage. For instance, assuming legume rotation could replace 100 kg fertiliser N/ha, fertiliser cost would be reduced by 100 kg N/1000/0.46×$500/t urea=$108/ha. If 12% of Australian sugarcane cropping areas could adopt legume rotation each year, this would save fertiliser N inputs for the industry by 400,000 ha×12%×$108/ha=$5.2 M/year. The N-efficient management strategies identified in this project would help further improve the profitability.

Given the considerable environmental benefits (mitigation of N_2O emissions and downstream water pollution), the results should be of interest to government agencies as well as policy makers. The data and knowledge should also be of considerable value for refinement of the existing nutrient management programs, tools and models.

Overall, the findings from this research should help the sugar industry improve farming profitability, mitigate N_2O emissions and reduce possible N losses to the downstream water including the Great Barrier Reef.

4. INDUSTRY COMMUNICATION AND ENGAGEMENT

4.1. Industry engagement during course of project

- Trial demonstration and exposition to 60 growers, agronomists and agribusiness people, *Peanut and Soybean Field Day*, Bundaberg, 15/03/2016.
- Trial demonstration and exposition to 100 sugarcane industry growers, representatives and stakeholders including Senator Matt Canavan (minister for Northern Australia) and his advisors, the Herbert Walk & Talk Day, Ingham, Herbert, 21/04/2016.
- Showcase of field trial to Alvean sugar marketers, Ingham, Herbert, 27/04/2016.
- Trial demonstration and exposition to about 30 growers and industry stakeholders including staff from other productivity boards, Ingham, Herbert, 05/05/2016.
- On-site meeting and discussion with Isis Cane Productivity Services staff and management, Bundaberg, 06/2016.
- Presentation of the Bundaberg trial to approximately 25 participants in the 'Southern Group' meeting organised by SRA, Childers, 23/08/2016.
- Communication of the Burdekin trial concept and results at the bimonthly BBIFMAC General Meetings & the BCEG (Burdekin Cane Extension Group) meetings, Burdekin, approximately bimonthly.
- Communication and engagement with local extension officers and growers through recurrent face-to-face discussions and site visits, Bundaberg, Herbert and Burdekin.
- Project seminar once peer review of this final report is complete (tentatively September 2018??)

4.2. Industry communication messages

Growing legume crops during the fallow season can add substantial amounts (70-280 kg N/ha) of biomass nitrogen (N) into the farming system. The amount of nitrogen input from legume crop residues generally increases with increasing length of the growing season. If site and seasonal conditions suit and the fallow period is less than six months, the legume crop should be planted as early as possible to maximise its nitrogen benefits. Soybean green manure contains about two time more nitrogen than peanut green manure. With grain/pod harvest, soybean residues can contribute approximately three times more nitrogen than peanut residues. However, farming profit was markedly higher for growing peanut than soybean thanks to higher market values of peanut pods.

About 74-88% of the soybean biomass nitrogen derived from biological nitrogen fixation and 22-26% from soil nitrogen recovery. This means that legume crop rotation can (i) substantially alleviate the risk of soil mineral nitrogen losses to waterways as nitrate or to the atmosphere as nitrous oxide during the fallow period; and (ii) reduce or eliminate fertiliser nitrogen application for the subsequent sugarcane crop, thus mitigating greenhouse gas emission during fertiliser manufacture.

The legume residue nitrogen is released into soil gradually, with the rapid release occurring in the first 2-3 months. Apart from soil and weather conditions and the legume crop species and age, management practices can substantially affect the dynamics of nitrogen release. Based on a laboratory study, 15-55% of the biomass nitrogen was recovered in the plant-available mineral nitrogen form after ten months of incubation at 25°C and optimal soil moisture. On average, nitrogen release is about two time faster from incorporated than unincorporated residues, and about one and a half times faster for soybean than peanut residues. These factors should be taken into account when making decisions on fertiliser nitrogen application rates in circumstances with legume crop residue retention. In particular, care should be taken that not all the legume residue nitrogen would be available to the following plant cane.

If cane is not planted shortly after the legume crop harvest or spray out, it is a good practice to leave the crop residues on the soil surface to slow down the nitrogen release and thus minimise the risk of nitrogen loss after high rainfall events. If time allows, growing a nitrogen catch crop or allowing volunteer crop (e.g., soybean) to re-grow can also reduce the risk of N loss when cane crops are absent, particularly under high rainfall conditions.

5. METHODOLOGY

Field trials were conducted in three different sugarcane cropping regions with different climatic conditions and farm management practices: (i) Herbert (wet tropics with no irrigation), Burdekin (dry tropics with irrigation) and Bundaberg (subtropics with irrigation). In consultation with project partners, local extension professionals and growers, the legume species and the subsequent crop residue management strategies that were included in the field trial in a specific region were determined by taking into account local soil properties, climate conditions, other farming practices, machinery availability and market of product etc. Consequently, the experimental protocols for different regions differed to various extents.

A laboratory incubation study was undertaken with an objective to improve understanding of the interactive effects of legume species, legume residue placement (surface application vs. incorporation to simulate no-till vs. tillage) and treatment of legume residues with a nitrification inhibitor on N mineralisation and nitrification dynamics. This experiment was designed to complement the field trials where only a limited number of treatments could be included and the sensitivity of soil mineral N measurements was relatively low due to spatial variability.

5.1. Field trial in the Herbert region

5.1.1. Experiment site

The field trial was established on a sugarcane farm near the townships of Ingham in the Herbert (wet tropics) region (18° 41'56"S, 146°0'59"E). This region has a tropical monsoon climate. Long-term (1968-2017) annual mean temperature is 24.1 °C (the Bureau of Meteorology, Australia; Station # 032078), with the lowest monthly mean temperature in July (19.4 °C) and the highest in January (27.7 °C). Mean annual rainfall is 2126 mm, with c.a. 60% received from January to March.

The soil was a silty clay loam in the surface 0-10 cm, transitioning to clay below 30 cm depth (Table 1). The 0-60 cm of soil was acidic with pH 5.4 to 6.4, but the deep soil was alkaline with pH ≥7.6. In addition, BSES-P (0.005M H_2SO_4-extractable phosphorus) and exchangeable K (1 M NH_4OAc-extractable K) contents in the top 10 cm soil were 13.2 mg/kg (low) and 0.14 cmol/kg (low), respectively.

Sugarcane had been grown on this farm since trees were cleared in 1995, with green cane trash blanketing adopted from the commencement. The previous cane crop was harvested on 15 July 2015, and the millable cane yield was 58 t/ha.

Table 1. Initial physiochemical properties of soil in the profile at the Herbert site.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	TOC[a] (g/kg)	TN[b] (g/kg)	pH_{H2O}	$EC_{1:5}$[c] (ds/m)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)
0-10	43.6	26.0	30.4	10.6	0.83	5.4	0.08	6.4	12.3
10-30	36.1	24.8	39.1	8.0	0.67	5.6	0.08	9.6	18.0
30-60	36.8	20.4	42.8	5.5	0.47	6.4	0.12	2.7	4.2
60-90	21.8	21.6	56.5	3.7	0.33	7.6	0.24	0.0	0.0
90-120	31.8	19.3	48.9	2.1	0.23	8.5	0.29	0.0	0.0

[a]TOC: total organic carbon; [b]TN: total nitrogen; [c]EC: electric conductivity.

5.1.2. Legume cropping and residue management

Beds were re-shaped in November 2015 with a bed width of approximately 120 cm and each in-row space measuring about 70 cm. The paddock was then divided into four blocks (reps) and forty eight plots with twelve plots per block. Each plot measured 20 m in length and 11.4 m in width, including 6 beds.

Two plots in each block were randomly chosen for bare fallow, and the remaining ten plots were sown with soybean (*Glycine max*) on 04 January 2016, with two rows 50 cm apart on each bed. The soybean seeds were inoculated with a peat-based group H inoculant to maximise inoculation. Aboveground plant samples were taken from two 1-m sections of the crop rows in each block on 23 March 2016 for determination of biomass yield and N contents (see Section 1.5.4). Non-N_2 fixing weeds that grew during approximately the same period with the soybean crop were sampled from the bare fallow plots as reference plants for estimating the $\delta^{15}N$ value of soil N taken up to plants, which was then used to calculate soybean biomass N derived from soil and air through N fixation (Section 5.1.4). On 28 March 2016, the soybean crop was sprayed out as green manure.

Following spray out of the soybean crop, twelve treatments were implemented to compare different management strategies in terms of N availability and efficiency, fertiliser N saving, cane and sugar yield, N uptake by cane and profitability. These management strategies included bare fallow (BF) *vs*. soybean rotation (SB), full tillage (FT) *vs*. no-till (NT) vs. minimum tillage (MT), with *vs*. without nitrification inhibitor (NI) spray onto the soybean residues before tillage, and various fertiliser N application rates (base + side dressing; see Section 5.1.3) for the subsequent sugarcane crop as follows:

1) BF – FT –Cane + 0N
2) BF – FT – Cane + (30 + 100)N
3) SB – FT – Cane + 0N
4) SB – NT – Cane + (30 + 25)N
5) SB – MT– Cane + (30 + 25)N
6) SB – FT – Cane + (30 + 25)N
7) SB – NT – Cane + (30 + 60)N
8) SB – MT – Cane + (30 + 60)N
9) SB – FT – Cane + (30 + 60)N
10) SB – NI – NT – Cane + (30 + 25)N
11) SB – NI – MT – Cane + (30 + 25)N

12) SR – NI – FT – Cane + (30 + 25)N

After consultation with local extension officers and growers, the N-catch-crop treatment mentioned above was not included in this experiment because cane is normally planted soon after the legume crop in the Herbert region. However, extraordinarily wet conditions after spray out of the soybean crop delayed cane planting, which created a situation where an N-catch crop treatment would be desirable.

The nitrification inhibitor DMPG (3,4-dimethylpyrazole glycolate, supplied by Incitec Pivot Ltd) was sprayed onto the soybean crop residues at 0.91 kg DMP/ha (Wang et al. 2015) on 12 May 2016. The FT, MT and NT treatments were implemented using a bed renovator, wavy disc and no cultivation on 13 May, which resulted in full, partial and no incorporation of the crop residues into soil, respectively (Fig. 1). The NT plots were sprayed with glyphosate (Roundup®) at 4 L/ha to control weeds. Herbicides (1.5L Gramoxone®/ha plus 0.5 kg Diuron®/ha) were applied to all plot on 25 July.

Figure 1. Crop beds before cane planting at the Herbert site.

5.1.3. Sugarcane planting and fertiliser application

According to the 6ES guidelines, the recommended fertiliser N application rate for plant cane following bare fallow in this region with a yield potential of 120 t/ha was 120-130 kg N/ha, after a discount of 20 kg/ha for the bare fallow. However, soil profile samples collected before cane planting indicated that the soil mineral N contents in all treatments were not relatively low (< 15 mg N/kg soil, Fig. 7. a) most likely due to high rainfall in the months prior to cane planting. Consequently, 130 kg N/ha was applied to the plant cane following the bare fallow (treatment 2).

The aboveground soybean biomass yield was 2.9 t/ha. The green manure (including roots) contained about 87 kg N/ha (see Section 6.1.1). Assuming 85% of the soybean residue N would be mineralised before and during the sugarcane cropping season, N fertiliser application rate for the cane following soybean cropping was reduced by about 60% (75 kg N/ha) to 55 kg N/ha.

Given the very low mineral N contents in soil (<1.5 mg N/kg soil), 30 kg N/ha was used as a base in all treatments except the 0N treatments. Treatments 7-8 with a higher fertiliser N application rate at 90 kg N/ha were included because considerable legume crop residue N might have been lost during the unexpected wet period between the spray out of soybean crop and cane planting.

Sugarcane was planted with two rows 50 cm apart on each bed on 04-05 August 2016. Basal fertilisers (DAP and urea) were applied at 30 kg N/ha and 20 kg P/ha to a depth of approximately 10

cm below the soil surface in the cane row immediately before planting. Phosphoric acid was applied at 20 kg P/ha to about 10 cm depth between the cane rows in the 0N treatments. On 18 October 2017, fertiliser N as urea was side dressed approximately 10 cm deep between the cane rows at 25, 60 or 100 kg N/ha in accordance to the treatments specified above. In the meantime, potassium sulphate was side dressed at 200 kg/ha in all plots.

The cane crops were mechanically harvested on 28 August 2017. Sugarcane yield was measured by harvesting the middle two rows (2 × 20 m) with a plot harvester and a weighing bin (Fig. 2).

Figure 2. Sugarcane harvesting at the Herbert site.

5.1.4. Soil and plant sampling and analyses

Deep soil sampling down to 120 cm was undertaken from beds (close to cropping rows) at soybean planting, before cane planting, before the high N uptake season, and post cane harvest. Soil samples were also collected from the 0-10 cm and 10-30 cm depths intermittently during the fallow period and the sugarcane growing season to monitor soil mineral N dynamics under different treatments. The soil samples were transported to the laboratory in insulated boxes with ice blocks, where they were stored at 4 ^{o}C in a fridge until extracted with 2 M KCl solution in a 1:5 ratio within about one week. The extracts were then analysed for mineral nitrogen N, including ammonium (NH_4^+) and nitrate (NO_3^-), concentrations using colorimetric techniques (Rayment and Lyons 2010). Soil moisture contents were also determined by drying at 105 ^{o}C in an oven for more than 24 hours. Soil mineral N contents were reported on a dry-mass basis.

As mentioned in Section 5.1.2, aboveground soybean samples were taken on 23 March 2016 shortly before the spray out. After weighing the fresh biomass, the vegetative parts and bean pods were separated and subsampled for determination of moisture contents by drying in an oven at 60^{o}C for >48 hours. The aboveground weed samples were processed in a similar manner. The dried biomass subsamples were cut into small pieces (~2 cm long) and then pulverised in a ball mill to < 0.25 mm. The fine ground plant samples were analysed for total N (TN) contents and δ^{15}N values using an isotope ration mass spectrometer (Thermo Fisher Scientific Australia Pty Ltd, VIC). The non-N_2 fixing weeds were used as reference plants, assuming they accessed the same soil N pool over the same

growing period as the N_2 fixing legumes. The percentage of N that the legumes fixed from air (%Ndfa) was calculated by the following equation:

$$\%\text{Ndfa} = \frac{(\delta^{15}\text{N of reference plant} - \delta^{15}\text{N of } N_2\text{fixing legume})}{(\delta^{15}\text{N of reference plant} - B)} \times 100\% \quad (1)$$

where *B* is the δ^{15}N of legume shoots that were completely reliant on N_2 fixation for N. Mean soybean and peanut specific *B* values reported by Unkovich *et al* (2008) were used to calculate %Ndfa in this study.

Sugarcane biomass samples were taken and analysed for total nitrogen contents three times throughout the cropping season. In January 2017, aboveground plant samples were taken from a 1-m section on beds 2 and 5, avoiding the central beds (3 and 4) that were to be used for yield determination at harvest. The number of plants and the weight of total biomass in the 1-m sections were recorded. In the meantime, the number of plants was counted from a 10-m section on beds 3 and 4 each. In April 2017 when the cane stalks were of considerable size, twenty plants were randomly taken from each of beds 2 and 5. The plant samples were segregated into stalks and leaves & cabbage by cutting between the 5th and 6th dewlaps. All live and dead leaves and sheaths were included in the leaves & cabbage fraction. The stalk and leaves & cabbage were weighed separately. Immediately after weighing, 6 stalks and about 500-1000 g of leaves & cabbage samples were mulched and then subsampled for determination of dry matter content at 60 ^{o}C for >48 h. The dried sub-samples were sent to the DES laboratory, fine ground and analysed for total N contents as described above. Total above-ground N uptake by the crops was calculated using plant number per ha, the average fresh stalk and cane/cabbage mass per plant, dry matter content and total N content.

At harvest, six whole plants were randomly cut from each plot (Fig. 3) and separated into millable stalk and leaves & cabbage as described above. The millable stalks were crushed and sub-sampled for determination of commercial cane sugar (CCS) content using an infra-red spectrometer. Sugar yield was calculated by multiplying fresh cane yield and the CCS content. Dry matter and total N contents of the millable stalk and leaf & cabbage were determined as described above. Total above-ground N uptake by the crops was calculated using the fresh cane yield, cane to leaves & cabbage ratio, their dry matter contents and total N contents.

Figure 3. Plant sampling at harvest at the Herbert site.

5.1.5. Field incubation study on mineralisation of soybean residue N

Aboveground soybean plant samples (including any visible material lying on the ground) were collected from a 2 m section in each block on 31 March 2016. The fresh plant samples were cut into 15 cm pieces, and subsamples of approximately 300-400 g were weighed and then placed into nylon mesh bags (30cm × 40 cm with a mesh size of ~1.5 mm). The bag size and mass of crop residues in each bag were such that created a layer of crop residue with similar thickness as on the open ground. Subsamples were also taken for determination of moisture content by drying at 60 °C. The dried subsamples were fine ground and analysed for total N content as described in Section 5.1.4.

Twelve of the mesh bags with soybean residues were pinned on the soil surface and another twelve were buried at about 10 cm below the ground in each block on 31 March 2016. The surface-laid and buried mesh bags were sampled with one bag per placement per replicate after 28, 62, 90, 119, 151, 182 and 210 days. The mesh bag samples were cleaned by carefully removing soil particles. The remaining soybean residues in each mesh bag were transferred into a paper bag, which were then dried at 60 °C for about 48 hours before weighing for dry matter mass. The dried biomass samples were transported to laboratory for determination of total N contents as described above.

5.2. Field trial in the Burdekin region

5.2.1. Experiment site

The field trial was established on a sugarcane farm near the townships of Ayr in the dry tropical Burdekin region (19°48'2"S, 147°9'59"E). Long-term (1970-2017) annual mean temperature in this region is 23.7 °C (the Bureau of Meteorology, Australia; Station # 033002), with the lowest monthly mean temperature in July (18.6 °C) and the highest in January (27.4 °C). Mean annual rainfall is 946 mm, with c.a. 75% received from December to March.

The farm was on a clay soil with small variations in texture throughout the 0-120 cm profile (Table 2). The soil was non-saline but neutral in the surface, progressing to highly alkaline with increasing depth.

The previous cane crop was harvested on 24 October 2015, with a millable cane yield of 90.5 t/ha. The cane was burnt to remove leaves before harvesting.

Table 2. Initial physiochemical properties of soil in the profile at the Burdekin site.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	BD[a] (g/cm^3)	TOC[b] (g/kg)	TN[c] (g/kg)	pH_{H2O}	$EC_{1:5}$[d] (ds/m)	CEC[e] (cmol/kg)
0-10	33.0	21.2	45.8	1.19	10.8	0.88	7.3	0.33	24.3
10-30	33.0	21.4	45.6	1.38	9.0	0.75	8.0	0.14	25.3
30-60	27.2	22.2	50.5	1.63	5.7	0.47	8.9	0.21	25.0
60-90	29.8	21.9	48.3	1.66	5.6	0.40	9.3	0.36	23.5
90-120	31.8	22.3	45.9	1.68	5.9	0.28	9.5	0.40	24.8

[a]BD: bulk density; [b]TOC: total organic carbon; [c]TN: total nitrogen; [d]EC: electric conductivity; [e]CEC: cation exchange capacity.

5.2.2. Soybean cropping and residue management

Beds were formed on 12 November 2015 with a bed width of approximately 100 cm and furrow width of about 65 cm. The paddock was then divided into four blocks (30 m × 40.5 m each). Each block was then divided into nine plots (30 m × 4.95 m each). Guano containing 13% P (nil N) was applied at 120 kg/ha on 26 November 2015. Seven plots in each block were randomly chosen and

planted with soybean (*Glycine max*; Bunya variety; seeds inoculated with a peat-based group H inoculant) on the next day, leaving the remaining two plots unplanted as bare fallow. Two rows of soybean were planted on each bed with a row spacing of 50 cm (Fig. 4). The site was irrigated with 80 mm of water on 28 November and 22 December 2015. Aboveground plant samples were taken from two 1-m sections of the crop rows in each block on 14 March 2016 for determination of biomass yield and N contents as described in Section 5.1.4. The soybean crops were sprayed out as green manure (no grain harvesting) on 20 March and slashed on 28 march 2016 to prepare for cane planting. The yield of the aboveground biomass was 6.9 t/ha. The soybean crop residues (including roots) contained about 300 kg N/ha.

Nine treatments with contrasting soybean residue and fertiliser N management practices were used to compare bare fallow (BF) *vs*. soybean rotation (SB), heavy tillage (HT) *vs*. minimum tillage (MT), with *vs*. without nitrification inhibitor (NI) spray onto the soybean residues before tillage, and with *vs*. without a N catch crop (NCC; mung bean). These treatments are as follows:

1) BF – MT – Cane + 0N
2) BF – MT – Cane + (0 + 200)N
3) SB – MT – Cane + 0N
4) SB – MT – Cane + (0 + 60)N
5) SB – NI – MT – Cane + 0N
6) SB – NCC – MT – Cane + 0N
7) SB – HT – Cane + 0N
8) SB – NI – HT – Cane + 0N
9) SB –NCC – HT – Cane + 0N

Nitrification inhibitor DMPG was sprayed onto the soybean crop residues at 0.91 kg DMP/ha on 29 March 2016. The MT and HT tillage treatments received one and three wavy disc passes, respectively, on 29 March 2016.

Figure 4. Soybean cropping and bare fallow plots at the Burdekin site.

5.2.3. Sugarcane planting and fertiliser application

The famer's fertiliser N application rates for plant cane following bare fallow were 200 kg N/ha. Therefore, no N fertiliser was applied for the treatments with soybean rotation except Treatment 4. Soil samples prior to cane planting were collected in early March and mineral N contents were determined. The bare fallow soil contained 34 mg N/kg soil, while the soybean fallow soil had 9 mg N/kg soil in the top 0-10 cm depth. The soil test confirmed that fertiliser N application was unnecessary at cane planting.

Sugarcane crop was planted on 01 April 2016 and irrigated immediately afterward. A deep soil sampling was undertaken on 18 April to monitor soil mineral N contents. Excessive mineral N accumulation in the 0-10 cm depth of soil was detected in the soybean fallow treatments (> 40 mg N/kg). Consequently, mung bean (*Vigna radiate*) was sown as an N-catch crop in treatments 6 and 9 on 06 May, on both sides of the cane row with a spacing of about 20 cm between the adjacent cane and mung bean rows. The Mung Bean catch crop was sampled from two 1-m section per plot and sprayed out on 03 July 2016 with 2-4D to prevent restriction to cane growth from competition for nutrients and water. A side dress of urea was applied at 200 kg N/ha to treatment 2 [BF – MT – Cane + (0 + 200)N], and at 60 kg N/ha to the treatment 4 [SB – MT – Cane + (0 + 60)N], on 07 September 2016. The plots were mechanically harvested on 26 July 2017. Sugarcane yield was measured by harvesting the middle 20 m of the central row with a plot harvester and a weighing bin.

Over the sugarcane cropping season, the site was irrigated seven times, at approximately 80 mm of water each time on 02 April, 10 May, 14 August, 11 September, 10 and 27 October, 08 November 2016.

5.2.4. Soil and plant sampling

Soil sampling was conducted thirteen times during the experiment, including one before soybean planting, two during the soybean growing season, one between the soybean spray out and cane planting, and nine during the sugarcane cropping season. Among these activities, deep soil sampling down to 120 cm (0-10, 10-30, 30-60, 60-90 and 90-120 cm) was undertaken with steel sampling tubes (6.0 cm in diameter) from 3 points on the beds on 19 November 2015 prior to soybean planting, 18 April 2016 shortly after cane planting, 09 December 2016 before high N uptake by cane crops, and 27 July after cane harvest. The remaining soil samplings were conducted from the 0-10 cm and 10-30 cm depths across the beds using a shovel. All samples were analysed for mineral N (NH_4^+ and NO_3^-) and moisture contents as described in Section 5.1.4.

Aboveground soybean plant samples were taken from two 1-m sections in each block on 14 March 2016, shortly before spray out of the crop. In the meantime, non-N_2 fixing weeds were sampled from the bare fallow plots as described in Section 5.1.2. The mung bean N-catch crop was sampled on 03 July 2016 immediately before the spray out. Sugarcane plant sampling was undertaken from the two 5-m ends of each plot for three times on 02 December 2016, 03 May 2017 and 26 July 2017 using the methods described in Section 5.1.4. The plant samples were processed and measured for fresh and dry matter yield, total N content, and CCS (at harvest only) following the methods given in Section 5.1.4.

Figure 5. Post-harvest deep soil sampling at the Burdekin site.

5.3. Field trial in the Bundaberg region

5.3.1. Experiment site

This field experiment was located 18 km south of Bundaberg in the subtropics (S25°03', E152°24'). The long-term (1959-2017) annual mean temperature in this region is 21.6 °C (the Bureau of Meteorology, Australia; Station # 039128), with the lowest monthly mean temperature in July (16.2 °C) and the highest in January (25.9 °C). Mean annual rainfall is 1027 mm, with ca. 56% of rainfall received from December to March.

The soil is a Redoxic Hydrosol (Isbell 2002) with a texture marginal between sandy loam and loamy sand in the 0-60 cm depth and a texture of loamy sand in the 60-100 depth. The mail soil properties are given in Table 3. In addition, the surface 20 cm of soil contained 71 mg BSES-P/kg (high) with a Ps buffer index of 37 (low), 0.1 meq exchangeable K/100g (very low), 0.88 meq exchangeable Ca/100g (moderately low), 0.26 mg hot $CaCl_2$-extractable B/kg (low), with a CEC of 1.6 meq/100g (low) and a aluminium saturation of 15% (very high).

The last sugarcane crop was fertilised with about 150 kg N/ha as urea in October 2014 and was green harvested in October 2015 with cane trash retained on the ground.

Table 3. Initial physiochemical properties of soil in the profile at the Bundaberg site.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	BD[a] (g/cm^3)	TOC[b] (g/kg)	TN[c] (g/kg)	pH_{H2O}	$EC_{1:5}$[d] (ds/m)	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)
0-10	78.0	12.0	9.9	1.276	10.5	0.82	5.6	0.123	7.4	28.1
10-20	77.2	11.6	11.2	1.468	10.4	0.79	5.4	0.127	6.1	29.1
20-30	77.3	12.4	10.3	1.591	9.0	0.73	5.2	0.125	6.2	26.3
30-60	75.8	13.8	10.4	1.707	4.6	0.42	5.2	0.047	4.5	32.6
60-100	76.2	17.1	6.8	1.840	1.4	0.24	5.4	0.023	0.0	16.0

[a]BD: bulk density; [b]TOC: total organic carbon; [c]TN: total nitrogen; [d]EC: electric conductivity.

5.3.2. Legume cropping and residue management

After harvest of the previous sugarcane, lime was applied at 2.0 t/ha on 27 November 2015 to correct low soil pH and high aluminium saturation. Sodium molybdate and sodium borate (15% B) were also applied at 500 g/ha and 2 kg/ha, respectively, to aid in symbiotic N fixation and correct boron deficiency. A fertiliser blend (Legume Max, Incitec Pivot Ltd) was surface-applied at 300 kg/ha (containing 12 kg N, 26 kg P, 57 kg K, 15.6 kg S and 19 kg Ca per ha) and then incorporated into soil with a rotary hoe.

Thirty six plots (11 m wide × 20 m long) were marked out in the paddock, which were grouped into four blocks (replicates). Peanut (*Arachis hypogaea*; variety Holt) and soybean (*Glycine max;* variety A6785) were sown in dual rows (90 cm apart) on raised beds (~120 cm wide with a centre-to centre spacing of 183 cm) on 17 and 18 December 2015, respectively. Peanut inoculant (group P) and or soybean inoculant (group H) were applied in solution into the planting furrow. Non N_2-fixing peanut and soybean were planted in the buffer zones without inoculation as reference plants to measure $\delta^{15}N$ of the legume biomass N derived from soil only. Bare fallow plots were used for comparison.

Both crops were fully irrigated with a high pressure travelling irrigator. Weeds were controlled with combination of herbicides and tillage. The peanut crop was sprayed with fungicides on a 10-14 day basis to prevent foliar disease. The soybean crop was sprayed with insecticides to control pod-sucking insects.

Figure 6. Bare fallow (BF), soybean (SB) and peanut rotation (PN) plots at Bundaberg.

The mature soybean and peanut crops were harvested for grain and pods on 10 May and 17 June 2016, respectively (see Section 5.3.4). The yield was 3.5t/ha for soybean and 4.3t/ha for peanut pods. The soybean and peanut crop residues after harvest of grain or pods had a dry matter yield of 7.1 and 6.8 t/ha, containing 281 and 88 kg N/ha, respectively.

Nine treatments were used to compare bare fallow (BF) *vs*. soybean rotation (SB) *vs*. peanut rotation (PN), full tillage (FT) *vs*. no-till (NT) after the legume crop harvesting, with *vs*. without spray of nitrification inhibitor (NI) onto the legume crop residues before tillage, with *vs*. without a N-catch crop during late fallow period, and different application rates of fertiliser N (base N + side dressing N) for the subsequent cane crop as follows:

1) BF – FT – Cane + 0N
2) BF – FT – Cane + (25+125)N
3) SB – FT – Cane + 0N
4) SB – NI – FT – Cane + 0N
5) SB – NT – Cane + 0N
6) SB – NT – NCC – Cane + 0N
7) PN – FT – Cane + (0+42)N
8) PN – NI – FT – Cane + (0+42)N
9) PN – NT – Cane + (0+42)N

Nitrification inhibitor (DMPG, Incitec Pivot Ltd, Australia) was applied on 14 July 2016 for Treatments 4 and 8 at 0.92 kg DMP-equivalent per ha by spraying water solution onto the legume crop residues. The treatment plots with tillage were tilled a few hours after the application of DMPG using a rotary hoe, incorporating the crop residues to a depth of approximately 10 cm. Weeds including re-emerged soybean and peanut volunteers in the direct drill plots were controlled using herbicides. The treatments were arranged in a randomised block design with four replicates per treatment. The plots measured 20 m in length and 10 m in width comprising 6 crop beds.

5.3.3. Sugarcane planting and fertiliser application

The industry average N application rate is 150 kg/ha. Fertiliser N for sugarcane following the peanut crop rotation was applied at 42 kg N/ha, which brought the total N application rate to 130 kg/ha including 88 kg N/ha from the peanut residues. This N rate was the recommended rate for the plant cane following bare fallow. No N fertiliser was applied following the soybean rotation.

Sugarcane (variety Q208) was planted on 19 August 2016. Pesticide and herbicide were applied at planting to protect the emerging eyes from insect and fungal attack, respectively. Based on the pre-plant soil tests, mineral-mulch (a Si source) was surface-applied at 6 t/ha immediately prior to planting to correct potential Si deficiency. Sulphate of potash was applied at 72 kg/ha to the soil surface in the open drill for all treatments on 22 August 2016. On the same day, urea was applied to Treatment 2 at 25 kg N/ha and was raked into soil immediately.

All plots received a fertiliser application of 70 kg K/ha and 13 kg S/ha immediately prior to fill-in on 16 November 2017, 89 days after planting. A further 125 kg N/ha was applied as urea in the planting drill to the plots of Treatment 2. Urea was also applied to supply 42 kg N/ha in the peanut plots. The planting furrow was closed and a row profile was formed using a set of 'ratooning disc' in one pass. This process minimised the amount of stubble incorporation.

The sugarcane crop was manually harvested on 15 August 2017 by cutting canes close to the ground in a quadrat encompassing 3 rows × 5 m in each plot. The total biomass was recorded, sub-samples were taken and partitioned into trash and millable stalk, and a six-stalk sample was used for CCS determination with a near-infrared (NIR) spectroscopic analyser at SRA. A sub-sample of millable stalk and trash (consisting of dry trash, green leaf and cabbage) was mulched, weighed wet and dried at 60 ^{o}C.

5.3.4. Soil and plant sampling

Soil sampling was conducted on fifteen occasions during the experiment, including one at sowing of the legume crops, three to four during the legume growing season, one to two between the legume crop harvest and cane planting, and eight during the sugarcane cropping season and one after cane harvesting. Among these activities, deep soil sampling down to 100 cm (0-10, 10-30, 30-60 and 60-100 cm) was undertaken immediately after the legume crop sowing (from the bare fallow plots), between soybean and peanut harvesting, before cane planting, before rapid N uptake in summer, after summer, and after cane harvest. The remaining soil samplings were conducted from the 0-10 cm and 10-30 cm depths. All samples were analysed for mineral N (NH_4^+ and NO_3^-) and moisture contents as described in Section 5.1.4.

In addition, soil samples were collected from the 0-10 cm depth in the legume root zone or a similar position in the bare fallow plots on 27 April 2016, approximately at the maximum biomass stage of the legume crops. The soil samples were used to examine effects of legume cropping on soil microbial community in the rhizosphere. Eight separate samples of soil were taken from each plot and bulked (~300 g), resulting in four replicates per treatment. The soil samples were transported to the laboratory on the same day in insulated boxes filled with ice blocks, stored in a fridge at 4°C overnight and sieved through a sterilised 2 mm sieve. Sub-samples were stored at −20°C for DNA isolation. DNA extraction, metagenome analysis, and quantitative polymerase chain reaction (qPCR) analysis were conducted to measure soil microbial community composition and abundances of microbial genes related to nitrification. A detailed description of the methods were given in a published paper from this study (Paungfoo-Lonhienne *et al.* 2017).

The legume crops were sampled from two 1-m sections in each block at their maximum biomass stages. Aboveground soybean samples were taken on 13 April 2016, and both below- and aboveground peanut samples were taken on 31 May. The plant samples were dried at 60 ^{o}C until a constant weight was achieved. Subsamples of the crop biomass was taken and analysed for N contents as described in Section 5.4.1. The grain yield of soybean was measured by harvesting the middle 5-m section from each of three central crop beds on 10 May 2016. The pod yield of peanut was determined by harvesting the middle 4-m section from each of two central crop beds on 17 June. The soybean grain and peanut pods were sampled for determination of water and N contents, which were used to calculate the amount of N removed from grain or pod harvesting. The crop

residue N was calculated as the difference between the maximum biomass N (including roots) and the N removed from grain or pod harvest.

Sugarcane plant sampling was undertaken at four and six months after planting and at harvest using similar methods described in Section 5.1.4. The methods used for measuring fresh and dry matter yield, total N content, and CCS (at harvest only) were also given in Section 5.4.1.

5.3.5. Study on effects of legume crop rotation on nitrifying microbial community

Total dsDNA was extracted from 0.25 g of soil using the PowerSoil® DNA isolation kit following manufacturer's instructions (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). DNA libraries were prepared using an Illumina® Nextera XT Library Prep Kit following manufacturer's protocol. Shotgun metagenomic sequencing was used to determine the relative abundances of nitrifying microorganisms and nitrification-related genes in the soil samples.

The methods used for metagenome analysis, microbial community profile data processing, classification of *amo*A sequences and qPCR analysis were described in detail by Paungfoo-Lonhienn et al. (2017).

5.4. Laboratory incubation study on nitrogen mineralisation of legume residues

5.4.1. Legume Residue and soil preparation

Above ground components of three legume crops were collected from the Herbert and Bundaberg sites. Mature soybean samples were collected immediately after grain harvest and consisted of the leaves, stems and empty pods. Green manured soybean samples were taken at early pod development and consisted of the leaves, stems and pods. Peanut samples were harvested at maturity and consisted of leaves and stems. The collected legumes were dried at 60^{o}C, then cut into 10-20 mm pieces. A sub sample was ground for total carbon (TC) and total nitrogen (TN) analysis. Properties of the legumes are given in Table 4.

Table 4. Chemical properties of legume residues

Legume residue	Total C %	Total N %	C:N ratio
Soybean – mature residue	44.4	2.5	17
Soybean – green manure	44.1	2.0	22
Peanut – mature residue	43.5	1.7	26

Bulk soil samples were collected to a depth of 0-10 cm from multiple points at the Herbert, Burdekin and Bundaberg sites in October 2016. The field moist samples were passed through a 4 mm sieve, thoroughly mixed and stored at 4^{o}C until the start of the pre-incubation. Soil water holding capacity (WHC) for the soils from Herbert, Burdekin and Bundaberg was 44%, 55% and 25% (w/w), respectively.

5.4.2. Incubation setup

Field moist soils equivalent to 80-90 g of dry matter were weighed into 120 ml polythene jars (height: 107 mm; diameter 44 mm). The weighed soils were pre-incubated for 3 days at 25^{o}C with a punctured lid on to minimise moisture loss and maintain aeration. Soil moisture was determined gravimetrically at 105^{o}C, to enable calculation of water required to reach 50% WHC. After the pre-incubation period, ten treatments were applied to each soil (Table 3).

Table 5. Incubation Treatments

Treatment ID*	Legume	Management
Control	nil	nil
S-MR-Un	Soybean MR	Unincorporated
S-MR-In	Soybean MR	Incorporated
S-MR-In+DMPG	Soybean MR	Incorporated+DMPG
S-GM-Un	Soybean GM	Unincorporated
S-GM-In	Soybean GM	Incorporated
S-GM-In+DMPG	Soybean GM	Incorporated+DMPG
P-MR-Un	Peanut MR	Unincorporated
P-MR-In	Peanut MR	Incorporated
P-MR-In+DMPG	Peanut MR	Incorporated+DMPG

*MR: mature residues/biomass; GM: green manure/biomass; DMPG: 3 4-dimethylpyrazole glycolate (nitrification inhibitor).

Treatments with residue addition received 0.83 g of crop residue per jar. This application rate of crop residues was equivalent to dry matter of 4 t/ha applied on cropping beds that usually account for about 70% of a cane field. For the unincorporated treatments, the pre-incubated soil was mixed and packed in jars to field bulk density. The soil NO_3^- pool was labelled with $K^{15}NO_3$ (99% atm%) solution equivalent to 1 mg N/kg soil, by pipetting the solution on to the soil surface of each jar. This also increased the soil water content to 50% WHC. Legume residues were then applied to the surface of the soil. For the incorporated treatments, soil samples were transferred from the jars into bowls, where the crop residue was added and mixed with the soil, before being added back into the jar, to ensure the residues were evenly distributed. The same $K^{15}NO_3$ solution as used for other treatments was then added to reach 50% WHC and label the NO_3 pool. The legume residues used in the In+DMPG treatment were sprayed with the nitrification inhibitor equivalent to 3.2 L/ha, and then allowed to dry before being mixed into the soil and $K^{15}NO_3$ solution added. The control treatment had only $K^{15}NO_3$ solution applied without residue addition. All soil jars were placed in the dark in an incubator at 25°C. Soil moisture was adjusted by weight difference, at least twice per week, to maintain the soil moisture at 50% WHC.

5.4.3. Calculation of carbon and nitrogen mineralisation

Three replicate jars of each treatment were destructively sampled on day 0, 10, 30, 60, 100, 150, 200, 252 and 302. Soil mineral N (NH_4^+ and NO_3^-) contents were determined as described in Section 5.14. Net N mineralisation from soil and crop residues was calculated as the change in (NH_4^+ + NO_3^-)-N content from time zero to each sampling day. Net N mineralisation from legume residues was calculated as the difference in mineral N between the legume residue-applied treatments and the control treatment on a particular sampling day.

Carbon mineralisation (as CO_2-C respired) and N_2O flux were measured on a subset of jars at day 1, 2, 4, 6, 8, 11, 15, 23, 32, 45, 58, 73 and then every 28 days thereafter. Glass jars (1.5 L) with a rubber seal and septum were sequentially flushed with compressed air for 30 seconds, then a soil jar was placed into each jar and the lid closed. Background air was sampled before closure and analysed for CO_2 and N_2O concentration at time zero. After five to seven hours closure, a 26 ml sample was extracted from the jar headspace using a syringe, and injected into a 12 ml evacuated vial (Exetainer; Labco, UK). The gas samples were analysed for N_2O and CO_2 concentrations on a gas chromatograph, equipped with an ECD and FID with methaniser (GC 2010, Shimadzu Co., Kyoto, Japan). Standards of 500, 1000, 2000 and 4000 µL/L for CO_2 and 0.5, 5.0, 12.0 and 20.0 µL/L for N_2O (BOC Ltd, Sydney, Australia) were used to calibrate the gas chromatograph. Flux rate of CO_2 or N_2O was calculated as the increase in CO_2 or N_2O concentration, expressed as mg CO_2-C/kg soil/day or mg N_2O-N/kg

soil/day. The values for days between sampling days were estimated by linear interpolation. Cumulative C mineralisation and N_2O flux were calculated by summing the daily measurements. Carbon mineralisation and N_2O flux attributed to residue addition were calculated as the difference in CO_2 and N_2O fluxes, respectively, between the residue treatment and the control treatment.

5.4.4. ^{15}N recovery

To measure ^{15}N abundance in the soil and plant residues at the end of the 302-day incubation, the soil pellet remaining from the 2M KCL extraction (explained above), was washed three times with 50 ml of 0.01M $CaCl_2$. The pellet was resuspended each time, mixed for 5 minutes and centrifuged at 2500 rpm for five mins, before being filtered through the same filter paper. This process removed residual mineral N from the soil. The particles retained on the filter paper were added back into the soil pellet after each filtration, and the filtrate was discarded. The soil pellets were then dried at 60°C, before being fine ground to <150 µm and weighed into tin capsules (5 x 7 mm) for analysis on an Isotope Ratio Mass Spectrometer (IRMS).

The ^{15}N abundance of mineral N in the KCl extracts at Day 302 of the incubation was quantified using a diffusion method (Brooks *et al*. 1989; Stark and Hart 1996) and analysed on an IRMS. Briefly, an aliquot of the extracted KCL solution was added into a 100 ml plastic jar, containing a glass bead. To diffuse NH_4^+, magnesium oxide (0.2g) was added into the solution and then a stainless steel wire attached with an acidified filter disk (7 mm diameter; Whatman Grade 1, 10 uL of 2.5 M $KHSO_4$) was suspended across the mouth of the jar. The cap was quickly closed and the solution carefully mixed. The samples were left for six days with daily mixing, before the wire and filter paper were removed, and allowed to dry overnight in a desiccator containing H_2SO_4. The jars were left open overnight, before Devarda's alloy (0.4 g) was added into each solution to convert NO_3^- to NH_4^+, and a new wire and an acidified filter disk was suspended in the jar. The jars were immediately capped and mixed daily for six days, before the wire and filter paper were removed and placed in a desiccator with H_2SO_4 overnight to dry. After drying, the filter paper disks were carefully removed from the wire and placed into tin capsules (5mm x 7mm) for analysis. Blank KCL solutions were also spiked with ^{15}N solution of known abundance and diffused to correct for possible contamination.

5.5. Economic analysis

An economic analysis of the different management scenarios at each site was conducted using the Farm Economic Analysis Tool (FEAT; Department of Agriculture and Fisheries; https://www.daf.qld.gov.au/business-priorities/plants/field-crops-and-pastures/sugar/farm-economic-analysis-tool). The gross margin per hectare was used to compare the relative profitability of the different management scenarios and calculated as the gross income less all variable costs, not taking into account fixed costs and capital expenditure. As fixed costs and expenditure may differ between sites, comparisons between the three sites should be exercised with caution. Variable costs included fertilisers, herbicides, insecticides, fungicides, seeds, irrigation, sowing, harvesting, labour and machinery costs. Labour is costed at $35/hr. Machinery costs associated with management operations included fuel usage, field efficiency, width of machinery, speed of operation and ongoing repairs and maintenance. All of the analyses assumed a sugar price of $400/tonne and an on-farm diesel price of $1/L, net of GST and diesel rebate. The soybean and peanut price was $510/tonne and $856/tonne, respectively. The management scenarios used in the study reflected the actual management operations undertaken throughout each experiment, from fallow through to the harvest of the plant cane.

5.6. Statistical analysis

Statistical analyses were performed using GenStat V.18 (VSN International Ltd., UK). One way analysis of variance (ANOVA) was conducted to test general differences among all treatments. Two

or three way ANOVA was performed to test effects of legume crop species, tillage, nitrification inhibitor and/or N-catch crop as well as their interactions using relevant treatments following legume crop rotation. Prior to ANOVA, data were tested for normal distribution and log-transformed where appropriate. Treatment effects were assessed using Duncan's multiple range test at $P < 0.05$ unless indicated otherwise.

6. RESULTS AND DISCUSSION

6.1. The Herbert trial

6.1.1. Soybean biomass yield and N benefit

Yield of the soybean green manure was low at 2.94 t dry matter equivalent/ha (Table 4). The average N content of the biomass was also low (2.31%), compared to 3.0-3.5% for typical soybean crops (Garside and Bell 2001;Schroeder et al. 2005). The low biomass yield and low N content were probably due to the relatively short growing time (84 days). The aboveground crop residues contained 67.8 kg N/ha. Using a shoot N to root N ratio of 3.5 (Schroeder et al. 2005), the total amount of N that could be supplied from the green manure was estimated to be 87 kg N/ha. To maximise the N benefit from legume rotation, legume crops should be planted early as long as the site conditions (e.g., soil moisture) and seasonal conditions are suitable for sowing, germination and early plant growth. About 74.4% of the biomass N derived from biological N_2 fixation from air, and the remaining 25.6% from soil. In spite of the relatively short soybean growing period, these results demonstrated that the soybean rotation added a considerable amount of N from N_2 fixation (65 kg/ha) into the cropping system and recovered 22 kg N/ha from soil that could otherwise be lost following rainfall.

Table 6. Soybean green manure yield (dry biomass equivalent) and N inputs at the Herbert site.

Crop parts	Biomass (t/ha)	N content (%)	Biomass N (kg/ha)	Ndfa** (%)	Ndfs***
Pods	0.10	4.14	4.3	78.9	21.1
Leaves/Stems	2.84	2.25	63.5	74.2	25.8
Total	**2.94**	**2.31***	**67.8**	**74.4***	**25.6***

*Weighted average of pods and vegetative parts; **Ndfa: N derived from air through N_2 fixation; ***Ndfs: N derived from soil.

6.1.2. Dynamics of soil mineral N under different management practices

Shortly after the soybean crop spray out, soil mineral N contents in the top 10 cm depth were slightly lower in the bare fallow soil than in the soybean cropped soil (Fig. 7). The soil mineral N contents increased by 4.4 to 21.2 mg/kg during the period between the spray out of soybean crop and shortly before cane planting, with no significant differences between treatments. Nitrate was the predominant form of mineral N during this period, which could be easily lost through denitrification and/or leaching as a results of the heavy rainfall events. From cane planting onwards, soil mineral N contents in the 0-10 cm depth in the unfertilised treatments were similar (21-24 mg/kg) following both bare fallow and soybean rotation. This demonstrated that the soybean crop residues in the FT treatment did not release a substantial amount of N during this period perhaps because most of the crop residue N had been mineralised and then lost during the wet period between the tillage and cane planting. In contrast, the treatments that received fertiliser N had higher mineral N contents until approximately February to March 2017. Thereafter, mineral N contents in all treatments remained low.

Soil mineral N in the 0-10 and 10-30 depths under bare fallow declined from 18.7 mg/kg and 13.8 mg/kg in early January, respectively, to 12.5 mg/ka and 9.4 mg/kg at cane planting (Fig. 8 c, f). This

decline indicated the risk of soil mineral N loss under bare fallow during a wet fallow period. The soybean rotation treatments tended to have higher mineral N stocks in the 0-30 cm at cane planting compared to the bare fallow treatment. By the summer rapid cane growing season, there were no significant differences in mineral N stock in the soil profile between the unfertilised BF and the unfertilised SB treatment (Fig. 8 i). Fertiliser application increased soil mineral N contents, and fertiliser N downward movement was evident in the 30-60 cm depth but not in the deeper soil. At cane harvest, soil mineral N contents dropped to <3 mg/kg throughout the soil profiles in all treatments Fig. 8 l), indicating that residue effects of the fertiliser N to cane crops in the next season would be minimal.

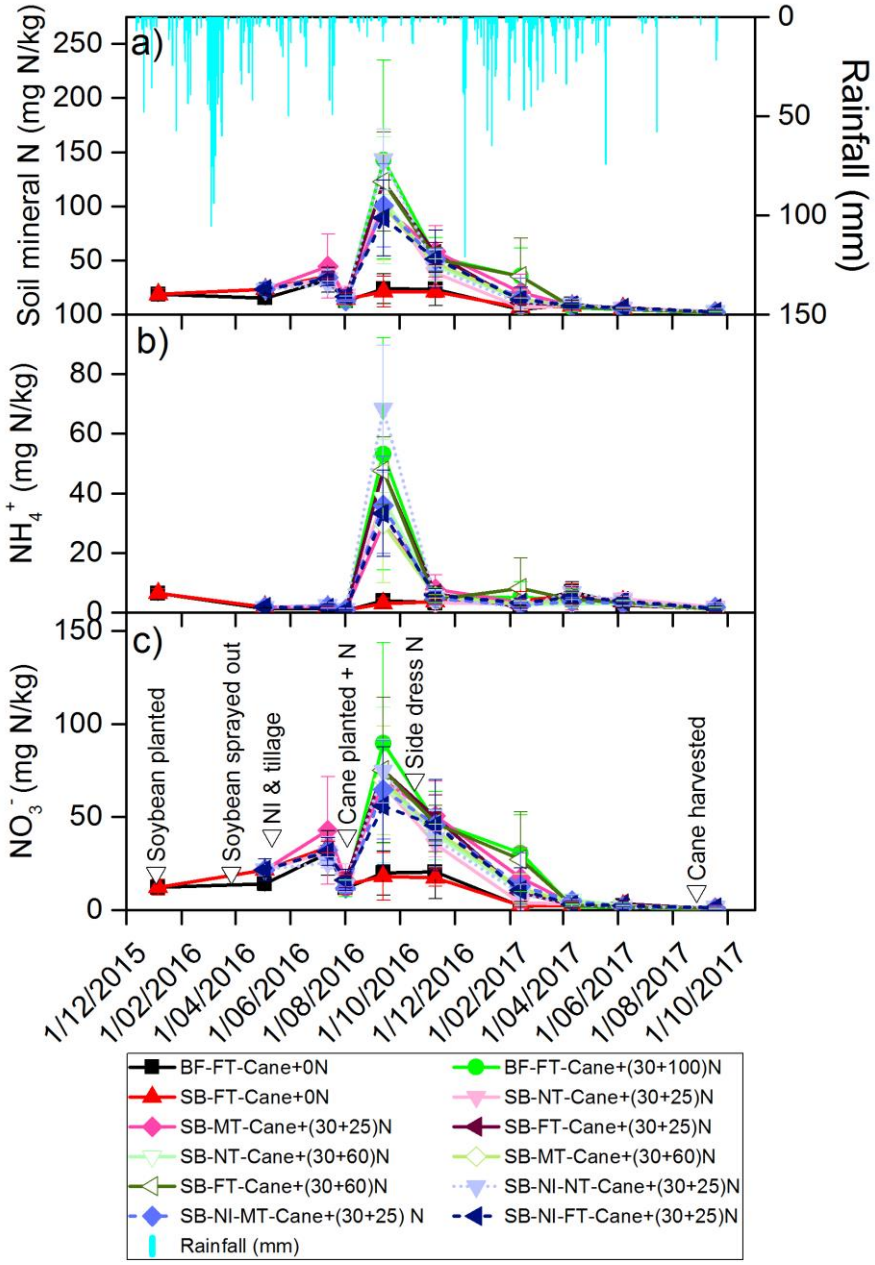

Figure 7. Mineral N dynamics in the top 0-10 cm soil in relation to rainfall and management practices at the Herbert site. Error bars are standard deviation of four replicates. BF, bare fallow; SB, soybean fallow; FT, full tillage; NT, no-till; MT, minimum tillage; NI, nitrification inhibitor spray on soybean residues; 0N, nil fertiliser N applied. Note soil samples were collected around the crop rows and might have somewhat missed the central area where fertiliser N was side dressed, particularly in the soil sampling event shortly after the side dressing in October 2016.

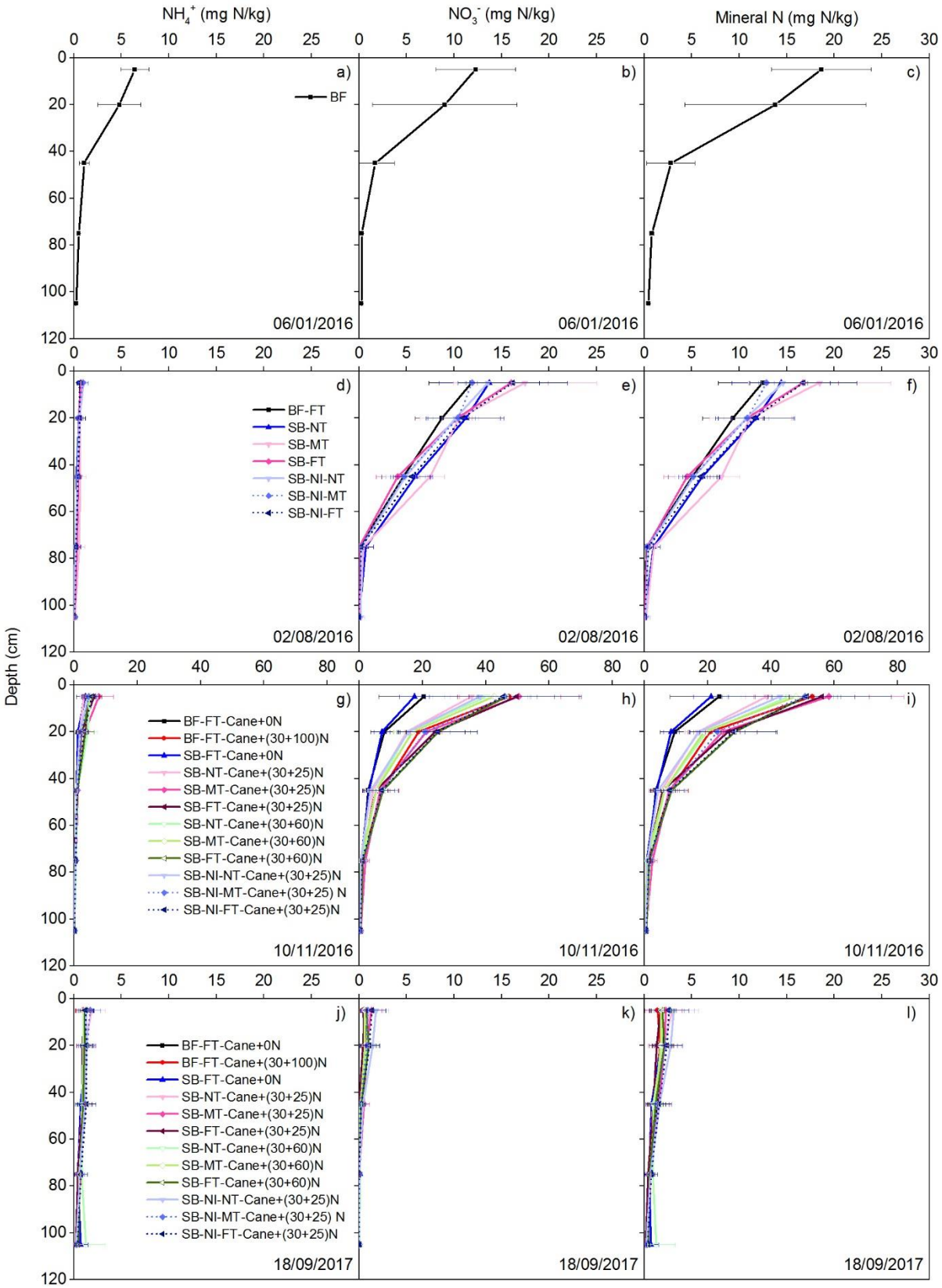

Figure 8. Mineral N contents in the soil profile at Herbert (a). BF: bare fallow; SB: soybean fallow; FT: full tillage; MT: minimum tillage; NT: no tillage; NI: nitrification inhibitor spray; (30+100)N: fertiliser applied as base at 30 kg N/ha and as side dressing at 100 kg N/ha.

6.1.3. Decomposition of soybean residues under field conditions

The soybean residue N vanished rapidly and exponentially during the first two months after incorporation into the ground, with about 75% of the total N unrecovered (Fig. 9). In comparison, N vanishing of the surface-retained soybean residues during the same period was significantly slower (43% of the total N released), and the rapid decomposition phase continued for about three months with approximately 61% of the biomass N released. Given the lack of significant differences in soil mineral N contents between the main plots with vs. without soybean residues or tillage vs. no till (Fig. 7 c) about two months after implementation of the treatments, the unrecovered soybean residue N during this period perhaps largely transformed into soil organic matter or lost in association with the high rainfall events. After the first 2-3 months, decomposition of the soybean residue N exhibited a linear pattern, at substantially slower rates with no significant differences between placement methods. At the end of the seven-month field incubation, about 93% and 69% of the soybean residue N was released under the incorporation and surface retention treatments, respectively.

The dynamics of soybean residue N vanishing under field conditions could be described with exponential plus linear models (Fig. 9). Based on these models, the incorporated soybean residues could release all the N in approximately 290 days within the plant cane growing season, while the surface-retained biomass could release about 83% of the total N within 12 months. However, as demonstrated by the results from laboratory incubation (Section 6.4.1), the crop residue N unrecovered might have partly entered into soil organic N pool rather than mineral N. Further studies using ^{15}N-labelled plant materials would help monitor the fate of legume residue N. Regardless, the differences in decomposition dynamics of the legume crop residues under different placement methods demonstrated that selection of a proper tillage method (e.g., no-till vs. rotary tillage) can be used to maximise synchronisation between legume residue N release and N demand by the subsequent plant cane. If cane is not to be planted shortly after harvest or spray out of the legume crop, leaving the crop residue on the soil surface could reduce the risk of N loss following high rainfall events. In contrast, if cane is to be planted soon and soil mineral N stock is low, incorporating the legume residues into soil may improve N supply to the cane.

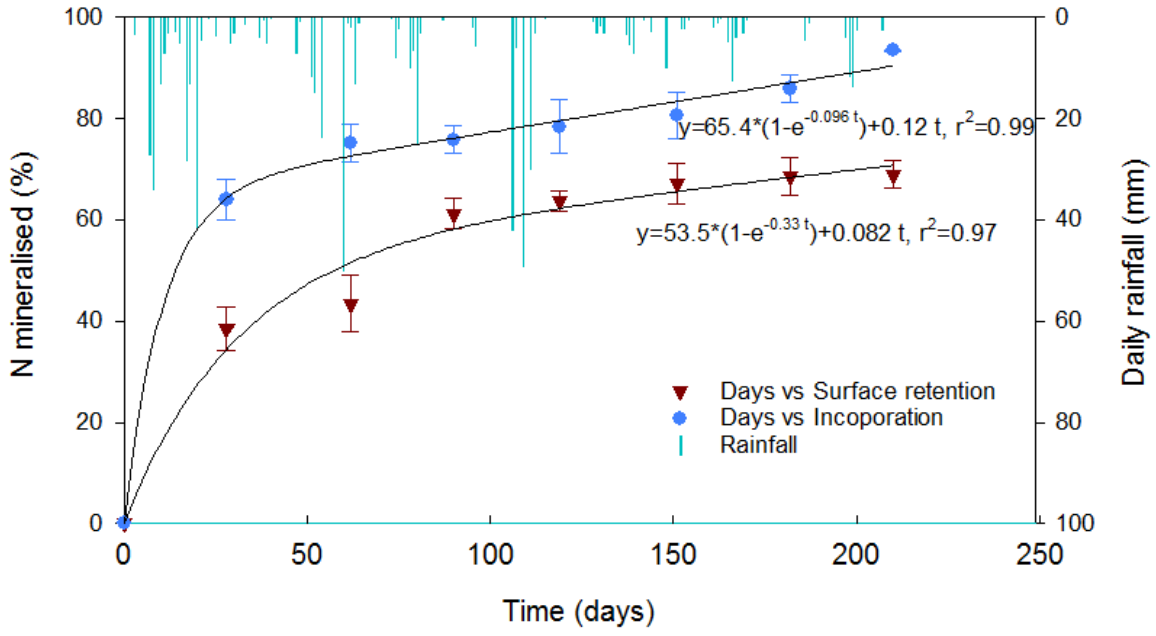

Figure 9. Dynamics of N mineralisation of soybean residues in litter bags placed on the ground or buried 10 cm below the ground surface under field conditions from 31 March (t = 0) to 27 October (t = 210 days), 2016.

6.1.4. Dynamics of cane biomass and N uptake under different management practices

The above ground dry biomass accumulation followed a sigmoidal pattern, while the biomass N exhibited a parabolic pattern and peaked around eight and a half months after cane planting (Fig. 10). The decline in the aboveground biomass N was probably attributable to re-allocation and transportation of the plant tissue N from the aboveground to the below ground and or loss of dead leaves.

At five and a half months after planting with basal application and side dressing of N fertiliser, the bare fallow with no fertiliser N application had the lowest dry biomass (8.8 t/ha). Among the SB-cane+(30+25)N treatments, the dry biomass increased in the order of FT (10.0 t/ha) < MT (11.5 t/ha) < NT (12.6 t/ha) with a significant difference between FT and NT treatments ($P < 0.05$). A similar trend was also observed for the plant N uptake. No significant differences in biomass and N uptake were recorded between the SB-cane+(30+25)N and (30+60)N treatments. This indicated that increasing fertiliser N application rates from the recommended rate to a higher rate was unnecessary. It also implied that the benefits of NT compared to FT might be due to non-N-related effects. There were no significant differences in biomass and N uptake between the treatments with NI and without NI spraying.

The aboveground biomass sampling at eight and a half months after planting showed significant ($P < 0.05$) differences between treatments (Fig. 10 a). At this stage, the dry biomass was lowest for the unfertilised bare fallow (30.2 t/ha) and the unfertilised soybean fallow (31.0 t/ha) treatments, which were consistent with the low soil mineral N for these treatments. Application of fertiliser increased the dry biomass, on average, by 13% following the bare fallow and by 8-19 % following the soybean fallow ($P < 0.05$). However, spraying DMPG on the soybean residues, different tillage management practices and different fertiliser N application rates (55N vs. 90N) did not significantly affect cane biomass at the 8.5-month stage.

The crop N uptake peaked at eight and a half months after planting (Fig. 10 b). The unfertilised treatments consistently had the lowest and similar N uptake throughout the cropping season, although the differences between these treatments and some others were not statistically significant. The different management practices of tillage, nitrification inhibitor application and even N fertiliser application rate did not significantly affect the crop N uptake. The lack of treatment effects was in line with the generally low and similar soil mineral N contents in different treatments at cane planting (Fig. 7), most likely due to N loss during the wet period between the spray out of the soybean crop and cane planting as mentioned above. Application of fertiliser N tended to improve crop N uptake, but increasing fertiliser application rate from 55 to 90 kg N/ha did not significantly increase crop N uptake.

At harvest, the aboveground dry biomass reached 42.1-50.1 t/ha, with the total N uptake in the range of 102-123 kg/ha. There were no significant differences in the biomass yield and N uptake between different treatments, including between fertilised and unfertilised treatments, at harvest.

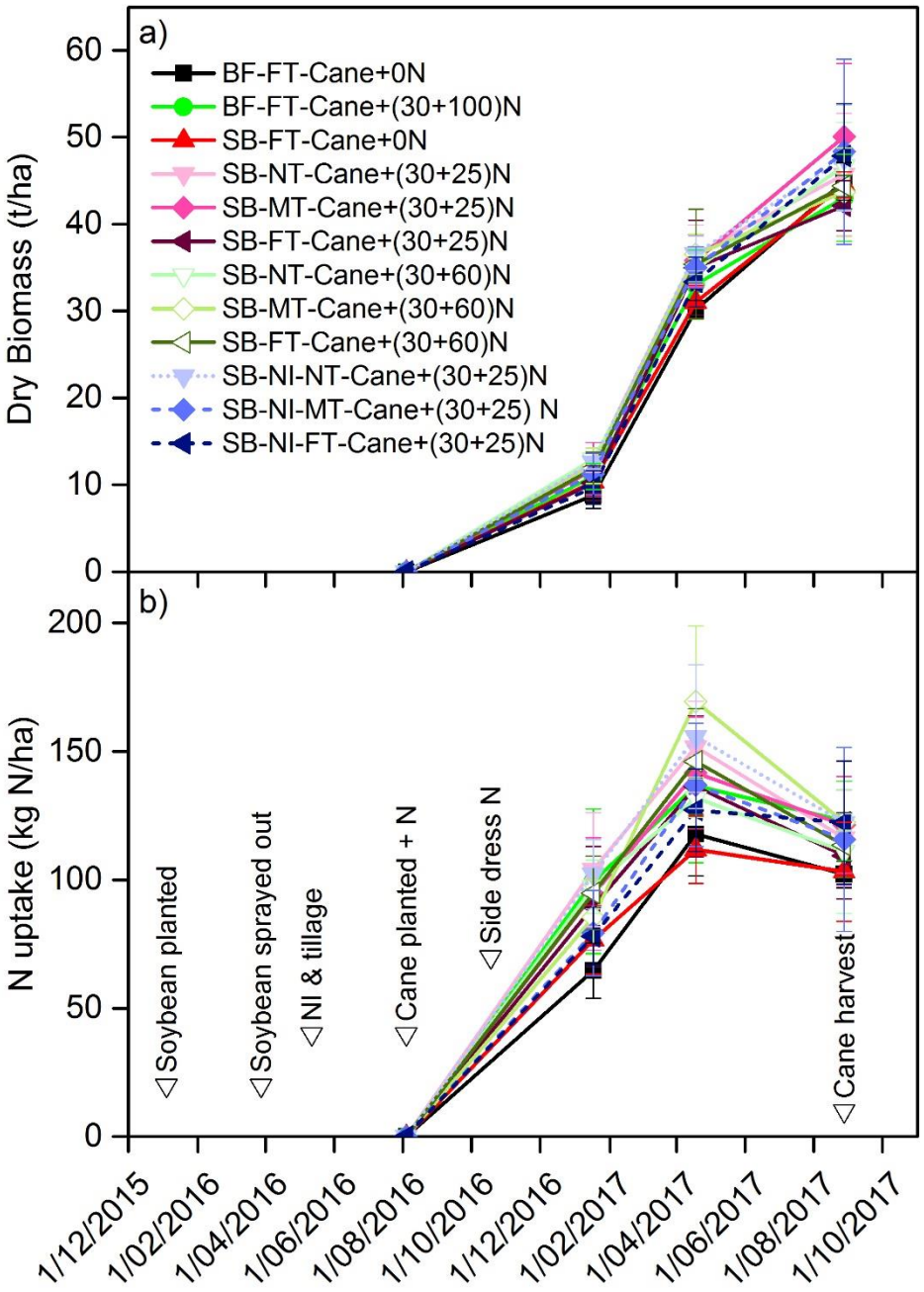

Figure 10. Dynamics of aboveground sugarcane biomass and N uptake under different treatments at the Herbert site.

6.1.5. Cane yield, CCS, sugar yield and profitability

The cane yield, CCS, sugar yield and gross margin were not significantly different between treatments (Table 5). In particular, N fertiliser application at 130 kg N/ha following bare fallow and at 55 and 90 kg N/ha following soybean rotation did not significantly increase sugar yield, compared to the unfertilised treatments. However, the 0N treatments had significantly lower soil mineral N contents during the first four to five months following cane planting (Figs. 7, 8) and consistently lower crop N uptake throughout the cropping season (Fig. 9; Table 5). The SB-cane+0N treatment had lower sugar yield and profit, although not statistically significant, than eight out of nine fertilised BF-cane treatments. From a risk management perspective, following BF with a low legume residue N

(87 kg/ha) and possibly substantial N loss before cane planting, fertiliser application would be preferred. Although it was difficult to determine the optimum N application rate in such circumstance, 30+25N appeared to have supplied enough N as the higher N application rate at 30+60N did not generate benefits in terms of sugar yield and profitability. The lack of yield and economic benefits at the N application rate of 130 kg N/ha following bare fallow, combined with the slightly lower CCS and higher N uptake ($P > 0.05$) compared to the unfertilised treatment, indicated that the recommended N rate based on 6ES guidelines was too high and thus should be reduced to a lower level.

Table 7. Cane yield, CCS, sugar yield, aboveground crop N uptake and profitability (mean±SD) under different management practices at the Herbert site.

Treatment†	Cane (t/ha)	CCS (%)	Sugar (t/ha)	N uptake (kg/ha)	Goss margin ($000s/ha)
1. BF – FT – Cane + 0N	118±6	15.9±0.7	18.8±1.2	102±5	2.72±0.35
2. BF – FT – Cane + (30+100)N	120±6	15.4±2.3	18.3±2.5	123±15	2.61±0.90
3. SB – FT – Cane + 0N	116±12	16.0±1.2	18.5±0.7	103±19	2.48±0.16
4. SB – NT – Cane + (30+25)N	125±17	16.3±0.2	20.4±2.8	116±19	3.16±0.64
5. SB – MT – Cane + (30+25)N	128±21	16.3±0.8	21.0±3.7	121±19	3.31±0.86
6. SB – FT – Cane + (30+25)N	111±12	16.0±0.7	17.7±1.3	109±17	2.48±0.23
7. SB – NT – Cane + (30+60)N	122±12	16.0±0.9	19.5±1.3	111±24	2.88±0.30
8. SB – MT – Cane + (30+60)N	113±8	16.4±0.7	18.6±1.2	122±3	2.76±0.30
9. SB – FT – Cane + (30+60)N	116±2	16.2±0.3	18.8±0.6	114±12	2.70±0.18
10. SB – NI – NT – Cane + (30+25)N	126±18	15.5±1.2	19.7±3.6	122±3	2.87±0.90
11. SB – NI – MT – Cane + (30+25)N	121±27	16.1±1.8	19.3±3.5	116±36	2.83±0.79
12. SB – NI – FT – Cane + (30+25)N	124±20	16.1±0.6	20.1±3.9	122±24	2.98±0.72
P value	*0.81*	*0.96*	*0.83*	*0.71*	*0.79*

†BF, bare fallow; SB, soybean rotation; FT, full tillage; NT, no tillage; MT, minimum tillage; NI, nitrification inhibitor sprayed on soybean residues before tillage; 0N, nil N applied; (30+100)N, basal plus side dressing fertilizer N at 30 and 100 kg N/ha, respectively.

6.2. The Burdekin trial

6.2.1. Soybean biomass yield and N benefit

The 3.5-month soybean crop yielded 6.3 t of aboveground biomass per hectare (Table 6). Total N in the aboveground crop residues was 213 kg/ha, and total N in both shoots and roots was about 273 kg N/ha, which was more than the industry fertiliser N application rate of 200 kg/ha. Therefore, there was no need for N fertilisation for the subsequent sugarcane crop following the soybean green manure retention.

The average $\delta^{15}N$ of the soybean residues was 0.048‰ and that for the reference grass plants was 10.02‰. Based on Eq. 1, about 88% (263 kg N/ha) of the soybean residue N was derived from biological N_2 fixation, while 12% (37 kg N/ha) from soil N.

Table 8. Aboveground soybean crop biomass and N yield at the Burdekin site.

Crop parts	Biomass (t/ha)	N content (%)	Biomass N (kg/ha)	Ndfa* (%)	Ndfs** (%)
Shoot	6.3	3.4	213	87.7	12.3

*Portion of biomass N derived from air through N_2 fixation; **Portion of biomass N derived from soil.

6.2.2. Dynamics of soil mineral N under different management practices

The soil mineral N contents changed with time, fallow management schemes as well as fertiliser N application. At the late fallow period, significantly higher mineral N was observed in bare fallow than in soybean rotation treatments in both 0-10 cm depth (35 vs. 9 mg/kg; Fig. 11 a) and 10-30 cm depth (15 vs. 7 mg/ha; data not shown). The mineral N was predominantly in the NO_3^--N form (Fig. 11 b, c). Following a few rainfall events, the mineral N contents in the BF soil decreased significantly to levels similar to those in the SB soil, demonstrating the risk of N loss with rainfall particularly in absence of growing crops. In the three months from cane planting to side dressing of fertiliser N, soil mineral N contents increased substantially with time in all treatments, but the SB treatments had consistently higher mineral N than the BF treatment ($P < 0.05$). Different tillage management practices, NI spraying and short-term growing of the N-catch crop did not significantly affect soil mineral N contents in the SB treatments. The highest mineral N contents in the soybean rotation treatments occurred in mid-June 2016, three months after the soybean spray out, with 45-89 mg N/kg in the 0-10 cm depth and 16-41 mg N/kg in the 10-30 cm depth. The soybean rotation treatments had probably adequate soil mineral N contents (>8 mg/kg) until early December 2016, in the first eight months of the sugarcane cropping season. With commencement of the wet season in January 2017, the soil mineral contents dropped to low levels and remained low until the cane harvest. The low mineral N during this period could be the results of multiple factors including decreased mineralisation of soybean residue N (Fig. 9), rapid N uptake by cane crops during the wet and warm season, and N loss from denitrification, leaching and/or runoff associated with the high rainfall events.

The mineral N contents in soil profile decreased exponentially with depth until early summer during the sugarcane cropping season (Fig. 12) and significant differences between different treatments or times were detected mainly in the 0-30 cm depth. During the period between 11 November 2015 (pre-soybean planting) and 18 April 2016 (post cane planting), soil mineral N contents in the 0-30 cm depth increased substantially in both the bare fallow and the soybean rotation treatments (Fig. 12 c and f), in spite of possible N losses in conjunction with some high rainfall events (Fig. 11 c). This demonstrated partly the capacity of N supply from soil organic matter mineralisation. The higher mineral N contents in the surface 0-30 cm soil were evident until the early summer of the cane growing season. Mineral N movement to below 30 cm was not detected (Fig. 12 f, i, l) in this clayed soil in the dry tropical region. The deep soil layers had consistently low mineral N (< 2.5 mg/kg) over the fallow period and the sugarcane cropping season. Therefore, the mineral N losses from the 0-10 cm soil as mentioned above were most likely due to the denitrification and perhaps lateral runoff rather than leaching. By the harvesting time, soil mineral N contents were low throughout the profile with little differences between depths, demonstrating severe depletion of mineral N in the surface 30 cm soil during the sugarcane growing season.

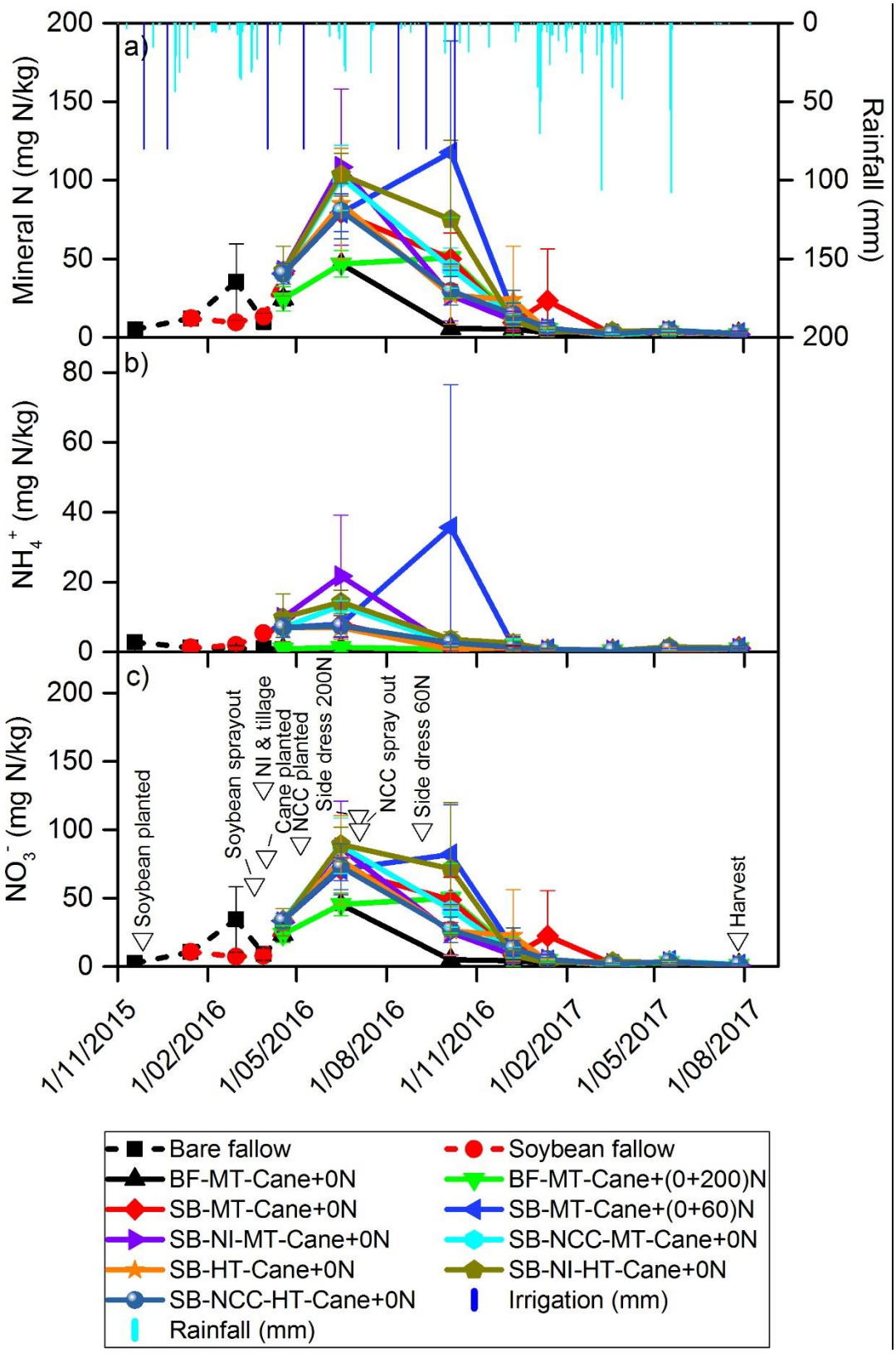

Figure 11. Mineral N dynamics in the top 0-10 cm soil in relation to rainfall and management practices at the Burdekin site. Error bars are standard deviation of four replicates. BF, bare fallow; SB, soybean fallow; FT, full tillage; NT, no-till; MT, minimum tillage; NI, nitrification inhibitor spray on soybean residues; 0N, nil fertiliser N applied. Note soil samples were collected around the crop rows and might have somewhat missed the central area where fertiliser N was side dressed, particularly in the soil sampling event shortly after the side dressing in October 2016.

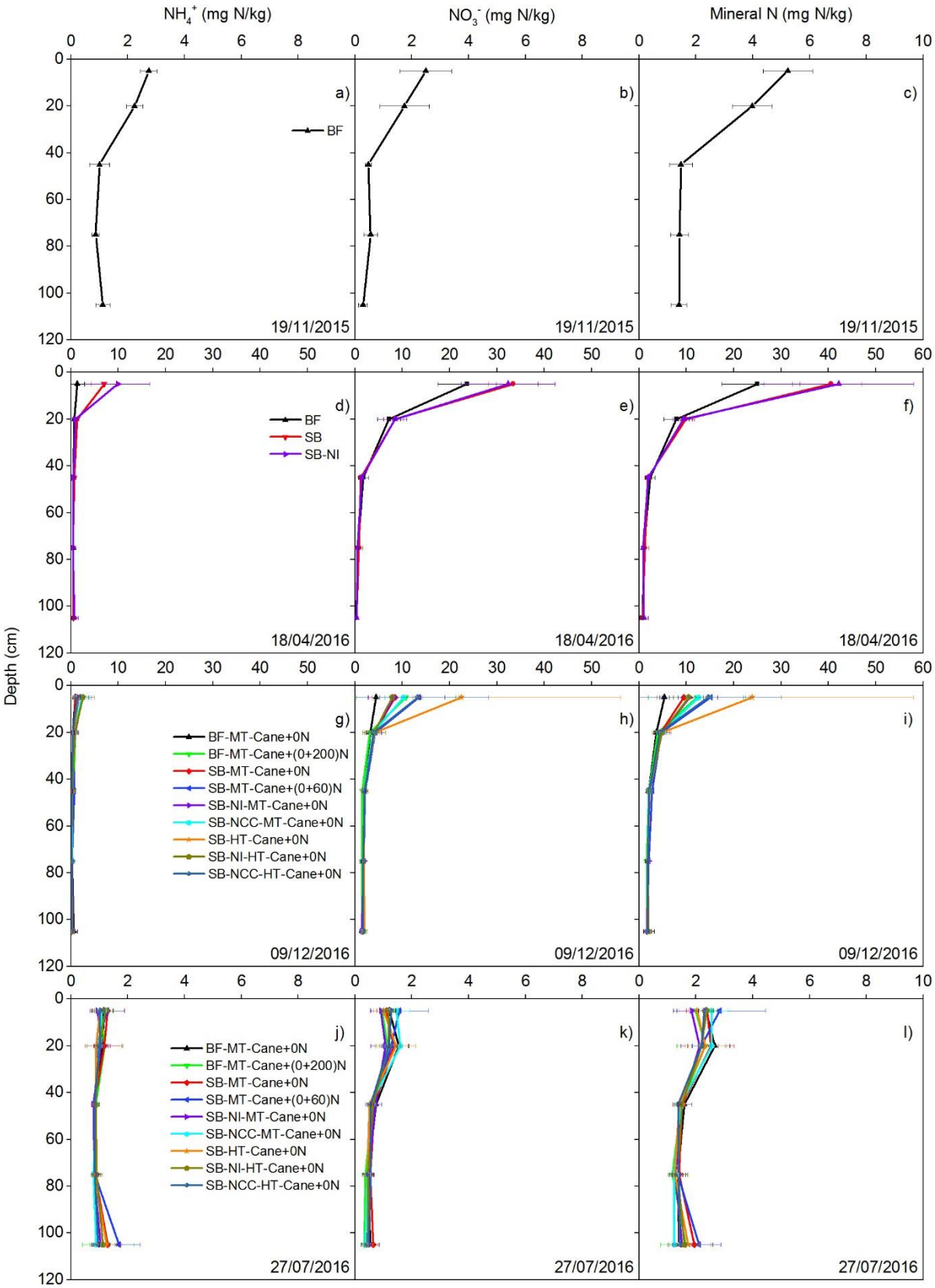

Figure 12. Mineral N contents in the soil profile at Burdekin. BF: bare fallow; SB: soybean fallow; MT: minimum tillage; HT: heavy tillage (three passes of wavy disc); NI: nitrification inhibitor spray; (0+200)N: fertiliser applied as side dressing at 200 kg N/ha.

6.2.3. Dynamics of cane biomass and N uptake under different management practices

The aboveground biomass increased linearly in the first thirteen months after cane planting (Fig. 13 a). The biomass in some treatments decreased during the last three months of the cropping season. This decrease was perhaps partly due to the different plant sampling methods used within the cropping season (manually cut from a 1 m section per plot) and at harvest (machine harvested from a 15 m section in each plot), and partly due to respiration exceeding photosynthesis of the aged cane crop. No biomass decline was observed with the 13-month crop at Herbert and the 12-month crop at Bundaberg (see below). There were no statistically significant differences in dry biomass between the treatments before harvest. However, the BF-cane +0N treatment and the BF-cane+200N treatments resulted in the lowest and the highest biomass at harvest ($P < 0.05$), respectively. The biomass at harvest also tended to be lower under HT than MT ($P = 0.088$). DMPG and N-catch crop did not significantly affect the crop biomass throughout the season.

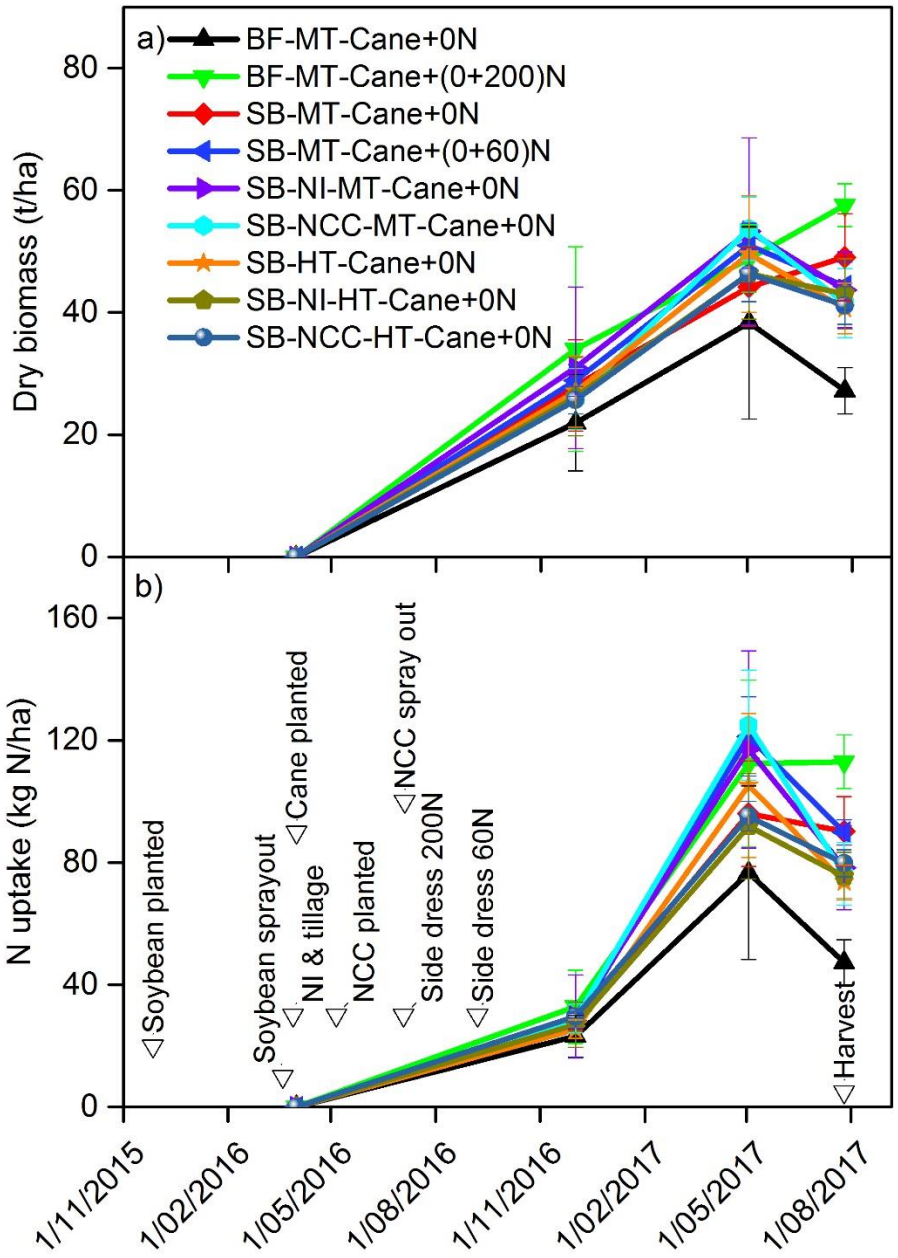

Figure 13. Dynamics of aboveground sugarcane biomass and N uptake under different treatments at Burdekin. BF: bare fallow; SB: soybean fallow; MT: minimum tillage; HT: heavy tillage (three passes of wavy disc); NI: nitrification inhibitor spray; (0+200)N: fertiliser applied as side dressing at 200 kg N/ha.

The crop N uptake appeared more sensitive than biomass to the different soybean residue and N management practices (Fig. 13 b). At the thirteenth month plant sampling, cane plants in the BF+0N treatment had the lowest amount of biomass N ($P < 0.05$). There were significant interaction between tillage and NCC. Among the unfertilised SB treatments without DMPG, the crop N uptake was significantly higher in the MT (125 kg N/ha) than in the FT treatments (95 kg N/ha) when the NCC was grown but had no significant differences without NCC. Growing NCC also increase N uptake (125 kg N/ha) by the cane compared to the nil NCC treatment (96 kg N/ha) under MT but not under FT. At harvest, the lowest and highest N uptakes in the aboveground biomass were recorded in the BF-cane+0N and BF-cane+(0+200)N treatments ($P < 0.05$), respectively. Without fertiliser N application or N-catch crop, SB-MT resulted in significantly higher crop N uptake than the SB-HT treatments. Growing N-catch crop substantially decreased sugarcane N uptake under MT, in contrast to the observation thirteen months after cane planting. However, NCC did not reduce cane N uptake under HT.

6.2.4. Cane yield, CCS, sugar yield and profitability

Both cane yield and sugar yield differed significantly between different treatments at Burdekin (Table 7). The conventional BF-MT-Cane+(0+200)N treatment resulted in the highest cane yield (136.5 t /ha), sugar yield (21.0 t/ha) and gross margin ($3430/ha). The lowest yields and gross margin were recorded for the unfertilised bare fallow treatment. With no fertiliser N application, the soybean fallow increased sugar yield by 48-73 % and gross margin by $1120/ha compared to the bare fallow. However, soybean rotation alone with zero or 60 kg N/ha fertiliser decreased sugar yield by 21-32% compared to the BF+200N treatment. These results indicated that the large amounts of soybean residue N (272 kg N/ha) failed to supply sufficient N to the cane crop and that a proper fertiliser application scheme would be needed following the soybean rotation. This appears consistent with the findings from the laboratory study (Section 6.4.1) that only 16.0% and 33.5% of the soybean residue N could be counted in the soil mineral N pool after 10 months of incubation at 25^{o}C and optimal soil moisture. However, interpretation of this result needs to be cautious as the treatment effects on crop biomass at harvest differed substantially from those obtained three months earlier (Fig. 13 a). Different tillage management practices, spraying DMPG onto the soybean residues before tillage and growing mungbean as a N-catch crop during the early cane cropping season did not significantly affect cane or sugar yield.

Table 9. Cane yield, CCS, sugar yield, aboveground crop N uptake and profitability (mean±SD) under different management practices at the Burdekin site.

Treatment†	Cane* (t/ha)	CCS (%)	Sugar* (t/ha)	N uptake* (kg/ha)	Goss margin ($000s/ha)
1. BF – MT – Cane + 0N	62.7^{a}	15.4	9.6^{a}	47.3^{a}	0.93^{a}
2. BF – MT – Cane + (0 + 200)N	136.5^{d}	15.4	21.0^{c}	113.0^{d}	3.43^{c}
3. SB – MT – Cane + 0N	108.4^{c}	15.2	16.5^{b}	90.3^{c}	2.05^{b}
4. SB – MT – Cane + (0 + 60)N	103.5^{bc}	14.5	14.9^{b}	89.9^{c}	1.45^{ab}
5. SB – DMPG – MT – Cane + 0N	97.9^{bc}	15.8	15.5^{b}	78.3^{bc}	1.85^{b}
6. SB – NCC – MT – Cane + 0N	94.8^{bc}	16.0	15.2^{b}	76.3^{b}	1.64^{ab}
7. SB – HT – Cane + 0N	96.2^{bc}	15.4	14.9^{b}	73.7^{b}	1.62^{ab}
8. SB – DMPG – HT – Cane + 0N	97.1^{bc}	15.8	15.4^{b}	75.5^{b}	1.71^{b}
9. SB – NCC – HT – Cane + 0N	92.9^{b}	15.7	14.6^{b}	79.7^{bc}	1.39^{ab}
P value	< 0.001	0.062	< 0.001	< 0.001	<0.001

†BF, bare fallow; SB, soybean rotation; HT, heavy tillage; MT, minimum tillage; NI, nitrification inhibitor sprayed on soybean residues before tillage; 0N, nil N applied; (0+160)N, fertilizer side dressed at 160 kg N/ha. *Values followed by the same letter were not significantly different at $P < 0.05$.

6.3. The Bundaberg trial

6.3.1. Soybean and peanut yield and N input from crop residues

The total biomass yield was slightly higher for peanut than soybean (Table 8). However, the total amount of N in soybean plants was about two times that in peanut plants. Of the biomass N, biological N_2 fixation contributed 73.9% and 87.3% for the soybean and peanut crops, respectively. The soybean acquired more N from both biological N_2 fixation and soil than the peanut crops. After harvest of grain/pod, the soybean residues contained 281 kg N/ha, while the peanut residues contained 88 kg N/ha. Regional typical N fertiliser application rate for the plant cane following bare fallow was 150 kg N/ha at this site. Based on the 6ES guidelines, there was no need for N fertiliser application following the soybean rotation, but 42 kg N/ha was needed following the peanut rotation.

Table 10. The legume crop biomass and N yield at different trial sites.

Site	Biomass (t/ha)	N content (%)	Total biomass N (kg/ha)[c]	Crop residue N (kg/ha)	Ndfa (%)	Ndfs (%)
Soybean[a]	10.6	3.92	531*	281	73.9	26.1
Peanut[b]	11.1	2.40	264	88	87.3	12.7

*Only the aboveground biomass was sampled; total biomass N was calculated assuming 22% of the total biomass N being roots. Ndfa: N derived from air; Ndfs: N derived from soil.

6.3.2. Impacts of legume crop rotation on nitrifying microbial community

There was no significant difference in the estimated microbial species richness (Chao1) between bare fallow (633 ± 18), peanut cropping (650 ± 19) and soybean cropping (499 ± 152). Similarly, Shannon's index also indicated no significant difference in community evenness between bare fallow (7.5 ± 0.1), peanut cropping (7.7 ± 0.1) and soybean cropping (7.8 ± 0.0).

Quantitative PCR results demonstrated significantly higher ($P < 0.05$) 16S rRNA gene abundances in the peanut (63.5±13.3 $\times$ 10^8/g soil) and soybean (79.0±4.0 $\times$ 10^8/g soil) treatments compared to the bare fallow (40.9±4.80 $\times$ 10^8/g soil). Thus, the legume cropping increased abundances of bacteria and archaea by 1.6 (peanut) and 2.0 (soybean) times compared to the bare fallow ($P < 0.05$).

While the richness and evenness of microbial species were similar in different treatments, the overall microbial community composition significantly differed between the treatments (Fig. 14). In addition, soil microbial community composition differed between soybean cropping and peanut cropping, suggesting that the crops imparted species-specific selective pressure on the surrounding soil microbial communities.

The 16S rRNA gene sequence-based community composition indicated that the ammonia oxidisers responsible for conversion of ammonia to hydroxylamine in the first step of nitrification were mainly archaea rather than bacteria in this sugarcane cropping soil (Table 9). Relative abundances of these ammonia oxidisers were significantly lower ($P < 0.05$) in both the peanut (0.26 ± 0.08%) and soybean (0.20 ± 0.10%) cropped soils compared to the bare fallow (0.54 ± 0.15%). After taking into account the number of 16S rRNA genes measured in the soils, the absolute abundance of ammonia oxidisers in peanut and soybean treatments were 24% and 44% lower ($P < 0.05$), respectively, compared to the bare fallow. This result indicates that legume cropping supressed the proliferation of known ammonia oxidisers.

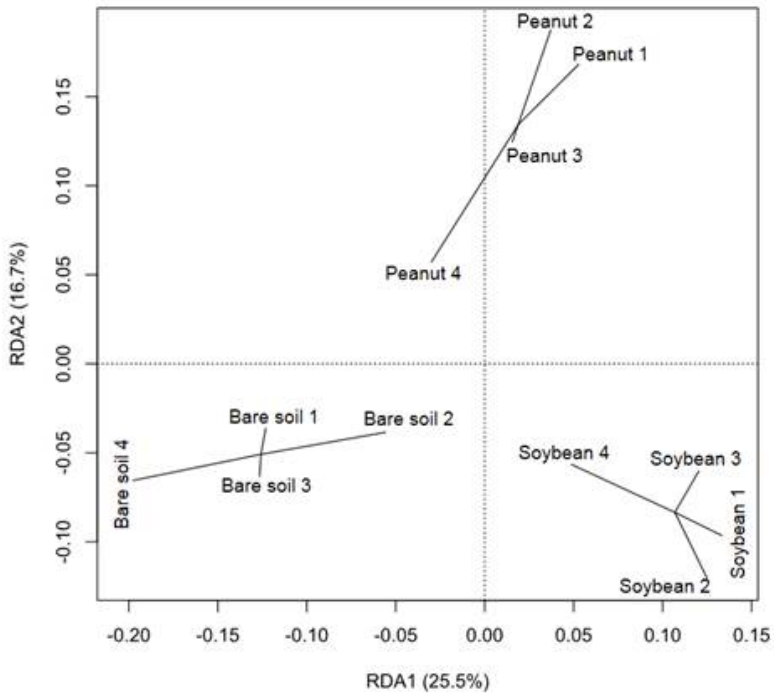

Figure 14. Ordination of soil microbial community composition in soils under different fallow management practices (Redundancy analysis, RDA). Replicates are connected to their respective group centroid. Components 1 and 2 represent 25.5% and 16.7% of the communities' variance, respectively.

Table 11. Relative abundance (%, mean±SD) of ammonia oxidisers and nitrite oxidisers in BF, PN and SB treatments.

Genus identification (Phylum)*	Bare**	Peanut**	Soybean**
Ammonia oxidising archaea			
Nitrosopumilus (Thaumarchaeota)	0.03 ± 0.04[a]	0.00 ± 0.01[a]	0.00 ± 0.01[a]
Nitrososphaera (Thaumarchaeota)	0.48 ± 0.22[a]	0.24 ± 0.14[b]	0.14 ± 0.14b
Ammonia oxidising bacteria			
Nitrosomonadaceae (Proteobacteria)	0.03 ± 0.04[a]	0.02 ± 0.02[a]	0.06 ± 0.06[a]
Sub-total	**0.54 ± 0.30[a]**	**0.26 ± 0.16[b]**	**0.20 ± 0.10[b]**
Nitrite oxidising bacteria			
Nitrospira (Nitrospirae)	0.28 ± 0.14[a]	0.29 ± 0.12[a]	0.25 ± 0.12[a]
Nitrobacter (Proteobacteria)	0.04 ± 0.04[a]	0.01 ± 0.02[a]	0.00 ± 0.01[a]
Sub-total	**0.32 ± 0.18[a]**	**0.30 ± 0.14[a]**	**0.25 ± 0.12[a]**

*All taxa listed are genus level except for the family *Nitrosomonadaceae*. **Numbers within a row followed by same letters were not significantly different at P < 0.05.

There were significantly more archaeal than bacterial *amo*A sequences (P < 0.05; Fig. 15). In addition, the relative abundances of archaeal *amo*A gene in the peanut and soybean soils were only about 22% and 15%, respectively, of that in the bare fallow soil (*P* < 0.05; Fig 15). As the total microbial dsDNA in the peanut (4.9±0.5) and soybean (6.2±0.2) cropping soils was 1.6 and 2.0 times higher, respectively, than in bare fallow (3.1±0.4), the total abundances of archaeal *amo*A in the peanut and soybean treatments were 35% and 30% of that in the bare fallow, respectively (*P* < 0.05).

These *amo*A abundance profiles corroborated (r = 0.77, P < 0.01; n = 12) the 16S-based measurements of AOA and AOB (Table 9).

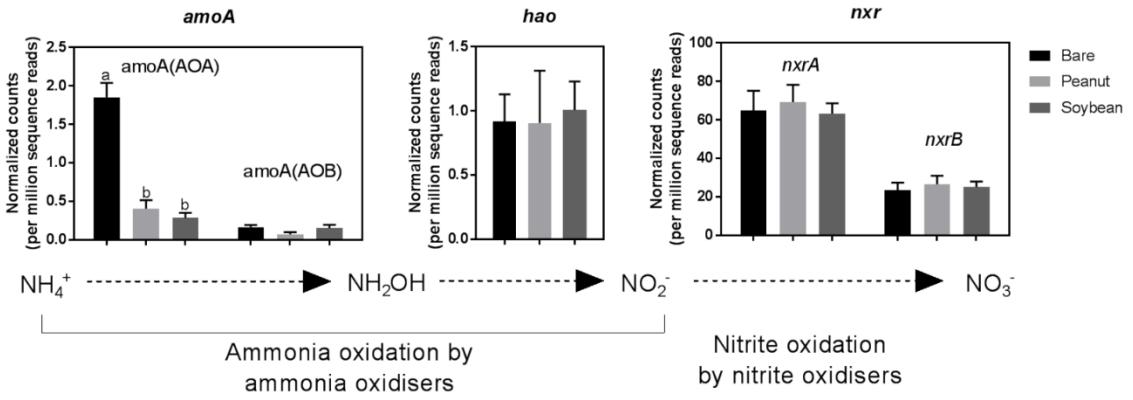

Figure 15. Effects of legume crop rotation on the abundance of nitrification genes (mean±SE). The genes and their encoded enzymes are: *amoA*(AOA), archaeal ammonia monooxygenase; *amoA*(AOB), bacterial ammonia monooxygenase; *hao*, hydroxylamine oxidoreductase; *nxrA*, nitrite oxidoreductase α subunit; *nxrB*, nitrite oxidoreductase β subunit. Different letters indicate significant differences at *P* < 0.05.

6.3.3. Dynamics of soil mineral N under different management practices

Mineral N contents in the 0-10 cm depth varied substantially with time and management practices (Fig. 16). At legume planting, the BF soil had quite high mineral contents (35 mg/kg; dominated by NO_3^-). After few high rainfall events during the early fallow period, soil mineral N in this treatment decreased by about 50%. While increased with time in the BF soil in the following less wet period, most likely as a result of N mineralisation, the mineral N contents in the surface soil declined significantly after two high rainfall events following harvest of the legume grain/pods. In comparison, soil mineral N contents in the SB and PN treatments remained low throughout the legume cropping season, at least in part due to plan N uptake. These results indicated that the soil mineral N accumulated during the fallow period was susceptible to losses unless being taken up by plants, and legume crop rotation provides an effective means to retain the soil mineral N in plant biomass.

During the sugarcane cropping season, soil mineral N contents in the 0-10 cm depth were significantly affected by fertiliser application, as expected, as well as other management practices and rainfall. The BF treatment that received 25 kg N/ha at cane planting had higher soil mineral N contents than the unfertilised BF and SB treatments for approximately three months after cane planting, before decreasing and increasing again following side dressing of 125kg N/ha (Fig. 16). The PN+42N treatments also showed relatively higher mineral N contents following side dressing of urea. Although the BF+0N and soybean treatments received no N fertiliser, the mineral N contents in soil increased with time over the first 7 months following cane planting. The treatment that allowed volunteer soybeans to re-grow as a NCC had comparatively low mineral N contents. By April to May 2017, approximately 7-8 months after cane planting, soil mineral N contents decreased to low levels in all treatments following a few high rainfall events (>60 mm/day). Thereafter, soil mineral N contents remained low, with no significant differences between treatments.

Legume crop rotation and subsequent management practices including fertiliser application significantly affected mineral N distribution in the soil profile. At the beginning of the legume cropping season, there were substantial amounts of mineral N in the soil profile (Fig. 17 a, b, c). Growing legume crops substantially depleted mineral N contents throughout the profile (*P* < 0.01; Fig. 17 d, e, f), with significantly lower mineral N accumulated in the SB and PN treatments than in the BF soil at the late stage of the legume growing season. These observations demonstrated the capacity of the legume crops in retaining the excessive N through root uptake. Allowing volunteer

soybean plants to re-grow as N-catch crops also significantly decreased soil mineral N stock in the 0-30 cm depth (Fig. 17 i). Spraying DMPG onto the legume residues before incorporation slightly slowed down nitrification in the SB-FT treatment in the first month as indicated by higher NH_4^+ in the 0-10 cm depth (Fig. 17 g). FT increased N mineralisation from soybean residues in the first month (Fig. 17 i) and from peanut residues in the following 3 months (Fig. 17 l) in comparison to the NT treatments, as indicated by the higher mineral N contents. At the time of cane harvest, mineral N contents in the soil profiles declined to < 5 mg/kg in all treatments, with moderate but evident movement of NO_3^- to deep soil (30-100 cm) in the BF-cane+(25+125)N treatment (Fig. 17 p, q).

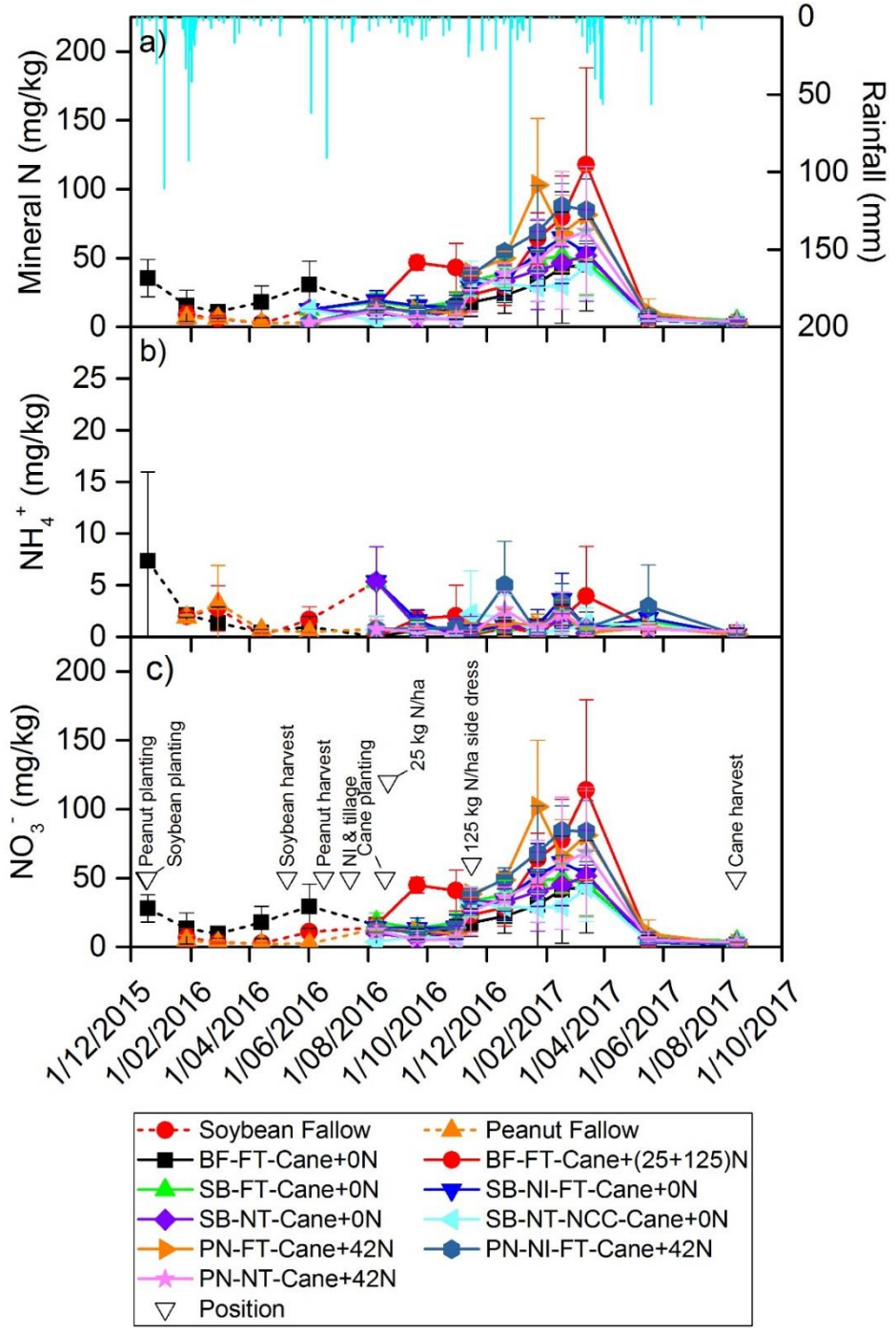

Figure 16. Mineral N dynamics in the top 0-10 cm depth of soil in relation to rainfall and management practices at Bundaberg. Hollow triangles indicate when management practices occurred. Error bars are standard deviation of four replicates. BF: bare fallow; SB: soybean rotation; PN: peanut rotation; FT: full tillage; NT: no tillage; NI: nitrification inhibitor sprayed on legume residues; 0N: nil fertiliser N; NCC: N-catch crop.

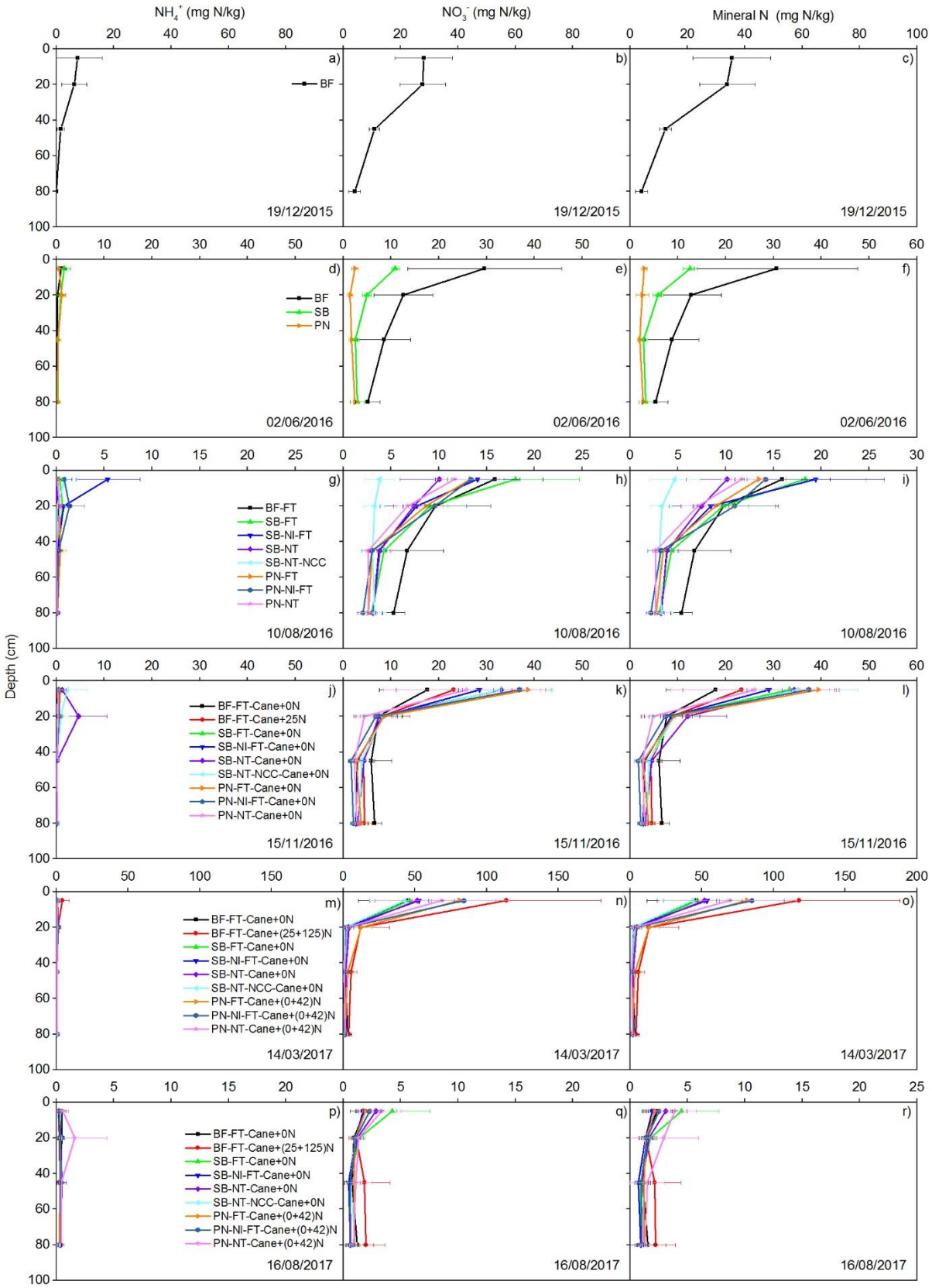

Figure 17. Mineral N contents in the soil profile at Bundaberg. Horizontal bars are standard deviation of four replicates. BF: bare fallow; SB: soybean rotation; PN: peanut rotation; FT: full tillage; NT: no tillage; NI: nitrification inhibitor spray; (25+125)N: fertiliser N applied as base and side dressing at 25 and 125 kg N/ha, respectively.

6.3.4. Dynamics of cane biomass and N uptake under different management practices

The aboveground cane biomass increased slowly in the initial four months and then almost linearly until harvest (Fig. 18). The treatments resulted in no significant differences in dry biomass at the fourth and seventh months after cane planting. At harvest, the amounts of biomass were the lowest in the PN-NT-cane+(0+42)N and PN-FT-cane+(0+42)N treatments (29.2-29.4 t/ha) and the highest in the SB-FT and SB-NI-FT treatments (36.5-37.2 t/ha; $P < 0.05$).

Nitrogen uptake by the sugarcane crop continued throughout the cropping season, but at markedly slower rates during the last five months (Fig. 18 b). The treatments did not show significant differences in N uptake at any stage of the cropping season. At harvest, the total amount of aboveground N uptake ranged from 101-102 kg/ha in the PN-FT or PN-NT treatments to 126.9-128.3 kg/ha in the BF+150N and SB-FT+0N treatments.

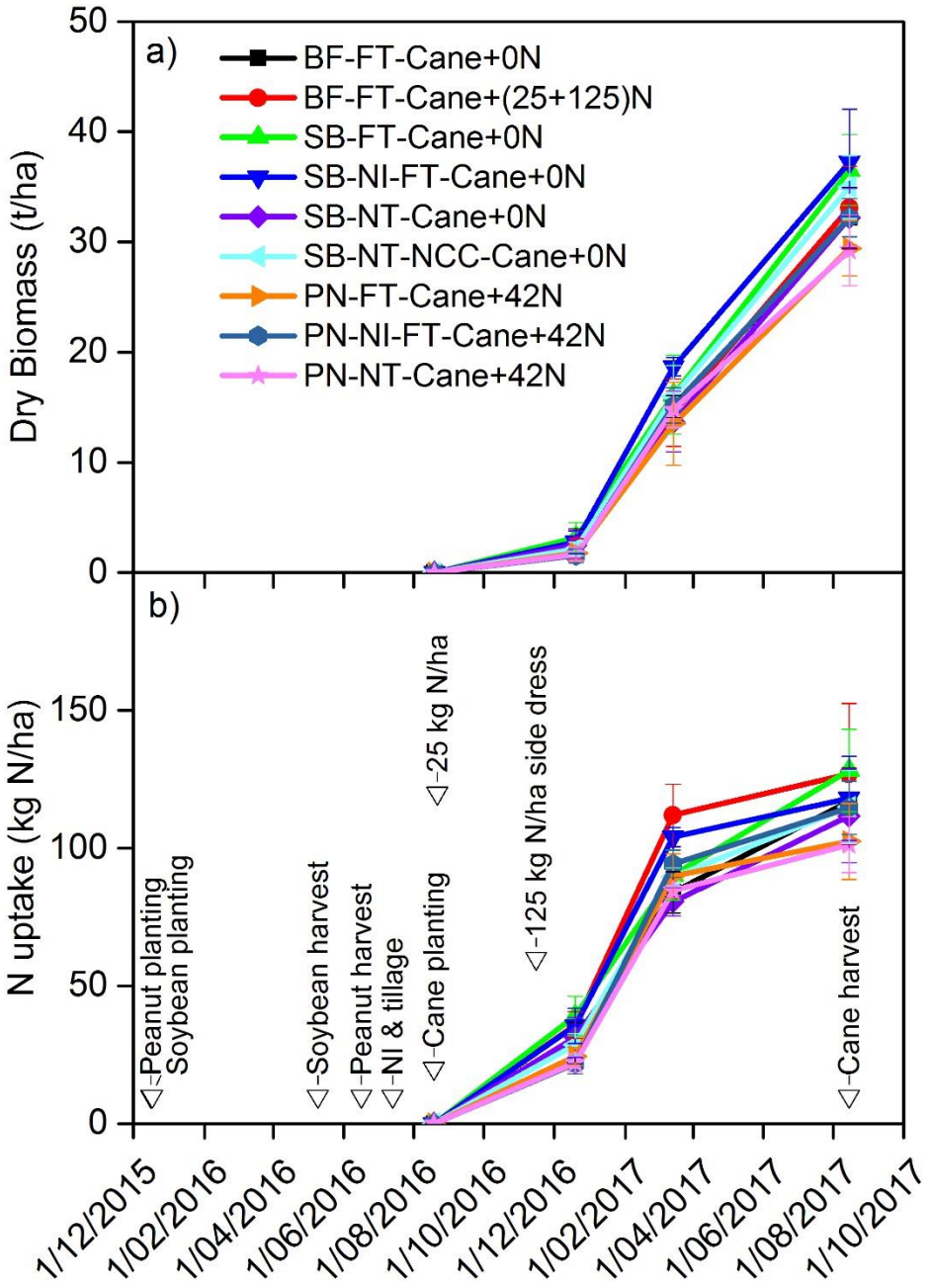

Figure 18. Dynamics of aboveground sugarcane biomass and N uptake under different treatments at Bundaberg.

6.3.5. Cane and sugar yield and profitability

The different management practices resulted in significantly different cane yields at Bundaberg (Table 12). Application of 150 kg N/ha to the bare fallow only increased cane yield by 9% compared to nil N addition ($P > 0.05$). Soybean fallow generally resulted in higher cane yield than peanut fallow although no N fertiliser was applied following soybean fallow. For example, the SB-FT+0N treatment had 23% higher cane yield compared to the PN-FT+42N treatment. The application of DMPG to soybean and peanut residues prior to tillage increased cane yield by only 3.9% and 12%, respectively, which were not statistically significant. The direct drill technique (NT) did not have benefit on cane yield regardless of legume species. In the SB-NT-NCC+0N treatment where soybean was allowed to re-establish to capture surplus soil mineral N and also potentially fix N_2, the cane yield was higher but not significantly different from the SB-NT+0N treatment.

The difference in sugar yield between treatments were marginally non-significant ($P = 0.076$). Multiple comparison results of sugar yield between the treatments were generally consistent to those of cane yield as the CCS contents were not significantly influenced by the management practices. On average, the sugar yield increased in the order: PN + 42N (13.7 t/ha) < BF-FT with or without N fertiliser application (15.3 t/ha) = SB+0N (15.6 t/ha).

Different land use schemes (BF vs. SB vs. PN) during the fallow period generated significantly different economic outcomes. The land use effects on profitability were mainly due to cash values of soybean grain or peanut pods. In spite of the relatively lower N benefits and sugar yield resulting from peanut rotation, the PN-cane scheme generated the highest income on average ($2.58K/ha) compared to SB-cane ($1.82K/ha) and the BF-cane schemes ($1.28K/ha). The gross margins were not significantly different between different management practices (tillage, NCC, NI and N application rates) under the same fallow land use schemes.

Table 12. Cane yield, CCS, sugar yield, aboveground crop N uptake and profitability (mean±SD) under different management practices at the Bundaberg site.

Treatment†	Cane yield* (t/ha)	CCS (%)	Sugar (t/ha)	N uptake (kg/ha)	Goss margin ($000s/ha)
1. BF – FT – Cane + 0N	88±9.1^{ab}	17.0±0.5	15.0±1.4^{ab}	110±18	1.34±0.15^{a}
2. BF – FT – Cane + 25N+125N	95.6±7.8^{bc}	16.2±0.3	15.5±1.3^{ab}	127±26	1.21±0.14^{a}
3. SB – FT – Cane + 0N	99.3±9.5^{bc}	16.1±0.5	16.0±1.8^{b}	128±15	1.82±0.35^{abc}
4. SB – NI – FT – Cane + 0N	103.2±16.6^{c}	15.9±0.6	16.3±2.4^{b}	118±15	1.78±0.42^{abc}
5. SB – NT – Cane + 0N	84.9±12.5^{ab}	16.6±0.6	14.1±2.46^{ab}	112±17	1.57±0.39^{ab}
6. SB – NT – NCC – Cane + 0N	95.6±8.2^{bc}	16.9±0.6	16.1±1.5^{b}	115±13	2.10±0.16^{abcd}
7. PN – FT – Cane + 42N	80.9±4.8^{a}	16.3±0.6	13.2±1.2^{a}	102±14	2.43±0.29^{bcd}
8. PN – NI – FT – Cane + 42N	90.6±3.2^{abc}	16.5±0.7	14.9±0.7^{ab}	115±10	2.81±0.09^{d}
9. PN – NT – Cane + 42N	77.9±10.3^{a}	16.7±0.2	13.0±1.9^{a}	101±10	2.60±0.32^{cd}
P value	*0.022*	*0.086*	*0.076*	*0.285*	*0.003*

†BF, bare fallow; SB, soybean rotation; FT, full tillage; NT, no tillage; MT, minimum tillage; NI, nitrification inhibitor sprayed on soybean residues before tillage; 0N, nil N applied; (30+100)N, basal plus side dressing fertilizer N at 30 and 100 kg N/ha, respectively. *Values followed by the same letter were not significantly different at $P < 0.05$.

6.4. Laboratory incubation study: N mineralisation from different legume residues and impacts of placement and nitrification inhibitor

6.4.1. Effects of legume residue types

The three legume residues studied demonstrated large variability in N mineralisation dynamics. Soil NH_4^+ contents initially increased substantially in mature soybean treatments, and to smaller extents in other residues (Fig. 19 a, b, c), as a result of N mineralisation of the legume residues. Soil NO_3^- contents initially decreased in treatments added with peanut and green manure soybean residues, whilst increased in the treatment with mature soybean (Fig. 19 d, e, f). Decreases in mineral N contents were evident in the peanut and green manure soybean treatments at Day 10, perhaps due to immobilisation or denitrification of NO_3^-. Compared to the Control, lower mineral N contents continued up to Day 30 in most peanut treatments and up to Day 100 in the unincorporated peanut treatment.

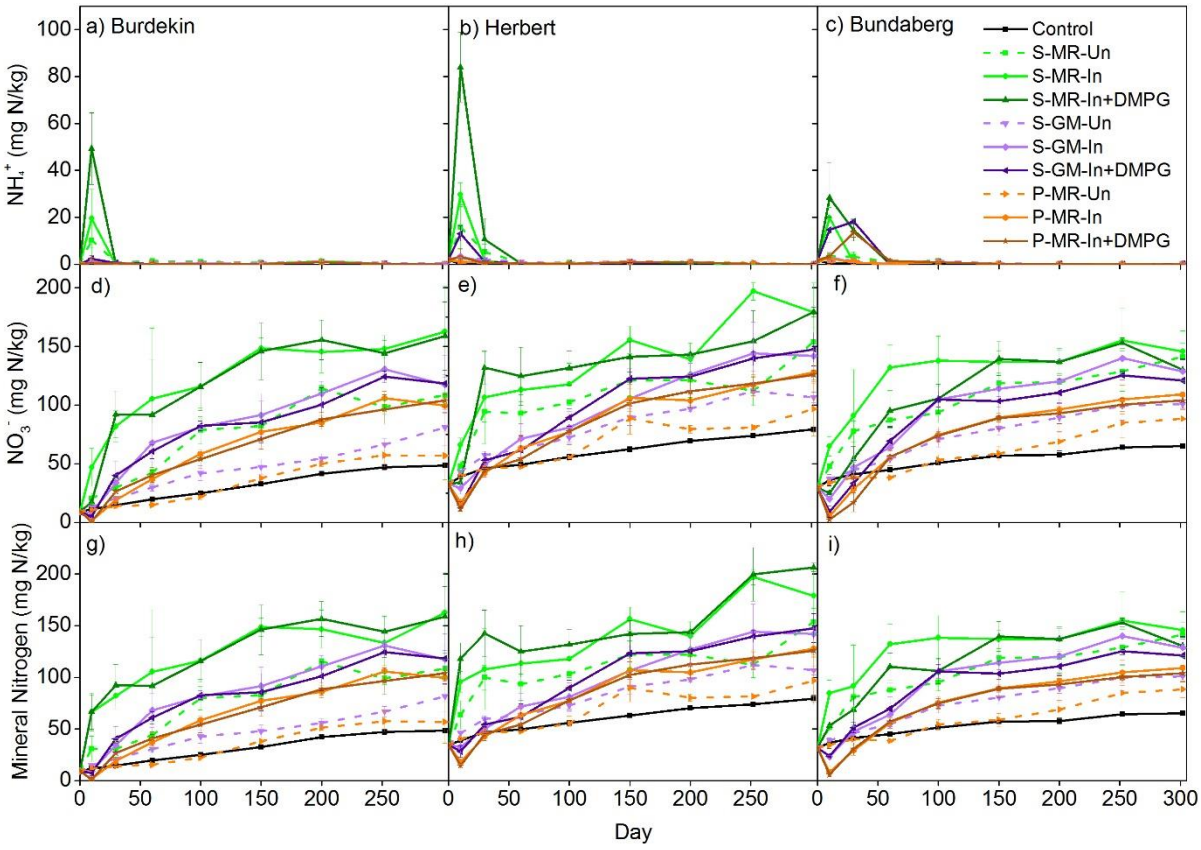

Figure 19. Mineral N dynamics (mean ± SD kg N/kg soil) in three soils applied with different legume residues and different placements, over 302 days of incubation. S: soybean; P: peanut; MR: mature residue; GM: green manure; Un: unincorporated; In: incorporated; In+DMPG: incorporated with nitrification inhibitor applied.

Cumulative mineral N in soil (Fig. 19) and net N mineralisation from legume residues (Table 13) increased evidently in the order of peanut residues < green manure soybean residues < mature soybean residues. The mature soybean mineralised 2.3 and 1.6 times more N than peanut and soybean green manure residues, respectively, over the 302 days (Table 13). There was a clear trend that net N mineralisation from the legume residues increased with decreasing C:N ratio or increasing total N content in the plant materials.

A C:N ratio of between 20-40 is generally regarded as the switch over point between net immobilisation and mineralisation (Cabrera et al. 2005). The C:N ratio of mature soybean (C:N ratio:

17) used in the current study fell below this range, which might explain why mature soybean exhibited net mineralisation continuously throughout the 302 days. In comparison, the other two legume types had C:N ratios within 20-40, which could result in initial net N immobilisation before net mineralisation. Similar trends were observed when net N mineralisation was expressed on a residue N basis and on a dry soil basis (Table 13). This indicates that it was not only the high N content that caused the increased net N mineralisation, but also other residue quality factors. A review by Cabrera et al. (2005) highlighted groups of compounds (e.g. polyphenols, proteins and lignin) that further affect residue mineralisation rates in addition to the C:N ratio.

Table 13. Net crop residue N mineralisation, CO_2 and N_2O flux during 302 days of incubation.

Factors	Net N mineralisation from legume residue		Net Carbon mineralisation	Cumulative N_2O flux
	mg N/kg soil	mg/g residue N	mg CO_2-C/kg soil	µg N_2O-N/kg soil
		*Means of individual effects**		
Soil				
Burdekin	63.5	289	2098[a]	611[c]
Herbert	60.2	278	2583[b]	562[b]
Bundaberg	53.8	279	2868[c]	345[a]
Residue				
Soybean mature	86.6[c]	345[c]	2668[b]	1087[b]
Soybean green manure	54.0[b]	273[b]	2481[a]	237[a]
Peanut mature	37.0[a]	228[a]	2400[a]	193[a]
Management				
Unincorporated	39.6[a]	183[a]	2739[b]	256[a]
Incorporated	70.4[b]	338[b]	2398[a]	754[b]
Incorporated+DMPG	67.6[b]	325[b]	2413[a]	507[b]
		Source of Variation (P value)		
Soil	0.09	0.83	<.001	<.001
Residue	<.001	<.001	0.01	<.001
Management	<.001	<.001	<.001	0.001
Soil x residue	0.406	0.736	0.17	0.573
Soil x management	0.02	0.034	<.001	<.001
Residue x management	0.856	0.263	0.131	0.028
Soil x residue x management	0.831	0.923	0.616	0.341

*After subtracting values for the Control; **Within a column and subheading, values followed by the same letter are not significantly different at $P < 0.05$. Where there is no statistical difference between any means, lettering is not shown.

Although net N mineralisation was evident in the mature soybean treatment throughout the incubation, immobilisation of initial soil mineral N still occurred (Fig. 20). On average across soils and placements, mature soybean had 17% of the initial soil $^{15}NO_3^-$ immobilised in the soil and plant residues at day 302. In comparison, green manure soybean and peanut residues had 31 and 41% of the initial soil $^{15}NO_3^-$ immobilised, respectively. It is evident that the legume residues with higher C:N ratios not only had lower net N mineralisation, but also had significantly higher immobilisation of initial soil $^{15}NO_3^-$. The implication for the significant increases in immobilisation with increasing C:N ratios in legume crop residues is that a large proportion of any pre-existing soil inorganic N will

potentially not be available for the sugarcane crop within 302 days. Therefore, whether a residue is sprayed out and used as a green manure or left until maturity to harvest grain, could greatly affect the N susceptibility to loss and availability to the following crops. Franzluebbers *et al*. (Franzluebbers et al. 1994) also found mature cowpea mineralised more than double green manured cowpea, due to greater N contents and more rapid mineralisation. These outcomes should be taken into account when making decision on fertiliser application rates following legume crop rotation.

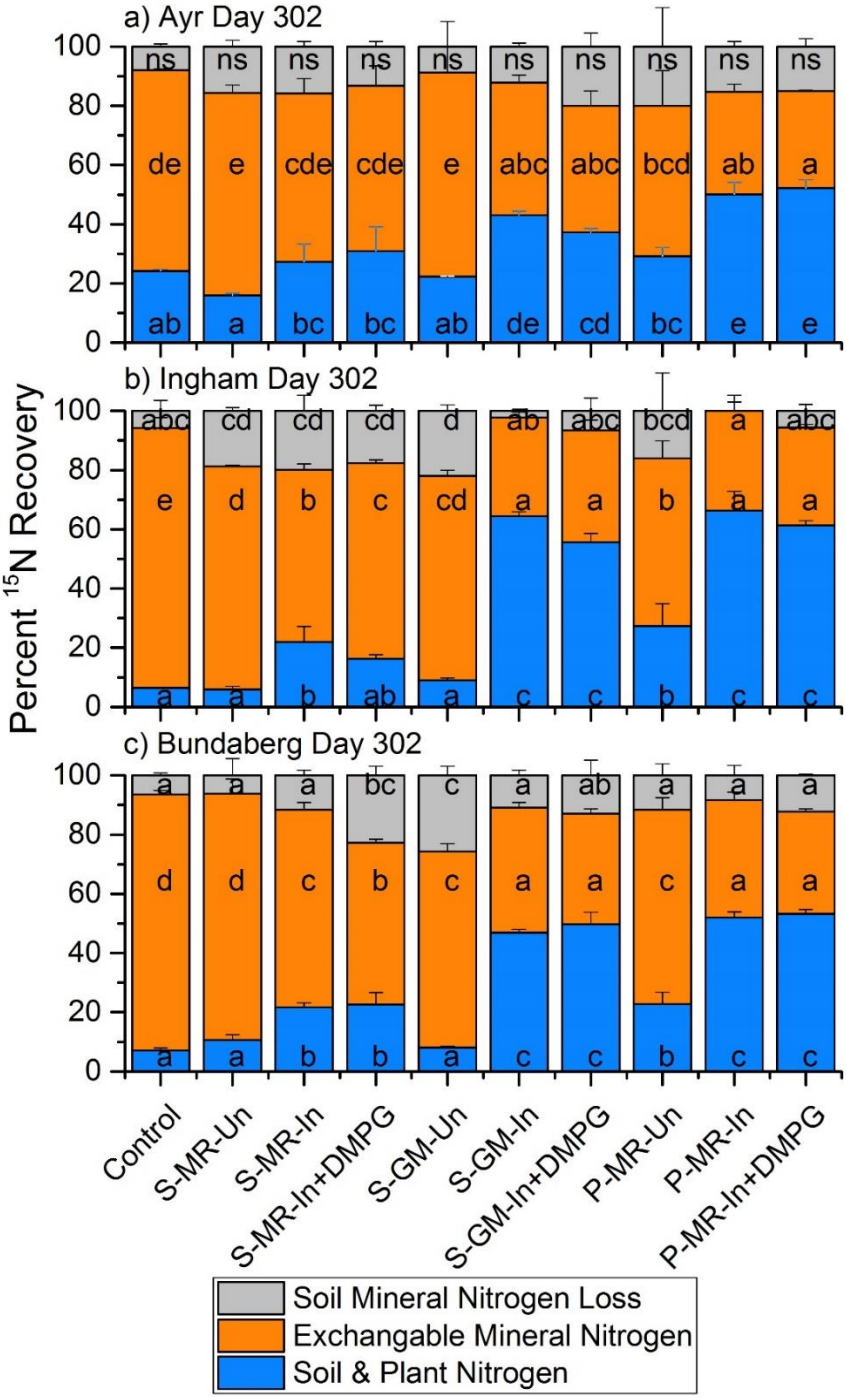

Figure 20. Percent recovery of $^{15}NO_3^-$ in the crop residue and non-exchangeable soil N pool and exchangeable mineral N pool in three soils at the end of a 302-day incubation. Loss of $^{15}NO_3^-$ was calculated as the percentage of $^{15}NO_3^-$ unrecovered in soil and crop residues. Within a $^{15}NO_3^-$ pool, treatments with the same lettering were not significantly different at $P < 0.05$. S: soybean; P: peanut; MR: mature residue; GM: green manure; Un: unincorporated; In: incorporated; In+DMPG: incorporated with nitrification inhibitor applied.

6.4.2. Effects of legume residue placement: incorporation vs. surface retention

Residues retained on the soil surface are generally reported to mineralise more slowly than incorporated residues due to less contact with soil and microbes (Garside & Berthelsen 2004). Averaged across different soils and residue types, the unincorporated legume residues had significantly lower (39.6 mg N/kg) net N mineralisation compared with incorporated treatments (In: 70.4 mg N/kg; In+DMPG: 67.6 mg N/kg; Table 19). N_2O flux exhibited a significant ($P < 0.05$) interactive effect of residue type and management, with only the mature soybean releasing significantly higher emissions when incorporated (1839 µg N_2O-N/kg) than unincorporated (387 µg N_2O-N/kg), while the green manure soybean and mature peanut treatments emitted similar amounts of cumulative N_2O whether incorporated or unincorporated (161-235 µg N_2O-N/kg; Table 19). If cane is not planted shortly after incorporation of the legume crop residues in a paddock situation, the accumulated inorganic N in the soil would also be prone to leaching or denitrification (Garside & Bell 2001). Thus, incorporation of the legume residues should be avoided in such situation.

As well as higher residue N mineralisation rates, there was increased immobilisation of initial soil $^{15}NO_3^-$ when the legume residues were incorporated (Fig. 20). At the end of the 302-day incubation, between 1.7 and 7.2 times more initial soil $^{15}NO_3^-$ was immobilised in incorporated treatments than unincorporated treatments. This suggests that a large proportion of soil mineral N present at the time of incorporation can be immobilised for a significant length of time into the cane crop cycle.

Carbon mineralisation initially proceeded at a slower rate in unincorporated treatments, compared to incorporated residues (Fig. 21). However, from approximately day 50 onwards the C mineralisation rate was higher in unincorporated than incorporated residues. Overall, this resulted in higher total C mineralisation from the unincorporated residues after 302 days of incubation (Table 19). These observations might be due to: (1) higher C incorporation into microbial biomass and soil organic matter during the initial phase of rapid C mineralisation in the incorporated treatments, therefore reducing the overall C respired at the late stages in these treatments; and/or (2) fungi requiring aerobic conditions were able to decompose more recalcitrant carbon in the remaining residues near the surface of the soil (Mosier et al. 2017).

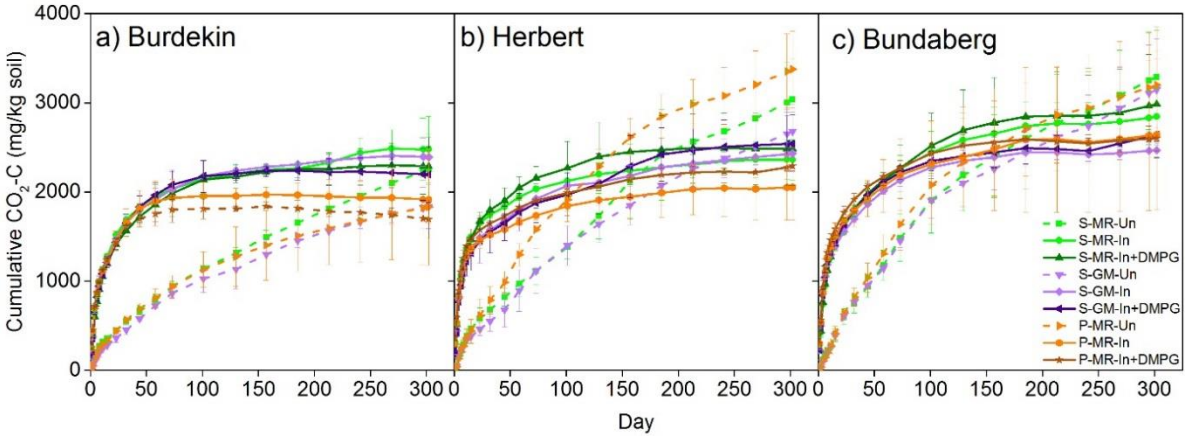

Figure 21. Cumulative C mineralisation (mean ± SD) from three soils applied with different legume residues and different placements. S: soybean; P: peanut; MR: mature residue; GM: green manure; Un: unincorporated; In: incorporated; In+DMPG: incorporated with nitrification inhibitor applied.

6.4.3. Effects of nitrification inhibitor spray onto legume residues before incorporation

The nitrification inhibitor applied on incorporated residues affected both nitrogen mineralisation dynamics and N_2O flux from residues, although it had no effect on the total net N mineralised the end of the experiment (Fig. 19; Table 13). Nitrification was effectively reduced by the inhibitor in all soils for at least 10 days after incorporation. At day 10, treatments with a nitrification inhibitor

applied to legume residues had a higher proportion of mineral nitrogen as NH_4^+ (22-74%) compared with incorporated residues without an inhibitor (6-31%). By Day 30 the effect of the nitrification inhibitor was only present in the Bundaberg soil, where 21-44% of the mineral nitrogen was in the NH_4^+ form when an inhibitor was applied, compared with 1-3% without an inhibitor. The prolonged effect of the nitrification inhibitor in the sandier Bundaberg soil, supports other studies that have found higher sand contents increases efficacy of the nitrification inhibitor (Barth et al. 2001).

The increased NH_4^+ concentration could potentially reduce N leaching and denitrification. The addition of a nitrification inhibitor significantly reduced N_2O flux from the incorporated mature soybean treatment in the Ayr soil, but there was no significant effect in other cases. By maintaining N in the NH_4^+ form, NO_3^- was effectively reduced, therefore there was less substrate for denitrification and associated N_2O emissions. This was probably because the green manured soybean and mature peanut both produced net N immobilisation soon after incorporation, reducing the N available for nitrification and perhaps subsequent denitrification during this time period when most N_2O emissions occurred over the 302 days. In comparison, soil with mature soybean incorporated had net N mineralisation directly following incorporation, providing higher concentrations of inorganic N for nitrification and perhaps denitrification. Where residue incorporation may likely cause initial net N immobilisation, the benefits of applying an inhibitor may not be significant, compared with residues that cause net N mineralisation.

The supply of N is only one factor required for denitrification to occur in soil, the others are a C supply and anaerobic conditions. In this study excess bio-available C was supplied by the residues. However, it is often assumed that anaerobic conditions do not prevail in soil at 50% WHC. With the addition of residues, the microbial community could proliferate quickly, consuming oxygen and likely creating anaerobic microsites within the soil, where denitrification could occur. The rapid proliferation of the microbial population was evidenced by the rapid increase in C mineralisation in the incorporated treatments at the beginning of the incubation (Fig. 21). Most incubation studies examining plant residue N mineralisation did not consider possible N loss from denitrification, and therefore N mineralisation could be underestimated. Furthermore, whether or not in-situ residue incorporation creates anaerobic conditions can be affected by soil properties and conditions such as texture and water contents, and needs to be investigated.

7. CONCLUSIONS

Growing soybean break crops during the fallow period between two sugarcane cropping cycles could add 70-280 kg N/ha to the cropping systems. The amount of soybean biomass N increased with increasing length of growth. Therefore, as long as site, weather and operations permit, the cropping season should be extended by sowing early and harvesting or spraying out late. About 74-88% of the soybean biomass N derived from biological N_2 fixation and 22-26% from recovery of soil N. The N inputs from soybean residues significantly reduced or eliminated N fertiliser N application, thus saving on fertiliser cost by $75-215/ha.

There was clear evidence that substantial amounts of mineral N (predominantly NO_3^-) in bare soil accumulated during the fallow period were lost following rainfall. Growing legume crops significantly decreased accumulation of mineral N in soil due to plant N uptake. Thus, legume crop rotation provided an effective means to retain soil mineral N in plant biomass. In addition, 16S DNA analysis indicated that abundances of soil microbes were significantly higher in soybean or peanut cropping soil than in bare fallow soil at the maximum biomass stage. However, abundances of nitrifying archaea and bacteria and the *amo*A gene that encodes the enzyme responsible for the first step of nitrification were significantly lower in the legume cropping soil than the bare soil. Further research is required to assess long-term effects of legume cropping on soil microbial community and nitrification under field conditions.

With grain or pod harvest, mature soybean crop residues contained 3 times more N (280 kg N/ha) than peanut residues (88 kg N/ha) at Bundaberg. However, farming profit was markedly higher for growing peanut than soybean thanks to higher market values of peanut pods. However, if circumstances do not allow the legume crops to grow to maturity (taking approximately 5 months for soybean and 6 months for peanut), soybean is a better green manure crop because of its greater N benefit.

The litter bag incubation study under field conditions demonstrated that rapid N release from soybean residues occurred in the first 2-3 months. About 73% and 43% of the total residue N were released from the crop residues within two months after incorporation into soil and placement on the soil surface, respectively. Therefore, N-efficient management of legume residues is critical during the early months after harvest or spray out of the legume crops. However, the laboratory isotope tracing study indicated that 45-65% of the initial soil NO_3^- (generally the major form of mineral N) was immobilised into non-exchangeable soil N pools 302 days after incorporation of legume residues, compared to 7-24% in the control without legume residues. Only 23% of the peanut residue N and 27-35% of soybean residue N (depending on C:N ratios) were found in the soil mineral N pool after 302 days of incubation at 25 ^oC and optimal soil moisture. These findings have implications for estimating plant-available N from the legume residues, and care should be taken to avoid overestimation of N supply from the legume residues.

Results from laboratory incubation, field litter bag incubation and field soil sampling consistent indicated that leaving legume residues on the soil surface by practising no-till could slow down N mineralisation compared to incorporating crop residues into soil with tillage. Therefore, if sugarcane is not planted for a prolonged period and high rainfall is expected after legume crop harvest or spray out, no-till provides an effective management strategy to slow down N mineralisation thus reduce the risk of soil mineral N loss. In cases where cane is planted shortly after the legume cropping, tillage can improve N supply to the cane crop and may benefit crop growth. Selection of tillage method need to take into account seasonal conditions, time and practicality.

Spraying nitrification inhibitor onto legume residues before tillage could slow down conversion of ammonium to nitrate for at least 10-30 days. However, this did not result in significant improvement of cane or sugar yield and crop N uptake.

Allowing volunteer soybean to re-grow after grain harvest as N-catch crops significantly decreased mineral N accumulation in soil profile and thus could potentially reduce the risk of N loss. This technique also resulted in a non-significant increase in sugar yield by 2 t/ha. Growing mungbean during the early months of sugarcane cropping season at Burdekin did not have any benefits to the cane growth and productivity. Therefore, efficacy of N-catch crops may depend on the length of time between legume crop harvest/spray out and cane planting, species of the N-catch crops, N mineralisation from soil and previous legume crop residues as well as site and weather conditions. If sugarcane cannot be planted for a prolonged period during a high rainfall season, allowing the volunteer legume crop to grow or delaying spray out of the legume crop for green manure can minimise the risk of N loss.

8. DECISION TREE

Based on existing knowledge, previous research and findings in this project, a decision is given below with an aim to assist with selection of fallow and legume crop residue management practices. Broad consultation will be made with growers, extension officers and researchers to further improve the suitability and practicality by taking into account circumstances in different regions.

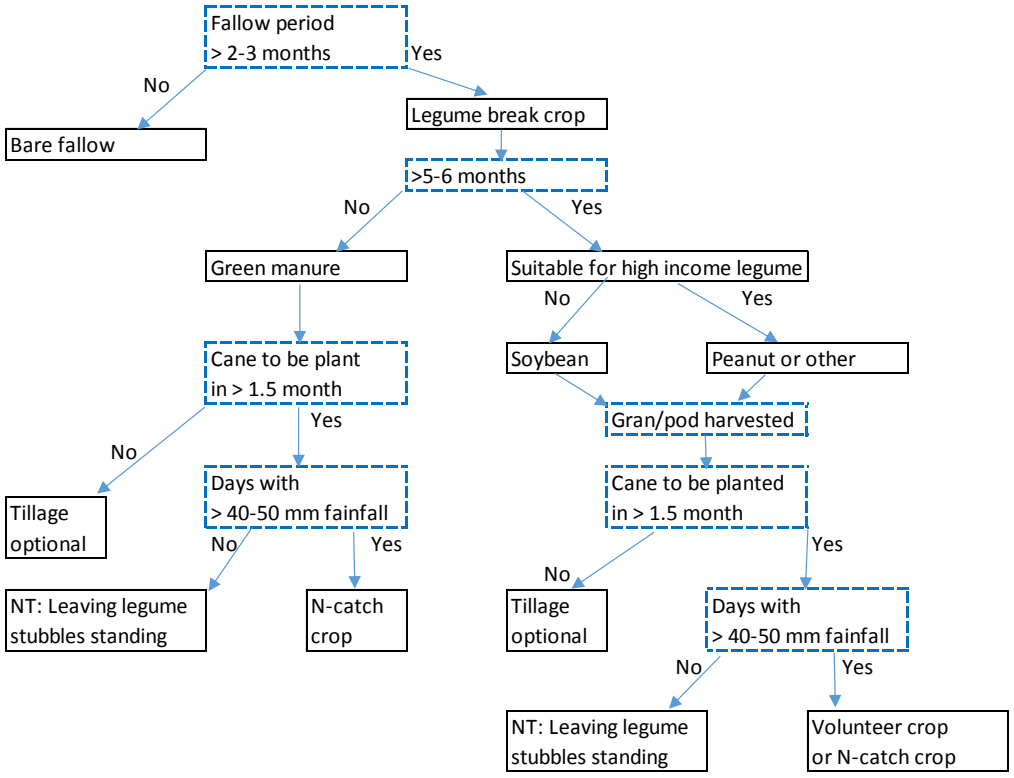

Figure 22. A decision tree to assist in fallow and legume residue management.

9. PUBLICATIONS

Paungfoo-Lonhienne C., Wang W., Yeoh Y., Halpin N. 2017. Legume crop rotation suppressed nitrifying microbial community in a sugarcane cropping soil. *Scientific Reports* 7: 16707, DOI:10.1038/s41598-017-17080-z

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Reeves S., Wang W., Heenan M., Halpin N., Mcshane T., Rickert A., Royle A. 2018. Nitrogen mineralisation of different legume crop residues in relation to soil properties, application methods and nitrification inhibitor (under preparation).

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12. APPENDIX

12.1. Appendix 1 METADATA DISCLOSURE

Table 14 Metadata disclosure

Data	All project data
Stored Location	Science Division, Department of Environment and Science, QLD G:\Soil_Processes\WangW\SRA Legume Management
Access	With permission from project leader.
Contact	Dr Weijin Wang Principal Scientist Science Division Department of Environment and Science P (07) 3170 5768 Post: GPO Box 2454, Brisbane, QLD 4001 Email: weijin.wang@des.qld.gov.au